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Photobioreactor Engineering: Design and Performance

In Soo Suh and Choul-Gyun Lee*

Institute of Industrial Biotechnology, Department of Biological Engineering, Inha University, Incheon 402-751, Korea

Abstract This review summarizes the recent advances in high-density algal cultures in the field of algal biotechnology. Photobioreactor engineering for economical and effective utilization of algae and its products has made impressive and promising progress. Bioprocess engineers have expedited the design and the operation of algal cultivation systems. Many of them in use today are open systems due to cost considerations, and closed photobioreactors have recently attracted a considerable attention for the production of valuable biochemicals or for special applications. For high-density cultures, the optimization of environmental factors in the photobioreactors have been explored, including light delivery, CO_2 and O_2 gas transfer, medium supply, mixing and temperature. It is expected that further advanced photobioreactor engineering will enable the commercialization of noble algal products within the next decade.

Keywords: algal biotechnology, photobioreactor engineering, production system, environmental factor

INTRODUCTION

Photosynthetic cells are of great importance as primary producers of organic matter and their ability to produce oxygen. Many algal biotechnologists have studied the utilization of their photosynthetic machinery for the production of new bioproducts and possible environmental applications over the last several decades. Dozens of algal species are produced on a commercial scale for single-cell proteins, polysaccharides, health food compounds such as polyunsaturated fatty acids and vitamins in the pharmaceutical and dietetic industries. In recent years, there has been considerable interest in the production of many clinically and medically important biochemicals from algae, mainly those that are not obtainable by chemical synthesis [1-3]. Numerous attempts have also been made to exploit algal usage for the construction of closed environmental life support system (CELSS) [4,5] and environmental applications, including tertiary wastewater treatment [6-8] and accumulation of heavy metals [9,10]. With increasing energy costs, its application is being extended to the fixation of carbon dioxide [11-16] and the production of hydrogen gas [17-20].

Despite their recognized utility, algal biotechnologies have progressed slowly, compared with the rapid and significant advances in the commercial usages of bacteria, yeast and mammalian cells. In the industrial scale application of algae, the main problem is to justify the high cost of installation and operation. In order to solve this problem, algal biologists have isolated high value bioproducts using a high throughput screening system from diverse and large groups of algae, which are estimated at between 22,000 and 26,000 species. Bioprocess engineers, however, have focused on reducing the production costs and realizing the potential of algae, which has been accelerated by the recent advances in photobioreactor engineering.

***Corresponding author**
Tel: +82-32-860-7518 Fax: +82-32-872-4046
e-mail: leecg@inha.ac.kr

ALGAL PRODUCTION SYSTEMS

The history of the commercial use of algal cultures spans about 60 years with various applications [21]. So far, two major approaches to the design of mass cultivation of algae have emerged: (1) relatively simple open units, which are easy to maintain, in order to compete with other methods of production, and (2) more sophisticated closed photobioreactors for the cultivation of the specialized algal strains for the production of specific biochemicals.

Open Cultivation Systems

Many open cultivation system configurations, using natural sunlight, were constructed after the late 1940s. The pioneering experiments on outdoor open algal mass production were reported from Germany [22] and the United States [23]. Commercial algal productions are mainly performed in these open systems, particularly raceway and circular ponds [24-26], with the principal advantage of using free light energy from the sun. However, large-scale open algal cultures typically result in low cell densities, resulting in expensive harvesting procedures and unfavorable economics in addition to difficul-

ties with species control [27].

These systems are easily contaminated by other organisms, so it is particularly difficult to maintain a monoculture in open systems [28]. Thus, they have only proven adequate for the cultures of specific species that grow in highly selective environments that exclude contaminating organisms. Furthermore, settling causes low yields, unstable algal populations and difficulty in distributing nutrients. Therefore, various mixing systems have been added, such as propellers, paddle-wheels, rotating arms and pumps *etc.*, which increase the capital and operating costs of these systems. Even considering these additional costs, pond systems are among the least expensive to operate, but are also frequently among the least productive.

Closed, Outdoor Systems

Until now, the outdoor mass culture of algae has been undoubtedly the hottest topic in algal biotechnology, but the overemphasis on open culture systems has undoubtedly retarded the development of algal biotechnology. While open cultivation technology is more or less at a standstill, closed photobioreactors in open spaces (usually outdoors) have been installed for the cultivation of specialized algal strains for the production of specific biochemicals [29]. The most widely adopted enclosed photobioreactors are rigid tubular, thin-panel, or flat-plate photobioreactors [30-33].

The advantages of these photobioreactors are: (1) improved environmental control of important physicochemical variables (temperature, pH, pCO_2, *etc.*); (2) increased biomass concentration which makes harvesting easier; and (3) effective sterilization of the system and easier maintenance of a monoculture, due to the prevention of major contamination from the environment. However, these systems are still subjected by outdoor conditions and settling problems. Seasonal, latitudinal and diurnal variations in light conditions, ambient temperature and dissolved oxygen make cultivation reproducibility especially problematic [34,35]. Thus, for outdoor cultivation systems, it is technically difficult to establish high-density photoautotrophic algal cultures, if not impossible.

Closed, Indoor Systems

In order to upgrade the level of algal biotechnology, to those of bacterial and fungal biotechnologies, bioprocess engineers have developed closed indoor photobioreactors, using electric light for irradiation. As the usage of algae expands to the manufacture of products for human consumption, the manufacturers should guarantee the quality of their algal products. Thus, conventional fermentors, such as stirred tank bioreactors and vertical cylindrical columns, have been modified by employing artificial light sources: fluorescent lights (light tubes) [36,37], optical fibers [38,39] or light emitting diodes [40,41] and plates [42]. Internal radiators are especially known to distribute light energy more efficiently inside photobioreactors than external radiators. The complex geometries of these photobioreactors can monitor and control the culture environmental conditions and growth parameters, and the systematic design and scale-up strategy of these photobioreactors have been examined for the successful industrialization to a commercial scale [43]. However, these closed, indoor photobioreactors are presently restricted to laboratory scale operations, and their widespread use has been limited by the high installation cost and scale-up problems.

It is rather difficult to directly compare the performance characteristics of open systems, closed outdoor systems and closed indoor photobioreactors. Each system has both advantages and disadvantages, and the choice of system depends on the production costs, value of the desired products and the production quantity of the products. The performances of previously reported systems are enumerated in Table 1, which clearly shows that the light is the major limiting factor. Higher productivities and cell concentration can be achieved in the photobioreactors with high S/V ratio.

FACTORS AFFECTING ALGAL GROWTH

When culturing a specific alga for commercial production, the following factors should be considered: algal life cycle, growth rate, productivity of the desired product, genetic stability, nutritional requirement and tolerance to shear stress. The interrelation between algal characteristics and the design factors of a photobioreactor should start by conceptualizing the algal microenvironment, and a schematic representation for this is shown in Fig. 1. Important factors in achieving high-density productive algal cultures are: light energy supply (delivery and distribution), CO_2 enrichment, O_2 removal, medium supply, mixing and other control of environmental parameters (temperature, pH, *etc.*). Each aspect will be discussed separately in the following sections.

Supply of Light Energy

Light energy is essential for the phototropic growth of algae, which is a particular concern for industrial applications. Thus, the provision of light energy is undoubtedly a key design challenge for an efficient and economical photobioreactor. When selecting a light source, both the spectral quality and intensity must be considered. The spectral quality of light utilized by algae is defined by the absorption spectrum in the range of 400 to 700 nm for the chlorophylls and other photosynthetically active pigments, and the algal photosynthetic efficiency is a function of the spectral quality of the light sources [44,45]. The light sources available are incandescent lamps (tungsten or halogen lamps), discharge lamps (such as mercury, xenon, fluorescent lamps), light-emitting diodes (LEDs) and lasers. A detailed discussion on these light sources can be found elsewhere [46,47]. To effectively exploit the commercial potential of algae, a powerful and efficient light source is desired, with a high ratio of energy supplied to that source, to energy released in photons. Furthermore, a reliable and defined light source is

Table 1. Comparison of the performance of various algal photobioreactors

Reference	Culture chamber design	Total vol. L	S/V Ratio/m	Productivity		Max. biomass g/L
				per area g m^{-2} day^{-1}	per vol. g L^{-1} day^{-1}	
Davis, 1953 [93]	Tubular	1	170[a]	11.7	1.3	18.5
Tamiya, 1953 [94]	Tubular	40	40[a]	17		17.5[a]
Juttner, 1977 [95]	Column	30	28.6[a]			1.85[a]
Pirt, 1983 [30]	Tubular	4.6	127	52.8		20
Mori, 1985 [38]	Tank	2.4	580		1.65[a]	5
Roubicek, 1986 [96]	Falling-film	190	7.5[a]			1.2
Torzillo, 1986 [97]	Tubular	8,000	10	25		1.2
Driessens, 1987 [98]	Column	2	1[a]		10.41[f]	4.19
Miyamoto, 1988 [99]	Column	4.6	80	23	0.57	
Treat, 1989 [100]	Tank	2.5				8.2
James, 1990 [101]	Column	200	0.66[a]		0.246	
Lee, 1990 [102]	Helix	0.315	127			4.6
Javanmardian, 1991 [39]	Cylinder	1	320		1.51	7.5[a]
Ratchford, 1992 [31]	FPARL[c]	10	50			2.27
Tredici, 1992 [32]	VAP[b]		80	23.9		7
Takano, 1992 [103]	LDOF[d]	2.5	692[e]		1.94	11.2
Yongmanitchai, 1992 [104]	Tank	5.6	19.3		0.51	2.67
Burgess, 1993 [105]	LDOF	2.5				1.9
Torzillo, 1993 [106]	Tubular	145	54	27.8		6.3
Lee, 1995 [69]	Slab	0.1	100	44	3.15	25
Hu, 1996 [107]	Inclined slab	6	85	51.1	4.3	15.8
Hu, 1998 [108]	Flat-plate	0.34	132		28.8	26.6
Degen, 2001 [55]	Flat-panel	1.5	56		2.64	4.8

[a]Values not given by the authors. The values estimated indirectly from other parameters based on the article. [b]VAP means vertical alveolar panel. [c]FPALR represents flat plate, air-lift reactor. [d]LDOF stands for light-diffusing optical fibers. [e]Corrected value from [109]. [f]Value from a photomixotrophic culture.

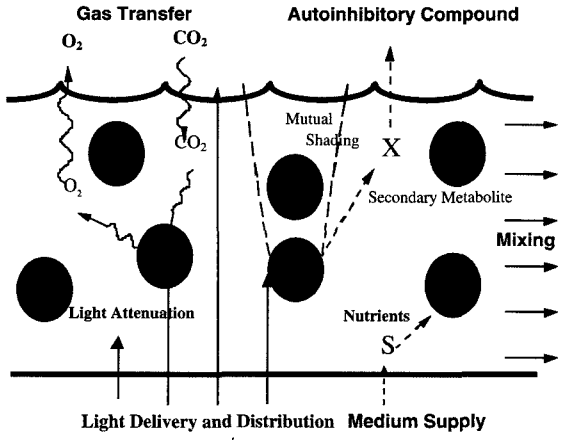

Fig. 1. Schematic representation of the factors influencing the metabolic and growth performance of algae.

required for algal growth studies and mass cultivation.

The effect of light intensities (irradiance or illumination) has been studied in the greatest detail in connection with growth and photosynthesis [48,49]. The total amount of organic material that can be produced from light energy, by growing algal cells, increases with the intensity of light up to a certain point (saturation intensity), but beyond that point, the amount produced per unit of light energy decreases rapidly when the intensity of light increases. Light of high intensity apparently stimulates a process of photooxidation, which partially offsets some of the photosynthesis. Higher light intensities beyond a certain threshold, normally damage the photosystems; the damage caused by excess light is described by using the term 'photoinhibition', which is a function of the temperature, and shows a coupled response with the photorespiratory carbon metabolism [50,51].

Light energy is readily absorbed, as the antenna pigments of the reaction centers of algal cells are very efficient. As the light penetrates the algal culture, its intensity is dramatically decreased by mutual shading. Thus, the light intensity emitted from the light sources should be increased in order to overcome this light limitation [52,53]. However, the exposure of cells to excess light often leads to a decline in the algal growth rates. One solution to this problem is the use of flashing light [54-56]. The use of flashing light or flashing light effect with proper parameters can significantly increase the overall light utilization efficiency [57].

In order to maintain the light condition at an appropriate level during cell cultivation, lumostatic operation has been introduced during the culturing various photoautotrophic microalgae. The use of the lumostatic operation may allow not only the efficient utilization of light

energy but also the production of cells in the same physiological state. Lumostatic approaches have been attempted based on the incident light intensity [58,59], average light intensity [60] and specific light uptake rate [61]. For lumostatic operation, the major premise is the accurate quantitative expression of the light conditions inside photobioreactors. Hence, more study is required for discriminating the light conditions, of either photoinhibition or light limitation, in order to maintain the optimal light condition during cell cultivation.

Carbon Dioxide Enrichment

Carbon dioxide (or bicarbonate after it dissolves into the culture medium) is the source of the carbon in photoautotrophically growing cells. Even when intense mixing of the culture is provided, simple diffusion of CO_2 from the air (0.03% CO_2) into the water is too slow to replace the CO_2 assimilated by rapidly growing algae. Thus, the algal cultures in photobioreactors are generally CO_2-limited and additional CO_2 must be supplied to the medium to ensure satisfactory growth. Typically, CO_2 concentrations above 1% (by volume) are generally considered to have an adverse effect on algal growth, and the maximum growth is essentially the same regardless of the CO_2 concentrations in the range 1 to 5%, balanced with air. However, a gas supply with a high CO_2 partial pres sure leads to a decrease in the specific growth rate of algae which had been adapted to a low CO_2 partial pressure [62]. The upper and lower limits of CO_2 are not well defined [63], but aeration of algal cultures with 5~15% CO_2 (or even pure CO_2) is fairly routine laboratory practice.

Enclosed photobioreactors require a continuous supply of soluble inorganic carbon to provide a sufficient carbon source to the growing algal cells. The required inorganic carbon is most commonly introduced in the form of bubbles of enriched CO_2 gas mixture. CO_2 gas may be expensive if purchased commercially, even though it can be obtained by the combustion of fossil fuels [13]. Thus, an efficient sparging system and mixing pattern have been sought, which will retain the bubbles until they are completely absorbed into the medium. It is known that the dissolving rate of gaseous CO_2 depends on the contact time of the bubble with the medium, bubble size and the state of saturation of the medium for CO_2, as well as the pressure and temperature of the reactor. Sodium bicarbonate and similar salts can also be used as an alternative carbon source. However, such salts usually cost more than three times that of gaseous CO_2 per unit of carbon.

Oxygen Removal

Oxygen is a product of photosynthesis, but a high level of dissolved oxygen inhibits algal growth, even at high concentrations of CO_2 [64,65]. Particularly, in enclosed photobioreactors, oxygen supersaturation reaches no more than 400~500%, even under sufficiently intense mixing conditions. Respiration in the dark region, or during the night, also builds up the level of dissolved oxygen in an algal suspension. However, many algal species cannot withstand the exposure of 2~3 hours to oxygen levels much above air-saturation (7.5 mg/L at 30°C). This O_2 inhibition of photosynthesis is precisely discussed in terms of the Warburg effect, photorespiration or photoreduction.

The removal of excess O_2 is a mass transfer problem comparable to that of the CO_2 supply. The principal control possibilities are decreased pressure of oxygen, higher agitation, and higher temperature. At present, two major solutions of, (1) increasing turbulence, and (2) O_2 stripping with air, are open to the photobioreactor design and operation. Vigorous mixing decreases the O_2 tension in the culture, particularly when mixing is administered by a properly designed device. Efficient gas exchange systems are also installed such as a degassing station, in photobioreactor systems.

Nutrient Supply

Nutrient availability is an important factor controlling the levels of the primary productivity of photosynthetic organisms [66,67]. Media with different compositions are supplied to the cells in the form of carbon dioxide, water and mineral salts in macro or micro quantities. The macronutrients considered essential for normal growth include carbon, nitrogen, phosphorus, hydrogen, oxygen, sulfur, calcium, magnesium, sodium potassium and chlorine. The micronutrients needed in trace quantities of micro-, nano- or even picograms per liter are iron, boron, manganese, copper, molybdenum, vanadium, cobalt, nickel, silicon and selenium. Some micronutrients may require a chelating agent to allow them to be dissolved or to minimize their toxicity. The nutrient requirements can be estimated from the elemental biomass composition or stoichiometry of growth [68].

Algae may secrete autoinhibitory compounds when the major nutrients are exhausted during batch cultivation. Thus the spent medium is removed and replenished by perfusion with fresh medium, or by on-line ultrafiltration with highly concentrated medium [39,40,69]. The on-line replenishment of medium can be achieved either by continuous culture or perfusion (including dialysis and ultrafiltration). The major difference between the two methods is whether the reactor loses biomass during the replenishment process. A semi-batch or fed-batch modes cannot be used when the accumulation of autoinhibitors retards or inhibits the algal growth and photosynthesis.

Mixing

Continuous mixing of an algal suspension is important and necessary, to keep the cells in suspension, to enhance light utilization efficiency, to improve gas exchange, to eliminate thermal stratification, and to help nutrient distribution. The degree of mixing has already been shown to significantly influence the algal productivity [70,71]. Settling occurs when the flow is too slow and will be particularly severe in the areas where turbulence is smallest. The accumulation of cells in dead zones will affect cell

deterioration, anaerobic decomposition and the quality of the product. However, mechanical agitation and bubble break-up often lead to hydrodynamic stress, resulting into restrictions to the algal growth and metabolic activity [70,72]. Turbulence also causes a continuous shift in the relative positions of the cells with respect to the photic zone which relates to the flashing light effect [56]. In a deliberately designed mixing system with an optimal time scale, each cell can absorb light energy while exposed to the light, and then uses up the absorbed photons while in the dark layer [55].

When the nutritional requirements are satisfied, and the other environmental conditions are not growth-limiting, mixing designed to create turbulent flow constitutes the most important requisite for consistently high yields of algal mass. Both the productivity of algal systems and the cost of their construction and operation are determined to a great extent by the nature of the mixing system employed. Practically, mixing is achieved by gas bubble dispersion and broth mixing and the cost for construction and operation should also be considered. Aeration-induced hydrodynamic stress can also be alleviated by the addition of carboxymethylcellulose (CMC) to increase the viscosity of the culture [73,74].

Temperature Control

One of the most distinctive environmental factors is the seasonal and diurnal variations in the ambient temperature. Payer *et al.* [75] investigated the temperature responses of 34 different green and blue-green species. The majority of the algae exhibited a temperature growth response curve with a wide plateau in the optimal temperature range, with a sudden decline outside of this range. The change of the culture temperature also profoundly influences the overall productivity or the cellular composition, as photosynthesis and respiration are fundamentally enzyme-based reactions [76,77]. The effects of temperature on algal photosynthesis have been reviewed, focusing on the phenotypic and genotypic differences, variations in the photosynthetic metabolism and in the maximum specific growth rate, as a function of temperature [78,79].

Temperature is almost invariably measured and controlled during the operation of photobioreactor. The algae in outdoor photobioreactors are exposed to seasonal and diurnal variations in the temperature and light intensity. Without temperature control equipment, the temperature, during the summer, in a closed photobioreactor can reach 10~30°C higher than ambient temperature for several hours. Thus, evaporative cooling and shading are conventionally used, by the spraying water onto the photobioreactor surface and by blocking the sunlight to a certain degree, respectively. Heat exchangers are mainly incorporated for the heating of cultures in colder climates. In order to reduce the cost of cooling and increase the various reaction rates within the cell, the cultivation temperature should be operated at the maximum possible for not induce stress in the organism.

Other Factors

The pH in autotrophic algal cultures increases continuously as a result of the depletion in carbon through photosynthesis. The pH of the culture medium affects both the liquid chemistry of polar compounds and the availability of many algal nutrients such as CO_2, iron and organic acids [80,81]. Variation in the pH also exerts ion transport systems at the plasmalemma, electrical charge of the cell wall surface and the associated membrane potentials [82]. Thus, the use of commercial pH controllers is required to maintain the optimal pH range for algal growth or product formation.

Algae are reported to be relatively insensitive to pressure. They can grow at the vapor pressure of water, to an upper limit that probably exceeds 1 atm. However, the pressure, together with the temperature and pH, has a significant effect on the solubilities of gases essential to algae, and hence, could have an indirect effect on algal growth [83]. The possibility of applying culture pressure monitoring has also been explored for the estimation of the biomass production in closed photobioreactors [84].

The osmotic pressure in algae is regulated by organic metabolites such as polyhydric alcohols (glycerol, mannitol or sorbitol, cyclohexanetetraol), glycosides (galactoglycerides, floridoside, and isofloridoside), amino acids (proline and glutamic acid) and other osmoregulators [85]. The most outstanding example of osmoregulation in algae is *Dunaliella* [86,87].

Strain Development

The molecular tools of modern genetic biotechnology are being applied for enhancing the tolerance to chilling and salt stress, and are now becoming available for allowing their low-cost, large-scale, commercial production [88,89]. Potentially practical mutants that are not light saturated or inhibited at high light intensities, could be derived by reducing antenna sizes, and they exhibited increased photosynthetic rates under high light intensities [90,91]. By introducing a human glucose-transporter gene, researchers have genetically modified the alga, *Phaeodactylum tricornutum*, to thrive on exogenous glucose in the absence of light [92]. This remarkable progress can be applied to make other commercially valuable algae also grow in the dark. The impact will be absolutely remarkable when conventional fermentation technology is successfully applied to the industrial production of these algae.

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REFERENCES

[1] Metting, B. and J. W. Pyne (1986) Biologically active compounds from microalgae. *Enzyme Microb. Technol.* 8:

386-394.

[2] Glombitza, K. W. and M. Koch (1989) Secondary metabolites of pharmaceutical potential. pp. 161-238. In: R. C. Cresswell, T. A. V. Rees, and H. Shah (eds.). *Algal and Cyanobacterial Biotechnology*. Longman Scientific & Technical, Harlow, UK.

[3] Cannell, R. J. P. (1993) Algae as a source of biologically active products. *Pestic. Sci.* 39: 147-153.

[4] Javanmardian, M. and B. O. Palsson (1992) Design and operation of an algal photobioreactor system. *Adv. Space Res.* 12: 231-235.

[5] Lee, C.-G. and B. O. Palsson (1995) Light emitting diode-based algal photobioreactor with external gas exchange. *J. Ferment. Bioeng.*, 79: 257-263.

[6] Borowitzka, M. A. and L. J. Borowitzka (1988) *Micro-algal Biotechnology*. Cambridge University Press, Cambridge, UK.

[7] Lee, K. and C.-G. Lee (2002) Nitrogen removal from wastewaters by microalgae without consuming organic carbon sources. *J. Microbiol. Biotechnol.* 12: 979-985.

[8] Eroglu, E., U. Gunduz, M. Yucel, L. Turker, and I. Eroglu (2004) Photobiological hydrogen production by using olive mill wastewater as a sole substrate source. *Int. J. Hydrogen Energy* 29: 163-171.

[9] Kuyucak, N. and B. Volesky (1990) Biosorption by algal biomass. pp. 173-198. In: B. Volesky (ed.). *Biosorption of Heavy Metals*. CRC Press, Boca Raton, FL, USA.

[10] Wilde, E. W. and J. R. Benemann (1993) Bioremmoval of heavy metals by the use of microalgae. *Biotechnol. Adv.* 11: 781-812.

[11] Karube, I., T. Takeuchi, and D. J. Barnes (1992) Biological reduction of CO_2 emissions. *Adv. Biochem. Eng./ Biotechnol.* 46: 63-79.

[12] Keffer, J. E. and G. T. Kleinheinz (2002) Use of *Chlorella vulgaris* for CO_2 mitigation in a photobioreactor. *J. Ind. Microbiol. Biotechnol.* 29: 275-280.

[13] Negoro, M., A. Hamasaki, Y. Ikuka, T. Makita, K. Hirayama, and S. Suzuki (1993) Carbon dioxide fixation by microalgae photosynthesis using actual flue gas discharged from a boiler. *Appl. Biochem. Biotechnol.* 39/40: 643-653.

[14] Zhang, K., N. Kurano, and S. Miyachi (2002) Optimized aeration by carbon dioxide gas for microalgal production and mass transfer characterization in a vertical flat-plate photobioreactor. *Bioprocess Biosys. Eng.* 25: 97-101.

[15] Olaizola, M. (2003) Microalgal removal of CO_2 from flue gases: Changes in medium pH and flue gas composition do not appear to affect the photochemical yield of microalgal cultures. *Biotechnol. Bioprocess Eng.* 8: 360-367.

[16] Lee, J.-S. and J.-P. Lee (2003) Review of advances in biological CO_2 mitigation technology. *Biotechnol. Bioprocess Eng.* 8: 354-359.

[17] Akkerman, I., M. Janssen, J. Rocha, and R. H. Wijffels (2002) Photobiological hydrogen production: photochemical efficiency and bioreactor design. *Int. J. Hydrogen Energy* 27: 1195-1208.

[18[Benemann, J. (1996) Hydrogen biotechnology: Progress and prospects. *Nature Biotechnol.* 14: 1101-1103.

[19] Hallenbeck, P. C. and J. R. Benemann (2002) Biological hydrogen production; fundamentals and limiting processes. *Int. J. Hydrogen Energy* 27: 1185-1193.

[20] Melis, A. (2002) Green alga hydrogen production: progress, challenges and prospects. *Int. J. Hydrogen Energy* 27: 1217-1228.

[21] Benemann, J. R., D. M. Tillett, and J. C. Weissman (1987) Microalgae biotechnology. *Trends Biotechnol.* 5: 47-53.

[22] Gummert, F., M.-E. Meffert, and H. Stratmann (1953) Non-sterile large-scale culture of *Chlorella* in greenhouse and open air. pp. 166-176. In: Burlew, J. S. (ed.). *Algal Culture from Laboratory to Pilot Plant*. Carnegie Institution of Washington Publication, Washington, DC, USA.

[23] Cook, P. M. (1951) Chemical engineering problems in large scale culture of algae. *Ind. Eng. Chem.* 43: 2385-2389.

[24] Terry, K. L. and L. P. Raymond (1985) System design for the autotrophic production of microalgae. *Enzyme Microb. Technol.* 7: 474-487.

[25] Oswald, W. J. (1988) *Large-scale Algal Culture Systems (Engineering Aspects)*. Cambridge University Press, Cambridge, UK.

[26] Chaumont, D. (1993) Biotechnology of algal biomass production: a review of systems for outdoor mass culture. *J. Appl. Phycol.* 5: 593-604.

[27] Avron, M. (1989) The efficiency of biosolar energy conversion by aquatic photosynthetic organisms, pp. 385-387. In: Y. Cohen and E. Rosenberg (eds.). *Microbial Mats: Physiological Ecology of Benthic Microbial Communities*. American Society for Microbiology, Washington, DC, USA.

[28] Richmond, A. (1986) Outdoor mass cultures of microalgae. pp. 285-330. In: A. Richmond (ed.). *Handbook of Microalgal Mass Culture*. CRC Press, Boca Raton, FL, USA.

[29] Lee, Y.-K. (1986) Enclosed bioreactors for the mass cultivation of photosynthetic microorganisms: the future trend. *Trends Biotechnol.* 5: 186-189.

[30] Pirt, S. J., Y.-K. Lee, M. R. Walach, M. W. Pirt, H. H. M. Balyuzi, and M. J. Bazin (1983) A tubular photobioreactor for photosynthetic production of biomass from CO_2: Design and performance. *J. Chem. Tech. Biotechnol.* 33B: 35-58.

[31] Ratchford, I. A. J. and H. J. Fallowfield (1992) Performance of a flat plate, air-lift reactor for the growth of high biomass algal cultures. *J. Appl. Phycol.* 4: 1-9.

[32] Tredici, M. R. and R. Materassi (1992) From open ponds to vertical alveolar panels: The Italian experience in the development of reactors for the mass cultivation of phototrophic microorganisms. *J. Appl. Phycol.* 4: 221-231.

[33] Olaizola, M. (2000) Commerçial production of astaxanthin from *Haematococcus pluvialis* using 25,000-liter outdoor photobioreactors. *J. Appl. Phycol.* 12: 499-506.

[34] Lee, Y.-K. and C.-S. Low (1992) Productivity of outdoor algal cultures in enclosed tubular photobioreactor. *Biotechnol. Bioeng.* 40: 1119-1122.

[35] Lee, Y.-K. and C.-S. Low (1993) Productivity of outdoor algal cultures in unstable weather conditions. *Biotechnol. Bioeng.* 41: 1003-1006.

[36] Takano, H., R. Takei, E. Manabe, J. G. Burgess, M. Hirano, and T. Matsunaga (1995) Increased coccolith production by *Emiliania huxleyi* cultures enriched with dissolved inorganic carbon. *Appl. Microbiol. Biotechnol.* 43:

460-465.
[37] Ogbonna, J. C., H. Yada, H. Masui, and H. Tanaka (1996) A novel internally illuminated stirred tank photobioreactor for large-scale cultivation of photosynthetic cells. *J. Ferment. Bioeng.* 82: 61-67.
[38] Mori, K. (1985) Photoautotrophic bioreactor using solar rays condensed by fresnel lenses and transmitted through optical fibers. *Biotechnol. Bioeng. Symp.* 15: 331-345.
[39] Javanmardian, M. and B. O. Palsson (1991) High-density photoautotrophic algal cultures: Design, construction, and operation of a novel photobioreactor system. *Biotechnol. Bioeng.* 38: 1182-1189.
[40] Lee, C.-G. and B. O. Palsson (1994) High-density algal photobioreactors using light-emitting diodes. *Biotechnol. Bioeng.* 44: 1161-1167.
[41] Matthijs, H. C. P., H. Balke, U. M. van Hes, B. M. A. Kroon, L. R. Mur, and R. A. Binot (1996) Application of light-emitting diodes in bioreactors: Flashing light effects and energy economy in algal culture (*Chlorella pyrenoidosa*). *Biotechnol. Bioeng.* 50: 98-107.
[42] Hirata, S., M. Hayashitani, M. Taya, and S. Tone (1996) Carbon dioxide fixation in batch culture of *Chlorella* sp. using a photobioreactor with a sunlight-collection device. *J. Ferment. Bioeng.* 81: 470-472.
[43] Vernerey, A., J. Albiol, C. Lasseur, and F. Godia (2001) Scale-up and design of a pilot-plant photobioreactor for the continuous culture of *Spirulina platensis*. *Biotechnol. Prog.* 17: 431-438.
[44] Prokop, A., M. F. Quinn, M. Fekri, M. Murad, and S. A. Ahmed (1984) Spectral shifting by dyes to enhance algae growth. *Biotechnol. Bioeng.* 26: 1313-1322.
[45] Simmer, J., V. Tichy, and J. Doucha (1994) What kind of lamp for the cultivation of algae? *J. Appl. Phycol.* 6: 309-313.
[46] Geider, R. J. and B. A. Osborne (1992) Light Sources and Related Accessories. pp. 93-106. In: R. J. Geider and B. A. Osborne (eds.). *Algal Photosynthesis*. Chapman & Hall, Inc., New York, NY, USA.
[47] Pulz, O. and K. Scheibenbogen (1998) Photobioreactors: design and performance with respect to light energy input. pp. 123-152. In: T. Scheper (ed.). *Bioprocess and Algae Reactor Technology, Apoptosis.* Vol 59. Springer-Verlag, Berlin, Germany.
[48] Richardson, K., J. Beardall, and J. A. Raven (1983) Adaptation of unicellular algae to irradiance: An analysis of strategies. *New Phytol.* 93: 157-191.
[49] Camacho Rubio, F., F. Garcia Camacho, J. M. Fernandez Sevilla, Y. Chisti, and E. Molina Grima (2003) A mechanistic model of photosynthesis in microalgae. *Biotechnol. Bioeng.* 81: 459-473.
[50] Powels, S. B. (1984) Photoinhibition of photosynthesis induced by visible light. *Ann. Rev. Plant Physiol.* 35: 15-44.
[51] Reuter, W. and C. Muller (1993) Adaptation of the photosynthetic apparatus of cyanobacteria to light and CO_2. *J. Photochem. Photobiol. B: Biol.* 21: 3-27.
[52] Lee, C.-G. (1999) Calculation of light penetration depth in photobioreactors. *Biotechnol. Bioprocess Eng.* 4: 78-81.
[53] Suh, I. S. and S. B. Lee (2003) A light distribution model for an internally radiating photobioreactor. *Biotechnol. Bioeng.* 82: 180-189.
[54] Park, K.-H., D.-I. Kim, and C.-G. Lee (2000) Effect of flashing light on oxygen production rates in high-density algal cultures. *J. Microbiol. Biotechnol.* 10: 817-822.
[55] Degen, J., A. Uebele, A. Retze, U. Schmid-Staiger, and W. Trosch (2001) A novel airlift photobioreactor with baffles for improved light utilization through the flashing light effect. *J. Biotechnol.* 92: 89-94.
[56] Terry, K. L. (1986) Photosynthesis in modulated light: Quantitative dependence of photosynthetic enhancement on flashing rate. *Biotechnol. Bioeng.* 28: 988-995.
[57] Park, K.-H. and C.-G. Lee (2001) Effectiveness of flashing light for increasing photosynthetic efficiency of microalgal cultures over a critical cell density. *Biotechnol. Bioprocess Eng.* 6: 189-193.
[58] Eriksen, N. T., T. Geest, and J. J. L. Iversen (1996) Phototrophic growth in the lumostat: a photo-bioreactor with on-line optimization of light intensity. *J. Appl. Phycol.* 8: 345-352.
[59] Hata, J.-I., Y. Toyo-Oka, M. Taya, and S. Tone (1997) A strategy for control of light intensity in suspension culture of photoautotrophic liverwort cells, *Marchantia paleacea* var. *diptera*. *J. Chem. Eng. Japan* 30: 315-320.
[60] Suh, I. S. and S. B. Lee (2001) Cultivation of cyanobacterium in an internally radiating air-lift photobioreactor. *J. Appl. Phycol.* 13: 381-388.
[61] Choi, S.-L., I. S. Suh, and C.-G. Lee (2003) Lumostatic operation of bubble column photobioreactors for *Haematococcus pluvialis* cultures using a specific light uptake rate as a control parameter. *Enzyme Microb. Technol.* 33: 403-409.
[62] Lee, Y.-K. and H.-S. Tay (1991) High CO2 partial pressure depresses productivity and bioenergetic growth yield of *Chlorella pyrenoidosa* culture. *Appl. Phycol.* 3: 95-101.
[63] Kodama, M., H. Ikemoto, and S. Miyachi (1993) A new species of highly CO2-tolerant fast-growing marine microalga suitable for high-density culture. *J. Mar. Biotechnol.* 1: 21-25.
[64] Aiba, S. (1982) Growth kinetics of photosynthetic microorganisms. p. 85-156. In: Fiechter, A. (ed.). *Microbial Reactions,* vol 23. Springer-Verlag, Berlin, Germany.
[65] Lee, Y.-K. and S.-Y. Ding (1995) Effect of dissolved oxygen partial pressure on the accumulation of astaxanthin in chemostat cultures of *Haematococcus lacustris* (Chlorophyta). *J. Phycol.* 31: 922-924.
[66] Borowitzka, M. A., J. M. Huisman, and A. Osborn (1991) Culture of the astaxanthin-producing green alga *Haematococcus pluvialis*: I. Effects of nutrients on growth and cell type. *J. Appl. Phycol.* 3: 295-304.
[67] Gong, X. and F. Chen (1998) Influence of medium components on astaxanthin content and production of *Haematococcus pluvialis*. *Process Biochem.* 33: 385-391.
[68] Mandalam, R. K. and B. O. Palsson (1998) Elemental balancing of biomass and medium composition enhances growth capacity in high-density *Chlorella vulgaris* cultures. *Biotechnol. Bioeng.* 59: 605-611.
[69] Lee, C.-G. and B. O. Palsson (1995) Continuous medium perfusion leads to long-term cell viability and oxygen production in high-density photobioreactors. *Biotechnol. Lett.* 17: 1149-1154.
[70] Gudin, C. and D. Chaumont (1991) Cell fragility: The key

problem of microalgae mass production in closed photobioreactors. *Bioresource Technol.* 38: 145-151.
[71] Bosca, C., A. Dauta, and O. Marvalin (1991) Intensive outdoor algal cultures: How mixing enhances the photosynthetic production rate. *Bioresource Technol.* 38: 185-188.
[72] Garcia Camacho, F., A. Contreras Gomez, T. Mazzuca Sobezuk, and E. Molina Grima (2000) Effects of mechanical and hydrodynamic stress in agitated, sparged cultures of *Porphyridium cruentum*. *Process Biochem.* 35: 1045-1050.
[73] Silva, H. J., T. Cortinas, and R. J. Ertola (1987) Effect of hydrodynamic stress on *Dunaliella* growth. *J. Chem. Tech. Biotechnol.* 40: 41-49.
[74] Garcia Camacho, F., E. Molina Grima, A. Sanchez Miror., V. Gonzalez Pascual, and Y. Chisti (2001) Carboxymethyl cellulose protects algal cells against hydrodynamic stress. *Enzyme Mircrob. Technol.* 29: 602-610.
[75] Payer, H. D., Y. Chiemvichak, K. Hosakul, C. Kongpanichkul, L. Kraidej, M. Nguitragul, S. Reungmanipytoon, and P. Buri (1980) Temperature as an important climate factor during mass production of microscopic algae. pp. 389-399. In: G. Shelef and C. J. Soeder (eds.). *Algae Biomass. Production and Use*. Elsevier/North Holland Biomedical Press, Amsterdam, The Netherlands.
[76] Torzillo, G., A. Sacchi, and R. Materassi (1991) Temperature as an important factor affecting productivity and night biomass loss in *Spirulina platensis* grown outdoors in tubular photobioractors. *Bioresource Technol.* 38: 95-100.
[77] Thompson, P. A., M.-X. Guo, and P. J. Harrison (1992) Effects of variation in temperature. I. On the biochemical composition of eight species of marine phytoplankton. *J. Phycol.* 28: 481-488.
[78] Raven, J. A. and R. J. Geider (1988) Temperature and algal growth. *New Phytol.* 110: 441-461.
[79] Davison, I. R. (1991) Environmental effects on algal photosynthesis: Temperature. *J. Phycol.* 27: 2-8.
[80] Coleman, J. R. and B. Colman (1981) Inorganic carbon accumulation and photosynthesis in a blue-green alga as a function of external pH. *Plant. Physiol.* 67: 917-921.
[81] Lee, Y.-K. and S. J. Pirt (1984) CO_2 absorption rate in an algal culture: Effect of pH. *J. Chem. Tech. Biotechnol.* 34B: 28-32.
[82] Falkner, G. and F. Horner (1976) pH Changes in the Cytoplasm of the blue-green alga *Anacystis nidulans* caused by light-dependent proton flux into the thylakoid space. *Plant Physiol.* 58: 717-718.
[83] Yegneswaran, P. K., M. R. Gray, and B. G. Thompson (1990) Kinetics of CO2 hydration in fermentors: pH and pressure effects. *Biotechnol. Bioeng.* 36: 92-96.
[84] Cogne, G., C. Lasseur, J.-F. Cornet, C.-G. Dussap, and J.-B. Gros (2001) Growth monitoring of a photosynthetic microorganism (*Spirulina platensis*) by pressure measurement. *Biotechnol. Lett.* 23: 1309-1314.
[85] Cohen, Z. (1986) Products from microalgae, pp. 421-454. In: A. Richmond (ed.). *Handbook of Microalgal Mass Culture*. CRC Press, Boca Raton, FL, USA.
[86] Ben-Amotz, A. and M. Avron (1990) The biotechnology of cultivating the halotolerant alga *Dunaliella*. *Trends Biotechnol.* 8: 121-126.
[87] Avron, M. and A. Ben-Amotz (1992) *Dunaliella: Physiology, Biochemistry, and Biotechnology*. CRC Press, Boca Raton, USA.
[88] Wada, H., Z. Gombos, and N. Murata (1990) Enhancement of chilling tolerance of a cyanobacterium by genetic manipulation of fatty acid desaturation. *Nature* 347: 200-203.
[89] Deshnium, P., D. A. Los, H. Hayashi, L. Mustardy, and N. Murata (1995) Transformation of *Synechococcus* with a gene for choline oxidase enhances tolerance to salt stress. *Plant Mol. Biol.* 29: 897-907.
[90] Melis, A., J. Neidhardt, and J. R. Benemann (1999) *Dunaliela salina* (Chlorophyta) with small chlorophyll antenna sizes exhibit higher photosynthetic productivities and photon use efficiencies than normally pigmented cells. *J. Appl. Phycol.* 10: 515-525.
[91] Nakajima, Y. and R. Ueda (2000) The effect of reducing light-harvesting pigment on marine microalgal productivity. *J. Appl. Phycol.* 12: 285-290.
[92] Zaslavskaia, L. A., J. C. Lippmeier, C. Shih, D. Ehrhardt, A. R. Grossman, and K. E. Apt (2001) Tropical conversion of an obligate photoautotrophic organism through metabolic engineering. *Science* 292: 2073-2075.
[93] Davis, E. A., J. Dedrick, J. H. C. Smith, and H. A. Spoehr (1953) Laboratory experiments on *Chlorella* culture at the Carnegie Institution of Washington, p. 105-153. In: Burlew, J. S. (ed.). *Algal Culture from Laboratory to Pilot Plant*. Carnegie Institution of Washington Publication, Washington, DC, USA.
[94] Tamiya, H., K. Shibata, T. Sasa, T. Iwamura, and Y. Morimura (1953) Effect of diurnally intermittent illumination on the growth and some cellular characteristics of *Chlorella*. pp. 76-84. In: J. S. Burlew (ed.). *Algal Culture from Laboratory to Pilot Plant*. Carnegie Institution of Washington Publication, Washington, DC, USA.
[95] Juttner, F. (1977) Thirty liter tower-type pilot plant for the mass cultivation of light and motion sensitive planktonic algae. *Biotechnol. Bioeng.* 19: 1679-1687.
[96] Roubicek, R. V., K. H. Patton, K. H. McCorkle, and A. L. Rakow (1986) Cultivation of microalgae in a photobioreactor. pp. 218-221. In: W. R. Barclay and R. P. McIntosh (eds.). *Algal Biomass Technologies: An Interdisciplinary Perspective*. J. Cramer, Berlin, Germany.
[97] Torzillo, G., B. Pushparaj, F. Bocci, W. Balloni, R. Materassi, and G. Florenzano (1986) Production of *Spirulina* biomass in closed photobioreactors. *Biomass* 11: 61-74.
[98] Driessens, K., J. Liessens, S. Masduki, W. Verstraete, H. Nelis, and A. De Leenheer (1987) Production of *Rhodobacter capsulatus* ATCC 23782 with short residence time in a continuous flow photobioreactor. *Process Biochem.* 22: 160-164.
[99] Miyamoto, K., O. Wable, and J. R. Benemann (1988) Vertical tubular reactor for microalgae cultivation. *Biotechnol. Lett.* 10: 703-708.
[100] Treat, W. J. and C. R. Engler (1989) Culture of photomixotrophic soybean and pine in a modified fermentor using a novel impeller. *Biotechnol. Bioeng.* 34: 1191-1202.
[101] James, C. M. and A. M. Al-Khars (1990) An intensive continuous culture system using tubular photobioreac-

tors for producing microalgae. *Aquaculture* 87: 381-393.
[102] Lee, E. T.-Y. and M. J. Bazin (1990) A laboratory scale air-lift helical photobioreactor to increase biomass output rate of photosynthetic algal cultures. *New Phytol.* 116: 331-335.
[103] Takano, H., H. Takeyama, N. Nakamura, K. Sode, J. G. Burgess, E. Manabe, M. Hirano, and T. Matsunaga (1992) CO2 removal by high-density culture of a marine cyanobacterium *Synechococcus* sp. using an improved photobioreactor employing light diffusing optical fibers. *Appl. Biochem. Biotechnol.* 34/35: 449-458.
[104] Yongmanitchai, W. and O. P. Ward (1992) Growth and eicosapentaenoic acid production by *Phaeodactylum tricornutum* in batch and continuous culture systems. *JAOCS* 69: 584-590.
[105] Burgess, J. G., K. Iwamoto, Y. Miura, H. Takano, and T. Matsunaga (1993) An optical fiber photobioreactor for enhanced production of the marine unicellular alga *Isochrysis* aff. *Galbana* T-Iso (UTEX LB 2307) rich in docosahexaenoic acid. *Appl. Microbiol. Biotechnol.* 39: 456-459.
[106] Torzillo, G., P. Carlozzi, B. Pushparaj, E. Montaini, and R. Materassi (1993) A two-plane tubular photobioreactor for outdoor culture of *Spirulina*. *Biotechnol. Bioeng.* 42: 891-898.
[107] Hu, Q., H. Guterman, and A. Richmond (1996) A flat inclined modular photobioreactor for outdoor mass cultivation of photoautotrophs. *Biotechnol. Bioeng.* 51: 51-60.
[108] Hu, Q., Y. Zarmi, and A. Richmond (1998) Combined effects of light intensity, light-path and culture density on output rate of *Spirulina platensis* (Cyanobacteria). *Eur. J. Phycol.* 33: 165-171.
[109] Takano, H., H. Furu-Une, J. G. Burgess, E. Manabe, M. Hirano, M. Okazaki, and T. Matsunaga (1993) Production of ultrafine calcite particles by coccolothophorid algae grown in a biosolar reactor supplies with sunlight. *Appl. Biochem. Biotechnol.* 39/40: 159-167.

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