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IMPROVEMENT OF PHOTOSYNTHETIC EFFICIENCY THROUGH REDUCTION OF CHLOROPHYLL ANTENNA SIZE

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Abstract

We have previously presented a graphical illustration of a strategy to improve photosynthetic conversion efficiencies by a reduction of the antenna size in photosynthetic reaction centers. During the current reporting period, we have made progress in demonstrating the conceptual correctness of this idea. Light-saturation studies for CO_2 in air were performed with an antenna-deficient mutant of *Chlamydomonas* (DS521) and the wild type (DES15). The light-saturated rate for CO_2 assimilation in mutant DS521 was about two times higher (187 μmol·h^{-1}·mg chl^{-1}) than that of the wild type, DES15 (95 μmol·h^{-1}·mg chl^{-1}). Significantly, a partial linearization of the light-saturation curve was also observed. The light intensities that give half-saturation of the photosynthetic rate were 276 and 152 μE·m^{-2}·s^{-1} in DS521 and DES15, respectively. These results confirmed that DS521 has a smaller chlorophyll antenna size and demonstrated that the reduction of antenna size can indeed improve the overall efficiency of photon utilization. Corresponding experiments were also performed with CO_2 in helium. Under this anaerobic condition, no photoinhibition was

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observed, even at elevated light intensities. Photoinhibition occurs under aerobic conditions. The antenna-deficient mutant DS521 can also provide significant resistance to photoinhibition, in addition to the improvement in the overall efficiency in CO_2 fixation.

Introduction

Photosynthesis is the foundation for algal hydrogen production. Improvement of overall photosynthetic efficiency in a mass algal culture must be achieved to develop a viable biohydrogen production process. We have previously presented a graphical illustration of a strategy to improve photosynthetic conversion efficiencies by reduction of chlorophyll antenna size in photosynthetic reaction centers (Greenbaum and Lee 1998). The concept is that the overall photosynthetic efficiency of an algal population can be improved by reduction of the size of the light-harvesting chlorophyll antenna. In full sunlight (2000 $\mu E \cdot m^{-2} \cdot s^{-1}$), a kinetic imbalance exists between the rate of photon excitation of photosynthetic reaction centers and the ability of the thermally activated electron transport chains to process photogenerated electrons (Fig. 1A). Typically, a functional photosystem (PS) I or II has about 200 chlorophyll molecules per reaction center. At full sunlight intensity, therefore, the reaction centers can receive photoexcitation at a rate of about 2000/s. However, the operation of the electron transport chain is, at most, only about 200/s because of the relatively slow diffusive motion of the electron carriers that shuttle between PSII and PSI and/or in the Calvin cycle (Fig. 1A). Therefore, normal photosynthesis saturates at much less than full sunlight, typically about 10%. Reduction of the antenna size can effectively reduce the rate of photon absorption per reaction center and allow actinic photons to penetrate more deeply into the algal culture, thus improving the overall efficiency of sunlight photosynthetic utilization.

This theoretical consideration was clearly stated by Clayton (1977). However, very few experimental studies were conducted to test this theory. In a developmental study of photosynthesis, Herron and Mauzerall (1972) first observed a nearly linear light-saturation curve in a dark-grown greening mutant culture of *Chlorella*, which is remarkably consistent with the prediction of the theory. Using high-light treatment, which physiologically reduces the chlorophyll antenna size in *Dunaliella salina* (Chlorophyta), Melis et al. (1999) recently demonstrated that the high-light-grown algal culture exhibited a threefold higher P_{max} (light-saturated rate of photosynthesis) than the normally pigmented low-light-grown culture, suggesting that algal strains with small antenna size could exhibit higher productivity than that currently achieved with normally pigmented cells. However, the smaller antenna sizes in both the dark-grown greening mutant and the high-light-grown *Dunaliella* used in the previous studies cannot be sustained because they readily revert to that of the normally pigmented cells upon returning to normal light intensity. Here, we report the results of an experimental study with a stable antenna-deficient mutant of *Chlamydomonas* DS521 and demonstrate the validity of the theory.

Results and Discussion

The mutant DS521 was created by chemical metagenesis using fluorodeoxyuridine and ethylmethanesulfate (Galloway and Mets 1989). Briefly, colonies grown from mutagenized cells were subjected to selection in the presence of metronidazole in the light. This procedure kills cells capable of full rates of photosynthetic electron transport but allows the survival of strains that have lower photosynthetic rates under the conditions used. DS521 is a nuclear gene mutant characterized by a high chlorophyll a/b ratio, though it is not completely deficient in chlorophyll b. It was estimated that DS521 contained only 5–10% of wild-type levels of the mRNA for chlorophyll a/b–binding (cab) proteins (Steve Howell, unpublished and personal communication to L. Mets). As a result, a portion of the antenna chlorophyll molecules in DS521 are removed by the mutation (Fig. 1B). Therefore, DS521 has fewer chlorophyll molecules per reaction center than wild-type DES15 (Fig. 1A).

Antenna-deficient mutant DS521 and wild-type DES15 were comparatively assayed for photoassimilation of CO_2 under various actinic intensities. These algal strains were grown under a light intensity of about 20 $\mu E \cdot m^{-2} \cdot s^{-1}$ in minimal-plus-acetate liquid medium. The rationale for growing these strains photoheterotrophically is that the selection pressure for algal cells to make antenna would be minimal under such a growth condition. When the cultures grew to a density of about 10^6 cells/mL, the algal cells were harvested by gentle centrifugation (3000 rpm). They were then washed and resuspensed in fresh minimal medium for photosynthetic assays.

The CO_2 fixation assays were performed with 700 ppm CO_2 in helium or air using our unique dual-reactor-flow detection system (Fig 2). The actinic illumination was provided by a red (peak wavelength at 670 nm) light-emitting diode (LED) light source that was controlled by a computerized step motor. Because of the automatic control of the LED light source, precise and reproducible step functions of actinic intensity were generated. The actinic intensity was monitored and recorded by a computer simultaneously with the rates of CO_2 fixation. Analysis of experimental data showed that the maximal rate of CO_2 fixation in DS521 with 700 ppm CO_2 under air was about two times higher (187 $\mu mol \cdot h^{-1} \cdot mg\ chl^{-1}$) than that of the wild type, DES15 (95 $\mu mol \cdot h^{-1} \cdot mg\ chl^{-1}$). A comparative illustration of the light-saturation curves in DS521 and DES15 is presented in Fig. 3. Photoassimilation of CO_2 in DS521 saturated at about twice the actinic intensity of that in DES15. The light intensities that give half-saturation of the photosynthetic rate was 276 and 152 $\mu E \cdot m^{-2} \cdot s^{-1}$ in DS521 and DES15, respectively. This is an important result since it confirmed that DS521 has a smaller chlorophyll antenna size and demonstrated that the reduction of antenna size can indeed improve the overall efficiency of photon utilization.

DS521 also had significantly more resistance to photoinhibition than the wild type. As illustrated in Fig. 4, the simultaneous monitoring of CO_2 photoassimilation and actinic intensity showed that the photosynthetic activity in the wild type was photoinhibited almost completely when the LED actinic light reached its full intensity [red (670nm) photons: 2000 $\mu E \cdot m^{-2} \cdot s^{-1}$], while DS521 retained about 25% of its maximal photosynthetic activity. The photoinhibited wild-type cells released more CO_2 by respiration than the amount of CO_2 fixation, while DS521 was still capable of photosynthesizing. These are important results since they demonstrate that reduction of

photosynthetic antenna size in green algae can also provide resistance to photoinhibition, in addition to improvement of the overall efficiency in CO_2 fixation.

Photoassimilation of CO_2 was also measured with 700 ppm CO_2 in helium. As illustrated in Fig. 5, the maximal rate of CO_2 photoassimilation in DS521 (165 μmol·h^{-1}·mg chl^{-1}) was about 37% higher than that in DES15 (120 μmol·h^{-1}·mg chl^{-1}). However, no dramatic photoinhibition was observed in either DS521 or DES15 under the anaerobic (helium) condition. This is expected since photoinhibition results primarily from the formation of singlet oxygen, which can react with many biomolecules and destroy their functionality. The anaerobic condition limits the formation of this destructive species. Based on the measured rates of photosynthesis, the antenna size in DS521 was estimated to be about 30–50% smaller than that in DES15. More experimental studies are under way to verify these findings.

Acknowledgments

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Figure Legends

Figure 1. A: Conventional Photosynthetic Antenna Size and Pathway in DES15; B: Antenna-Deficient Mutant DS521.

Figure 2. Schematic f a Dual-Reactor Flow Detection System for Simultaneous Detection of CO_2, H_2, O_2, and CH_4.

Figure 3. Light-Saturation Curve as Measured with Photosynthetic Fixation of CO_2 by DS521 and DES15 with 700 ppm CO_2 in Air.

Figure 4. Simultaneously Recorded Data of Actinic Intensity and CO_2 Photoassimilation by DS521 and DES15 with 700 ppm CO_2 in Air.

Figure 5. Light-Saturation Curve as Measured with Photosynthetic Fixation of CO_2 by DS521 and DES15 with 700 ppm CO_2 in Helium.

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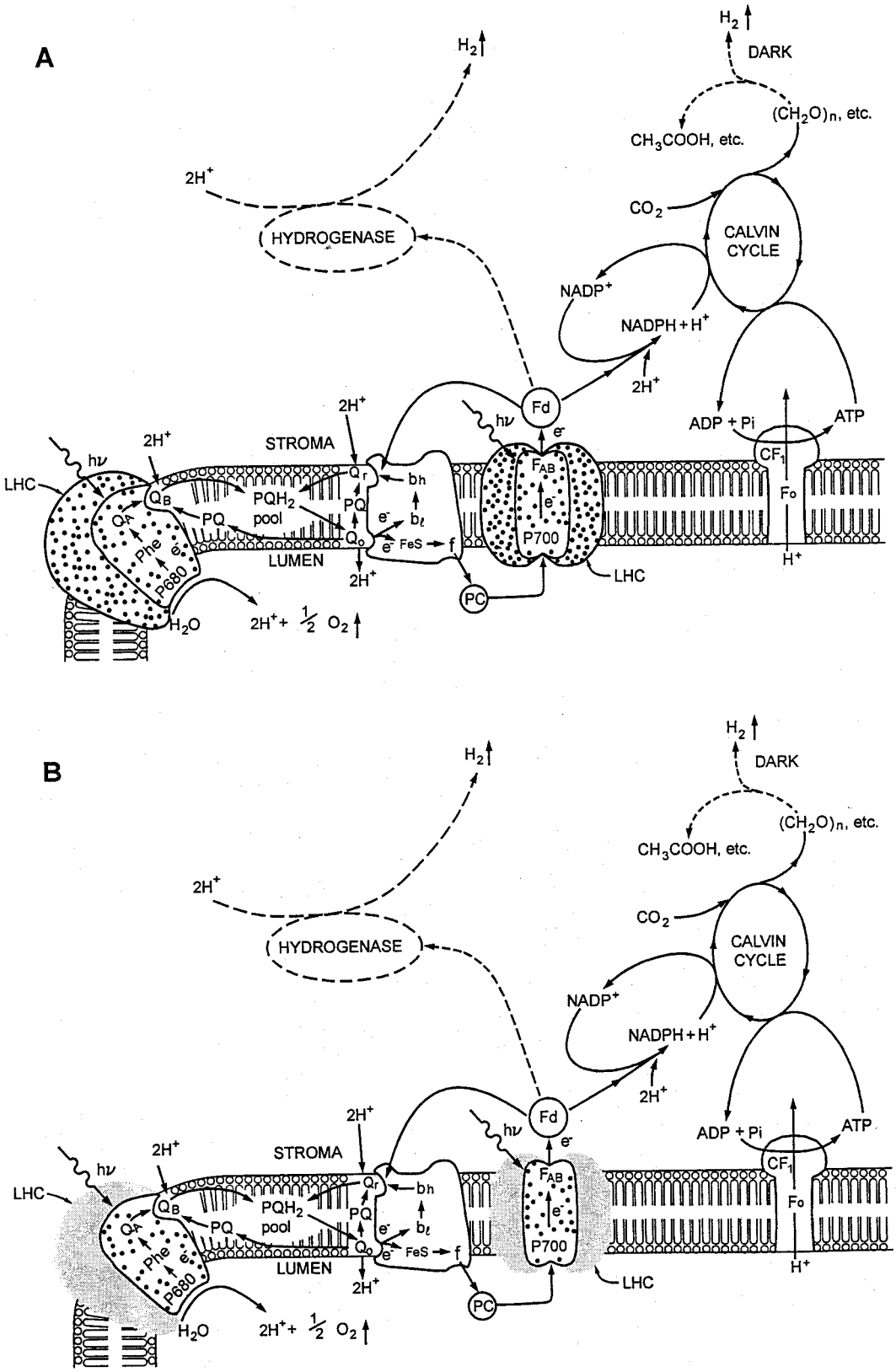

Figure 1–A: Conventional Photosynthetic Antenna Size and Pathway in DES15; B: Antenna-Deficient Mutant DS521

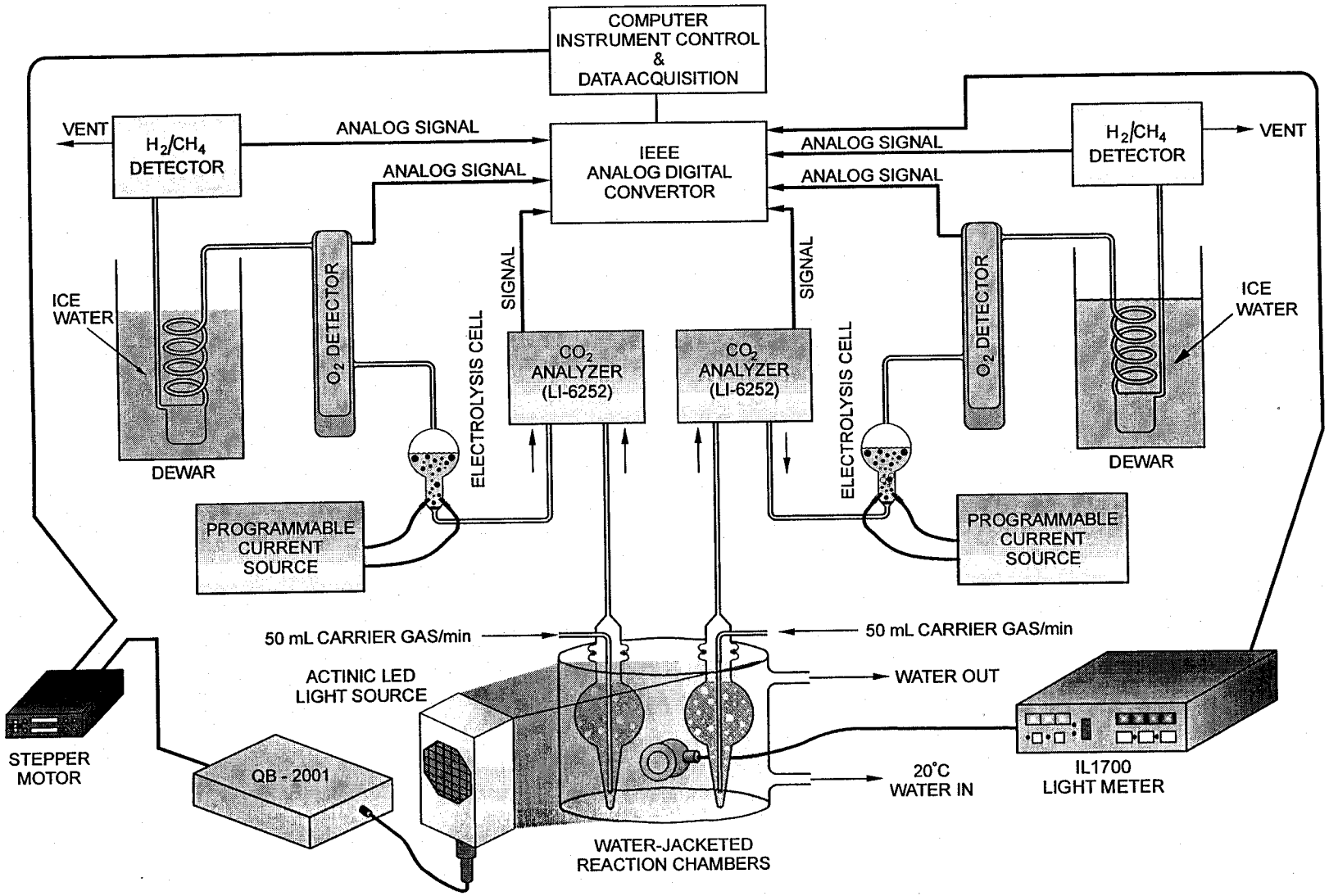

Figure 2–Schematic of a Dual-Reactor-Flow Detection System for Simultaneous Detection of CO_2, H_2, O_2, and CH_4

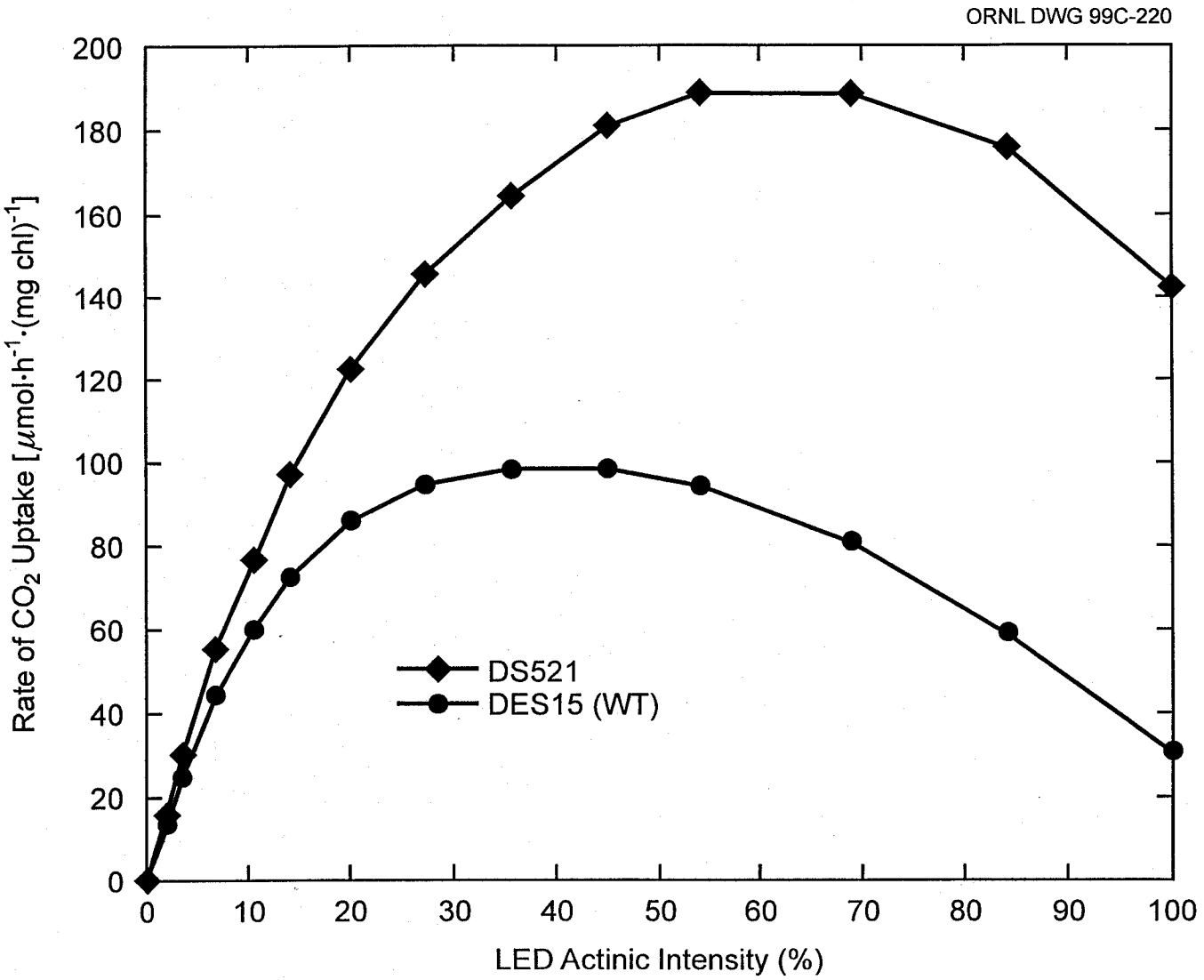

Figure 3–Light-Saturation Curve as Measured with Photosynthetic Fixation of CO_2 by DS521 and DES15 with 700 ppm CO_2 in Air

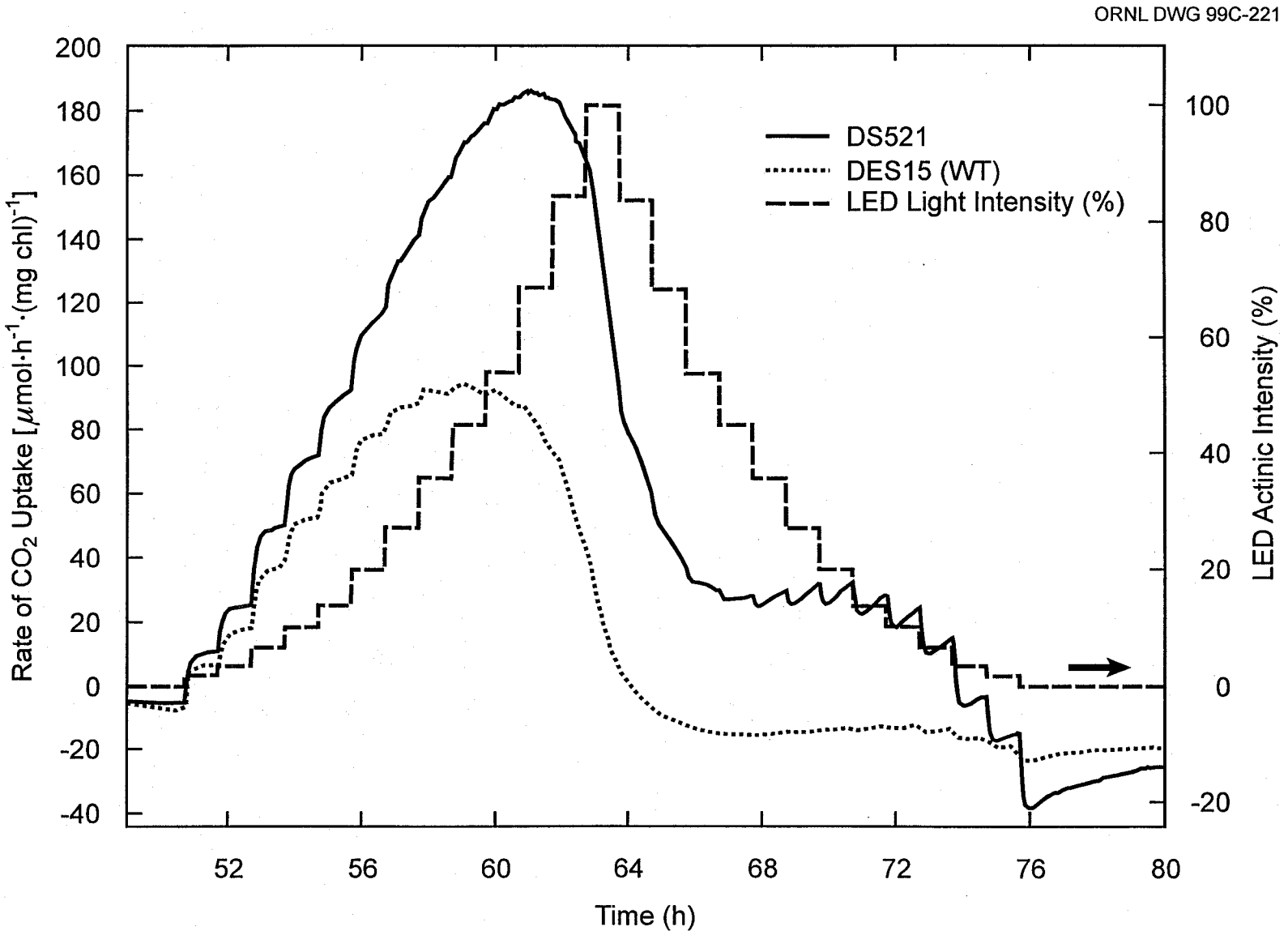

Figure 4–Simultaneously Recorded Data of Actinic Intensity and CO_2 Photoassimilation by DS521 and DES15 with 700 ppm CO_2 in Air

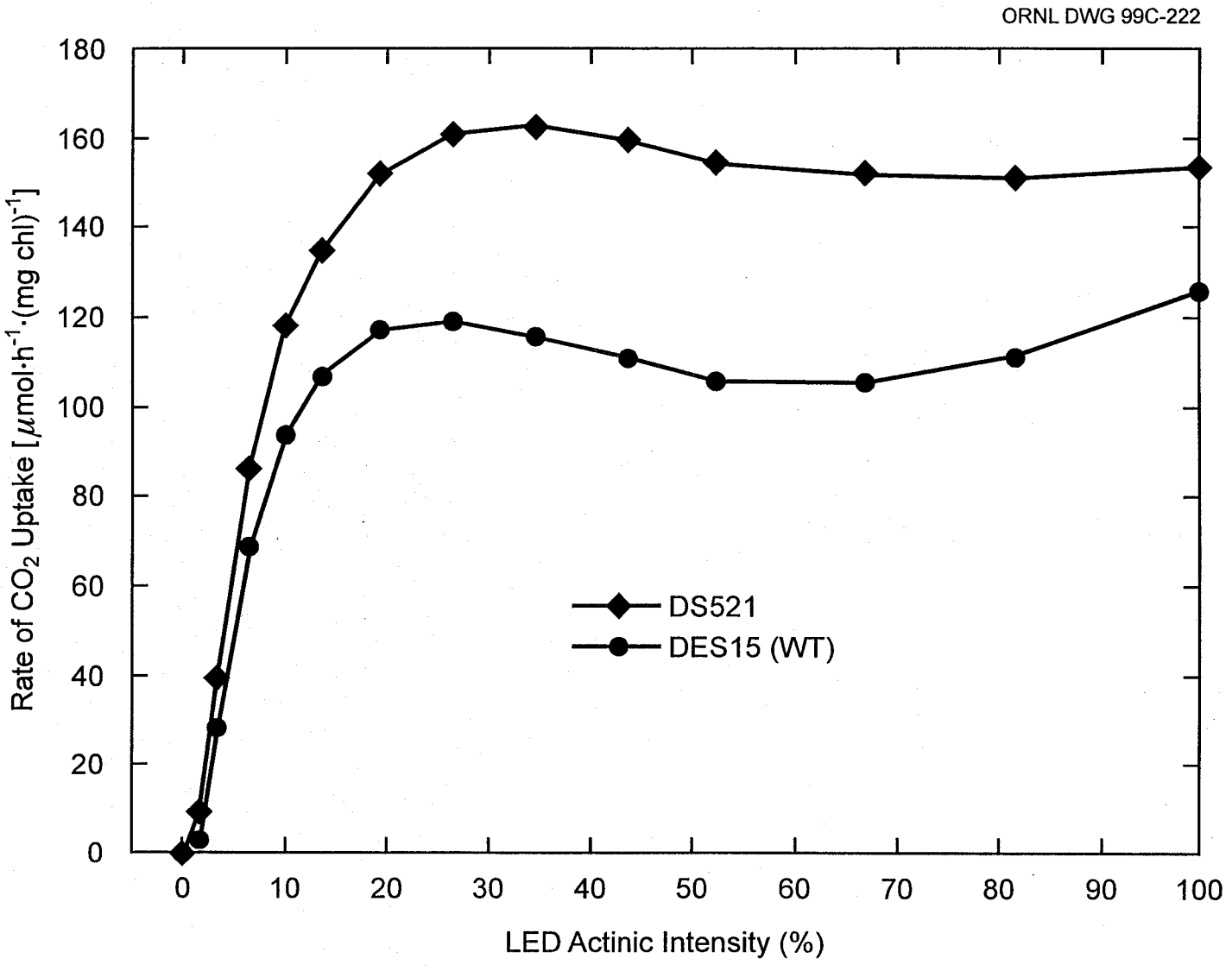

Figure 5–Light-Saturation Curve as Measured with Photosynthetic Fixation of CO_2 by DS521 and DES15 with 700 ppm CO_2 in Helium