Pigment Reduction to Improve Photosynthetic Productivity of *Rhodobacter sphaeroides*

KIM, NAG-JONG[1], JEONG KUG LEE[2], AND CHOUL-GYUN LEE[1]*

[1]*Institute of Industrial Biotechnology, Department of Biotechnology, Inha University, Incheon 402-751, Korea*
[2]*Department of Life Science, Sogang University, Seoul 121-742, Korea*

Abstract Improving the light utilization efficiency of photosynthetic cells in photobioreactors (PBRs) is a major topic in algal biotechnology. Accordingly, in the current study we investigated the effect and suitability of photosynthetic pigment reduction for improving light utilization efficiency. The light-harvesting complex II (LH-II) genes of *Rhodobacter sphaeroides* were removed to construct a mutant strain with less pigment content. The mutant strain exhibited a slower growth rate than the wild-type under a low light intensity, while the mutant grew faster under a high light intensity. In addition, the specific absorption coefficient was lower in the mutant due to its reduced pigment content, thus it seemed that light penetrated deeper into its culture broth. However, the distance (light penetration depth) from the surface of the PBR to the compensation point did not increase, due to an increase in the compensation irradiance of the mutant strain. Experimental data showed that a reduced photosynthetic pigment content, which lessened the photoinhibition under high-intensity light, helped the volumetric productivity of photosynthetic microorganisms.

Key words: Pigment reduction, photosynthetic productivity, *Rhodobacter sphaeroides*, mutual shading effect, photoinhibition

Photosynthetic organisms, such as plants, algae, and photosynthetic bacteria, have developed efficient systems to harvest photons from the sun and then use their energy to reduce CO_2 into sugar [14]. Photosynthetic pigments, such as chlorophylls and carotenoids, absorb light energy and are contained in photosynthetic units (PSUs) [14], which are always organized so that the light-harvesting (LH) complexes surround the reaction center (RC) complex [12, 14].

However, if cells absorb more light than their photosynthetic ability to convert light into chemical energy, only a fraction of the light energy is used. As such, the portion of light energy utilized decreases rapidly as the light intensity increases [2, 16], meaning that the overall utilization of photosynthetic energy does not increase proportionally according to the light intensity [11]. One explanation for this decrease in efficiency is mutual shading, which becomes particularly intense in a high-density culture where there is only a very thin (shallow) photic zone through which cells can receive photons to reach a saturation point. With a high cell density, mutual shading also decreases the number of cells that are exposed to light, as only those cells close to the illuminated surface 'see' the light, regardless of the light intensity supplied [17]. To circumvent these mutual shading problems, various solutions have already been introduced, such as reducing the cellular contents of the light-harvesting pigment [2, 3, 25, 33], dispersing the incoming light throughout the culture using an optical fiber [2], redistributing the light with modified photobioreactor geometry [22], genetically manipulating the CO_2 fixation gene, RuBisCO (ribulosebisphosphate carboxylase/oxygenase), to improve the dark reaction rate in photosynthesis [36, 37], improving the heterotrophic ability of photoautotrophic cells [18, 39], or simply admitting the limitation of light transfer in high-density cultures.

In recent attempts to reduce photosynthetic pigments, Sukenik and Falkowski [33] presented a theoretical model where low-light-adapted *Dunaliella tertilecta* was determined to produce a smaller decrease in productivity than high-light-adapted *Dunaliella tertilecta* due to photoinhibition. In addition, Nakajima and Ueda [26–28] used pigment-deficient mutants of a cyanobacterium (*Synechocystis*) and green alga (*Chlamydomonas perigranulata*) to demonstrate increased productivities in continuous cultures at high light intensities, and reported 25 to 50% higher productivities for the mutants than the wild-type. As such, the fundamental

*Corresponding author
Phone: 82-32-860-7518; Fax: 82-32-872-4046;
E-mail: leecg@inha.ac.kr

validity of this approach has already been demonstrated, although the reported increases in productivity have only been modest compared to the maximum theoretical increase of over 200% [3].

There has also been dramatic progress in the current understanding of the reactions taking place during the early events of photosynthesis, largely due to research involving purple photosynthetic bacteria. Anaerobic photosynthetic prokaryotes have been and continue to be excellent model organisms for investigating the basic mechanisms of photosynthetic light-harvesting and reaction-center photochemistry [4]. The relatively simple photosystems of photosynthetic bacteria make them attractive models for studying light absorption and the transfer and trapping of the excitation energy. This simplicity also extends to the organization and expression of genetic information, thus it would be a missed opportunity if molecular genetics were not used to examine the assembly, organization, and function of light-harvesting complexes [15]. However, despite intensive research on light capture, its transfer, and dissipation, as well as pigment formation, the genetic engineering of light-harvesting complexes, and structural study of their photosynthetic units [4, 5, 12, 13, 19, 29–32], the application of the engineering concept of photosynthetic productivity to such organisms has been rare except for studies on hydrogen production [8, 23, 38]. Accordingly, in the current study we have selected the anoxygenic photosynthetic purple bacterium *Rhodobacter sphaeroides* as a model microorganism to effectively predict the effect of pigment reduction in conjunction with previous intensive research on the biophysical, biochemical, and genetic engineering of this strain.

MATERIALS AND METHODS

Rhodobacter sphaeroides 2.4.1 and its B800-850⁻ mutant *R. sphaeroides* KDC were provided by Lim et al. [19]. The mutant cells, which had Km^r DNA inserted at the *Xmn*I site within *pucB*, were constructed as described earlier [19]. Each *R. sphaeroides* strain was grown photoheterotrophically at 29°C in a modified Sistrom's medium [20] containing three times the amount of enriched $(NH_4)_2SO_4$ compared with the original composition. The photosynthetic conditions were achieved by illuminating an anaerobically-maintained PBR-A or PBR-B with 500 W linear tungsten-halogen lamps. The anaerobic conditions were achieved by either eliminating the head space (filled with a culture broth) or using helium gas. The light intensity was adjusted by changing the distance from the lamps and measured using a quantum sensor (model LI-190SA, LI-COR, Lincoln, NE, U.S.A.) with a DataLogger (model LI-1400, LI-COR, Lincoln, NE, U.S.A.). Two types of glass photobioreactors were used in the current study: a PBR-A with an inner

diameter of 2.5 cm and culture volume of 60 ml and PBR-B with a 7 cm diameter and 400 ml volume. The cultures in the PBR-B were mixed with magnetic stir bars at an agitation rate of 200 rpm.

The cell concentration (in cell/ml), total cell volume (in μm^3 cell/ml), and average cell volume (in μm^3/cell) were all measured using a Coulter Counter (model Z2, Coulter Electronics, Inc., Hialeah, FL, U.S.A.) with Coulter AccuComp® Software. To obtain the specific absorption coefficient, the culture broth was sampled at various growth phases and measured as described earlier [17] using a spectrophotometer (model HP8453E, Hewlett Packard, Waldbronn, Germany). The photopigments of the whole cells were determined after extracting with acetone-methanol (7:2, v/v), as described previously [6].

RESULTS AND DISCUSSION

Characteristics of Light Absorption

Light-harvesting complex II was responsible for the first and second peaks (800 and 850 nm), while the absorption by light-harvesting complex I appeared at 875 nm as shown in Fig. 1. The LH-II deficient mutant, KDC, did not exhibit the absorption maxima of B800-850 (lower lines in Figs. 1a and 1c, upper lines in Figs. 1b and 1d). Figures 1a and 1b were obtained from the cells cultured under a high light intensity of 302 $\mu E/m^2/s$, while the light absorption of the cells grown under a low intensity light of 74 $\mu E/m^2/s$ are represented in Figs. 1c and 1d. The high light intensity, which was the highest possible intensity with the available setup, was about 70 W/m^2, a photoinhibiting intensity [19], whereas the low light intensity was selected as a light-limiting intensity for high-density cell cultures.

The specific absorption coefficient based on the bacteriochlorophylls (BChls, Figs. 1a and 1c) was lower for the wild-type strain than for the mutant due to the high BChl content in the wild-type strain. However, the specific absorption coefficients based on the cell mass (cell volume, Figs. 1b and 1d) were almost identical between the strains, except for the 800– 850 nm peaks, due to LH-II, and the ~500 nm peaks, due to the difference in the carotenoid (Crt) quantity [14]. As such, within the range of 800– 850 nm and ~500 nm, the light penetrated deeper into the mutant cell cultures than into the wild-type cultures, indicating that the mutant cells were able to grow better at a higher density than the wild-type cells.

Light Response Curve

The profiles of the light response curves in Fig. 2 (specific growth rate vs. light intensity) were obtained from the PBR-A under different light intensities. The profiles exhibited a good agreement with the Aiba model [1] except for the origin of the curve. While the Aiba model
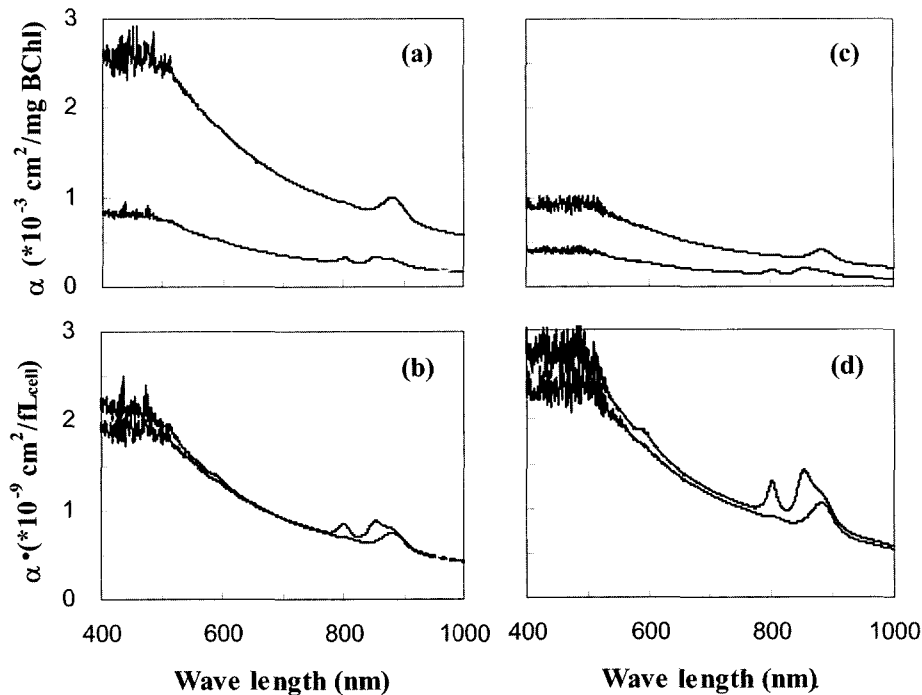
Fig. 1. Comparison of light absorption by wild-type and mutant cells based on Bchls (a and c) and cell volume (b and d). α is the specific absorption coefficient of the cells calculated using Beer-Lambert's Law based on the data measured with a UV spectrophotometer.

The light intensities were 302 μE/m^2/s for the high-intensity light (a and b) and 74 μE/m^2/s for the low-intensity light (c and d). Peaks appeared at 800, 850, and 875 nm. Only the wild-type strain (lower line in a and c, upper line in b and d) showed absorption maxima at 800 and 850 nm. To create the same conditions for comparison, both strains were grown with almost the same cell density.

cannot represent light compensation points, the profiles in Fig. 2 indicated a compensation irradiance based on a parallel shift in the x-axis or light intensity, where the displacement of the x-axis represents the compensation irradiance. As Nakajima et al. [24] reported previously, the strain with the reduced antenna size showed reduced
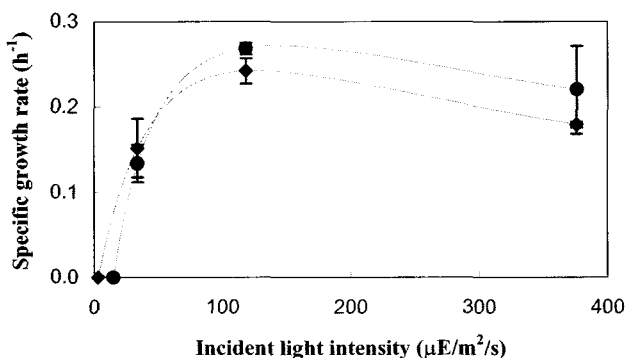

Fig. 2. Light response curves for wild-type ($\blacklozenge$) and mutant ($\bullet$) strains cultured in PBR-A.

The mutant and wild-type cells were cultured in a PBR-A with a small inner diameter to minimize the mutual shading effect. The specific growth rates were measured at a low cell density (<4×10^8 mm^3 cell volume/ml) during the initial stage of the exponential growth phase, and averaged based on two separate experiments.

photoinhibition. Under normal light conditions, it has been found that purple bacteria prefer to protect their PSUs from overheating by dissipation rather than achieve a higher efficiency [12]. As such, this could explain why the mutant exhibited a higher maximum specific growth rate than the wild-type. In addition, the compensation point, determined as the irradiance of no growth during two days after inoculation, was lower at 3 μE/m^2/s for the wild-type compared to 15 μE/m^2/s for the mutant. It should also be mentioned that the light response curves in Fig. 2 were obtained from the PBR-A with a small diameter, thus the same curve only fit at the surface of the PBR-B.

Pigmentation and Light Penetration Depth

To predict the light absorption profile, the specific absorption coefficient was determined. The pigment content per cell volume, the values of which were close to the pigment content per cell due to the same mean cell volume of both strains (data not shown), sensitively increased with a decrease in the light intensity (Fig. 3a). This is a common response of photosynthetic cells and considered to be an adaptive response, where the components of the light-harvesting apparatus increase in abundance when light is limited [9]. The specific light absorption coefficient based on the cell volume also exhibited the same pattern as the
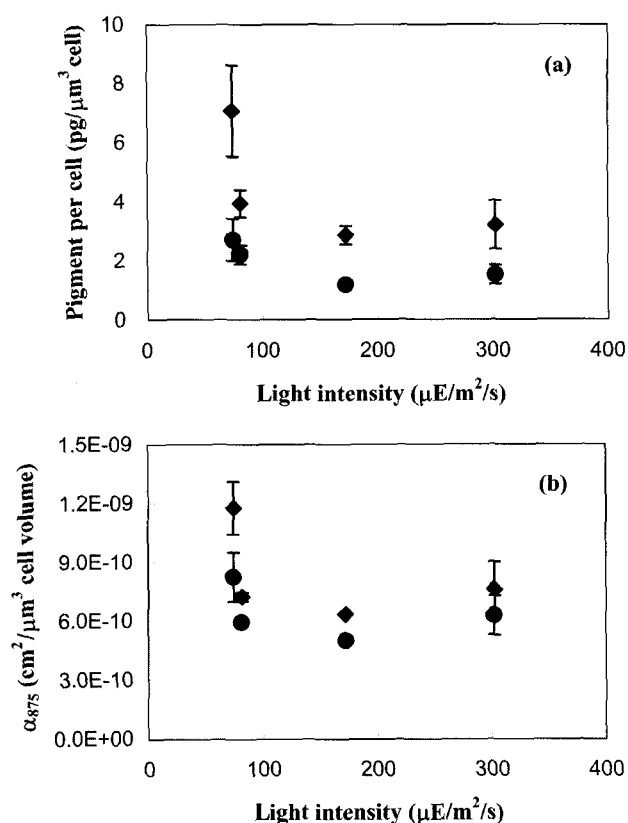
Fig. 3. (a) Plot of pigment contents per cell for wild-type (♦) and mutant (●) strains cultured in PBR-B relative to light intensity.

The pigment content per cell for the wild-type was higher than that for the mutant cells. (b) Light absorption coefficients for wild-type and mutant strains based on Beer-Lambert's law as a function of light intensity in PBR-B. Although the light absorption coefficient for the wild-type was higher than that for the mutant, the difference between the absorption coefficients was not as much as the difference between the pigment content per cell. The pigment content per cell and light absorption coefficients were measured for cells from all growth stages and the data averaged from two separate experiments.

Fig. 4. (a) Depth of photic zone (distance from surface to compensation point), (b) depth to half incident light intensity for cultures grown under high light intensity (302 $\mu E/m^2/s$), and (c) depth to half incident light intensity for cultures grown under low light intensity (74 $\mu E/m^2/s$) at 850 nm in PBR-B.

Closed symbols: cultures under high light intensity; open symbols: cultures under low light intensity; ♦ and ◇: wild-type; ● and ○: mutant cells.

pigment content per cell volume (Fig. 3b). The depth of the photic zone, shown in Fig. 4, was calculated based on Beer-Lambert's law using the experimental compensation points [17] of 3 and 15 $\mu E/m^2/s$ for the wild-type and mutant, respectively. The photic zone is defined as the upper layer of a body of water where the intensity of light is sufficient for net photosynthesis (photosynthesis exceeds respiration) [35], *i.e.*, the zone from the illuminating surface to the compensation point (irradiance). The depth of the photic zone was the same for both the wild-type and mutant cultures, regardless of the light intensities applied, as shown in Fig. 4a. However, the depth at which the light intensity became half the value of the incident light ($I_0/2$) slightly increased in the mutant cell culture compared to in the wild-type cell culture, as shown in Figs. 4b and 4c. Again, this increased depth of $I_0/2$ was observed with both
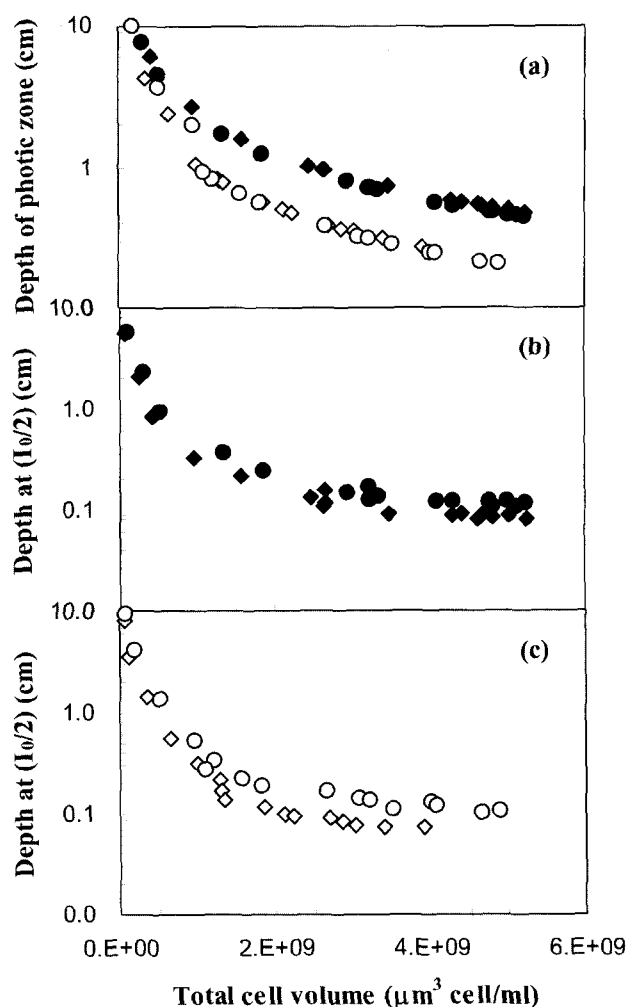
high and low light intensities, as confirmed by the profiles in Fig. 1.

Productivities in Photobioreactors

Although the photic zone for the mutant did not increase compared with that for the wild-type, due to the increase in the compensation point, the productivity of the mutant in the PBR-A was higher than that of the wild-type under high-intensity light (Fig. 5). As such, the sensitive adaptive response of the wild-type strain seemed to favor a low light intensity and low cell density. In nature, photosynthetic bacteria commonly grow under low light intensity and low cell density conditions [10]. However, although these adaptive processes may be advantageous for the purpose of evolution or survival in nature, the same processes were
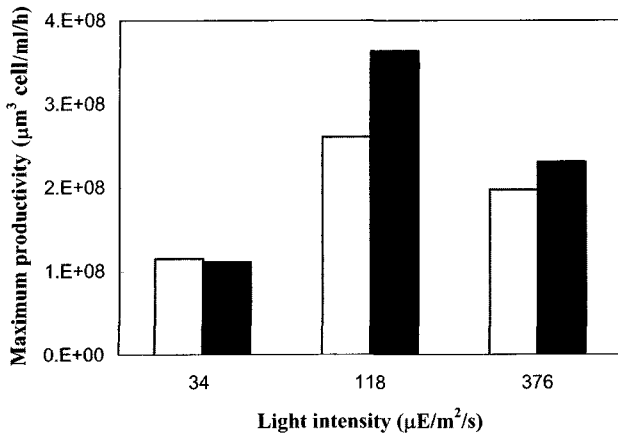
Fig. 5. Comparison of batch productivity under light-limiting conditions (> about 4×10^8 μm^3 cell volume/ml) in PBR-A.

The productivity was determined by the slopes of each growth curve under light-limiting conditions. The wild-type strain (□) exhibited a higher productivity than the mutant strain (■) under low-intensity light.

found to hamper light penetration in a high-density culture with an artificial light source.

To measure the productivities under light-limiting conditions, the PBR-B with an inner diameter of 7 cm was used. Due to the larger diameter of the PBR-B, there was a dark zone at the center of the PBR-B, especially with a high cell density. Under light-limiting conditions (>about 3×10^8 μm^3 cell volume/ml) or when the light intensity at the center of the reactor was below the compensation irradiance, the growth rate of the mutant (● in Fig. 6) was higher than that of the wild-type (◆) under a high light intensity of 302 $\mu E/m^2/s$. In contrast, the total pigment concentration (=sum of total BChl and Crt) was higher in the wild-type (▲) than in the mutant (■ in Fig. 6). Yet, when the light intensity was decreased from 302 to 74 $\mu E/m^2/s$ after 42 h (represented by arrow in Fig. 7), the mutant
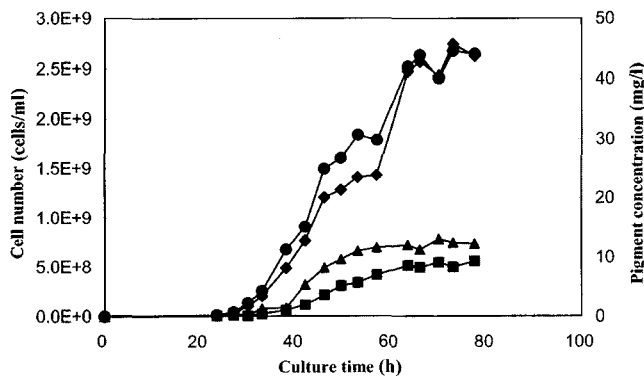

Fig. 6. Growth curves and pigment concentrations for wild-type and mutant in PBR-B under high light intensity (302 $\mu E/m^2/s$).
Growth curves for wild-type (◆) and mutant (●) under high light intensity; total pigment concentrations for wild-type (▲) and mutant (■) under high light intensity.
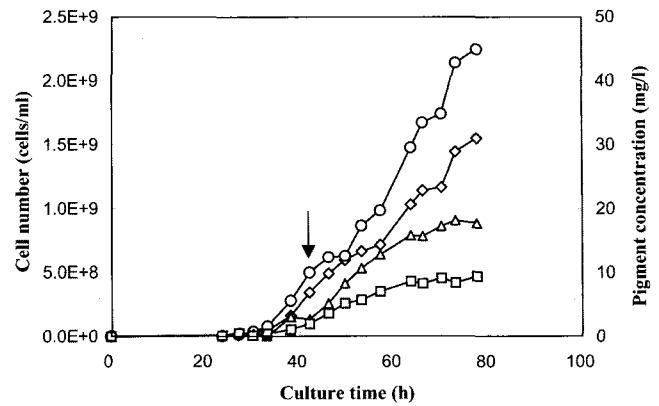

Fig. 7. Growth curves and total pigment concentrations for wild-type and mutant (KDC) when light intensity was changed from 302 to 74 $\mu E/m^2/s$ after 42 h (represented by arrow).
Growth curves for wild-type (◇) and mutant (○) under low light intensity; total pigment concentrations for wild-type (△) and mutant (□).

exhibited a slightly higher growth rate than the wild-type, indicating that the higher photosynthetic pigment content prevented the light penetration. As such, although a higher pigment content per cell may be advantageous for the survival of an individual photosynthetic cell, a high-density culture can be achieved more easily with reduced pigment contents.

Figures 8a and 8b show profiles of the total pigment content and ratio of total Crt and BChl, respectively. The total pigment content increased dramatically when the applied light intensity was decreased as in Fig. 8a (open markers), and this increase was higher in the wild-type cells (◇ in Fig. 8a). This adaptation process took about 2-3 generations for R. sphaeroides, during which time the growth rate decreased due to an energy limitation, while the amount of intracytoplasmic membrane (ICM) vesicles and number of photosynthetic units per cell increased [7]. Although the total pigment content was sensitive to a variation in the light intensity, the ratio of Crt/BChl remained relatively constant (Fig. 8b), which means that the composition of Crt and BChl in the PSUs did not change. However, the Crt/BChl ratio in the mutant cells (circles in Fig. 8b) was slightly higher than that in the wild-type (◆ and ◇ in Fig. 8b), because the mutant had a different ratio of the photosynthetic pigments.

Figure 9 shows a plot of the specific light absorption coefficient relative to the pigment content in the cells cultured in the PBR-B. From the slope, it is evident that reducing the pigment content per cell by half did not double the light penetration. Moreover, pigment reduction led to an increase in the compensation irradiance. Thus, the actual increase in the photic zone resulting from reducing the pigment content was minimal for the strain used in this study. Therefore, as regards the high-density culture of the KDC strain under a high light intensity, the increased
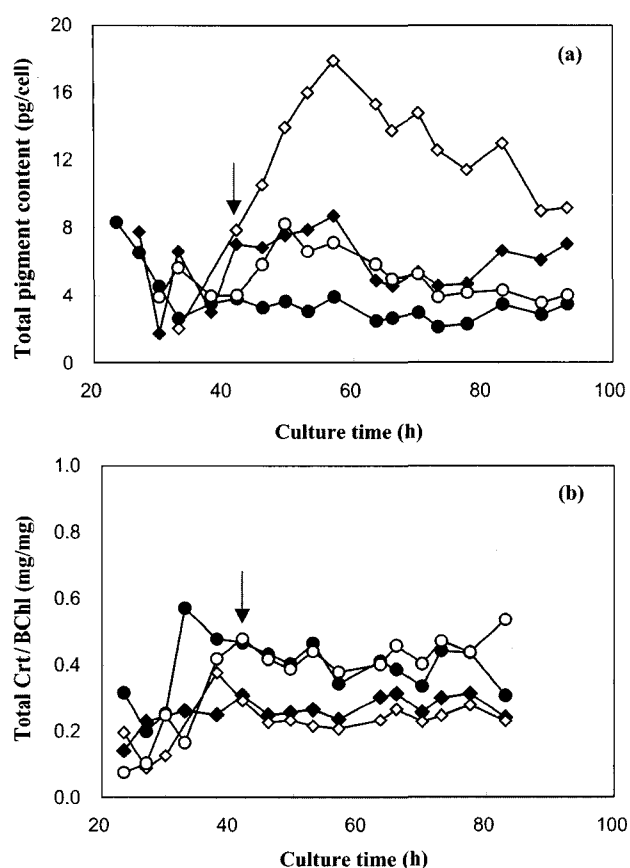
Fig. 9. Plot of specific light absorption coefficient at 875 nm versus pigment content in cells cultured in PBR-B.

The decrease in the light absorption coefficient was smaller than the reduction in the pigment content per cell, as the absorption coefficient could not decrease to zero. Closed symbols: cultures under high light intensity; open symbols: cultures under low light intensity; ◆ and ◇: wild-type; ● and ○: mutant cells.

Fig. 8. Profiles of (a) total pigment content per cell and (b) ratio of total carotenoid to bacteriochlorophyll (Crt/BChl) in cultures shown in Figs. 6 and 7.

Arrow indicates time when light intensity was reduced from 302 to 74 μE/m²/s. The wild-type strain responded sensitively to the decrease in the light intensity. The Crt/BChl ratio was maintained within a certain range during the culture. Closed symbols: cultures under high light intensity; open symbols: cultures under low light intensity; ◆ and ◇: wild-type; ● and ○: mutant cells.

biomass productivity was more likely due to an increase in the specific growth rate rather than an increase in the photic zone. Nonetheless, it was still evident that a reduced pigment content was beneficial in achieving a high-density photosynthetic culture.

Unlike purple and green bacteria, such as the current LH-II deficient mutant, heliobacteria cannot grow at low light intensities due to the small size of their antenna, while they can tolerate high light intensities quite well [21]. In fact, other mutant photosynthetic bacteria that lack antenna complexes [4, 15] can only grow photosynthetically at very high incident light intensities based on a report by Cogdell *et al.* [4]. As discussed earlier, since purple bacteria would appear to protect their PSUs before trying to achieve a higher efficiency [12], it would seem reasonable to suggest that the efficient absorption of light was less important to the wild-type bacteria under a high light intensity. High-density cultures require a high light
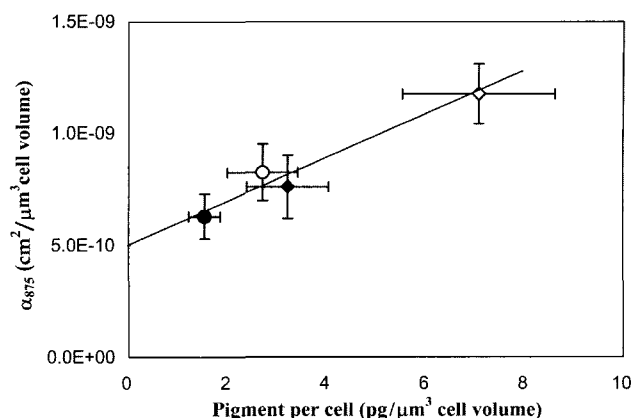
intensity to supply enough photons to the entire population inside the culture. As a result, in the high-density cultures, the cells near the illumination surface were exposed to a very high light intensity and absorbed most of the incoming light energy. Now, these cells with many more photons than they needed had to protect their PSUs from overheating by dissipation, because not all the absorbed energy could be converted into chemical energy in time. However, in the case of the mutants, the KDC cells could not absorb as many photons as the wild-type cells, as the mutant cells lacked certain antenna complexes. Thus, the lower absorption by the cells near the illumination surface of the high-density culture meant more light toward the center of the reactor, resulting in a higher productivity without the worry of damage from photoinhibition. Although the increased productivity of the LH-II deficient mutants with a high light intensity resulted from reduced pigments, understanding the actual process in a cell is difficult as discussed by Ritz *et al.* [32]. The *in vivo* architecture of a PSU, *i.e.*, the stoichiometry and arrangement of the various pigment-protein complexes in the photosynthetic membrane, is not known with certainty. In contrast to an intra-complex excitation transfer, the inter-complex excitation transfer steps LH-II↔LH-II, LH-II↔LH-I, and LH-I↔ RC have not been studied so much, even though these transfer steps account for the major amount of time spent in transferring excitation to the RC, thereby determining the efficiency and dynamics of the excitation trapping process in the PSU [32]. However, the more LH-II complexes exist, the more time spent in LH-II↔LH-II transfer steps, resulting in an increased trapping time and decreased quantum yield [32]. This may be one explanation for the higher productivity of the LH-II-deficient mutant compared to the wild-type

strain when cultured under a high-intensity light. The longest step in the energy-transfer sequence from LH-II to the RC is the step from LH-I to the RC, which takes 30–40 ps, and the comparative slowness of this step is clearly a consequence of the longer distance between the B875 ring and the primary electron donor, P, in the RC [5]. Consequently, this energy transfer step from the antenna to the reaction center is the rate-limiting step in the overall energy migration and trapping process [34]. In the step from LH-I to the RC, when the primary donor is preoxidized, the excitation energy is dissipated as heat in a radiationless process, and emitted as infrared fluorescence (>1,300 nm) [34]. If more energy is transferred from more LH-II, this increases the load on the process of dissipating the transferred energy. Thus, despite the above advantages of pigment reduction with a high light intensity, the full effect of pigment reduction is difficult to introduce. However, it is believed that a better understanding of the connection between the kinetics of light energy transfer and that of cell growth will help solve this problem.

Accordingly, although the current research is only in its initial stages, both theory and experimental data suggest the feasibility of significant productivity improvements in outdoor mass cultures of photosynthetic cells [3]. Yet, pigment reduction also induces various changes in the inherent properties of cells related to light, for example, the specific absorption coefficient, specific growth rate, compensation and saturation irradiance, quantity of photoinhibition, and organization of pigments in the membrane. Thus, pigment reduction is not always effective and has both positive and negative effects.

CONCLUSION

The photosynthetic productivity of photosynthetic cells can be increased by reducing the pigment content. As such, a mutant with reduced pigment content favored light-limiting conditions under a high light intensity. The reduction of photosynthetic pigments without the susceptibility of photoinhibition was found to be the key to the construction of a successful mutant for high-density cultures. In addition, when reducing the pigments of photosynthetic cells, damages to the inherent properties, especially the specific growth rate and compensation point, are needed to be minimized. Therefore, pigment reduction would appear to be a means of improving the light utilization efficiency of photosynthetic bacteria as well as green and blue-green microalgae [25].

Acknowledgment

REFERENCES

1. Aiba, S. 1982. Growth kinetics of photosynthetic microorganisms, pp. 85–156. In S. Aiba, H. W. Doelle, K. N. Ewings, L. T. Fan, M. M. Gharpuray, N. W. Hollywood, K. J. Lee, Y.-H. Lee, P. L. Rogers, M. L. Skotnicki, and D. E. Tribe (eds.). Microbial Reactions. Springer-Verlag, New York, U.S.A.
2. Benemann, J. R. 1997. CO₂ mitigation with microalgae systems. Energy Convers. Mgmt. **38**: S475–S479.
3. Benemann, J. R. 2000. Hydrogen production by microalgae. J. Appl. Phycol. **12**: 291–300.
4. Cogdell, R. J., N. W. Isaacs, T. D. Howard, K. Mcluskey, N. J. Fraser, and S. M. Prince. 1999. How photosynthetic bacteria harvest solar energy. J. Bacteriol. **181**: 3869–3879.
5. Cogdell, R. J. and J. G. Lindsay. 1998. Can photosynthesis provide a 'biological blueprint' for the design of novel solar cells? TIBTECH **16**: 521–527.
6. Cohen-Bazire, G., W. R. Sistrom, and R. Y. Stanier. 1957. Kinetic studies of pigment synthesis by non-sulfur purple bacteria. J. Cell. Comp. Physiol. **49**: 25–68.
7. Drews, G. and J. R. Golecki. 1995. Structure, molecular organization, and biosynthesis of membranes of purple bacteria, pp. 231–257. In R. E. Blankenship, M. T. Madigan, and C. E. Bauer (eds.). Anoxygenic Photosynthetic Bacteria. Kluwer Academic Publishers, Dordrecht, The Netherlands.
8. Eroglu, I., K. Aslan, U. Gunduz, M. Yucel, and L. Turker. 1999. Substrate consumption rates for hydrogen production by Rhodobacter sphaeroides in a column photobioreactor. J. Biotechnol. **70**: 103–113.
9. Geider, R. J. and B. A. Osborne. 1992. Chapter 7: The photosynthesis-light response curve, pp. 156–191. In: Algal Photosynthesis. Chapman & Hall, Inc., New York, U.S.A.
10. Gemerden, H. V. and J. Mas. 1995. Ecology of phototrophic sulfur bacteria, pp. 49–85. In: R. E. Blankenship, M. T. Madigan, and C. E. Bauer (eds.) Anoxygenic Photosynthetic Bacteria. Kluwer Academic Publishers, Dordrecht, The Netherlands.
11. Goldman, J. C. 1979. Outdoor algal mass cultures - II. Photosynthetic yield limitations. Water Res. **13**: 119–136.
12. Hu, X., T. Ritz, A. Damjanovic, F. Autenrieth, and K. Schulten. 2002. Photosynthetic apparatus of purple bacteria. Q. Rev. Biophys. **35**: 1–62.
13. Hu, X., T. Ritz, A. Damjanovic, and K. Schulten. 1997. Pigment organization and transfer of electronic excitation in the photosynthetic unit of purple bacteria. J. Phys. Chem. B **101**: 3854–3871.
14. Hu, X. and K. Schulten. 1997. How nature harvests sunlight. Phys. Today **50**: 28–34.
15. Hunter, C. N. 1995. Genetic manipulation of the antenna complexes of purple bacteria, pp. 473–501. In R. E. Blankenship, M. T. Madigan, and C. E. Bauer (eds.). Anoxygenic Photosynthetic Bacteria. Kluwer Academic Publishers, Dordrecht, The Netherlands.
16. Kim, N.-J. and C.-G. Lee. 2001. A theoretical consideration on oxygen production rate in microalgal cultures. Biotechnol. Bioprocess Eng. **6**: 352–358.

17. Lee, C.-G. 1999. Calculation of light penetration depth in photobioreactors. *Biotechnol. Bioprocess Eng.* **4:** 78-81.

18. Lee, Y.-K. 2001. Microalgal mass culture systems and methods: Their limitation and potential. *J. Appl. Phycol.* **13:** 307-315.

19. Lim, S.-K., I. H. Lee, and J. K. Lee. 1999. Role of OrfQ in formation of light-harvesting complex of *Rhodobacter sphaeroides* under light-limiting photoheterotrophic conditions. *J. Microbiol. Biotechnol.* **9:** 604-612.

20. Lueking, D. R., R. T. Fralely, and S. Kaplan. 1978. Intracytoplasmic membrane synthesis in synchronous cell populations of *Rhodopseudomonas sphaeroides*. *J. Biol. Chem.* **253:** 451-457.

21. Madigan, M. T. and J. G. Ormerod. 1995. Taxonomy, physiology and ecology of Heliobacteria, pp. 17-30. *In* R. E. Blankenship, M. T. Madigan, and C. E. Bauer (eds.). *Anoxygenic Photosynthetic Bacteria*. Kluwer Academic Publishers, Dordrecht, The Netherlands.

22. Morita, M., Y. Watanabe, and H. Saiki. 2000. Investigation of photobioreactor design for enhancing the photosynthetic productivity of microalgae. *Biotechnol. Bioeng.* **69:** 693-698.

23. Nakada, E., Y. Asada, T. Arai, and J. Miyake. 1995. Light penetration into cell suspensions of photosynthetic bacteria and relation to hydrogen production. *J. Ferment. Bioeng.* **80:** 53-57.

24. Nakajima, Y., M. Tsuzuki, and R. Ueda. 1999. Reduced photoinhibition of a phycocyanin-deficient mutant of *Synechocystis* PCC 6714. *J. Appl. Phycol.* **10:** 447-452.

25. Nakajima, Y., M. Tsuzuki, and R. Ueda. 2001. Improved productivity by reduction of the content of light-harvesting pigment in *Chlamydomonas perigranulata*. *J. Appl. Phycol.* **13:** 95-101.

26. Nakajima, Y. and R. Ueda. 1997. Improvement of photosynthesis in dense microalgal suspension by reduction of light harvesting pigments. *J. Appl. Phycol.* **9:** 503-510.

27. Nakajima, Y. and R. Ueda. 1999. Improvement of microalgal photosynthetic productivity by reducing the content of light harvesting pigment. *J. Appl. Phycol.* **11:** 195-201.

28. Nakajima, Y. and R. Ueda. 2000. The effect of reducing light-harvesting pigment on marine microalgal productivity. *J. Appl. Phycol.* **12:** 285-290.

29. Nishimura, K., H. Shimada, S. Hatanaka, H. Mizoguchi, H. Ohta, T. Masuda, and K.-I. Takamiya. 1998. Growth, pigmentation, and expression of the *puf* and *puc* operons in a light-responding-repressor (SPB)-disrupted *Rhodobacter sphaeroides*. *Plant Cell Physiol.* **39:** 411-417.

30. Papiz, M. Z., S. M. Prince, A. M. Hawthornthwaite-Lawless, G. McDermott, A. A. Freer, N. W. Isaacs, and R. J. Cogdell. 1996. A model for the photosynthetic apparatus purple bacteria. *Trends Plant Sci.* **1:** 198-206.

31. Ritz, T., A. Damjanovi, and K. Schulten. 2002. The quantum physics of photosynthesis. *Chemphyschem.* **3:** 243-248.

32. Ritz, T., S. Park, and K. Schulten. 2001. Kinetics of excitation migration and trapping in the photosynthetic unit of purple bacteria. *J. Phys. Chem. B* **105:** 8259-8267.

33. Sukenik, A. and P. G. Falkowski. 1987. Potential enhancement of photosynthetic energy conversion in algal mass culture. *Biotechnol. Bioeng.* **30:** 970-977.

34. Sundstrom, V. and R. V. Grondelle. 1995. Kinetics of excitation transfer and trapping in purple bacteria, pp. 249-372. *In* R. E. Blankenship, M. T. Madigan, and C. E. Bauer (eds.). *Anoxygenic Photosynthetic Bacteria*. Kluwer Academic Publishers, Dordrecht, The Netherlands.

35. Sze, P. 1998. *A Biology of the Algae*, 3rd Ed. WCB/ McGraw-Hill.

36. Tabita, F. R. 1999. Microbial ribulose 1,5-bisphosphate carboxylase/oxygenase: A different perspective. *Photosynth. Res.* **60:** 1-28.

37. Watson, G. M. F. and F. R. Tabita. 1997. Microbial ribulose 1,5-bisphosphate carboxylase/oxygenase: A molecule for phylogenetic and enzymological investigation. *FEMS Microbiol. Lett.* **146:** 13-22.

38. Yigit, D. O., U. Gunduz, L. Turker, M. Yucel, and I. Eroglu. 1999. Identification of by-products in hydrogen producing bacteria; *Rhodobacter sphaeroides* O.U. 001 grown in the waste water of a sugar refinery. *J. Biotechnol.* **70:** 125-131.

39. Zaslavskaia, L. A., J. C. Lippmeier, C. Shih, D. Ehrhardt, A. R. Grossman, and K. E. Apt. 2001. Trophic conversion of an obligate photoautotrophic organism through metabolic engineering. *Science* **292:** 2073-2075.