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Raceway Cultivation of *Spirulina platensis* Using Underground Water

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Abstract The semi-outdoor cultivation of *Spirulina platensis* was attempted using an underground-water-based medium. Occurrence of contaminant organisms such as *Chlorella* sp. and *Chlamydomonas* sp. was not found from a microscopic observation and bacteria were not detected from denaturing gradient gel electrophoresis (DGGE) analysis of PCR-amplified 16S rDNA during the cultivation, owing to pH control and the high quality of the underground water. The mean productivity was high at 10.5 g/m^2/d with a range of 4.2–12.3 g/m^2/d despite the unfavorable weather conditions of the rainy season. The cultivated *S. platensis* included a normal protein content of 58.9%. Consequently, the underground water improved the biomass productivity and the biomass quality because of an abundant supplementation of natural minerals and through a contaminant-free culture.

Keywords: Semi-outdoor cultivation, *Spirulina platensis*, underground water

The water used in the outdoor cultivation of *Spirulina platensis* is critical to the biomass quality. Many producers have adopted open raceway ponds using fresh water or seawater. For example, Earthrise Farms uses Colorado river water irrigated through long canals [2], whereas Cyanotech Co. uses deep seawater plus fresh water to use diverse minerals (see Web site: http://www.cyanotech.com). Although these cultivations are on a large scale, they are capital intensive and vulnerable to invasion by diverse contaminant organisms. The occurrence of contaminant organisms is fatal to the biomass quality, especially in relation to use as a food supplement. Moreover, the indraft of contaminated rainwater by air pollution can also deteriorate the biomass quality. In regard to production of high-quality biomass underground water characterized by constant water temperature, water quality and mineral contents can be used as culture water for *S. platensis*, as applied by Kim *et al.* [7], who reported that the mean biomass productivity was 7.8 g/m^2/d, using a half concentration of SOT medium [16] based on the underground water in a greenhouse. However, reports on *Spirulina* outdoor cultivation using underground water as culture water has been seldom documented.

Accordingly, this study used underground water for cultivation of *S. platensis* to produce a high quality biomass and to improve biomass productivity in a greenhouse (semi-outdoor cultivation). It is expected that use of underground water will minimize the contaminant organisms and have a synergetic effect on growth because of the abundant supplementation of natural minerals, thereby reducing the production costs.

The culture strain, *Spirulina platensis* NIES 46 (below *S. platensis*), was provided by the National Institute for Environmental Studies, Japan, and preculture in the laboratory followed the method by Kim *et al.* [8]. The culture vessel equipped with a preculture vessel was adapted for the semi-outdoor cultivation. As shown in Fig. 1, the maximum capacity of the main culture vessel was 10 metric tons (W 3.3 m, L 10 m, H 0.4 m), and the ends were rounded (1.5 m diameter semicircles), to allow the culture water to rotate freely without stagnation. A preculture vessel with a capacity of 0.8 metric tons (W 0.3 m, L 7.0 m, H 0.4 m) and rounded ends (0.15 m diameter semicircles) was also set in the middle of the main culture vessel. The culture vessels were made of semitranslucent polyvinyl chloride (PVC) with the bottom also covered by PVC, and placed on the ground. A water tap was fixed to one end of the main culture vessel to provide the water supply, and a paddle wheel (0.5 m diameter) was used for water circulation on one side. The culture vessels were

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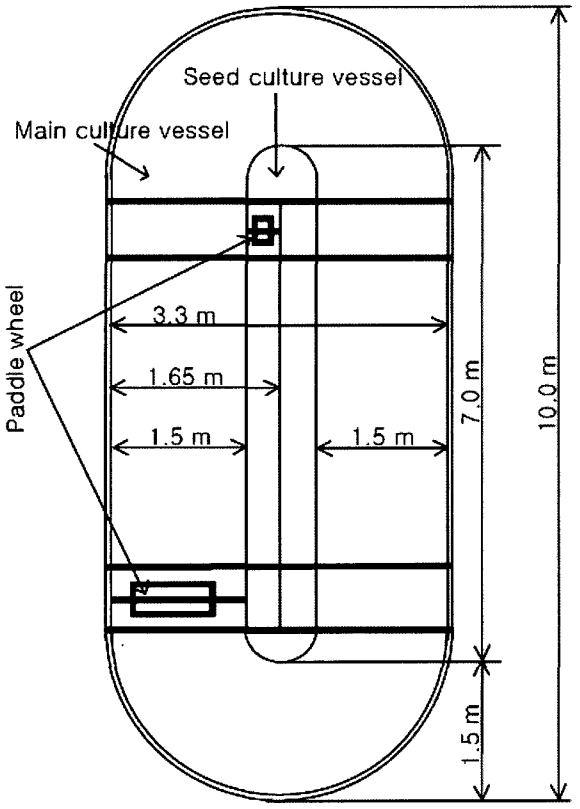

Fig. 1. Diagram of the culture vessel used in the semi-outdoor cultivation of *Spirulina platensis*.

placed in a greenhouse facility located in Gongju-Si, Choongchungnam-do, Korea.

The precultured *S. platensis* was then inoculated into the preculture vessel as the seed for the main culture and the working volume kept to 0.6 metric tones. On the basis of the cation analysis of the underground water, a SOT medium without $CaCl_2 \cdot 2H_2O$ and A_5 solution of minerals (H_3BO_3, $MnCl_2 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, MoO_3; hereafter SOT-$CaCl_2 \cdot 2H_2O$ and A_5) was used for the culture based on the underground water under natural conditions. The initial pH of the preculture was adjusted to 10.0 with Na_2CO_3 to minimize any contaminant organisms, and the culture was bubbled all day using 10 air stones (30 mm diameter and 80 mm length). The culturing was then continued for another 16 days.

On August 23, 2005, during the summer rainy season in Korea, the *S. platensis* grown in the preculture vessel was inoculated into the main culture vessel. The underground water was supplied to the main culture vessel along with the addition of SOT-$CaCl_2 \cdot 2H_2O$ and A_5, and adjusted to a height of 0.2 m (working volume, 5.7 metric tones; surface area, 27 m^2). The initial pH was adjusted to over 9.0, and then to 9.9 on day 6 of the culture, because adjustment of pH to over 9.5 is needed to avoid contamination by other algae, as reported by Belay [2]. The other culture conditions and operation followed the methods by Kim *et al.* [7].

To adjust the ingredients of the medium added to the underground water-based culture water, the cation composition of the underground water was analyzed using an ICP-AES (OPTIMA 4300 DV, PerkinElmer, U.S.A.) and ICP-MS (X-7, ThermoElemental, U.K.) under the following conditions: for the ICP-AES, RF power, 1,300 W; RF frequency, 40.68 MHz; Coolant gas flow, 15 l/min; Nebulizer gas flow, 0.7 l/min; for the ICP-MS, RF power, 1,200 W; RF frequency, 27.12 MHz; Coolant gas flow, 13 l/min; Nebulizer gas flow, 0.9 l/min.

For the semi-outdoor cultivation, measurement of the water temperature, pH, DO (dissolved oxygen), light intensity, and biomass followed those by the report of Kim *et al.* [7]. The chlorophyll *a* concentration was measured using 90% acetone [12]. Culture samples were observed everyday under a microscope to monitor the occurrence of contaminant organisms and culture states. For microbial contamination, culture samples collected on days 0, 7, and 15 were subjected to DGGE (denaturing gradient gel electrophoresis) analysis based on the PCR-amplified fragments of the16S rDNA. The primer set of 907R and 341FGC [5] for PCR amplification was used. The DGGE analytical methods followed those by Ahn *et al.* [1].

To analyze the nutritional chemical composition of the *S. platensis* produced from the present cultivation conditions, the cultured biomass was harvested using a harvesting machine (Hansol EME, Korea) and subjected to vacuum freezer drying. The analysis followed the methods of the Korea Food Code [9].

Table 1. Chemical composition (cations) of underground water used for cultivation of *Spirulina platensis*.

Component	Conc. (mg/l)	Component	Conc. (μg/l)	Component	Conc. (μg/l)
Na	16.20	Ni	1.20	V	0.60
Ca	27.02	Cu	0.59	Cr	1.10
Mg	4.93	Cd	<0.02	Mn	0.44
Si	15.44	Li	3.00	Co	0.15
K	0.69	B	22.00	Ba	2.30
Sr	0.38	Al	0.80	Pb	<0.05
Fe	<0.002	Ti	1.70	U	0.34
Zn	1.24				

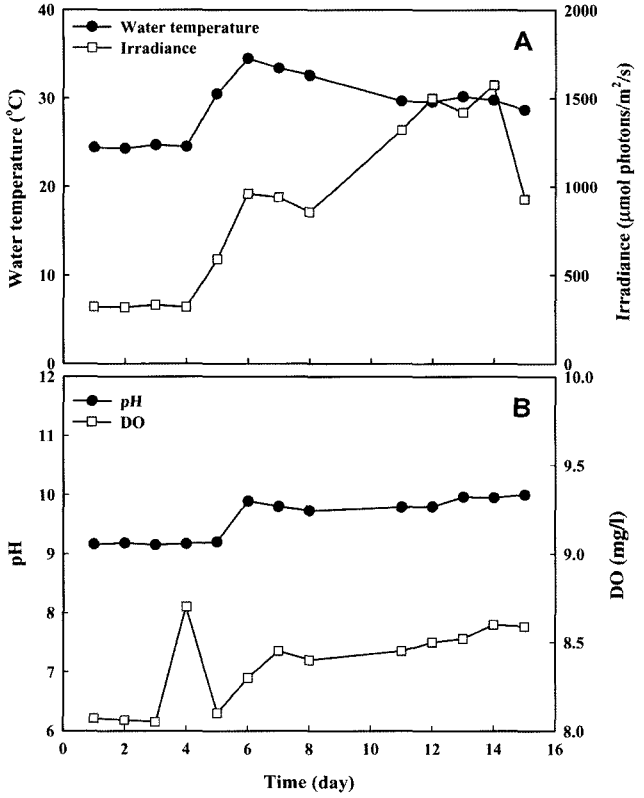

Fig. 2. Changes in water temperature and irradiance (**A**), and pH and DO (**B**) during outdoor cultivation of *Spirulina platensis*.

The result of cation analysis of the underground water is listed in Table 1. The underground water contained an abundance of sodium, calcium, silicate, magnesium, zinc, potassium, and strontium cations, over 0.38 mg/l, plus trace metals. The level of calcium and magnesium cations in the underground water was 27.02 mg/l and 4.93 mg/l, respectively, which was about 1.2 times higher and 3.9 times lower than that in the SOT medium, respectively. Underground water usually contains a lot of Ca^{2+}, which forms various precipitates when nutrients are added to the water. Since these precipitates can cause a nutrient deficiency, as suggested by Belay [2], it was determined not to add $CaCl_2 \cdot 2H_2O$ to the underground water-based culture to prevent any precipitates, and the A_5 micronutrient was replaced with the minerals in the underground water.

During the semi-outdoor cultivation of *S. platensis*, the mean water temperature was 29°C, ranging from 24–35°C during the day time (Fig. 2A). This was low, considering that *S. platensis* favors a high water temperature, ranging from 30 to 38°C for optimal growth [2], yet above 18°C at which growth is possible [10]. The mean sunlight intensity was 807 μmol photons/m^2/s, ranging from 319 to 1,500 μmol photons/m^2/s, the broad range also resulting from the meteorological conditions during the rainy season. As shown in Fig. 2B, the pH was adjusted to over 9.0 for the initial culture, and then to 9.9 on day 6 using Na_2CO_3 to minimize or delete any contaminant organisms. Thereafter, the pH increased to 10.0 on day 15 after a small fluctuation from 9.7 to 9.9. Adjusting the pH is the most popular countermeasure for contaminant organisms, such as *Chlorella* sp. and *Chlamydomonas* sp., as previously reported [2, 7]. However, an adjustment to too high a level can inhibit the *S. platensis* growth. Laboratory experiments showed a pH range of 8.0 to 10.5 to be suitable for the growth of *S. platensis*, whereas over 11.0 retarded growth (unpublished data). Therefore, the pH range of 9.2 to 10.0 during the outdoor cultivation was appropriate for *S. platensis* growth. The mean DO concentration was 8.3 mg/l with a stable range of 8.1–8.7 mg/l (Fig. 2B). A high DO concentration over 30 mg/l in the culture water retards the photosynthesis rate, as the excessive O_2 evolved from the culture organism competes with CO_2 for photosynthesis [13]. Jiménez *et al.* [6] reported a variance in the DO concentration from 10 mg/l in winter to 30 mg/l in summer during the year-round open raceway cultivation of *S. platensis* in Spain, and observed a partial loss of algal biomass at a DO concentration of over 25 mg/l. Belay [2] reported that small water circulation and turbulence in large ponds raise O_2 concentration as high as 500% of saturation. However, in the present cultivation, running the paddle wheel and air bubblation facilitated O_2 emission into the atmosphere, and thus a stable DO concentration was maintained, even in the summer period, as suggested previously [7].

The dry cell weight started at 0.04 g/l and increased to 0.80 g/l by day 16 (Fig. 3). The mean areal productivity in the semi-outdoor cultivation of *S. platensis* during 16 days was 10.5 g/m^2/d with a range of 4.2–12.3 g/m^2/d. This productivity was similar to 10.3 g/m^2/d under subtropical conditions [15] and higher than 5.2 to 7.3 g/m^2/d in Italy [13], yet lower than the 15 g/m^2/d obtained in July in Southern Spain [6]. Nonetheless, in a previous study of *S. platensis*

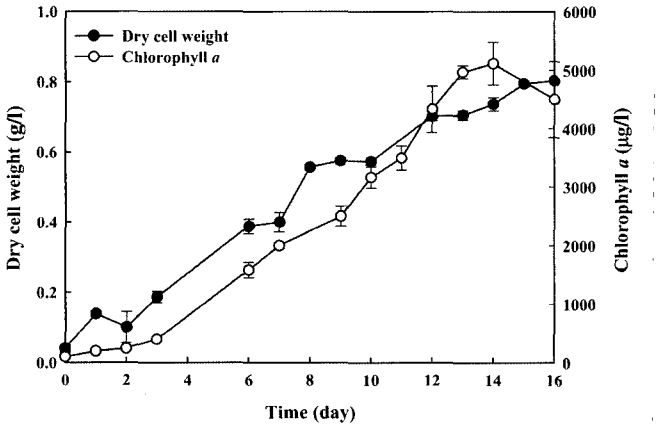

Fig. 3. Mean growth (n=3) curve based on dry cell weight and chlorophyll *a* of *Spirulina platensis* during outdoor cultivation.

by the current authors, using half the concentration of the SOT medium [16] and underground water, the maximum productivity was 7.8 g/m^2/d for 10 days [7]. Therefore, it is possible that the productivity of the present cultivation could have reached over 15.0 g/m^2/d under sunny conditions.

The chlorophyll *a* concentration increased linearly from day 4 to reach 5,117 μg/l on day 14, and then decreased sharply to 4,500 μg/l by day 16, yet no decreases were found, except for those during the initial days of culture (Fig. 3). Based on these results, the culture was harvested just after the chlorophyll *a* concentration started to decrease, as the chlorophyll *a* concentration directly reflects the culture status (r^2=0.955, P<0.0001), allowing it to be used as a criterion to determine the appropriate harvesting time.

The current study paid serious attention to the occurrence of any contaminant organisms. However, microscopic observation failed to find any contaminant organism during the cultivation, and DGGE analysis showed only *S. platensis* bands (Fig. 4), thereby confirming the high biomass quality of the *S. platensis* and emphasizing the advantage of using underground water and pH adjustment in semi-outdoor cultivation.

The nutritional composition of the cultivated *S. platensis* is given in Table 2. The moisture content was low at 4.7%, and the protein was 58.9%, although not high compared with the typical quantity of 60–70% (see Fox [4] and Belay [2]). It is assumed that the low protein content was due to inadequate washing with acid or water, leaving the ash content relatively high at 9.3%. The lipid content was 0.7%, which was ten times lower than that in the *Spirulina* from the Siam Algae Company [11] and the report of De Oliveira *et al.* [3], yet the carbohydrate (with fiber) content was high at 26.4%, corresponding to 1.4 times of the 19.4% content in the *Spirulina* from the Siam Algae Company [11]. It is possible that the low lipid content resulted from cell disruption during the vacuum filtration for harvesting, at which point some of the lipid component may have been lost, while the carbohydrate accumulated on the filter sieve, increasing the fiber. Among the mineral components, Na, K, and P were the predominant elements, and no potentially toxic heavy metals, such as As, Hg, Kd, and Pb, which are important check items for safety and quality standard of food grade *Spirulina* (see the Web site: http//: spirulina. com), were detected (Table 2).

In summary, the semi-outdoor cultivation of *S. platensis* using underground water was successfully carried out, resulting in a relatively high mean biomass productivity, even under poor weather conditions. The formation of precipitates, one of the most serious problems for the outdoor cultivation of *S. platensis* in an underground water-based cultivation, was avoided by the use of SOT-$CaCl_2$ and A_5. Furthermore, the underground water acted as a natural mineral source for *S. platensis* growth, thereby improving the biomass quality and reducing production costs.

Table 2. Chemical properties of *Spirulina platensis* produced by outdoor cultivation.

General composition (g/100 g)	
Moisture	4.7
Protein	58.9
Fat (lipid)	0.7
Carbohydrate (with fiber)	26.4
Mineral (ash)	9.3

Amino acid (g/100 g)	
Alanine	4.4
Arginine	3.6
Aspartic acid	5.6
Glutamic acid	8.7
Glycine	3.2
Histidine	0.8
Isoleucine	3.5
Leucine	5.2
Methionine	0.6
Phenylalanine	2.7
Proline	2.0
Serine	2.6
Threonine	2.1
Tyrosine	1.4

Amino acid (g/100 g)	
Valine	3.7

Essential fatty acid (g/1 kg)	
Linolenic acid	1.6
γ-Linolenic acid	0.7

Vitamins (mg/100 g)	
β-Carotene	8.0
Vitamin E	16.6

Minerals (mg/100 g)	
Ca	85.7
Cu	0.6
Fe	27.7
Mg	163.2
P	880.8
K	3,434.0
Na	8,235.8
Zn	2.6
Mn	1.5

Fig. 4. DGGE patterns of *Spirulina platensis* culture samples collected on different days.
Visible bands in the box were identified as *S. platensis* with 99% homology (512/516 sequence of AB074508)

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