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Principles for attaining maximal microalgal productivity in photobioreactors: an overview

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Abstract

Efficient management of mass algal cultures requires appreciation of the most important factors governing the light regime of the average cell, i.e. the interrelationships between the intensity of the light source – never the sole factor involved in mass culture productivity – and the optimal cell density affected by the optical path. The latter is a dominant factor in photosynthetic productivity of ultra high cell density cultures (UHDC) cultured in flat plate reactors. Indeed, a very short optical path (5–10 mm) permits a most efficient use of strong light by facilitating ultra-high cell densities (ca. 10–20 g dry cell mass l^{-1}), in which the condensed cells are exposed to very high frequency light/dark cycles. Another important feature of dense cultures concerns the very small but highly efficient light dose available to cells under extreme mutual shading. The low productivity of the single cell in the culture is well compensated, in terms of culture productivity, by the high culture cell mass exposed to very high frequency light/dark cycles. The combined effects of all these factors result in high efficiency of strong light-use for photosynthesis. UHDC are associated with growth inhibition which represents a severe production obstacle. Once this aspect is better understood and managed, UHDC in ultra short optical path reactors may become a useful production mode of photoautotrophic cell mass and secondary metabolites.

Introduction

Maximal culture productivity of phototrophic microorganisms is obtainable when light represents the sole limitation to productivity, i.e. the nutritional requirements are satisfied and the temperature not far from optimal. Therein rests the major challenge involved in microalgal biotechnology, both scientific and economic: devise efficient, cost-effective reactors and protocols by which to utilize best high irradiance such as solar energy for mass production of phototrophic algae.

Light zones in photobioreactors

Efficient utilization of light by the cells is associated with many constrains. One relates to the exponential attenuation of light energy in passing through the culture column. As cell density increases, light penetration into the culture, expressed as a percent of total incident light impinging on culture surface, decreases exponentially (Table 1). Two light zones are thereby affected in the photobioreactor: The illuminated photic volume in which light supports photosynthesis, and the dark volume, in which light intensity is below

Table 1. Light penetration depth[a] (cm) into cultures of *Nannochloropsis* sp. as effected by the concentration of cell mass (Gitelson, unpublished).

DW ($g\ l^{-1}$)	2	10	50
Blue (410-450 nm)	0.960115	0.192023	0.038405
Green (580-600 nm)	9.433679	1.886736	0.377347
Red (670-678 nm)	1.252403	0.250481	0.050096

[a]To the depth in which light energy is 10% of incident light.

the compensation point and net photosynthesis cannot take place. The higher the population density (and the longer the light path), the more complex it becomes to attain one basic requirement for efficient utilization of light, i.e. an even distribution of light to all cells in the reactor. Another difficulty in harnessing effectively solar, or any other strong light source for photosynthesis, rests upon the fact that the higher the light intensity, the lower, as a rule, the efficiency by which light energy impinging on the reactor surfaces is converted into chemical energy.

Adjusting the photon flux density to cell density

The difficulty in understanding the complex mode by which light effects mass cultures of phototrophic microorganisms has been augmented over the years by erroneous application of the so called 'light curve' (Goldman, 1979) to interpret the growth response of mass cultures to light. This model provides a generalized shape of the light response curve, relating the photosynthetic or growth rate of the culture to the intensity of the light source, i.e. the photon flux density (PFD) impinging on culture surface. The light source is thus placed as the sole rate-limiting factor in a light-limited system. This relationship, however, is only correct for optically thin cultures, of such low population density that mutual shading by the cells is essentially absent. In reality, however, mass cultures exposed to strong light cannot be maintained in optically thin concentrations with no mutual shading, since the photon flux density (PFD) outdoors is much higher, up to an order of magnitude or more, than the photosynthetic saturating light intensity. Excess light may cause photoinhibition followed by culture death and the most practical approach by which to cope with this is to increase cell density to the point in which mutual shading causes cells to receive strong light intermittently. The high PFD prevailing outdoors is thereby diminished or 'diluted' for the individual cells and the light energy each cell receives along time is thus not only a function of the intensity of the light source, but is also, and often more so, dependent on cell density. Any growth rate therefore may indeed be manifested in a culture in response to a given intensity of the light source, depending on the population density through its effect on modifying light availability in the culture. Second, the major parameter in mass culture is the output rate of cell mass or some specific product, per unit reactor-volume or illuminated reactor-area. The output rate in continuous cultures at steady- state is a function of both the growth rate and cell density, and both optically very thin or extremely dense cultures yield significantly below maximal output rates. As is in any biological phenomenon related to optimal exploitation of resources per unit area, there is a certain optimal algal 'stand' which results in the highest areal output rate. This is the 'optimal cell density' (OCD, $g\,l^{-1}$) which is species-specific and which is mandatory to maintain in a culture in order to exploit light most efficiently.

Light–dark (L–D) cycles

A salient feature of OCD is that at this cell concentration, except in optically thin cultures, light penetrates to only a small fraction of the reactor volume. At any given instant, therefore, most of the cells at OCD are exposed to darkness. For a given light-path length, the fraction of the photic volume over the entire, mostly dark reactor volume is a function of both the intensity of the light source and the extent of the population density. The length of the light-path (corresponding to the width of a plate reactor, perpendicular to the direction of the light source), greatly affects the frequency (measured in ms) of the light/dark (L–D) cycle which originates from the movement of cells in and out of the photic volume, in optimally stirred cultures. The shorter the light path, the higher the frequency of the L–D cycle which, as a rule, accelerates the photosynthetic rate. At optimal cell density (i.e. which results in highest productivity per irradiated area), cells are exposed to relatively short flashes of light followed by a relatively long period of darkness; the higher the frequency of this L–D cycle therefore, the more efficiently light (particularly high PFD) may be utilized for photosynthesis (Grobbelaar et al., 1996; Hu et al., 1998b; Janssen 2002; Richmond et al., 2003). Indeed, under specific laboratory conditions, reducing the light-path thereby increasing L–D cycle frequency results in a significant rise in areal productivity. Since the OPD is inversely related to the LP, using reactors with a very narrow LP (e.g. 1–2 cm) exposed to high PFD permits maintenance of cultures of extremely high cell concentrations, e.g. over 100 and up to 1000 mg chlorophyll l^{-1} (Zou et al., 2000).

Mixing the culture

This is yet another factor which affects the light regime to which the average cell in the culture is exposed in UHCD. Mixing represents the most practical means by which to attempt to distribute radiation evenly to all cells in the culture, as well as accelerate growth by reducing the diffusion barriers around the cells. Mixing affects a higher L–D cycle frequency, mandatory for efficient utilization of light for photosynthetic productivity. This may be readily observed in open raceways, in which insufficient stirring reduces the efficiency of solar energy utilization as the pattern of flow becomes increasingly laminar. In addition, dissolved oxygen (DO) builds up in cultures in which mixing is inadequate, inhibiting photosynthesis. The positive effect optimal stirring exerts on the output-rate of biomass is accentuated as the population density and light limitation in the culture increase. Inadequate mixing resulting in high O_2 tensions and in laminar – instead of turbulent – flows resulting in cell precipitation and wall growth, is at the root of colossal industrial failures well described recently by Tredici (1999).

The interrelationships existing between the intensity of the light source, the OCD, the mixing rate and the output rate are clearly evident in *Spirulina* cultures (Table 2). Varying the rate of mixing in cultures exposed to 'low' and 'high' incident light showed that at 'low' light and low OCD, mixing rate had no effect on productivity. Increasing light intensity (all other conditions kept constant), resulted in a significant shift-up of OCD, accompanied by an increase in the output rate. The magnitude of the mixing effect on productivity was strictly dependent on the strength of the light source through its effect on culture density. At 'high' PFD, the output rates of cell mass reflected strong dependence on the rate of mixing, and as the mixing rate was increased to optimal, the output rate doubled (Hu & Richmond, 1996). It should be stressed however, that the dominant effect of the mixing rate shown in Table 2, took place in a culture of *Spirulina platensis* ((Norst.) Gietler, a very large filamentous cell-aggregate requiring therefore high stirring energy for induction of optimal turbulent streaming. Small, single-cell species, e.g. *Nannochloropsis salina* D.J. Hibberd, require much less energy for optimal stirring. In such species, the effect of the mixing rate on the output rate would be considerably smaller than in *Spirulina* sp , and would become clearly manifested only at relatively high cell densities.

Growth inhibition

Mass production of ultra high cell-density cultures (abbreviated UHCD, Hu et al., 1996, 1998b) however, is presently unattainable due to inhibition of cell growth which unfolds in the culture as soon as cell concentration (of at least some species) increases above a certain, species specific, threshold (Javanmardian & Palsson, 1991; Imada et al., 1992; Zou et al., 2000). The economic advantages of growing phototrophic cultures at very high cell concentrations in terms of reduced production and capital costs, are presently curtailed by the necessity to continuously alleviate growth inhibition in such cultures.

Preliminary experiments conducted recently with *Nannochloropsis* sp. by Zhang Cheng-Wu in the author's laboratory showed that when the inhibitory activity is removed from UHDC, a decrease in the LP is associated with a marked increase in areal yield. If growth inhibition is not removed, which would practically be the case in large scale cultivation, a short 1.0 cm LP resulted in a sharp reduction in areal yield ($g\ m^{-2}\ h^{-1}$) compared with the areal yield of a 9.0 cm LP reactor (Richmond et al., 2003). The increase in volumetric yield ($g\ l^{-1}\ h^{-1}$) taking place when the LP is shortened does not represent a real increase in productivity, being simply proportional to the reduction in reactor volume. The contrasting response to reduction in optical path is depicted in Fig. 1 in which the effect of the light-path on areal yield of soluble polysaccharides in *Porphyridium* is compared to its effect on productivity of cell mass in *Spirulina*. In the latter, growth inhibition was routinely removed whereas it was not removed in the former. An increase in areal yield was associated with a decrease in LP in *Spirulina* (interpreted as due to a higher L–D cycle frequency), an effect which in *Porphyridium* was not manifested due apparently to growth inhibition (Fig. 1).

Considerations of cost-effectiveness

Efficient, cost-effective photobioreactors would be characterised by high areal- as well as volumetric-productivity. This may be obtained by establishing in the culture an optimal light regime, i.e. in which all parameters effecting the illumination of the average cell in the culture are optimised. Reactor efficiency in terms of cost effectiveness involves high volume- and areal yields of cell mass or products, coupled with a low annual investment cost and, very impor-

Table 2. Interrelationships between intensity of the light source, the optimal cell density, the rate of mixing and the output rate of cell mass in *Spirulina platensis* (after Hu & Richmond, 1996)

Light μmol photon m^{-2} s^{-1}	Mixing rate[a] L(air) l^{-1} min^{-1}	Optimal cell density g dw l^{-1}	Maximal output rate mg dw l^{-1} hr^{-1}
	0.6	2.0	70
500	2.0	5.0	100
	4.0	5.0	100
	0.6	6.0	200
1800	2.0	9.0	300
	4.0	17.0	400

[a]A linear relationship was found to exist between the rate of aeration and the rate of stirring.

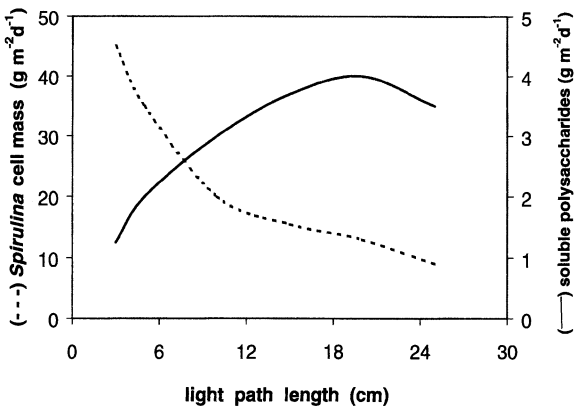

Figure 1. Area-output rates of *Spirulina* cell mass and *Porphyridium* polysaccharides as a function of light-path length (after Hu et al., 1998a; Singh et al., 2000).

tant, simple and easy procedures for reactor operation and maintenance. Some practical points in this context must be addressed in designing efficient reactors: Is the reactor illuminated on all its surfaces, displaying a high area to volume ratio? Is the reactor likely to sustain continuous, mono-algal cultures? I.e. would bio-fouling and wall-growth be readily checked and controlled, reducing the likelihood of culture deterioration? Is the oversaturated oxygen in the culture prevented from reaching prohibitive tensions? Is the cooling system optimised for the local weather conditions? Is turbulent streaming readily introduced and maintained? Is the cost of a unit reactor volume reasonable in terms of the amortisation as well as maintenance costs? Cost-effective photobioreactors may significantly augment the economic potential involved in microalga culture, representing therefore an important research goal.

Summary

Our recent researches (Hu et al., 1996, 1998b; Richmond, 2000; Richmond et al., 2003) permit an attempt to offer a unified concept with which to elucidate light-mediated growth limitations in photoautotrophic mass cultures related to reactor design and culture maintenance protocols, as follows: assuming all other growth conditions are optimal, the sole limitation to photosynthetic activity and cell growth in optically thin cultures is the intensity of the light source, as depicted by the 'light curve'. In high cell density mass cultures, in which cells receive light intermittently, cell density becomes ever more dominant in determining culture productivity, and the quantity light $cell^{-1}$, i.e. the light energy received by the average cell in the culture, becomes the significant growth parameter. Further up the cell concentration scale, the rate of mixing becomes ever more effective in controlling cell growth and culture productivity. Intensifying the PFD and shortening the length of the optical path facilitates maintenance of ultra high cell concentrations. The combined effects of short optical paths and high L–D cycle frequency which shortens the photosynthetically wasteful dark periods to which the cells are exposed as mutual shading is mounting, combined with high PFD and high cell concentrations result in efficient use of strong light, thus producing maximal areal yields of cell mass. Inhibitory substances or conditions however, arrest cell growth and development at high cell densities, and if not removed, bring about gradual deterioration of the culture. Limitation to growth at that stage is shifted from light, the major growth limitation in mass cultures, to an altogether different mode of growth limitation, which is not yet sufficiently understood.

References

Goldman, J. C., 1979. Outdoor algal mass cultures. 2; Photosynthetic yield limitations. Water Res. 13: 119–136.

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

Hu, Q. & A. Richmond, 1996. Productivity and photosynthetic efficiency of *Spirulina platensis* affected by light intensity, cell density and rate of mixing in a flat plate photobioreactor. J. Appl. Phycol. 8: 139–145.

Hu, Q., H. Guterman & A. Richmond, 1996. Physiological characteristics of *Spirulina platensis* (cyanobacteria) cultured at ultrahigh cell densities. J. Phycol. 32: 1066–1073.

Hu, Q., Y. Zarmi & A. Richmond, 1998a. Combined effects of light intensity, light-path and culture density on output rate of *Spirulina platensis* (cyanobacteria). Eur. J. Phycol. 33: 165–171.

Hu, Q., N. Kurano, M. Kawachi, I. Iwasaki & S. Miyachi, 1998b. Ultrahigh-cell-density culture of a marine green alga *Chlorococcum littorale* in a flat-plate photobioreactor. Appl. Microbiol. Biotechnol. 49: 655–662.

Imada, N., K. Kobayashi, K. Isomura, H. Saito, S. Kimura, K. Tahara & Y. Oshima, 1992. Isolation and identification of an autoinhibitor produced by *Skeletonema costatum*. Nippon Suisan Gakkaishi 58: 1687–1692.

Janssen, M., 2002. Cultivation of Microalgae: Effect of Light/Dark Cycles on Biomass Yield. Thesis, Wageningen University, Wageningen, The Netherlands.

Javanmardian, M. & B. O. Palsson, 1991. High-density photoautotrophic algal cultures: design, construction, and operation of a novel photobioreactor system. Biotechnol. & Bioengin. 38: 1182–1189.

Richmond, A., 2000. Microalgal biotechnology at the turn of the millennium: A personal view. J. Appl. Phycol. 12: 441–451.

Richmond, A., Zhang C.-W. & Y. Zarmi, 2003. Interrelationships between the optical path, the optimal population density and cell growth inhibition with reference to efficient use of strong light for photosynthetic productivity in high density algal cultures. Biomolecular Engineering (in press).

Singh, S., S. M. Arad & A. Richmond, 2000. Extracellular polysaccharide production in outdoor mass cultures of *Porphyridium* sp. in flat plate glass reactors. J. Appl. Phycol. 12: 269–275.

Tredici, M. R., 1999. Bioreactors, photo. In Flickinger, M. C. & S. W. Drew (eds), Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis and Bioseparation. John Wiley & Sons, New York.

Zou, N., C. W. Zhang, Z. Cohen & A. Richmond, 2000. Production of cell mass and eicosapentaenoic acid (EPA) in ultrahigh cell density cultures of *Nannochloropsis* sp. (Eustigmatophyceae). Eur. J. Phycol. 35:127–133.