Chapter 4

Cultivating *Spirulina maxima*: Innovative Approaches

Taeho Kim, Woo-Seok Choi, Bo-Ram Ye,
Soo-Jin Heo, Dongkyu Oh, SeongEun Kim,
Kwang-Sik Choi and Do-Hyung Kang

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.74265

Abstract

This chapter reports an annual production of *Spirulina* (*Arthrospira*) *maxima* in Ansan, South Korea (37.287°N, 126.833°E) with temperate four seasons climate for testing industrial application. Construction on pilot plant of semi-open raceway system (ORS) with each 20 ton culture volume has been established in early 2011 based on building information modeling (BIM). An optimized design of pilot culture system for microalgae scale-up culture in temperate area and details of culture was presented. In scale-up trials using two ORSs, the strain displayed satisfactory annual growth under batch condition. In an annual trial, average biomass concentration was recorded at 0.99 ± 0.16 g/L, which showed stable productivity in a year. Maximum concentration was estimated at 1.418 ± 0.09 g/L in August, while minimum production was estimated at 0.597 ± 0.05 g/L in October. Despite insufficient solar radiation and nutrients, ORS was favorable for *S. maxima* production. The technical strategies contribute to the annual production of *S. maxima* in this region: controlling the culture temperature, reducing production cost, and retrospective climatic data-based BIM construction of the greenhouse. Consequently, pilot production of *S. maxima* was feasible in Korean climates, a region previously thought to be outside its geographic limits.

Keywords: *Spirulina* production, temperate climate, BIM-based pilot plant construction, Korea

1. Introduction

Spirulina (*Arthrospira*) is a 3.6-billion-year-old cyanobacterium and inhabits alkaline aquatic ecosystems from freshwater to seawater [1, 2]. As sustainable primary producers, the cyanobacteria have been studied on various aspects centered by bio-industry and environmental

bioremediation [3–6]. *Spirulina* that highly contains various functional materials has been focused as an important nutrition source for the future of humans and animal feeds, so that related industry has been gradually growing [2, 7, 8, 12]. In addition, the biomass plays an important role in broad ranges of industries [9, 10] and functional foods using proteins [3, 11]. In particular, *S. platensis* has been mostly studied for industrial production, in which the most important factors for an increase of production could be nutrient concentration, light intensity, and optimal water temperature [12]. *Spirulina* sp. including other microalgae are cultured on a large scale worldwide for industrial use [13–17]. Proteins and phytopigments (phycocyanin and β-carotene) along with polyunsaturated fatty acids from *Spirulina* are one of the high value-added materials that bring health to humans [2, 13, 18].

Nowadays, microalgae are mostly cultured in photobioreactors (PBRs) and open raceway systems (ORSs) for industrial production [2, 19, 20]. Advantages of PBR include applicability of various designs in production, easy control of growth condition, prevention of biological contamination, and high productivity. On the contrary, it requires high expenses in initial investments, device maintenance, and expansion of mass production facility [20–22]. In contrast, ORS can directly use solar energy and CO_2 in the air though it has a low aerial productivity than PBR, and it is also advantageous due to inexpensive materials for facility (PVC, FRP, concrete, plastic, and soil) and easy to scale up structure. In respect of commercialization, ORS needs a low initial investment cost, while it has a highly efficient productivity, so that ORS has been attracting more interest [15, 20, 23–30]. However, there are still steps that need to be taken for the commercialization of ORS, which includes a control of water temperature and light intensity depending on season, elevation of aerial productivity, development of highly efficient microalgae species, development of low cost and highly efficient culture medium, technology of contamination improvement, and establishment of protocol for year-round culture and harvest [19, 20, 31–33].

Culture conditions of microalgae in ORS and designing of system are closely related with environment of selected area. *Spirulina* was the most widely used in outdoor cultivation trials and interests for commercial production have been increasing in many places based on the studies of intensive ecological and physiological research and development over four decades. However, this cyanobacterium needs high temperatures for optimal growth, thus commercial production has been limited until subtropical areas. In temperate regions, high temperatures are recorded in summer season, while the temperature is certainly low in fall and winter seasons. The overall temperature range should not be a suitable range for *Spirulina* growth in a year. Consequently, little information of commercial production has been reported in temperate areas [32, 34, 35]. Thus, an environmental analysis needs to be carried out on selected area for a culture system with maximal productivity based on which culture system needs to be constructed. Recently, the concept of building information modeling (BIM) developed in construction field was first introduced by the National Institute of Standards and Technology (NIST) of the Department of Commerce in the USA in 2007. BIM refers to a recent construction process that analyzes data with 3D modeling method using big data and information of the past and then applies it from designing to installation process, avoiding existing 2-D design. Therefore, BIM technology is advantageous due to cost saving for installation operation and prediction of future [36–38]. As shown in success cases such as the Disney Concert Hall of the US, the Olympic Main Stadium of Beijing, and Melbourne Recital Center, BIM technology has emerged as an important issue in architectural industry, so that it is considered beneficial to apply it to a microalgae production facility.

From 2008, small-scale (laboratory to 1.5 ton) experiments have been conducted to investigate the biomass production combined with culture conditions and low-cost medium of *S. platensis* and *Spirulina maxima* in Korea [33, 39, 40], but further progress was not reported. In 2011, the first pilot-scale *Spirulina* plant with semi-open raceway ponds was established in Ansan [41]. The purpose of this chapter is to present for construction technology of BIM-based ORS allowing year-round culture in the environment with four seasons, so that an ORS was constructed in a glass greenhouse structure and a culture study was performed on a pilot scale (173.5 m^2). In addition, in order to verify sustainability of the system, year-round biochemical quality analyses on year-round batch culture of *S. maxima* that has a characteristic of strong alkaline culture and on produced-dried biomass were performed at the same time.

2. System construction and strategies for culture plan

2.1. The production site

The city of Ansan was selected for the pilot study for production of *Spirulina*. Ansan is located in Western South Korea (37.287°N, 126.833°E), about 30 km south of Seoul, on the coast of the Yellow Sea. Recent average temperatures generally exceed 30°C in summer, and in winter are above −5°C. The average temperature is 12.5°C, and number of sunny days per year generally ranged from 86 to 124, with >10 h of sun per day for the whole year. Mean monthly temperatures are shown in **Figure 1A**. **Figure 1B** shows that weekly average solar radiation ranged from 1721 to 5671 Wh/m^2. From 2006 to 2010, the number of rainy days per year ranged from 99 to 138, and average annual rainfall was about 1150 mm in the locality of the cultivation area.

2.2. Construction of culture system and its structure

Figure 2 shows schematic processes for planning and construction of *Spirulina* culture system using BIM technology. A pilot system for microalgae production was constructed in the Korea Institute of Ocean Science and Technology based on BIM technology which analyzes atmospheric environment data (temperature, solar radiation, and shadow effect etc., **Figure 3**) of the past for a long time, and predicts the future based on the data in order to design an optimal eco-friendly structure.

Figure 4A is a vertical section of microalgae pilot system constructed based on BIM, in which the roof and side windows were designed with a maximal consideration of natural ventilation, and optimal construction cost and efficiency was realized by a four-way slide window at the side and the introduction of automatic opening and shutting system on the roof. **Figure 4B** is a horizontal section of the modified ORS culture facility. Each size of the culture facility was 10,000 (W) × 3250 (L) × 550 (H) mm, and the culture raceway was finished with concrete after vertical excavation of the ground as deep as 600 mm for the purpose of using geothermal heat as shown in **Figure 4**. Depth of medium for the culture was maintained as 400 mm. Boiler pipes were buried in the concrete floor of the modified ORS for maintenance of culture temperature in the winter.

Computational fluid dynamics (CFD) was conducted to analyze and optimize the mixing (e.g., water flow and paddle rotational speed) of medium with *S. maxima* cells in the ORSs. The main purpose is to find the suitable rotational speed of the paddle to maximize mixing in the flow field.

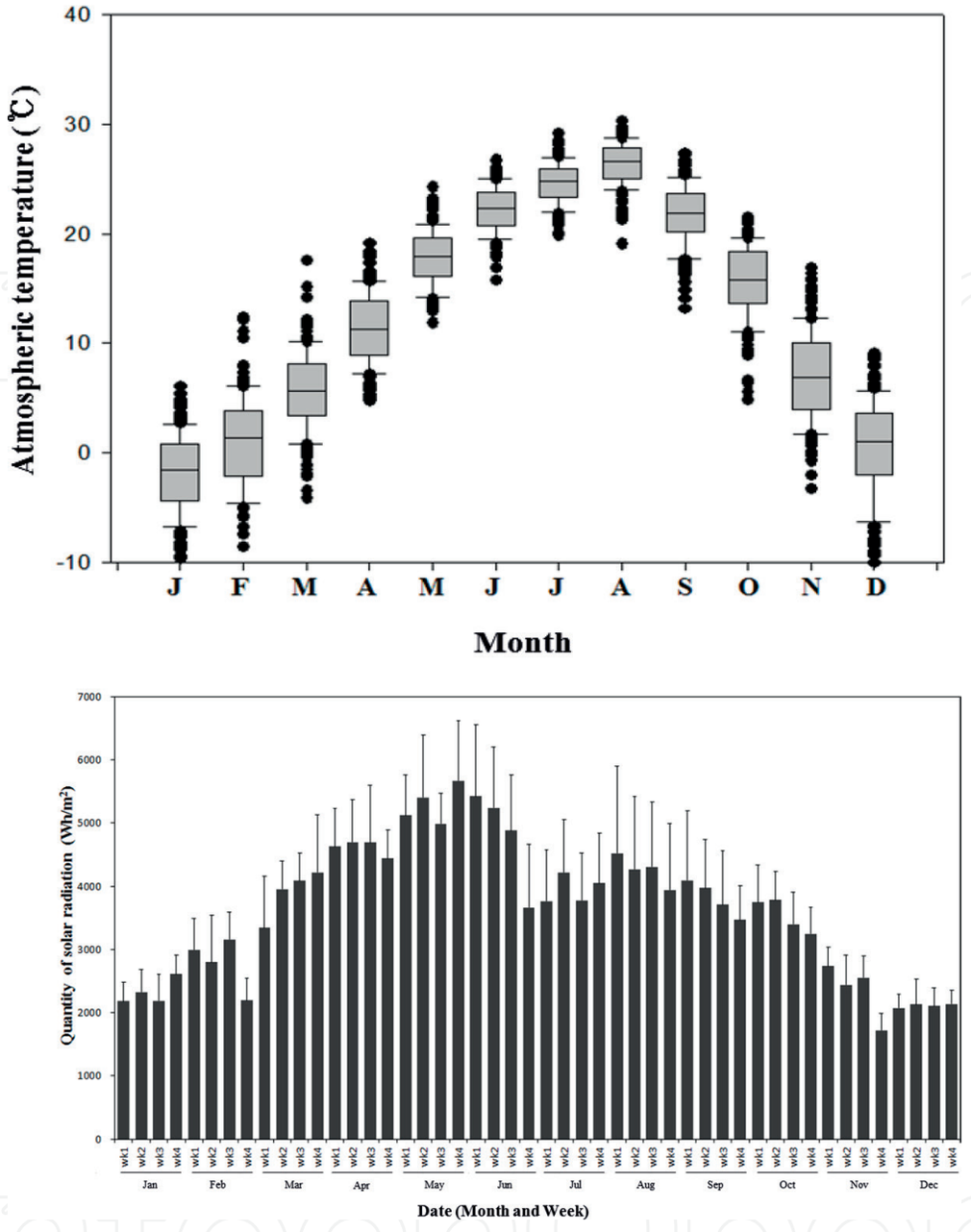

Figure 1. Mean monthly temperature (A) and integrated weekly solar radiation (Wh/m^2) (B) at the location of the study for past 5 years during 2006–2010.

The commercial software Flow-3D (version 10.0) was chosen for this analysis. The simulation was performed by ARA Consulting & Technology Co., by applying two rotational speeds (15 and 30 rpm) (**Figure 5**). As a result, rotational speed was selected as 15 rpm in the ORSs. Simplified structures of the ORS and the greenhouse for *S. maxima* cultivation were shown in **Figure 6**.

2.3. The microorganism, experimental design, and culture conditions

The axenic culture of *S. maxima* Cy-23, was obtained from Korea Marine Microalgae Culture Center (Pukyong National University, Busan, Korea) and was initially inoculated in a 5 L conical flask containing modified *Spirulina* medium [42] at plant room temperature (23–30°C). Thereafter, secondary culture with scale up was performed in a 300-L PE circular cylinder

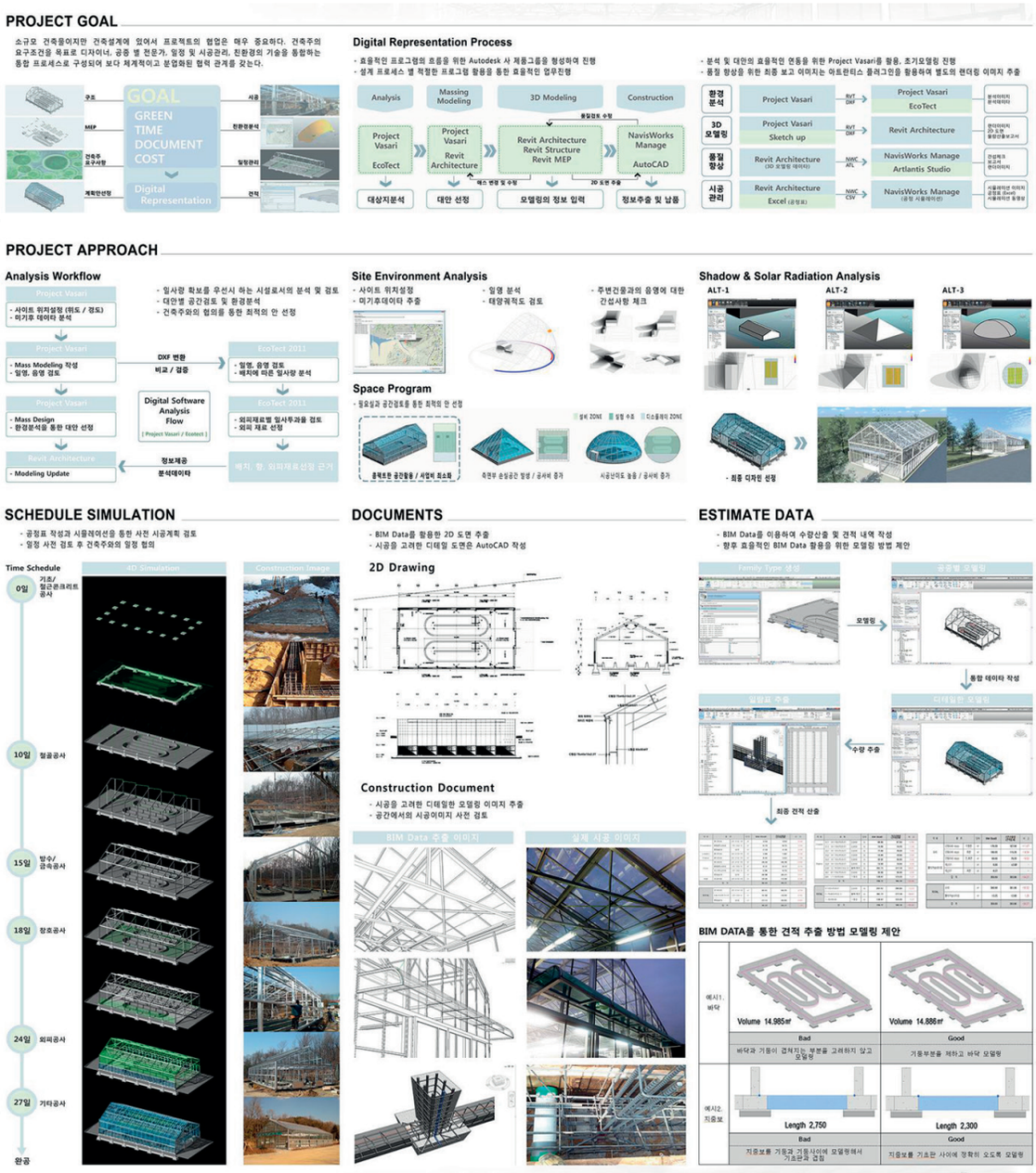

Figure 2. Schematic processes for planning and construction of *Spirulina* culture system using BIM technology.

raceway on the 10th day when the culture concentration of *S. maxima* reached to 0.5–0.8 g/L. Once concentration of secondary culture reached to 0.5–0.8 g/L on the 17th day, 20 tons of each culture medium with the same composition was made and added to the modified ORS. After 5 h, 300 L of sample from the secondary culture was inoculated. The rotational speed of the paddle wheel that circulates and mixes the culture medium was kept at 15 rpm. Batch culture was performed in the ORS for about 1 year from April 4, 2011 to March 16, 2012.

2.4. Maintaining culture system

The electric boiler that was installed for maintenance of temperature in the winter was operated from late October to early April to heat the system electrically and to maintain optimal

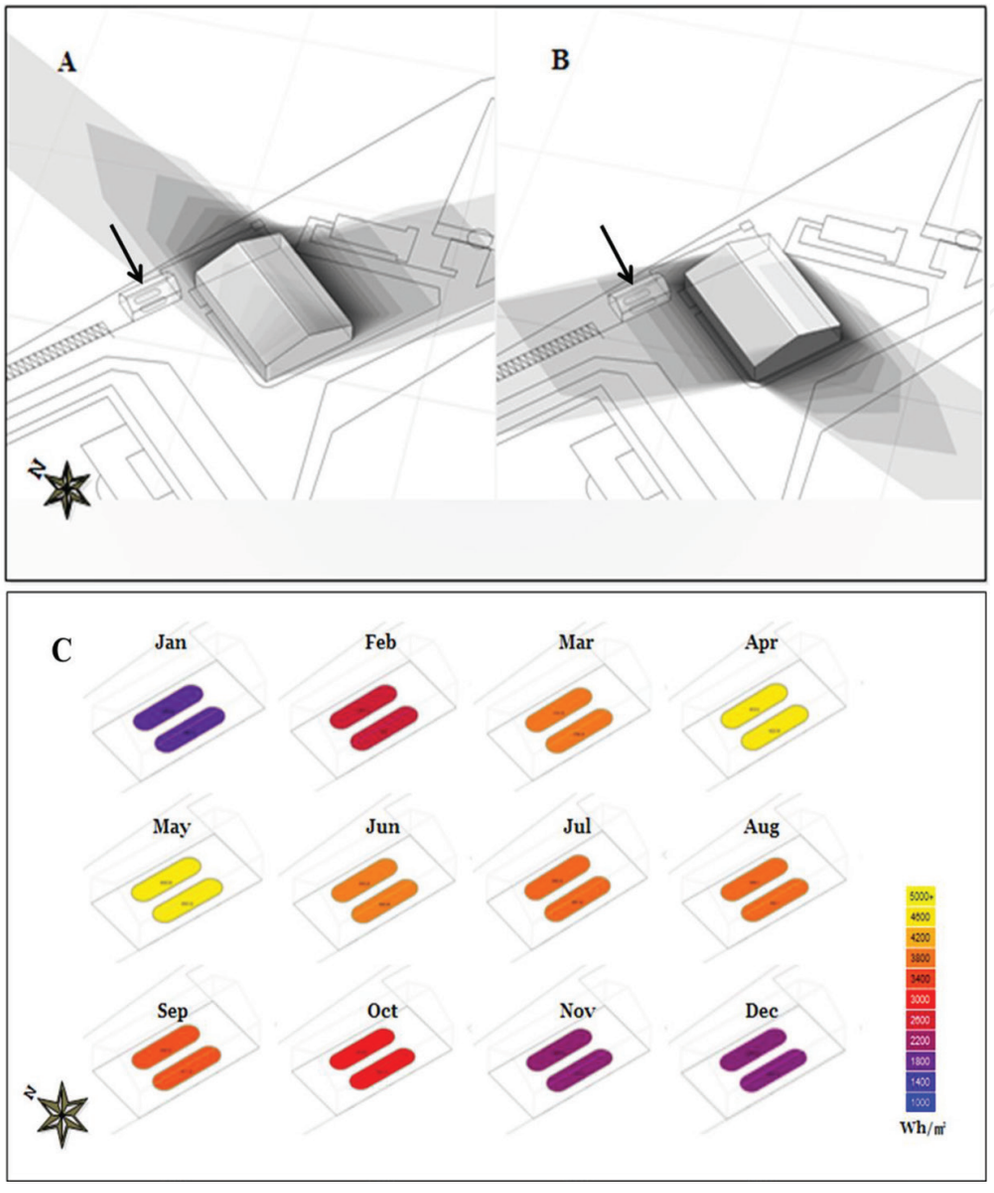

Figure 3. The retrospective analysis of BIM based 3D modeling for visualization of shadow area at summer (A) and winter (B) solstices and estimation of monthly solar radiation of two raceway pond (C) at the location of the study for past 5 years during 2006–2010. Arrows indicates the location of culture area.

water temperature. The temperature of the culture medium was maintained between 20 and 25°C, and the water temperature for October when its operation was initially started was in the range of 21–23°C. Total electricity consumption during the period of boiler operation was measured by using an electronic watt-hour meter (LD3410CT-005Te). The boiler operation was set as a variable type depending on variation in water temperature (on 30 min, off 30 min), and the maximum operation rate (on 50 min, off 10 min) was used to minimize fluctuation of water temperature during the period of rapid drop in room temperature in the winter. Daily measurement values were summed every week and expressed in mean value ± standard deviation for an analysis of each environment and electricity consumption data.

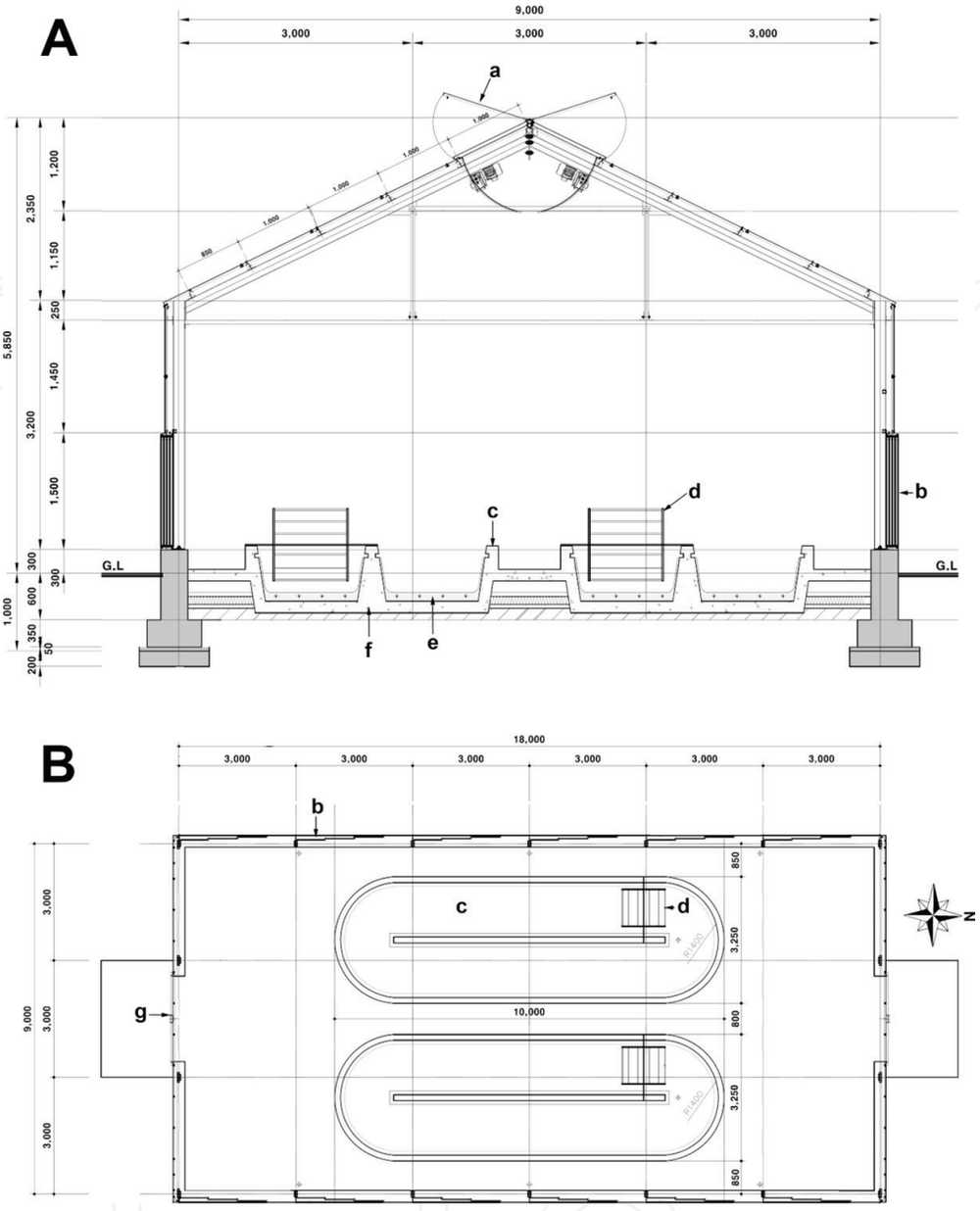

Figure 4. A cross sectional view of the designed open raceway system for pilot production of *Spirulina maxima* using BIM analysis (patent no. 10–1,142,358, 10–1,142,359, 10–1,110,068). A: Vertical view of the system; a: Automatically opening and shutting window, b: 4-way sliding windows including anti-insect nets, c: Raceway pond, d: Paddle-wheel, e: Electronic boiler pipe, f: Concrete structure. B: Horizontal view of the system; main gate (g) has located to the south.

2.5. Biomass concentration, harvesting, and productivity

Before harvesting, the biomass concentration was estimated by sampling and filtering 20 mL of each culture using a vacuum pump and GF/C filter paper (Whatman), and the filter paper was dried in a dry oven (65°C) for 24 h, followed by the measurement of biomass. Biomass was measured two times a week. For harvesting, cultured *S. maxima* samples in 3 tons of culture medium were harvested by using a Tubular Separator (GQLY series) at 15,000 rpm for 3 h in an interval of a week. Harvested samples were kept in a −50°C deep freezer (DEASAN) for a biochemical analysis and then freeze-dried by using a freeze dryer (ILSHIN).

Areal productivity was calculated by using the following equation. Data used for analysis were monthly mean value (mean value ± standard deviation) by adding the weekly measurement values.

$$\text{Areal productivity (AP, g/m}^2\text{/d)} = \text{Volumetric productivity (VP)} \times (\text{Odepth} \times 1000) \quad (1)$$

where Odepth is the pond operating depth.

2.6. Analysis of biochemical components and pigments

The crude biochemical composition of cultured *S. maxima* was determined according to the AOAC method [43]. Crude carbohydrate was determined by the phenol–sulfuric acid reaction, crude lipid was extracted by the Soxhlet method, and crude ash was prepared at 550°C

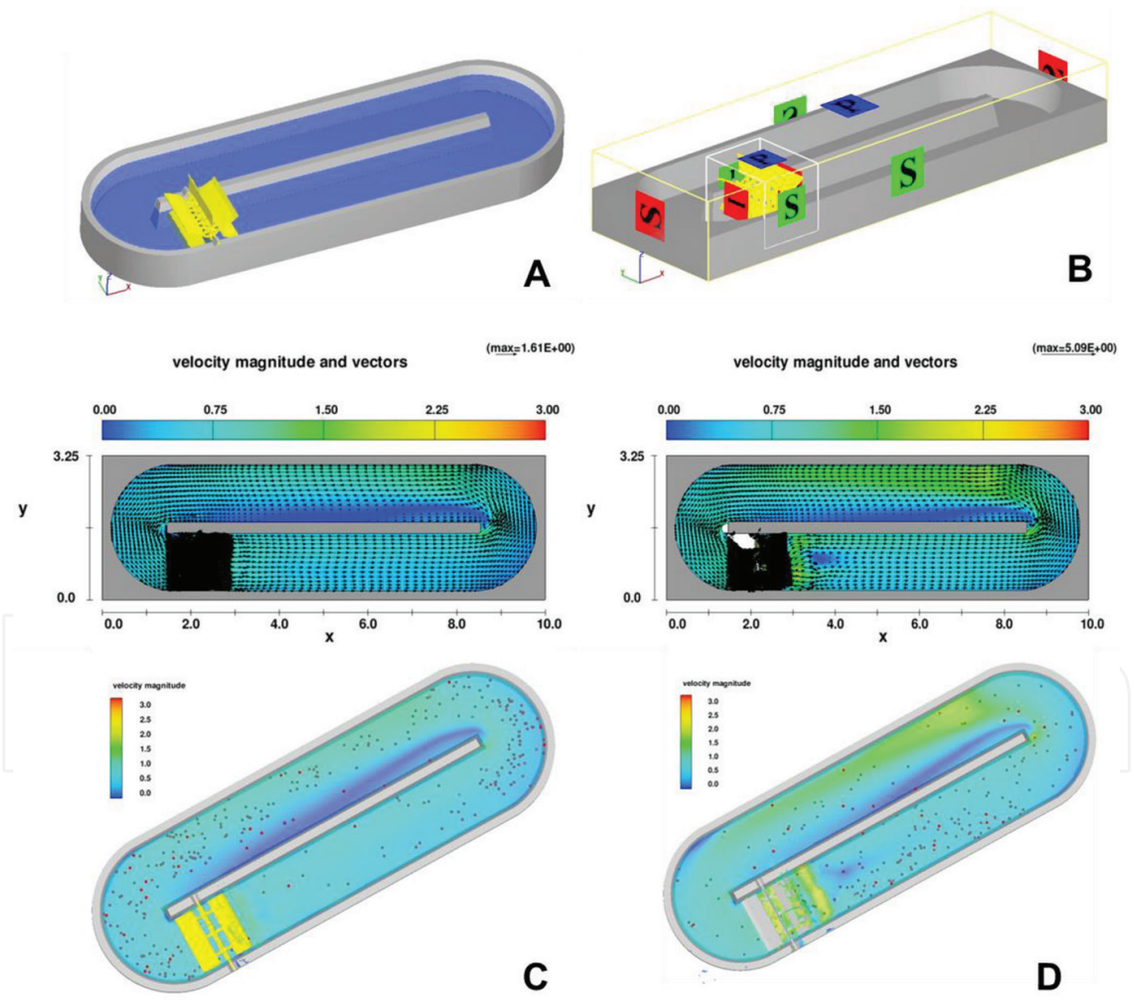

Figure 5. Summarized results on mixing simulation of medium with *S. maxima* cells in the ORSs by applying CFD of flow-3D software. A: 3D applied raceway pond (10 m in length, 3.25 m in width and 0.6 m in depth) with paddle, B: Interface condition of boundary line by interactive boundary layer modeling techniques, C and D: Velocity magnitude and particles maps at 60 second after operation for various aspect of particles with different rotational speeds (C: 15 rpm, D: 30 rpm).

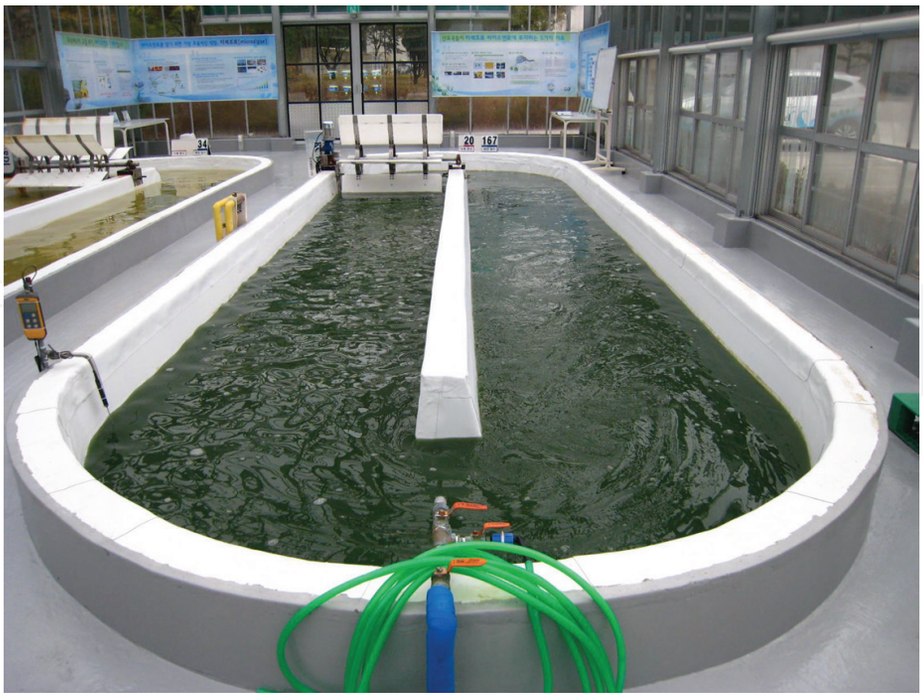

Figure 6. Simplified inner structure of ORS and the greenhouse for *S. maxima* cultivation at Ansan City, KIOST. The number of 20 was harvesting time and the number of 167 was the days after inoculation. Photographed by Taeho Kim in 2011.

in the dry-type furnace. The moisture was determined by keeping the sample in a dry oven at 105°C for 24 h and the crude protein was determined by the Kjeldahl method. Unfortunately, the samples of November and December 2011 were denatured by sudden trouble of refrigerator for biochemical and pigments analysis.

For phycocyanin analysis, dried *S. maxima* powder was weighted accurately to 50 mg, and then mixed with 10 mL sodium phosphate buffer (pH 7.0) in centrifuge tube. The mixed sample was sonicated for 1 min and stored in refrigerator overnight maintained at 20°C. The sample was mixed well using vortex and then was centrifuged for 20 minutes at 10°C at 3000 rpm. The supernatants were repeatedly collected until the color came off. After collecting all the phycocyanin, the supernatant was filtered using GF/C filter, and the red absorbency of each replicate was at 620 and 652 nm using phosphate blank buffer with spectrophotometer (Optizen POP bio). Concentration of phycocyanin was then calculated using below formula [44].

$$\mathrm{C-PC\,(mg/L)} = (\mathrm{A_{620}} - 0.474 \times \mathrm{A_{652}})/5.34 \tag{2}$$

$$\text{phycocyanin (mg/g)} = \frac{\mathrm{C-PC}\left(\frac{\mathrm{mg}}{\mathrm{L}}\right) \times \text{buffer volume (mL)}}{\text{sample weight (g)}} \times \frac{1\ \mathrm{L}}{1000\ \mathrm{mL}} \tag{3}$$

For chlorophyll-*a* analysis, dried *A. maxima* powder was weighted accurately at 50 mg and then mixed with 5 mL-90% acetone. The mixed sample was sonicated for 1 min and then stored in refrigerator for 30 min maintained at 4°C. The pigment extracts have to be kept on ice and in the dark. The sample was mixed well using vortex and then was centrifuged

for 20 minutes at 10°C at 3000 RPM. The supernatants were repeatedly collected until the color came off. After collecting all the chlorophyll, the supernatant was filtered using a 0.2 μm syringe filter, and the absorbency of each replicate was at 625, 647, and 664 nm using phosphate blank buffer with spectrophotometer (Optizen POP bio). Concentration of chlorophyll was then calculated using below formula [45].

$$C_a(\text{mg/L}) = (12.62 \times A_{664}) - (2.99 \times A_{647}) - (0.04 \times A_{625}) \quad (4)$$

$$\text{Chlorophyll} - a\ (\text{mg/g}) = \frac{C_a\left(\frac{\text{mg}}{\text{L}}\right) \times \text{acetone volume (mL)}}{\text{sample weight (g)}} \times \frac{1\ \text{L}}{1000\ \text{mL}} \quad (5)$$

2.7. Measurement of climatic and culture conditions

Various parameters of the system were measured on a daily basis including room temperature (TENMARS) and light intensity (Lux Meter TM-205), water temperature (UNIS thermometer), pH (pH METER D-51, HORIBA), and salinity (SALT MATER YK-31SA). Although humidity was not a variable of interest, it seems the humidity was dropping in the plant as the level of medium kept in the plant was consistently lowering by evaporation. Evaporation amount was measured during August when culture medium was highly evaporated. To specify the amount of water being evaporated each day per unit area (m^2), daily evaporation rate was measured and then averaged as $ml/m^2/h$. The amount of evaporation was then supplemented daily with underground tap water in KIOST ($HCO_3$4 6.4 mg/L, Ca 20 mg/L, Cl 13.6 mg/L, SO_4^{2-} 11.4 mg/L, Na 8.64 mg/L, Mg 3.99 mg/L, K 1.97 mg/L, T-N 1.66 mg/L, NO_3-N 1.61 mg/L, T-P 0.02 mg/L, Co, Mo, and B 0 mg/L while Fe, Zn, Cu and Mn were not detected, and pH was 7.3).

A statistical program (IBM SPSS, NY, USA) was used for statistical analysis in order to test significance of environmental factors and pigments and biomass of *S. maxima* (one way ANOVA, Tukey test, *P* values <0.05). Monthly variations among climatic and culture conditions, biomass, biochemical components, and pigment concentrations were statistically analyzed by one way ANOVA with Pearson's multiple range tests at $p < 0.05$ (SPSS version 12.0, NY, USA) for the identification of significant seasonal differences during the study period. All analyses were performed on triplicate samples.

3. A feasibility of *Spirulina* annual production in the area

Disadvantages for *Spirulina* production in this area are not only the average temperature (12.5°C), but putative aerial contamination of the medium by the dusts and microorganisms from reclaimed land in the City. The period of cultivation would be approximately <200 days for growing in open raceway ponds in this region without trials to control the disadvantages. In order to overcome the problems, the most important approach in annual cultivation in this area was to construct greenhouse over the raceway ponds. The greenhouse was made of a framework (mixture of steel and cement) and transparent glasses. The glass greenhouse enhances the culture temperature significantly (**Table 1**) and the growth period prolonged during a year.

The maximum daily medium temperature of the culture was over 20°C and up to 30°C during the period from January to August. **Table 1** and **Figure 7** present year-round change data of radiation amount (μmol/m^2/s), temperature of culture medium, room temperature, salinity, pH, and electricity consumption during the culture of *S. maxima* from April 2011 to March 2012. As for illumination intensity during the study period, the maximum value was 1590 μmol/m^2/s, and the minimum value was 7 μmol/m^2/s. Year-round water temperature of culture raceway for *S. maxima* was between 16.0 and 33.0°C, and mean water temperature was 23.6 ± 3.2°C.

Temperature of culture medium showed a change in a range of 20.2–26.8°C from April 4, 2011 to May 31. The highest water temperature of the year was 33.0°C on July 26 as an effect of increase in outer temperature. In addition, the average evaporation rate in August 2011 was 701 ± 136.4 ml/m^2/h. Water temperature gradually decreased from October, went below 20°C during the second week of December and recorded the lowest water temperature of the year at 16.0°C on February 23, 2012. Culture medium temperature for optimal growth of *Spirulina* was between 35 and 37°C, and should be kept at least at 15–20°C [2]. Since severe air temperature in this area was recorded from −18 to 36°C, and it was below 10°C on average in the winter, the ORS with glass greenhouse was electrically heated by the boiler during the period from late October to early April to maintain at least 15°C, the lowest temperature for *Spirulina* culture. These results should serve as fundamental data for setting temperature and running a boiler in order to maintain optimal medium temperature in the winter.

The mean initial salinity of May and June was 16.6 ± 0.9 psu due to the effect of added tap water. As culture days increase, salinity concentration showed a range of change between 13.1 and 18.4 psu during the year due to effects of evaporation of culture medium and supplementation of freshwater. In addition, the mean salinity concentration during the entire culture period was 16.5 ± 1.3 psu. The pH change during the culture period showed a relatively small variation between 9.9 and 11.9. Variation of pH from May 4, 2011 to September 9 was between 9.9 and 10.97, pH change between September 14 and September 30 was ranging between 11.15 and 11.90, and then it went down to below 11.0 from October 4. The ending pH on March 16 was 9.97. Internal room temperature of the plant during the culture period was in the range of 3.2–55.0°C, and the mean room temperature was 24.3 ± 10.5°C. The mean total electricity consumption (kWh) of the microalgae pilot plant was 10.3 ± 1.1 kWh per day during the initial culture between April 4, 2011 and May 4, during which the boiler was operated for maintenance of optimal medium temperature (20.2–26.1°C). Boiler operation was stopped between May 6, 2011 and October 16, 2011 for optimal temperature, during which 1.3 ± 0.6 kWh of electricity was used on average per day.

The range of biomass of *S. maxima* that was produced year-round using batch culture method in the raceway system with glass greenhouse structure in the present study was 5.68–37.67 g/m^2/d, in which the highest productivity of the year was recorded as 37.67 g/m^2/d in the summer when temperature increased. According to a study on *S. platensis* culture in a raceway system (750 L) by Richmond et al. [46], productivity was 15–27 g/m^2/d, whereas a study on culture of *S. platensis* in a raceway system (135,000 L) conducted in Spain showed 2–17 g/m^2/d [47]. Considering that limiting factors important for growth and productivity of microalgae are solar radiation, carbon supply, water temperature, and dissolved oxygen [5, 46, 48], a control system on these factors are also critical. BIM designing technology that was introduced in the present study to predict these effects had advantages including investigation of spatiotemporal

	2011									2012		
	Apr	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**
WT	22.4 ± 1.3	23.3 ± 1.7	25.2 ± 1.9	27.7 ± 2.4	29.0 ± 1.8	25.6 ± 1.9	21.2 ± 1.8	23.9 ± 2.4	21.3 ± 1.8	19.5 ± 1.4	20.4 ± 2.2	22.6 ± 1.6
RT	29.3 ± 4.1	26.4 ± 4.1	28.1 ± 3.8	34.8 ± 8.0	37.0 ± 4.6	35.9 ± 6.2	28.2 ± 3.5	22.9 ± 4.8	14.0 ± 3.6	10.8 ± 4.5	10.8 ± 4.8	15.7 ± 3.9
SR	822.2 ± 460.2	480.4 ± 397.6	519.4 ± 391.4	219.4 ± 179.4	351.0 ± 318.6	604.4 ± 377.1	382.2 ± 330.2	354.2 ± 351.8	328.0 ± 214.7	336.4 ± 310.8	371.8 ± 344.7	317.4 ± 404.6
Salinity	16.2 ± 0.1	15.6 ± 1.3	16.5 ± 0.8	18.1 ± 0.7	16.5 ± 1.6	16.3 ± 0.8	17.0 ± 0.4	16.2 ± 0.9	17.5 ± 1.3	16.4 ± 1.4	15.3 ± 1.9	13.5 ± 0.9
pH	9.69 ± 0.2	10.4 ± 0.1	10.3 ± 0.2	10.4 ± 0.3	10.4 ± 0.3	11.1 ± 0.4	10.5 ± 0.1	10.5 ± 0.1	10.6 ± 0.3	10.3 ± 0.3	10.2 ± 0.2	10.0 ± 0.1
TEC	290.6	72.5	42.0	38.9	39.1	38.5	144.4	331.6	475.3	453.2	440.6	389.9

WT: water temperature (°C); RT: room temperature (°C); SR: solar radiation (μmol/m²/s); TEC: total energy consumption (kWh).

Table 1. Monthly average data of water temperature (WT), room temperature (RT), solar radiation (SR), salinity, pH, and total energy consumption in the microalgae pilot plant.

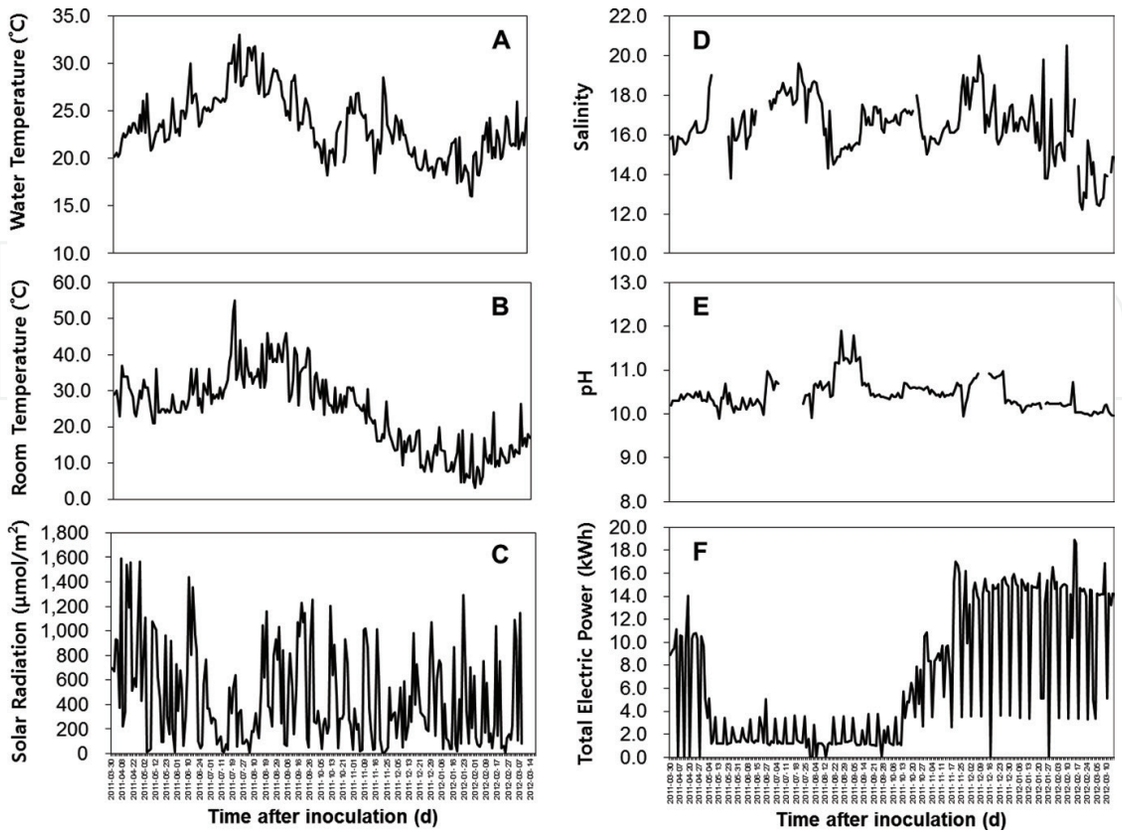

Figure 7. Annual variations of daily data on medium water temperature (A), room temperature (B), solar radiation (C), salinity (D), pH (E) and use of total electric power (F) in the *S. maxima* culture pond during the period from April 4, 2011 to March 16, 2012.

relevance of construction using 3D modeling, and prediction of problematic factors in advance including an analysis of environmental interference as known already [36–38], which resulted in desirable *Spiruina* biomass produced from the facility that designed based on an environmental analysis (atmospheric temperature and radiation amount) known as major factors of *S. maxima* growth, and predictions of environmental interference (e.g., shadow effect in **Figure 3**). **Table 2** and **Figure 8** show year-round biomass of *S. maxima* during the culture between April 8, 2011 and March 15, 2012.Variations of biomass and daily productivity were 0.227–1.507 g/L and 14.2 ± 9.6–31.42 ± 4.8 g/m^2/d, respectively, during the culture period, and mean values were 0.96 ± 0.24 g/L and 24.2 ± 5.90 g/m^2/d, respectively. The mean daily productivity of April when culture was started was 14.2 ± 8.1 g/m^2/d. The mean daily productivity gradually increased from May reaching to 23.92 ± 2.98 g/m^2/d. Thereafter, there were changes in productivity due to harvest of *S. maxima* for component analysis and supplementation of freshwater for evaporation of culture medium. The highest productivity of the year (31.42 ± 4.8 g/m^2/d) was achieved in August when temperature of culture water was high, which was statistically significant ($p < 0.05$), and the lowest productivity of the year (18.81 ± 4.3 g/m^2/d) was recorded in October. When comparing the results of this study with most preceeding studies, the raceway system with a glass greenhouse structure in the present chapter achieved the maximum aerial productivity (**Table 3**). In addition, despite the continuous study in batch culture method without resupply of nutrients, a high aerial productivity (24.2 g/m^2/d) in annual average was obtained.

	2011								2012			
	Apr	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**
Biomass (g/L)	0.57 ± 0.4	0.96 ± 0.2	1.02 ± 0.1	1.19 ± 0.2	1.26 ± 0.2	0.97 ± 0.1	0.75 ± 0.2	0.88 ± 0.2	1.01 ± 0.1	0.96 ± 0.1	0.96 ± 0.1	0.95 ± 0.2
Productivity (g/ m^2/d)	14.2 ± 9.6	23.92 ± 5.5	25.38 ± 3.5	29.65 ± 3.8	31.42 ± 4.8	24.26 ± 3.1	18.81 ± 4.3	21.95 ± 3.9	25.20 ± 3.3	24.09 ± 3.0	23.97 ± 3.3	23.65 ± 5.2
n.d.: not determined.												

Table 2. Monthly average data of biomass and productivity of ***S. maxima*** in the microalgae pilot plant.

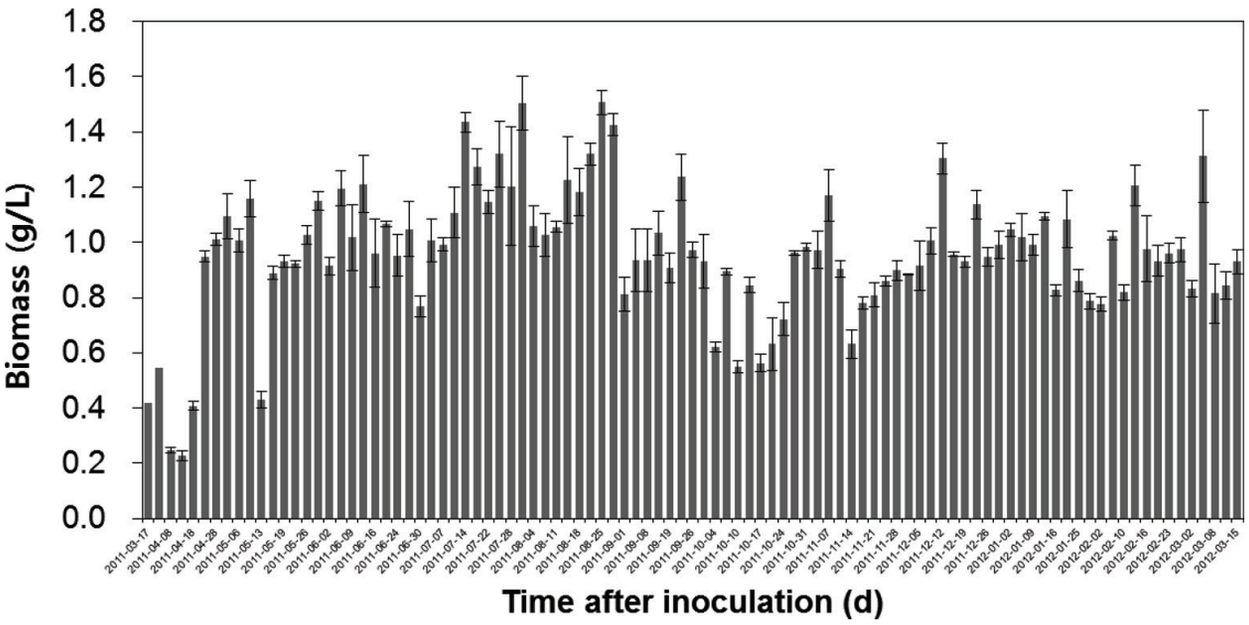

Figure 8. Annual variation on the biomass concentration of two times weekly-harvested *S. maxima* during a culture period.

Thus, it has confirmed a foundation to use a raceway system with a glass greenhouse structure or photobioreactor for countries with four distinct seasons.

Table 4 presents year-round ratios of protein, carbohydrate (CHO), and lipid contents in *S. maxima*. Protein content of *S. maxima* in April 2011 when culture was started was 40.08%, which gradually increased to 47.64% in July, the highest. As culture period became longer, ratio of protein gradually decreased and recorded 23.71% in February 2012, the lowest of the year. Protein content was higher from the spring to the early fall compared to other components, which had significant correlations with temperature of culture medium and solar radiation ($p < 0.05$). Ratio of CHO was 36.81% in April 2011 when culture was started, which decreased to 20.06% in June. Ratio of CHO highly increased from September 2011, and reached a peak of 42.19% in January in 2012, which was contrasted to the content of protein. Thus, protein and CHO contents exhibited a significant inverse correlation depending on season ($p < 0.05$, $r^2 = 0.8542$). Similar to the results of preceding studies, high protein contents were found in *S. maxima*, and changes in protein and CHO depending on season, and changes in

Cultivation system	Culture volume (L)	Productivity (g/m²/d)	Species	Location	References
Raceway	600	5–40	*Tetraselmis* sp.	Japan	Matsumoto et al. [63]
Raceway	—	1.6–3.5	*Dunaliella salina*	Spain	Garcia et al. [64]
Raceway	110	20–37	*Dunaliella salina*	Perth, Australia	Moheimani and Borowitzka [29]
Raceway	750	15–27	*Spirulina platensis*	Israel	Richmond et al. [46]
Raceway	—	8.2	*Spirulina platensis*	USA (California)	Belay [65]
Raceway	282	14.47 ± 0.16	*Spirulina platensis*	Italy	Pushparaj et al. [25]
Raceway	135,000	2–17	*Spirulina* sp.	Spain	Jimenez et al. [15, 37]
Raceway	—	9–13	*Spirulina* sp.	Mexico	Olguin et al. [66]
Raceway	500	11.2	*Tetraselmis* sp.	Japan	Matsumoto et al. [67]
Raceway	300–600	5–26	*Tetraselmis suecica*	Italy	Pedroni et al. [68]
Inclined thin layer pond	1000	10–30	*Chlorella* sp.	Czech Republic and Spain	Doucha and Livansky [69]
Inclined thin layer pond	~2500	19	*Scenedesmus obliquus*	Rupite, Bulgaria	Dilov et al. [70]
Circular central pivot pond	1960	1.61–16.47	*Chlorella* sp.	Japan	Kanazaqa et al. [71]
Open culture system	2400–16,200	19–22	*Chlorella* sp.	China	Tsukuda et al. [72]
Semi-open raceway	10,000–15,000	5.68–37.67	*Spirulina maxima*	Ansan, South Korea	In this study

Table 3. Comparison of biomass productivities of various microalgal species in outdoor open pond culture (modified from Borowitzka et al. [61]).

pigment contents were also identified [49–53]. Markou et al. [54] reported that although CHO content of *S. platensis* was between 10 and 20% in general, the limitation of phosphorus components in nutrition source resulted in an increase to 60–65%. In addition, Markou et al. [55] reported that a control of medium components for *S. platensis* caused elevation of CHO content among general components in a study on conversion of microalgae components to bioethanol. CHO content in the present study on culture of *S. maxima* showed a year-round change between 20.06–51.37%, and significantly increased at the later stage of culture. Since the present study performed a year-round experiment in batch–culture method, it seems that nutrition sources including phosphorus component was limited at the later stage, which might have caused elevation of CHO content and reduction of protein content. Batista et al. [56] reported protein content of *S. maxima* as 44.9 ± 1.8%, and Usharani et al. [57] reported protein content of *Spirulina* as 55–70%. Protein content in the present study was 40.08% in the beginning of culture and 23.71–47.64% in year-round content. Protein contents of *S. maxima* in year-round culture were the highest in July 2011 and the lowest in January 2012. In addition, protein content was significantly correlated with medium temperature and solar radiation. Jacob-Lopes et al. [58] reported that the change of light cycles (day/night) was closely related with microalgae production, and production decreased as the condition of darkness continued. Protein

	2011									2012		
	Apr	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**
Protein (%)	40.08	37.77	36.98	47.64	46.48	40.62	32.26	n.d.	n.d.	25.19	23.71	31.10
CHO (%)	36.81	28.04	20.06	25.16	28.52	37.47	47.35	n.d.	n.d.	51.37	42.19	42.92
Lipid (%)	7.16	20.68	11.03	9.13	8.01	7.29	5.90	n.d.	n.d.	4.43	5.77	8.17
Ash (%)	10.30	10.64	24.66	14.24	13.42	10.44	9.03	n.d.	n.d.	11.31	24.24	12.98
Moisture (%)	5.66	2.87	7.27	3.83	3.56	4.18	5.47	n.d.	n.d.	7.70	4.09	4.82
Chlorophyll a (mg/g)	6.1 ± 0.1	4.2 ± 0.1	3.3 ± 1.6	5.6 ± 1.8	6.3 ± 2.0	3.9 ± 0.6	3.0 ± 0.1	n.d.	n.d.	1.7 ± 0.1	1.8 ± 0.6	2.7 ± 0.1
Phycocyanin (mg/g)	28.5 ± 0.9	28.7 ± 8.5	14.8 ± 3.1	53.5 ± 11.6	80.7 ± 9.3	91.1 ± 4.6	64.6 ± 13.1	n.d.	n.d.	25.9 ± 0.8	24.5 ± 9.5	55.1 ± 0.9

n.d.: not determined.

Table 4. Results of biochemical analysis of dry powder of ***S. maxima***.

contents of *S. platensis* increased to 70.90 ± 2.37% at sunrise, and became 57 ± 0.69% at sunset, indicating that it tends to remarkably decrease compared to the daytime [59]. On the contrary, it was reported that CHO content was higher at the sunset time (33.81 ± 0.66%) than the sunrise time (19.48 ± 1.48%). Thus, it would be necessary to study the maintenance of protein content by application of phosphate-feed condition and LED illumination environment after the early fall when temperature of culture medium and amount of sunlight decline.

Year-round contents of phycocyanin *S. maxima* produced in the present study were 12–93 mg/g. Phycocyanin content (mg/g) of *S. maxima* was 28.5 ± 0.9 mg/g in April 2011 when culture was started. Then, it gradually decreased and the mean content became 14.8 ± 3.1 mg/g in June, which was the lowest. It showed a trend of increase from July, and the mean phycocyanin concentration of September 2012 was 91.1 ± 4.6 mg/g, which was the maximum of the year. Thereafter, phycocyanin contents again decreased and recorded 24.5 ± 9.5 mg/g in its mean value in February 2012. Chlorophyll-*a* content of *S. maxima* was 6.1 ± 0.1 mg/g in April 2011 when culture was started, and then the mean content was 6.3 ± 2.0 mg/g in August when radiation amount was relatively high, which was the highest of the year. Afterwards, chlorophyll-*a* concentration decreased to 1.8 ± 0.6 mg/g in February 2012, which was the lowest of the year. However, although content of chlorophyll-*a* was at the highest in the summer similar to the correlation between year-round contents of phycocyanin and amount of sunlight, there was no consistent year-round significance. In general, cell growth and phycocyanin production are closely related with light conditions [60, 61]. However, phycocyanin content was the highest in September 2011 when light conditions were the best in the present study, though there was no consistent significance throughout the year. Chlorophyll-*a* contents showed 6.3 ± 2.0 mg/g in August 2011, the maximum value, and 1.7 ± 0.1 mg/g in January 2012, the minimum value. It has been reported that as the light energy that microalgae received increased, chlorophyll contents also significantly increased [62], and the present study also showed the highest chlorophyll-*a* content in August when radiation amount was relatively high. Despite the significance of monthly pigment contents, however, there was no constant year-round significance between pigments and amount of sunlight.

4. Conclusion

A glass greenhouse pilot plant for microalgal culture fitting to temperate climate was designed based on 3D modeling designing BIM technology in KIOST. The bottom of the raceway system was placed 600 mm deep into the ground, and culture depth was kept at 400 mm, so that heat energy was efficiently stored in order to maintain thermal effects for a long time, and its structure was helpful in maintaining optimal temperature even in the winter. *S. maxima* was continuously cultured for a year in batch culture without further supply of nutrients, and the raceway system with a glass greenhouse structure in the present chapter achieved the maximum aerial productivity compared with most previous studies.

Acknowledgements

This research was supported by collective research grants from the Korea Institute of Ocean Science & Technology (PE99511). Also, this paper was studied with the support of 'Development of integrated technologies for developing biomaterials using by magma seawater' (PM60110) and the 'Marine Biotechnology Program' funded by Ministry of Ocean and Fisheries, Korea.

Conflict of interest

The authors declare no conflicts of interest.

Thanks

We would like to thank the staffs of the Research Group of Integrated Use of Marine Biomass of Jeju International Marine Science Center in Korea Institute of Ocean Science and Technology (KIOST), who supported and collected the annual data. Our research activities were strongly supported by the KIOST and Ministry of Ocean and Fisheries.

Nomenclature

AOAC	the association of official analytical chemists
BIM	building information modeling
CFD	computational fluid dynamics
CHO	carbohydrate
GF/C	glass microfiber

KIOST	Korea Institute of Ocean Science and Technology
KWh	kilowatt hour
NIST	National Institute of Standards and Technology
ORS	open raceway system
PBR	photobioreactor
PE	polyethylene
PSU	practical salinity unit
RPM	revolutions per minute

Author details

Taeho Kim[1], Woo-Seok Choi[1], Bo-Ram Ye[1], Soo-Jin Heo[1], Dongkyu Oh[3], SeongEun Kim[4], Kwang-Sik Choi[5] and Do-Hyung Kang[1,2]*

*Address all correspondence to: dohkang@kiost.ac.kr

1 Jeju International Marine Science Research and Education Center, Korea Institute of Ocean Science and Technology (KIOST), Jeju Special Self-Governing Province, Republic of Korea

2 Department of Marine Biology, KIOST Campus, Korea University of Science and Technology (UST), Republic of Korea

3 Alli Architectural Designer's Office, Jeju-do, Republic of Korea

4 Ara Consulting and Technology Co., Incheon, Republic of Korea

5 School of Marine Biomedical Science (BK21 PLUS), Jeju National University, Jeju, Republic of Korea

References

[1] Belov A, Giles J. Dynamical model of buoyant cyanobacteria. Hydrobiologia. 1997; **349**(1):87-97

[2] Gershwin ME. Belay A. Spirulina in human nutrition and health: CRC press; 2007. pp. 2-4

[3] Gantar M, Svirčev Z. Microalgae and cyanobacteria: Food for thought. Journal of phycology. 2008;**44**(2):260-268

[4] Flores FG. The cyanobacteria: Molecular biology, genomics, and evolution. Horizon Scientific Press; 2008. 424 p

[5] Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: A review. Renewable and sustainable energy reviews. 2010;**14**(1):217-232

[6] Prasanna R, Sood A, Jaiswal P, Nayak S, Gupta V, Chaudhary V, et al. Rediscovering cyanobacteria as valuable sources of bioactive compounds. Applied Biochemistry and Microbiology. 2010;**46**(2):119-134

[7] Estrada JP, Bescós PB, Del Fresno AV. Antioxidant activity of different fractions of Spirulina platensis protean extract. Il farmaco. 2001;**56**(5):497-500

[8] Miranda M, Cintra R, Barros S, Mancini-Filho J. Antioxidant activity of the microalga Spirulina maxima. Brazilian Journal of Medical and biological research. 1998;**31**(8):1075-1079

[9] Ducat DC, Way JC, Silver PA. Engineering cyanobacteria to generate high-value products. Trends in biotechnology. 2011;**29**(2):95-103

[10] Taton A, Lis E, Adin DM, Dong G, Cookson S, Kay SA, et al. Gene transfer in Leptolyngbya sp. strain BL0902, a cyanobacterium suitable for production of biomass and bioproducts. PloS one. 2012;**7**(1):e30901

[11] Habib MAB. Review on culture, production and use of Spirulina as food for humans and feeds for domestic animals and fish: Food and agriculture organization of the united nations; 2008

[12] Costa JAV, Colla LM, Duarte Filho P, Kabke K, Weber A. Modelling of Spirulina platensis growth in fresh water using response surface methodology. World Journal of Microbiology and Biotechnology. 2002;**18**(7):603-607

[13] Belay A, Ota Y, Miyakawa K, Shimamatsu H. Current knowledge on potential health benefits of Spirulina. Journal of applied Phycology. 1993;**5**(2):235-241

[14] Olaizola M. Commercial production of astaxanthin from Haematococcus pluvialis using 25,000-liter outdoor photobioreactors. Journal of Applied Phycology. 2000;**12**(3):499-506

[15] Jiménez C. Cossí BR, Niell FX. Relationship between physicochemical variables and productivity in open ponds for the production of Spirulina: a predictive model of algal yield. Aquaculture. 2003;**221**(1):331-345

[16] Zhang BY, Geng YH, Li ZK, Hu HJ, Li YG. Production of astaxanthin from Haematococcus in open pond by two-stage growth one-step process. Aquaculture. 2009;**295**(3):275-281

[17] Doucha J, Lívanský K. Production of high-density Chlorella culture grown in fermenters. Journal of applied phycology. 2012;**24**(1):35-43

[18] Belay A, Belay A. Production of high quality Spirulina at Earthrise Farms: c/o Institute of Advanced Studies; 1994

[19] Pulz O. Photobioreactors: Production systems for phototrophic microorganisms. Applied microbiology and biotechnology. 2001;**57**(3):287-293

[20] Kang D-H, Heo S-J, Oh C, Ju S-J, Jeon S-M, Choi H-W, et al. A Review on Major Factors for Microalgae Biofuel Commercialization. Ocean and Polar Research. 2012; **34**(4):365-384

[21] Tredici M, Materassi R. From open ponds to vertical alveolar panels: the Italian experience in the development of reactors for the mass cultivation of phototrophic microorganisms. Journal of Applied Phycology. 1992;**4**(3):221-231

[22] Torzillo G, Carlozzi P, Pushparaj B, Montaini E, Materassi R. A two-plane tubular photobioreactor for outdoor culture of Spirulina. Biotechnology and bioengineering. 1993;**42**(7):891-898

[23] Laws E, Terry K, Wickman J, Chalup M. A simple algal production system designed to utilize the flashing light effect. Biotechnology and bioengineering. 1983;**25**(10):2319-2335

[24] Richmond A. Open systems for the mass production of photoautotrophic microalgae outdoors: Physiological principles. Journal of Applied Phycology. 1992;**4**(3):281-286

[25] Pushparaj B, Pelosi E, Tredici MR, Pinzani E, Materassi R. As integrated culture system for outdoor production of microalgae and cyanobacteria. Journal of Applied Phycology. 1997;**9**(2):113-119

[26] Richmond A, Cheng-Wu Z. Optimization of a flat plate glass reactor for mass production of Nannochloropsis sp. outdoors. Journal of Biotechnology. 2001;**85**(3):259-269

[27] García-González M, Moreno J, Canavate J, Anguis V, Prieto A, Manzano C, et al. Conditions for open-air outdoor culture of Dunaliella salina in southern Spain. Journal of Applied Phycology. 2003;**15**(2):177-184

[28] Moreno J, Vargas MÁ. Rodríguez H, Rivas Jn, Guerrero MG. Outdoor cultivation of a nitrogen-fixing marine cyanobacterium, Anabaena sp. ATCC 33047. Biomolecular Engineering. 2003;**20**(4-6):191-197

[29] Moheimani NR, Borowitzka MA. The long-term culture of the coccolithophore Pleurochrysis carterae (Haptophyta) in outdoor raceway ponds. Journal of Applied Phycology. 2006;**18**(6):703-712

[30] Radmann EM, Reinehr CO, Costa JAV. Optimization of the repeated batch cultivation of microalga Spirulina platensis in open raceway ponds. Aquaculture. 2007;**265**(1):118-126

[31] Carvalho AP, Meireles LA, Malcata FX. Microalgal reactors: A review of enclosed system designs and performances. Biotechnology progress. 2006;**22**(6):1490-1506

[32] Lu Y-M, Xiang W-Z, Wen Y-H. Spirulina (Arthrospira) industry in Inner Mongolia of China: current status and prospects. Journal of applied phycology. 2011;**23**(2):265-269

[33] Affan MA, Lee D-W, Jeon S-M, Noh J-H, Heo S-J, Oh C, et al. Bituminous coal and sodium hydroxide-pretreated seawater stimulates Spirulina (Arthrospira) maxima growth with decreased production costs. Aquaculture. 2015;**436**:121-126

[34] Hargraves PE, Víquez R. Spirulina subsalsa Oersted en Costa Rica. Estructura y posible importancia comercial. Rev Biol Trop. 1980;**22**:304-307

[35] Oguchi M, Nitta K, Otsubo K, Shimada A, Miyazaki K, editors. Application of tubular photo-bioreactor system to culture spirulina for gas exchange and food production in CELSS. IAF, International Astronautical Congress, 40 th. Spain: Malaga; 1989

[36] Azhar S. Building information modeling (BIM): Trends, benefits, risks, and challenges for the AEC industry. Leadership and management in engineering. 2011;**11**(3):241-252

[37] Lee G, Sacks R, Eastman CM. Specifying parametric building object behavior (BOB) for a building information modeling system. Automation in construction. 2006;**15**(6): 758-776

[38] Goedert JD, Meadati P. Integrating construction process documentation into building information modeling. Journal of construction engineering and management. 2008;**134**(7):509-516

[39] Affan M-A, Lee D-W, Al-Harbi SM, Kim H-J, Abdulwassi NI, Heo S-J, et al. Variation of Spirulina maxima biomass production in different depths of urea-used culture medium. Brazilian Journal of Microbiology. 2015;**46**(4):991-1000

[40] Kim JH, Choi W, Jeon SM, Kim T, Park A, Kim J, et al. Isolation and characterization of Leptolyngbya sp. KIOST-1, a basophilic and euryhaline filamentous cyanobacterium from an open paddle-wheel raceway Arthrospira culture pond in Korea. Journal of applied microbiology. 2015;**119**(6):1597-1612

[41] Kim T, Jeon S-M, Kim JH, Park A, Lee JH, Oh C, et al., editors. The Feasibility of pilot production of Spirulina (Arthrospira) maxima cultivated newly constructed raceway pond in Republic of Korea. Proceedings on the 10th International Marine Biotechnology Conference; 2013 Nov 12-15; Brisbane, Queensland, Australia

[42] Ogawa T, Terui G, Kozasa H. Studies on growth of spirulina-platensis. 2. growth kinetics of an autotrophic culture. Journal of Fermentation Technology. 1972;**50**(3):143-149

[43] Horwitz W, Chichilo P, Reynolds H. Official methods of analysis of the Association of Official Analytical Chemists. Official methods of analysis of the Association of Official Analytical Chemists. 1970

[44] Bennett A, Bogorad L. Complementary chromatic adaptation in a filamentous blue-green alga. The Journal of cell biology. 1973;**58**(2):419-435

[45] Moran R. Formulae for determination of chlorophyllous pigments extracted with N. N-dimethylformamide. Plant physiology. 1982;**69**(6):1376-1381

[46] Richmond A, Lichtenberg E, Stahl B, Vonshak A. Quantitative assessment of the major limitations on productivity ofSpirulina platensis in open raceways. Journal of Applied Phycology. 1990;**2**(3):195-206

[47] Jiménez C. Cossío BR, Labella D, Niell FX. The feasibility of industrial production of Spirulina (Arthrospira) in Southern Spain. Aquaculture. 2003;**217**(1):179-190

[48] Wu H, Gao K, Ma Z, Watanabe T. Effects of solar ultraviolet radiation on biomass production and pigment contents of Spirulina platensis in commercial operations under sunny and cloudy weather conditions. Fisheries Science. 2005;**71**(2):454-456

[49] Holt TK, Krogmann DW. A carotenoid-protein from cyanobacteria. Biochimica et Biophysica Acta (BBA)-Bioenergetics. 1981;**637**(3):408-414

[50] Proteau P, Gerwick W, Garcia-Pichel F, Castenholz R. The structure of scytonemin, an ultraviolet sunscreen pigment from the sheaths of cyanobacteria. Experientia. 1993;**49**(9):825-829

[51] Zhang X-W, Zhang Y-M, Chen F. Application of mathematical models to the determination optimal glucose concentration and light intensity for mixotrophic culture of Spirulina platensis. Process Biochemistry. 1999;**34**(5):477-481

[52] De Oliveira M, Monteiro M, Robbs P, Leite S. Growth and chemical composition of Spirulina maxima and Spirulina platensis biomass at different temperatures. Aquaculture international. 1999;**7**(4):261-275

[53] Bonnet S, Webb EA, Panzeca C, Karl DM, Capone DG, Wilhelmy SAS. Vitamin B12 excretion by cultures of the marine cyanobacteria Crocosphaera and Synechococcus. Limnology and Oceanography. 2010;**55**(5):1959-1964

[54] Markou G, Chatzipavlidis I, Georgakakis D. Carbohydrates production and bio-flocculation characteristics in cultures of Arthrospira (Spirulina) platensis: improvements through phosphorus limitation process. BioEnergy research. 2012;**5**(4):915-925

[55] Markou G, Angelidaki I, Nerantzis E, Georgakakis D. Bioethanol production by carbohydrate-enriched biomass of Arthrospira (Spirulina) platensis. Energies. 2013;**6**(8):3937-3950

[56] Batista AP, Gouveia L, Bandarra NM, Franco JM, Raymundo A. Comparison of microalgal biomass profiles as novel functional ingredient for food products. Algal Research. 2013;**2**(2):164-173

[57] Usharani G. Spirulina Cultivation A Review. International Journal of Pharmaceutical & Biological Archive. 2012;**3**(6):1327-1341

[58] Jacob-Lopes E, Scoparo CHG, Lacerda LMCF, Franco TT. Effect of light cycles (night/day) on CO 2 fixation and biomass production by microalgae in photobioreactors. chemical engineering and Processing: Process Intensification. 2009;**48**(1):306-310

[59] Torzillo G, Sacchi A, Materassi R, Richmond A. Effect of temperature on yield and night biomass loss in Spirulina platensis grown outdoors in tubular photobioreactors. Journal of Applied Phycology. 1991;**3**(2):103-109

[60] Degen J, Uebele A, Retze A, Schmid-Staiger U, Trösch W. A novel airlift photobioreactor with baffles for improved light utilization through the flashing light effect. Journal of biotechnology. 2001;**92**(2):89-94

[61] Reichert CC, Reinehr CO, Costa JAV. Semicontinuous cultivation of the cyanobacterium Spirulina platensis in a closed photobioreactor. Brazilian Journal of Chemical Engineering. 2006;**23**(1):23-28

[62] Borowitzka MA, Moheimani NR. Algae for biofuels and energy: Springer; 2013

[63] Matsumoto H, Shioji N, Hamasaki A, Ikuta Y, Fukuda Y, Sato M, et al. Carbon dioxide fixation by microalgae photosynthesis using actual flue gas discharged from a boiler. Applied Biochemistry and Biotechnology. 1995;**51**(1):681-692

[64] García-González M, Moreno J, Canavate J, Anguis V, Prieto A, Manzano C, et al. Conditions for open-air outdoor culture of Dunaliella salina in southern Spain. Journal of Applied Phycology. 2003;**15**(2-3):177-184

[65] Belay A. Mass culture of Spirulina outdoors—the Earthrise Farms experience. Spirulina platensis. 1997:131-158

[66] Olguín EJ, Galicia S, Mercado G, Pérez T. Annual productivity of Spirulina (Arthrospira) and nutrient removal in a pig wastewater recycling process under tropical conditions. Journal of Applied Phycology. 2003;**15**(2-3):249-257

[67] Matsumoto H, Shioji N, Hamasaki A, Ikuta Y, Fukuda Y, Sato M, et al. Carbon dioxide fixation by microalgae photosynthesis using actual flue gas discharged from a boiler. Applied Biochemistry and Biotechnology. 1995;**51**(1):681

[68] Pedroni PM, Lamenti G, Prosperi G, Ritorto L, Scolla G. Capuano F, et al. -Enitecnologie R&D project on microalgae biofixation of CO_2: Outdoor comparative tests of biomass productivity using flue gas CO_2 from a NGCC power plant. Greenhouse Gas Control Technologies 7: Elsevier. 2005:1037-1342

[69] Doucha J, Lívanský K. Productivity, CO_2/O_2 exchange and hydraulics in outdoor open high density microalgal (Chlorella sp.) photobioreactors operated in a Middle and Southern European climate. Journal of Applied Phycology. 2006;**18**(6):811-826

[70] Dilov C, Georgiev D, Bozhkova M. Cultivation and application of microalgae in the People's Republic of Bulgaria. Arch Hydrobiol. 1985;**20**:35-38

[71] Kanazawa T, Fujita C, Yuhara T, Sasa T. Mass culture of unicellular algae using the open circulation method. The Journal of General and Applied Microbiology. 1958;**4**(3):135-152

[72] Tsukada O, Kawahara T. Mass culture of Chlorella in Asian countries. Biological solar energy conversion. 1977

IntechOpen