PHOTOSYNTHETICA 45 (2): 309-311, 2007

BRIEF COMMUNICATION

Influence of irradiance, dissolved oxygen concentration, and temperature on the growth of *Chlorella sorokiniana*

C.U. UGWU*, H. AOYAGI, and H. UCHIYAMA

Institute of Life Sciences and Bioengineering, Graduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba City, Ibaraki 305-8572, Japan

Abstract

The growth response of *Chlorella sorokiniana* to certain irradiance, DO, and temperature demonstrated the possible causes of low productivity with this strain in outdoor cultures. The growth (biomass productivity) and chlorophyll fluorescence (F_v/F_m) were substantially reduced when the dissolved oxygen (above 200 % of air saturation) and temperature were elevated.

Additional keywords: biomass; chlorophyll fluorescence; photoinhibition.

Growth of microalgae under photoautotrophic conditions is affected by changes in physicochemical factors such as irradiance, temperature, and dissolved oxygen (DO) concentration. Irradiance is one of the major factors, which control microalgae productivity (Terry 1986, Lee and Low 1992, Grobbelaar *et al*. 1996). In closed photobioreactors that are used for outdoor cultures, there are variations in solar irradiance and temperature during the day (Molina Grima *et al*. 1999, Acién Fernández *et al*. 2001, Ugwu *et al*. 2005). Furthermore, depending on the growth of the microalgae, high oxygen concentrations could accumulate in the culture. DO concentration (up to 70 to 80 g m^{-3}) is very common in *Arthrospira* (*Spirulina*) cultures grown in tubular photobioreactors (Vonshak *et al*. 1996). Torzillo *et al*. (1998) reported that the synergistic effect of high oxygen concentration and low temperature resulted to photosystem 2 (PS2) photoinhibition. A combination of high concentration of oxygen, high temperature, and high irradiance would drastically reduce the photosynthetic activities of alga during outdoor cultures. During the active growth of alga cells (in the midday), each of these physicochemical factors would affect the photosynthetic apparatus of the microalgae. Production models under laboratory conditions are necessary to understand the growth characteristics of microalgae in outdoor cultures. In an attempt to charac-terize the influence of solar irradiance of microalgae growth, irradiance modelling was investigated in a laboratory-scale photobioreactors (Wu and Merchuk 2004, Pottier *et al*. 2005). Our previous studies with outdoor tubular photobioreactor for mass cultivation of *Chlorella sorokiniana* (Ugwu *et al*. 2002) indicated that a thorough understanding of the interaction between the three mentioned factors is necessary for efficient outdoor cultivation of this microalga.

We used *C. sorokiniana* IAM-212, obtained from the Culture Collection Center of the Institute of Applied Microbiology, University of Tokyo. pH of the modified MM4 medium (Ugwu *et al*. 2005) was adjusted to 6.0. *C. sorokiniana* was inoculated into 1 200 cm^3 of the medium in Roux flask. Seven daylight fluorescent lamps (*8FL-40-s-PG*, *National Electric Tokyo*) arranged in parallel on a vertical plane were used for irradiation. The irradiance at the surface of the Roux bottles varied from 200 to 500 μmol(photon) m^{-2} s^{-1}. Aeration and mixing were achieved by sparging air enriched with 5 % CO_2 through a glass-ball filter, which was inserted to the bottom of the flasks, at 0.3 volume of air per volume of liquid per minute. All the experiments were carried out in the laboratory for 12 h (08:00–20:00). Each of the experiments was repeated three times to ensure reproducibility. Cell dry matter was determined by using duplicate samples of the

Received 14 October 2006, *accepted* 12 December 2006.
*Author for correspondence; fax (+81)-29-853-7212, e-mail: cugwu@sakura.cc.tsukuba.ac.jp
Abbreviations: Chl – chlorophyll; DO – dissolved oxygen concentration; F_v/F_m – maximum chlorophyll fluorescence; PS2 – photosystem 2.
Acknowledgment: The authors acknowledge the financial support provided to them by Japan Society for Promotion of Science (JSPS).

culture. The cells were washed with 0.5 M HCl to remove the precipitated salts and other non-organic substances, rinsed with distilled water, dried at 105 °C for 24 h, cooled over silica gel in a desiccator, and weighed (Ugwu *et al*. 2002). The optical density was measured at 680 nm using a spectrophotometer (*Spectronic 20A*; *Shimadzu*, Tokyo, Japan). Biomass productivity was defined as the increase in the cell dry mass between 08:00 and 20:00 (12-h culture). The solar irradiance on the surface of flask was measured using a photorecorder (*PHR-51*, Japan). Dissolved oxygen probe (*Mk-250 DO*; *Marubishi*, Japan) was inserted in the flask to measure the variation in DO at various times. In order to understand the effect of DO to the microalgae, various DO concentrations (120–300 % of air saturation) were maintained in the cultures. This was achieved by sparging either oxygen or nitrogen gases to the cultures to maintain the desired DO concentration. The DO regulator was equipped with valves to monitor and regulate the DO concentration to set values. Thus, when the DO in the culture drops or becomes elevated, the DO regulator pumps in either oxygen or nitrogen gases to the cultures to maintain the DO to the set value. Chlorophyll (Chl) fluorescence of the cultures (the maximum photosystem 2 photochemical yield, F_v/F_m) was measured using an induction fluorometer (*PEA*, *Hansatech*, UK) after incubation in dark for 10 min. 1 cm^3 of samples (triplicate samples) was dark-adapted for 10 min before measuring Chl fluorescence.

The growth of the microalga varied as the temperature was elevated from 20 to 40 °C. At 38 °C, the biomass productivity obtained was 0.85 kg m^{-3} d^{-1}, while at 40 °C it declined drastically (0.33 kg m^{-3} d^{-1}), which indicated that this temperature was not conducive for the growth of the microalga. Furthermore, accumulation of DO in the culture depended on the growth of the microalga at various temperatures. When the temperature was increased from 20 to 38 °C, the DO increased to about 200 % of air saturation. This increase in the DO could be attributed to increased photosynthetic activity of *C. sorokiniana*. Our preliminary studies on the effect of temperature on growth of *C. sorokiniana* also indicated that its optimum temperature falls within this temperature range. However, the DO decreased to less than 100 % of air saturation when the temperature was increased to 40 °C. Analysis of the Chl fluorescence showed that the photosynthetic activity of the cells was reduced drastically at high temperatures. Increase in irradiance from 50 to 150 μmol m^{-2} s^{-1} resulted in linear increase of biomass productivity and consequently led to increased accumulation of the DO in culture (Fig. 1). Both the biomass productivity and the Chl fluorescence (F_v/F_m) declined when the DO concentration was increased from 120 to 300 % of air saturation (Fig. 1). High degree of cell lysis and death was observed when the DO exceeded 200 % of air saturation. The decrease in biomass productivity as well as the reduction in F_v/F_m was an indication that high concentration of DO resulted in photochemical damage to the photosynthetic apparatus of *C. sorokiniana*. In terms of Chl fluorescence, it was reported that its parameters, ratios, and quenching provide information on the functionality of PS2 and the photosynthetic apparatus (Roháček and Barták 1999, Lichtenthaler *et al*. 2005).

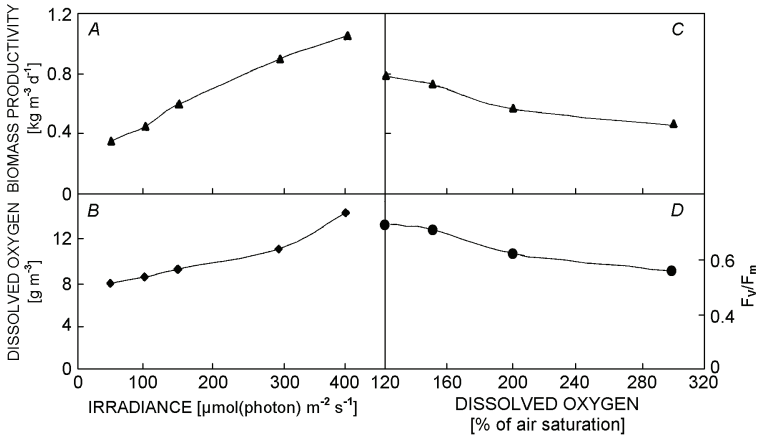

Fig. 1. *A*,*B*: Changes in the biomass productivity (▲) and accumulation of dissolved oxygen (♦) in *Chlorella sorokiniana* cultures at various irradiances, when temperature was maintained at 35 °C. *C*,*D*: Effect of dissolved oxygen on the biomass productivity (▲) and chlorophyll fluorescence (●) when irradiance was maintained at 400 μmol(photon) m^{-2} s^{-1}.

Increase in the irradiance favoured the growth of *C. sorokiniana* but resulted in increased accumulation of DO. Accumulation of DO is common in alga cultures, and the degree of its accumulation would depend on the mass transfer of photobioreactors used. Generally, efficient mixing would reduce the DO in cultures and ensure good mass transfer of oxygen and CO_2 in the culture system. In photoautotrophic cultures, a linear growth occurs provided the irradiance is sufficient and the nutrients are not limiting in the culture. When the irradiance exceeds the saturation value of the microalga, photoinhibition would occur. Aside from high irradiance, photoinhibition can also occur at suboptimal temperatures, even at relatively low irradiances (Vonshak and Torzillo 2004). This would also explain the decrease in the Chl fluorescence of *C. sorokiniana* when the temperature was maintained at 40 °C.

In conclusion, some outdoor variables that determine the growth of *C. sorokiniana* outdoors were characterized under laboratory conditions. Microalgae response to

various irradiances, DO, and temperature would help in optimization of their outdoor mass cultures. In outdoor cultures, combined effects of these factors would not only reduce the biomass productivity of *C. sorokiniana* but would also lead to low production of intracellular compounds, such as Chl and carotenoids. Our results showed that when one of these variables (for instance, irradiance) was set at the optimum, the adverse effect of other variables (such as DO and temperature) adversely affected the growth of *C. sorokiniana*. Furthermore, some preliminary studies showed that these outdoor variables are more problematic in tubular photobioreactors that are poorly mixed (data not shown). It is anticipated that a thorough understanding of the interaction between irradiance, DO, and temperature will be very helpful in optimizing the growth of *C. sorokiniana* in outdoor mass cultures.

References

Acién Fernández, F.G., Fernández Sevilla, J.M., Sánchez Pérez, J.A., Molina Grima, E., Chisti, Y.: Airlift driven external-loop tubular photobioreactors for outdoor production of microalgae: assessment of design and performance. – Chem. eng. Sci. **56**: 2721-2732, 2001.

Grobbelaar, J.U., Nedbal, L., Tichy, V.: Influence of high frequency light/dark fluctuations on photosynthetic characteristics of microalgae photoacclimated to different light intensities and implications for mass algal cultivation. – J. appl. Phycol. **8**: 335-343, 1996.

Lee, Y.-K., Low, C.-S.: Productivity of outdoor algal cultures in enclosed tubular photobioreactor. – Biotechnol. Bioeng. **40**: 1119-1122, 1992.

Lichtenthaler, H.K., Buschmann, C., Knapp, M.: How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio R_{Fd} of leaves with the PAM fluorometer. – Photosynthetica **43**: 379-393, 2005.

Molina Grima, E., Acién Fernández, F.G., García Camacho, F., Chisti, Y.: Photobioreactors: light regime, mass transfer, and scaleup. – J. Biotechnol. **70**: 231-247, 1999.

Pottier, L., Pruvost, J., Deremetz, J., Cornet, J.-F., Legrand, J., Dussap, C.G.: A fully predictive model for one-dimensional light attenuation by *Chlamydomonas reinhardtii* in a torus photobioreactor. – Biotechnol. Bioeng. **91**: 569-582, 2005.

Roháček, K., Barták, M.: Technique of the modulated chlorophyll fluorescence: basic concepts, useful parameters, and some applications. – Photosynthetica **77**: 339-363, 1999.

Terry, K.L.: Photosynthesis in modulated light: quantitative dependence of photosynthetic enhancement on flashing rate. – Biotechnol. Bioeng. **28**: 988-995, 1986.

Torzillo, G., Bernardini, P., Masojidek, J.: On-line monitoring of chlorophyll fluorescence to assess the extent of photoinhibition of photosynthesis induced by high oxygen concentration and low temperature and its effects on the productivity of outdoor cultures of *Spirulina platensis* (cyanobacteria). – J. Phycol. **34**: 504-510, 1998.

Ugwu, C.U., Ogbonna, J.C., Tanaka, H.: Improvement of mass transfer characteristics and productivities of inclined tubular photobioreactors by installation of internal static mixers. – Appl. Microbiol. Biotechnol. **58**: 600-607, 2002.

Ugwu, C.U., Ogbonna, J.C., Tanaka, H.: Characterization of light utilization and biomass yields of *Chlorella sorokiniana* in inclined outdoor tubular photobioreactors equipped with static mixers. – Proc. Biochem. **40**: 3406-3411, 2005.

Vonshak, A., Torzillo, G.: Environmental stress physiology. – In: Richmond, A. (ed.): Handbook of Microalgal Culture. Pp. 57-82. Blackwell Publ., Oxford 2004.

Vonshak, A., Torzillo, G., Accolla, P., Tomaselli, L.: Light and oxygen stress in *Spirulina platensis* (Cyanobacteria) grown outdoors in tubular reactors. – Physiol. Plant. **97**: 175-179, 1996.

Wu, X., Merchuk, J.C.: Simulation of algae growth in a bench scale internal loop airlift reactor. – Chem. eng. Sci. **59**: 2899-2912, 2004.