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Short Paper

Effects of solar ultraviolet radiation on biomass production and pigment contents of *Spirulina platensis* in commercial operations under sunny and cloudy weather conditions

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The cyanobacterium *Spirulina platensis* has been commercialized as a health food and feed due to its valuable constituents,[1] and has also been used as colorant in food and cosmetic industries for its components of carotenoids, chlorophyll and phycobiliproteins.[2] The economic value of this important cyanobacterium has led to an intensive large-scale cultivation in many countries, therefore, optimizing its growing conditions is of general concern. Growth-limiting factors for *S. platensis* have been considered as pH, contaminants, culture depth, stirring rate, and excessive solar radiation.[3] In contrast, more and more attention is being paid to increased levels of solar ultraviolet radiation (UVR) reaching the earth's surface due to industrial depletion of the stratospheric ozone.[4] Solar UVR may affect the growth, pigmentation and photosynthesis of organisms in aquatic ecosystems.[5,6] Primary production of phytoplankton was found to be reduced by increasing the solar energy of 290–400 nm wavelengths (UVA, 320–400 nm and UVB, 290–320 nm).[6] Solar UVR may also affect the physiological behavior and biomass production of *S. platensis*. However, little has been documented on this aspect. The present study evaluated the effects of UVR on the biomass production and pigmentation of commercially outdoor-cultured *S. platensis* under sunny and cloudy weather conditions.

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UV exposure experiments were carried out on a cloudy day (15 April 2003) and on a sunny day (16 April 2003) at the culture base of Hainan DIC Microalgae Co. Ltd, Hainan, China, where *S. platensis* has been produced in a total pond area of 100 000 m^2. Samples of *S. platensis* were collected from a culture pond and dispended in shallow glass containers (ϕ 12 cm, 6 cm high). The containers were covered with three different cut-off filters: Ultraphan 295 (Digefra, Munich, Germany), Folex 320 (Montagefolie, Nr. 10155099, Folex, Dreieich, Germany) and Ultraphan 395 (UV Opak, Digefra, Munich, Germany). These filter foils cut off the wavebands of <295 nm, <320 nm and <395 nm, respectively, and their transmission spectra have been published elsewhere.[5] The cultures were exposed for 4 h (11.00–15.00 hours) to full-spectrum solar radiation (PAR + UVA + UVB; PAB) with Ultraphan 295, solar radiation depleted of UVB (PAR + UVA; PA) with Folex 320, and deprived of total UV (PAR, photosynthetically active radiation; P) with Ultraphan 395. During the exposures from 11.00 hours to 15.00 hours, PAR was monitored every 10 min by using a PAR sensor (SKP-200, Skye Instruments Ltd, UK). The UVA and UVB radiations during this period were calculated according to their ratios to PAR, which were determined according to the DayLight model and the ozone concentrations at Hainan on the days of the experiments (274 and 279 DU on 15 and 16 April 2003, respectively; http//toms.gsfc.nasa.gov/ozone). The PAR, UVA and UVB radiations were used to estimate the biomass generation efficiencies per unit solar

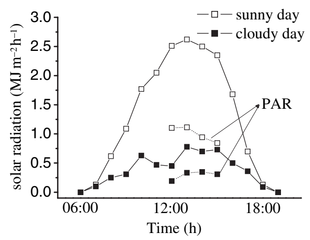

Fig. 1 Changes of the solar radiation (MJ/m^2/h^1) on 15 April (cloudy) and 16 April (sunny) 2003, in Hainan, China. Photosynthetically active radiation during the period of experiments from 11.00 hours to 15.00 hours is shown as dotted lines.

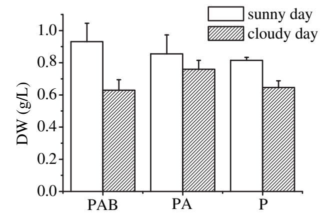

Fig. 2 Changes of biomass density in *Spirulina platensis* under full-spectrum solar radiation, solar radiation depleted of UVB, and solar radiation deprived of total UV on the sunny day or cloudy day. The initial biomass density before the experiments was 450 mg/L. Data are the mean ± standard deviation of triplicate cultures.

energy. Hourly changes in solar radiations from sunrise to sunset on 15 and 16 April 2003 (Fig. 1) were obtained from the weather bureau of Ding'an, Hainan. The temperature in the cultures was controlled at 32 ± 1°C by running water through a water bath. All the cultures were aerated with ambient air. Biomass densities were measured by filtering 50 mL of the culture on a predried filter, drying in an oven (105°C, 4 h), weighing on an electronic balance and subtracting the known weight of the dried filter. The biomass density was 450 mg/L for all the cultures at the start. The concentrations of chlorophyll *a* and carotenoids were determined according to Parsons and Strickland[7] by extracting in 100% methanol (>12 h), centrifuging at 5000 ×*g* for 10 min, and then measuring the absorbance of the supernatant with a spectrophotometer (Shimadzu UV-1206, Japan).

The biomass production was higher on the sunny day than on the cloudy day regardless of the radiation treatments (Fig. 2). However, such a difference was only significant in PAB and P treatments ($P < 0.05$, *t*-test). On the sunny day, solar radiation with UVR brought about 14% increase of the biomass yield, with UVA accounting for 5% and UVB for 9%, compared with that treated with PAR alone. On the cloudy day, PA treatment gave rise to 17% higher yield than PAB or P treatments. No significant difference ($P > 0.05$, *t*-test) was found in the biomass yield between PAB and P treatments. The biomass generation efficiency based on accumulated solar radiations was higher on the cloudy day than on the sunny day regardless of the treatments in view of the average values (Table 1), however, the difference between the sunny and cloudy days was only significant in PA and P treatments ($P < 0.05$). PA and P treatments brought about 159% and 82% increases, respectively, in the biomass generation efficiency on the cloudy day compared with the sunny day. PA treatment on the cloudy day showed the highest biomass generation efficiency.

Table 1 Accumulated solar radiations in the cultures exposed to PAB, PA and P, biomass yield and biomass generation efficiency of *Spirulina platensis* over the incubation period from 11.00 hours to 15.00 hours on the sunny and cloudy days

Radiation treatments	ASR (KJ)	BYD (mg/L)	BGE (mg/L/KJ)
PAB	52.2	481.3 ± 113.9	9.2 ± 2.2
	(15.4)	(179.3 ± 64.7)	(11.6 ± 4.2)
PA	52.0	405.3 ± 117.9	7.8 ± 2.3*
	(15.3)	(309.3 ± 55.6)	(20.2 ± 3.6)
P	45.3	364.7 ± 19.0	8.1 ± 0.4*
	(13.4)	(196.0 ± 42.0)	(14.6 ± 3.1)

*Indicates the significant differences between the sunny day and cloudy day ($P < 0.05$, *t*-test).

Data in the brackets represent the cloudy day. Data are the mean ± standard deviation of triplicate cultures.

ASR, accumulated solar radiations; BGE, biomass generation efficiency; BYD, biomass yield; P, solar radiation deprived of total UV; PA, solar radiation depleted of UVB; PAB, full-spectrum solar radiation.

UVR affected the photosynthetic pigments to different extents under the sunny and cloudy conditions. The content of chlorophyll *a* (Chl *a*) was about 14–53% lower on the sunny day than on the cloudy day regardless of the treatments (Fig. 3a). On the sunny day, PAB treatments caused 17% loss of Chl *a*, while PA treatments did not bring about significant ($P > 0.05$, *t*-test) changes compared with P treatments. In contrast, on the cloudy day, Chl *a* content increased by 47% with PAB and by 18% with PA treatment compared with P. The contents of carotenoids (Car) were not significantly ($P > 0.05$, *t*-test) changed among the treatments on the sunny day (Fig. 3b), however, on the cloudy day, the highest Car content was found in the cultures treated with PAB, being about 22% higher than PA or P-treated samples.

In the present study, UVR didn't bring about any inhibitory effects, instead it increased the biomass

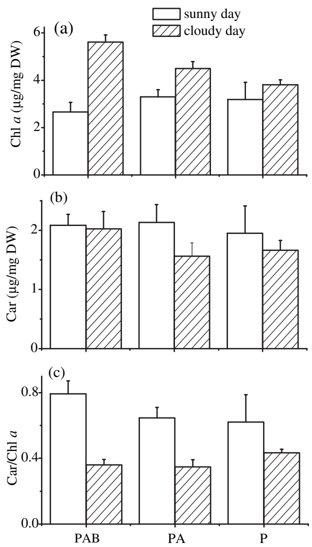

Fig. 3 Changes of the chlorophyll *a*, carotenoids contents and the ratio of carotenoids to chlorophyll *a* in *Spirulina platensis* under full-spectrum solar radiation, solar radiation depleted of UVB, and solar radiation deprived of total UV on a sunny day or cloudy day. Data are the mean ± standard deviation of triplicate cultures.

yields of the *S. platensis* strain that had been cultured under solar radiation for at least 10 years. This outdoor cultured *S. platensis* strain appeared to have adapted to the solar UVR, showing a strategy of UVR tolerance by increasing the Car/Chl *a* ratio (Fig. 3c). Increased proportion of carotenoids could play an important role in UVR protection; functioning as antioxidants and quenching photosensitization products. In addition, UV-absorbing compounds as sunscreens might be induced in *S. platensis*, as found in other cyanobacteria.[8] Although lower solar radiation resulted in less biomass on the cloudy day compared with the sunny day, the biomass generation levels normalized to the accumulated solar radiation were higher on the cloudy day than on the sunny day, indicating higher efficiency of light utilization by the cyanobacterium on the former. UVA, the ground-level flux of which is about 10-fold higher than UVB, was reported to be photosynthetically used by phytoplankton as an additional source of energy when light availability was low.[9] In the present study, the exposure to UVA resulted in higher biomass density than UVB + UVA exposures on the cloudy day. The discrepancy between UVA and UVA + UVB exposures could be attributed to the interactive effects of UVA and UVB, since the ratio of UVB to UVA has been shown to influence the growth of cyanobacteria.

Laboratory-maintained *Spirulina* strains may show different responses to outdoor UVR exposure compared with outdoor cultured *Spirulina* strains or those freshly isolated from nature, because having been grown in an environment free of UVR for years might have resulted in the loss of their UVR tolerance. The *S. platensis* strain in the present work that has been cultured under sunlight for decades must have acquired certain mechanisms to use solar UVR, especially the waveband of UVA. Whether laboratory-maintained *S. platensis* strains negatively respond to UVR remains a question, and comparison of the responses to solar UVR among strains of different backgrounds should be further studied.

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