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Biological Determinants of Photobioreactor Design

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SUMMARY

Microalgae is being considered for the capture and sequestration of CO₂ from power-plant flue-gases. High productivity of microalgae is necessary to make this process cost effective compared to the conventional methods used for reducing CO₂ levels in the atmosphere. This obviates the need for large-scale cultivation technologies and proper photobioreactor technology. The physical factors that influence the performance of a photoautotrophic microalgal culture are the quality and composition of light, inlet carbon dioxide concentration, nutrients, and secondary metabolites at high cell densities. In developing photobioreactor technology, balancing of biological processes to the physical rate process becomes important. The effect of various light compositions on the culture kinetics was studied. To determine the optimal composition, six wavelengths 470, 555, 560, 570, 580 and 605 nm, each supplemented with 680 nm of red light, were used to cultivate cultures. Based on the results obtained, it is concluded that a monochromatic red light of 680 nm is sufficient to obtain maximum capacity.

The intake rates of CO₂ were also investigated using 680 nm red light. Inlet CO₂ and O₂ concentration was varied and the growth kinetics were studied. It was found that 1% CO₂ concentration is sufficient to support growth of cells up to a concentration of 1×10^8 cells/mL in flask cultures. This experiment was repeated and the results were found to be consistent.

Proper mixing is very important for achieving high concentration photosynthetic cultures. A good mixing can keep the cells in suspension, eliminate thermal stratification, help nutrient distribution, and improve gas exchange. We have tested photobioreactors of various configurations and found a PBR with a vertical rectangular slab-shaped illumination chamber to be the best. This shape meets all the criteria of a successful photobioreactor and can support *Chlorella vulgaris* up to 10^8 cells/mL.

We have designed and constructed four different photobioreactor systems based on the results of the above experiments: (1) PBRc system with a conical shape illumination chamber and a silicone membrane for gas exchange; (2) PBRs system with a shallow cylindrically shaped growth chamber and a magnetic stirrer and the same gas transfer method as PBRc; (3) PBRe configuration with a rectangular shaped chamber and an external gas exchanger; and (4) PBRb system with the same illumination chamber as in the PBRe system but with internal sparging. The PBRb system was found to be the best in supporting higher cell concentrations. The main advantage of the PBRb system over the PBRc or PBRs configurations lies in the fact that the vertical slab chamber can effectively eliminate cell settling without damaging the healthy cells. The maximum cell concentration achieved in the PBRb configuration was $1.2 \cdot 10^9$ cells/mL (or 5% v/v), which was about 7% of the concentration of the closely packed cells.

The PBRb system above used light-emitting diodes (LEDs) as a sole light source. Direct internal illumination by 680 nm LEDs could deliver as high as 50 mW/cm² of light into the culture medium. Gas transfer by internal sparging had the capacity to transfer 250 mmol O₂/L culture/h. Nutritional limitations could be overcome by continuous perfusion, supplying the medium components to the culture without increasing osmolarity, while removing potentially inhibitory cellular wastes. When the PBR was operated in a continuous perfusion mode with a perfusion rate of 6 reactor volumes a day (6 VVD), it could support ultra high-density algal cultures up to cell concentrations of $4 \cdot 10^9$ cells/mL and total cell volume fractions of 9.4% v/v (about 25 g dry weight/L). The oxygen production rate at its peak was 13 to 15 mmol/L culture/h. Continuous perfusion allowed for long-term stable oxygen production, while oxygen production in batch mode ceased when stationary phase was reached. The results presented suggest that PBR technology can still be significantly improved.

I. INTRODUCTION

Design of photobioreactors (PBRs) relies on the balancing of biological dynamics with the physical rate processes. The key biological rates that govern and define the performance of PBRs are photosynthetic metabolism and cellular growth rates. Prior to designing PBR systems one needs to have detailed information on the achievable metabolic and growth performance of microalgae and how it is influenced by the process operating conditions and the algal microenvironment. Once these intrinsic kinetics are known, one can design a PBR system to a particular degree of performance under the known physicochemical constraints that govern the pertinent physical rate processes. The economics of the PBR system will be determined by the desired performance and the cost of providing light and the corresponding rates of supply and removal of various chemical constituents, such as gases and biomass.

The history of the commercial use of algal cultures spans about 50 years with applications to waste-water treatment, production of biomass, and production of specialty chemicals [Borowitzka and Borowitzka 1988, Faulkner 1986, Glombitza and Koch 1989, Hoppe et. al. 1979, Paul 1982, Shinho 1986]. Recently, interest has developed in using microalgae to fix carbon dioxide to produce oxygen and biomass for life-support in space, and for reducing CO₂ emissions from power plants. Unlike the rapid and significant advances in the biotechnological use of bacteria, yeast and mammalian cells, algal biotechnology and the development of photoautotrophic cultures has progressed slowly in spite of their recognized utility. Perhaps the most constraining factor is the lack of availability of efficient large scale cultivation techniques and the underdeveloped stage of photobioreactor technology.

Large-scale open algal cultures, such as lined and unlined ponds, typically result in low cell densities of 0.01% to 0.06% (w/v) [Avron

1989], resulting in expensive harvesting procedures and unfavorable economics, in addition to difficulties with species control [Benemann 1980, Richmond 1986]. Even with the development of closed tubular photobioreactors with controlled CO₂ supplementation, values of only 0.13% biomass concentrations have been reported [Chaumont et. al. 1988]. Recently, through the examination of the efficacy of light delivery, unique engineering design, and the recognition of the important role of secondary metabolites, values of 3% have been obtained [Javanmardian 1991].

This achievement demonstrates that photobioreactor technology may be vastly underdeveloped. Advances will most likely come with an improved understanding of underlying biological dynamics. The underlying rationale for this proposal is that photobioreactor technology can only be advanced by proper balancing of biological and physical rate processes. The latter are much better understood in terms of physicochemical constraints and engineering capability. The former are not well known and need to be studied.

Our laboratory has pioneered the development of high density PBRs. Our studies have shown that proper engineering of the physical rate process is insufficient to achieve maximal performance. We have found that complete understanding of the biological dynamics is critical to success. The results described here are focused on defining the intrinsic biological rate processes taking place in PBRs and how they are influenced by operating conditions. Based on this information, we will map out the performance versus economics of the full performance spectrum of possible PBR designs. The vehicle that we will use for experimentation are highly defined small light-emitting diode (LED) based PBRs. A large number of these LED-based PBRs can be operated simultaneously. In this fashion, controlled comparisons of performance can be obtained over a range of a particular operating variable.

II. DETERMINATION OF OPTIMAL LIGHT QUALITY

Introduction

In our previous experiments [Palsson 1992], advanced light emitting diodes (LEDs), constructed with double-power double-heterostructure (DDH) gallium aluminum arsenide (GaAlAs) chips [Barta et. al. 1990], were examined for their ability to support mass culture of the eucaryotic alga, *Chlorella vulgaris*. LEDs with peak emittance in the range of 630 to 730 nm (with half-power band width of 20 nm, purchased from Quantum Devices Inc., Barneveld, WI) were used as a sole light source for the cultivation of *C. vulgaris*. The results showed the monochromatic red lights from LEDs ranging between 660 and 700 nm could support the growth of *C. vulgaris*. Neither a series of experiments with various combinations of these growth-supporting wavelengths nor the experiment with these growth-supporting wavelengths supplemented with 630 nm LEDs showed significant difference in culture kinetics from the cells grown under 680 nm alone (data not shown, refer [Palsson 1992]). But the cells cultured under monochromatic red light and full spectrum showed significant differences in the oxygen production rate and growth rate. In an effort to identify the wavelengths responsible for the differences, all the available LEDs with different wavelengths were tested. Six wavelengths with peaks at 470, 555, 560, 570, 580, and 605 nm (from blue to orange, purchased from Stanley Co. Inc., Irvine, CA) were supplemented with 680 nm to determine the optimal composition. Unlike red ones, the other colored LEDs have much poorer electricity-to-light conversion efficiencies (due to poorer technology), and thus, they could not support the algal cultures without red LEDs (data not shown).

Materials and Methods

The detailed methods of the two sets of experiments are discussed below.

First Set: Supplements of other wavelengths

Eight air-tight sterile flasks were used in this experiment. All 7 flasks which were illuminated by LEDs (flasks L470, L555, L560, L570, L580, L605, and L680) had 3 of 680 nm LEDs each. All of these flasks were wrapped completely (except the bottom surface of the flask) to block stray light. Red light intensity was matched to around $30 \mu\text{W}/\text{cm}^2$ at the bottom of the flask by powering each unit separately with different voltages.

The intensities from colored LEDs were also matched to that of blue (470 nm), which had the lowest intensity (about $5.7 \mu\text{W}/\text{cm}^2$). Each colored LED unit consisted of 65 LEDs of that specific wavelength and was illuminating the flask through the bottom. The detailed composition, and the intensity of each flask, as well as other culture parameters, are shown below. The eighth flask, illuminated by a 22 W circline fluorescent light (flask marked as FL), served as a control.

L470 : 250 lux of red (680 nm) plus 33 lux of blue (470 nm)
L555 : 250 lux of red (680 nm) plus 39 lux of green (555 nm)
L560 : 250 lux of red (680 nm) plus 39 lux of green (560 nm)
L570 : 250 lux of red (680 nm) plus 40 lux of yellow (570 nm)
L580 : 250 lux of red (680 nm) plus 41 lux of yellow (580 nm)
L605 : 250 lux of red (680 nm) plus 42 lux of orange (605 nm)
L680 : 250 lux of red (680 nm) only
FL: 4000 lux of white light

Gas composition used : 5% CO_2 and 10%-15% O_2 .

Inoculum density : 1.9×10^5 cells/mL

Culture volume : 55 mL

Temperature : room temperature

Second Set: The effect of blue light

Five airtight sterile flasks were prepared. The light intensities of all 3 flasks which were illuminated by red LEDs (flasks named RO, RB, and RI) were matched again to $30 \mu\text{W}/\text{cm}^2$. The light intensities of the two flasks which were mainly illuminated by fluorescent light (FO and FI) were also matched to $30 \mu\text{W}/\text{cm}^2$. The intensities of blue

LEDs were again matched to $5.7 \mu\text{W}/\text{cm}^2$ (in the case of RB, RI, and FI). Blue lights for RI and FI was turned on for a day and off for the next day to see the adaptation of the algae to blue light. Please refer to the Table below for more information.

- RO: Red Only (250 lux of 680 nm)
- RB: 250 lux of red (680 nm) plus high intensity blue (33 lux)
- RI: 250 lux of red plus intermittent high intensity blue (33 lux)
- FO: 250 lux of white (Fluorescent light Only)
- FI: Fluorescent light plus Intermittent blue

Gas composition used : 5% CO_2 and 10%-15% O_2 .

Inoculum density : 2.4×10^5 cells/mL

Culture volume : 55 mL

Temperature : room temperature

Results and Discussion

The growth of L470 was comparable to that of fluorescent light (Fig. 2.1). This means *Chlorella vulgaris* needs a certain amount of blue light to grow since the growth in L470 was much higher than in any other flask with LEDs. However, the oxygen production of L470 was about the same as other LED-lit flasks, despite the higher cell concentration (Fig. 2.2).

In order to determine whether the better growth observed under full spectrum was due to light intensity or light quality, another set of experiments was performed by adjusting the distance of fluorescent light from the flask to give a light intensity similar to that from LED units.

Figure 2.1 Effect of various wavelength supplements on the growth of *C. vulgaris*.

As can be seen in Figure 2.3, no distinguishable difference in culture kinetics was found between the flasks with LEDs and those with fluorescent light when the intensities were matched, except the larger average cell size (and thus slightly higher per cell chlorophyll content, *i. e.*, chlorophyll concentrations per unit cell volume were constant, data not shown) observed under full spectrum. Furthermore, no difference in growth was observed between cultures with and without blue light. After repeating this experiment three times (to further investigate the discrepancy between the first set and the second one on blue light effects), it was certain that there was little difference in the culture kinetics with and without blue light supplement. The oxygen production rate (as an indirect measurement of photosynthetic rate) did not show any significant difference either (refer to Figure 2. of our first report).

Figure 2.2 Specific oxygen production rate in the cultures with different colored light supplements.

From a practical point of view, an LED light source has several important advantages over colored LEDs. The power conversion efficiency of red LEDs is high enough to give better use of electricity and less heat dissipation. LEDs are small and thus offer significant flexibility in the design of culture apparatus for photosynthetic organisms. Further, solid state technology is reliable and offers interesting possibilities with respect to temporal and spectral control of the light source. Given all these advantages, it is likely that new generations of PBRs can be developed that should accelerate the practical use of algal cultures.

Figure 2.3. Growth curves under red light with different amounts of blue light supplement.

Conclusion

In conclusion, the monochromatic red light from 680 nm DDH GaAlAs LEDs can successfully replace conventional full spectrum light sources.

III. EFFECTS OF CARBON DIOXIDE CONCENTRATION

Introduction

Algae cells need carbon dioxide to grow autotrophically. Carbon dioxide is the sole source of carbon for the process of photosynthesis and biomass production and oxygen is liberated as a product. Thus, higher carbon dioxide concentration and lower oxygen concentration is desirable. To investigate the effect of carbon dioxide and oxygen concentrations in inlet gas stream on algal cell growth kinetics, the following experiment was designed.

Materials and Methods

Five identical flasks, each with 22W fluorescent light, were prepared. Each flask was supplied with a different composition of inlet gas concentration. The detailed compositions are shown below.

F1 :	0.03% CO ₂	20% O ₂	(air, control)
F2 :	0.03% CO ₂	10% O ₂	
F3 :	5.0% CO ₂	10% O ₂	(same as F4 after 98.5 hrs)
F4 :	5.0% CO ₂	20% O ₂	
F5 :	0.5% CO ₂	10% O ₂	
Actual concentration	F1:	0.06 CO ₂	20.70 O ₂
	F2:	0.05 CO ₂	09.96 O ₂
	F3:	4.84 CO ₂	10.21 O ₂
	F4:	5.17 CO ₂	20.53 O ₂
	F5:	0.52 CO ₂	10.04 O ₂

Gas flow rate is 80 mL/min

Inoculum density around 1.0×10^5 cells/mL

Culture volume: 55 mL

Temperature : room temperature

Results and Discussion

0.05% CO₂ was not enough to support the cultures in shaker flasks. 0.5% CO₂ seems to provide sufficient carbon dioxide transfer to the medium. The growth rates and cell concentrations of cultures

provided with 0.5% CO₂ are highly comparable to cultures provided with 5% CO₂. However, the lower concentration of O₂ did not help in the growth of the cultures (Fig. 3.1).

Figure 3.1. The effect of carbon dioxide mass transfer rate on *C. vulgaris* growth..

The initial poor growth in flask #1 (0.05% CO₂ and 20.7% O₂) was either due to harmful wavelengths emitted from an old fluorescent light or lack of carbon dioxide from an old air cylinder. It began to grow like flask #2 after both the fluorescent light and gas cylinder were changed.

Even though the oxygen production rate and carbon dioxide consumption rate could not be measured due to gas analyzer constraints, the effects of inlet carbon dioxide concentration could be monitored by the growth curve. Furthermore, the higher carbon dioxide concentration apparently induced chlorophyll synthesis (data

not shown). The per cell chlorophyll contents were about two times higher in flasks number 3 to 5 (0.5 - 5.0% CO₂) than those in flasks #1 and #2 (0.03% CO₂).

It is interesting to see the pH increase was the highest in flask #5, which had 0.5% CO₂ and 10% O₂ inlet gas composition. From this graph, we can deduce two things. Firstly, the higher the activity (higher cell concentration), the higher is the pH as can be observed when comparing flask #1 and flask #2 to other flasks. Secondly, the higher the inlet carbon dioxide concentration, the more stable the pH (we can see this from the comparison of flasks #3 and #4 to flask #5). Since there are some reports that higher pH can inhibit the cell cycle via bicarbonate accumulation [Mandalam 1994], the inlet carbon dioxide concentration should be higher than a certain value. The result of this experiment suggests that it should be higher than 0.5%.

Final cell concentrations of each flask (total biomass) at the end of the run (211.5 hr) were as followings:

F1 :	0.03%	CO ₂	20% O ₂	0.09119	v/v	%
F2 :	0.03%	CO ₂	10% O ₂	0.08682	v/v	%
F3 :	5.0%	CO ₂	10% O ₂	0.63574	v/v	%
F4 :	5.0%	CO ₂	20% O ₂	0.62340	v/v	%
F5 :	0.5%	CO ₂	10% O ₂	0.49108	v/v	%

This data clearly shows that 0.03% of CO₂ is not enough to support the flask cultures. However there was a small improvement in the culture kinetics under 5.0% CO₂ over that under 0.5% CO₂. Based on these observations, 1% CO₂ should suffice to support algal cultures to over 1.0×10^8 cells/mL in the flask cultures.

Conclusion

From this observation, we can conclude that about 1% CO₂ should be enough to support the *C. vulgaris* cultures up to 10^8 cells/mL in flask cultures.

IV. EFFECTS OF MIXING ON MICROALGAL PRODUCTION

Introduction

Good fluid mixing is important for achieving high cell concentration in a PBR. Good mixing can keep the cells in suspension, eliminate thermal stratification, help nutrient distribution, and improve gas exchange. Since the algae cells are so efficient in absorbing the light, only the top layer of the cell will see the light and go through the photosynthesis once cells are settled down. Furthermore, mixing can reduce the degree of mutual shading and lower the probability of photoinhibition. The last reason is perhaps the most important one for requiring the right extent of mixing to achieve a successful PBR. Proper mixing will significantly reduce the mutual shading and thus increase the portion of the cells exposed to the light. This mixing will also move the cells close to the illuminated surface into the center of the PBR, which will give these photon-saturated cells the opportunity to use all the absorbed light energy for photosynthesis before they are exposed to the light again. This mixing, in turn, will also provide the same opportunity to the cells in the center (that already used up the absorbed photons) to "see" the light. As a result, a proper mixing may significantly improve overall light utilization efficiency.

Materials and Methods

Several different types of prototype photobioreactors were designed and tested with different recycling rates to see the effect of mixing and transit time on microalgal production.

Conical shape photobioreactor, PBRc (Fig. 4.1)

Cylindrical shape photobioreactor with magnetic stirrer, PBRs (Fig. 4.2)

Rectangular slab shape photobioreactor with external gas exchanger, PBR_e (Fig. 4.3)

	Volume	Flow rates
PBRc	10 mL	40 - 150 mL/min
PBRs	14.5 mL	0 - 80 mL/min
PBRe	66 mL	66 - 1000 mL/min

Results and Discussion

For the first generation prototypes (PBRc), no matter what the flow rate was the cells were settled on the bottom of the reactor. The cell concentration curves were non-smooth due to the settling of the cells inside the reactor. Further, the cell concentration after 2 days after the inoculation was always lower than the inoculum concentration. However, a sample withdrawn after vigorous shaking of the PBR gave a higher cell concentration than the inocula. This became clear when we opened the reactor as we could see the cells settled on the surface at the bottom. This occurred over a wide range of recirculation rates (40-150 mL/min in 40 mL working volume reactor).

To achieve better mixing, we introduced a magnetic stirrer to the PBR (PBRs). Nonetheless, similar results with PBRc were obtained

This implied that more efficient mixing is definitely required for the PBR system. Thus a PBR with a totally different geometry was designed. The inner shape of the reactor was a vertical rectangular slab. By this way, the cell settling could be prevented, since the flow direction was directly against gravity, which causes cell settling. With this design, a very high flow rate damaged the cells due to the accompanying high shear stress. Around 1 VVM of recirculation was found to be enough to support high cell densities. One of the growth curves from a PBRe run is shown in Figure 4.4.

Conclusion

From a series of simple preliminary experiments with various geometries, a PBR with a vertical rectangular slab-shaped illumination chamber was found to be suitable. This shape meets all the criteria such as good light distribution, proper mixing without damaging cells by shear stress, and minimization of wall growth.

Further, this geometry made the resulting PBR less sensitive to the transit times.

Figure 4.1. Schematic diagram of PBRc.

Figure 4.2. Schematic diagram of PBRs.

Figure 4.3. Schematic diagram of PBRe.

Figure 4.4. Typical growth curve in PBR (RII, see the next chapter for more details).

V. EVALUATION OF DIFFERENT PHOTOBIOREACTOR SYSTEM DESIGNS

Introduction

Algal biotechnology is drawing increased interest due to its vast potential as a source of valuable pharmaceuticals, pigments, carbohydrates, and other fine chemicals. In the 1990s, increasing attention will be paid to algae due to their suitability in food production, waste treatment and atmosphere regeneration. To exploit their potential efficiently, high density algal photobioreactors (PBRs) are required. Recent developments of highly efficient light emitting diodes (LEDs) have opened a new era in the development of a small novel ultra-high density PBR using LEDs as the sole light source.

Study of the following parameters is necessary for the design of a successful algal photobioreactor: (i) the potential of LEDs as light sources for algae (ii) light intensity, its quality and distribution inside the reactor (iii) mixing and mass transfer rate inside the PBR (iv) the effects of the above parameters on the algal growth and the oxygen productivity. The resulting PBR will not only have significant application in the area of algal biotechnology, but may also be a promising solution to some urgent environmental problems such as the green house effect and water pollution.

The performances of each of the photobioreactors (Figs. 4.1 through 4.3) and some modifications are presented.

Materials and Methods

The prototype photobioreactors whose designs were reported earlier were tested for their photosynthetic capacities.

(1) Conical shape photobioreactor, PBRc (Fig. 4.1).

- Four PBRc all with 680 nm LED units
- MS Mesh + Silicon coating (SigmaCoat)

UD Upside Down

SD Side Down

CX Cross Array LED Unit

- Recycle Rate : 50 mL/min
- 5% CO₂ balanced with Air
- LED units are powered with 1.8 V (after 190 h increased to 1.9 V and after 285 h increased to 2.0V)
- Inoculum density around $6 \cdot 10^5$ cells/mL

(2) Cylindrical shape photobioreactor with stirrer, PBRs (Fig. 4.2)

- Four PBRs (same as 4" PBR except a magnetic stirrer inside the PBR)
- 680S 680 nm LED unit (Spread)
- 700S 700 nm LED unit (Spread)
- 680X 680 nm LED unit (Cross)
- LED units are powered at 1.7 V
- stirrer speed at 4 without recirculation
- Inoculation density $7.5 \cdot 10^5$ cells/mL

(3) Rectangular shape photobioreactor with external gas exchange, PBRe (Fig. 4.3)

- Two PBR units with external gas transfer units
- RI unit has 66 mL inner volume with 48 LEDs and an external gas exchanger (the same as PBRe configuration in PBRe Run #1)
- RII unit has two of PBRe units in series (66 mL inner volume and 48 LEDs per unit) and an external gas exchanger
- F is a flask culture of the same inoculum as a control
- Light intensity from 1.8 V to 2.1 V
- Flow Rate from 100 mL x 4 to 125 mL x 4
- Inoculum density between $1.7 \cdot 10^7$ to $4.3 \cdot 10^7$ cells/mL

(4) Rectangular shape photobioreactor with sparging, PBRb (Fig. 5.1)

Figure 5.1. Schematic diagram of PBRb.

- Four PBR units with gas bubbling.
- CT unit with 4 narrow orifices (1.6 mm dia.). Gas enters the reactor through the nozzles after mixed with circulating culture broth. The same configuration as NR in PBRb Run #1.

1T unit is the same configuration as above except it has four 1-turn in-line mixers.

2T unit is the same configuration as CT except it has four 2-turn in-line mixers.

HD unit is also the same configuration as CT but it has almost four times more LEDs (180 LEDs, while others have only 48 LEDs).

- Light intensity from 1.8 V to 2.5 V
- Liquid recirculation rate 100 mL/min, Gas bubbling rate is also 100 mL/min
- Inoculum density around $1.0 \cdot 10^6$ cells/mL

Results and Discussion

The PBRc system was mixed by vortex motion induced by recirculation of the culture liquid. Mass transfer was made by a thin silicone membrane which also served as an illumination surface. The LED unit was located on top of the silicone membrane. The LED unit also served as a support of the flexible silicone membrane while the emitted light from it transferred into the culture through the membrane. The PBRs system was similar to the PBRc system except the vessel shape. It was mixed by a magnetically coupled stirrer and had the same silicone membrane. The salient feature of these systems (PBRc and PBRs) was the dual-function membrane.

The PBRe and PBRb systems had identical illumination chambers and mixing was made by recirculation of the culture. However, the PBRe system used an external hollow fiber gas exchanger to exchange CO_2 and O_2 while the PBRb system used a bubble swarm generated by sparging.

The growth curve inside PBRc is shown in Figure 5.2. All configurations had a certain degree of cell settling problems. Application of steel mesh and silicon coat (MS) or installation to upside down (UD) configuration didn't help much in the cell settling problem. However, the UD configuration looks like having more gas transfer than other configurations (data not shown). The SD

configuration was most effective in cell settling. But once the cells are settled, those cells cannot see the light any more. As a result it showed a worse cell growth. The CX configuration produces (reproducibly) better results in cell growth for unknown reasons (as of 213 h, cell mass is over 2% v/v ratio). It also showed less cell settling.

Figure 5.2. Typical growth curve in PBRc. Legends are as shown.

A little better growth was observed in the PBRs systems (Fig. 5.3). However maximum cell concentration was lower than 10^8 cells/mL. Increasing light intensity doesn't help cell growth, which means they are not light-limited. The reason for the low final cell concentration was found to be due to the shear stress inside the PBRs system, which didn't have any free surface. Vigorous stirring within a vessel without a free surface generated high enough shear stress to kill the cells inside the chamber.

Figure 5.3. Typical growth curve in PBRs. Legends are as shown.

The RII unit of the PBR system could maintain a logarithmic growth phase for a longer time (Fig. 4.4). The performance of the RI unit was even poorer. In this PBR system, the light itself was not limited but the cells were exposed to the light for a relatively short time in comparison to the residence time inside a dark hollow fiber unit (please refer to our 4th quarterly report for a detailed explanation on the effect of dark volume). As expected, the specific oxygen productivity showed a maximum of 35 fmole/cell/hr at the concentration of $1.4 \cdot 10^7$ cell/mL and monotonously decreased as the cells grew even with increased light intensities and higher flow rates.

The best prototype PBR was found to be PBRb system (Fig. 5.4). HD (3.75 times more LEDs than control) is able to support the growth better. The highest cell concentration reached was 4.95% v/v at the end of this experiment compared to the 2.29% v/v of the control.

Ultrafiltration (remove conditioned media by force) is more effective than dialysis (exchange conditioned media with fresh media by diffusion through hollow fiber cartridge). PBRb with 1 turn helix in-line mixer (1T) showed slightly better growth than control (but virtually the same at the end). This may be due to light limitation. However, 2T showed worse growth than control probably due to shear stress. The average per cell chlorophyll contents of both 1T and 2T were lower than that of CT. The maximum suspended cell concentration was around $1.2 \cdot 10^9$ cells/mL. An interesting observation here was at the late stationary phase of HD. The cell concentration didn't increase further (while others were still going up); instead, the cell size increased to reach about 5% v/v (resulting in a similar biomass growth rate as others, whose average cell size was maintained around 30 fL/cell). This observaton may indicate a spatial limitation at high cell concentration.

The maximum cell concentration at the end of the run could be calculated as follows, assuming that cells are a perfect sphere and rigid and the same size (the average cell volume was 30 fL/cell) and thus the void fraction of closest packing is about 0.3.

$$\frac{1 \cdot \text{mL} \times (1 - \epsilon)}{30 \cdot \frac{\text{fL}}{\text{cell}}} = 2.3 \times 10^{10} \cdot \frac{\text{cell}}{\text{mL}}$$

The cell concentration achieved in HD configuration of PBRb was 7.1% of the closest packing.

Figure 5.4. Typical growth curve in PBRb. Legends are as shown.

Conclusion

Among the photobioreactor configurations tested in this study, the vertical rectangular slab-shaped illumination chamber was found to be the best in supporting higher cell concentration. The main advantage of the PBRb system over the PBRc or PBRs configurations lay in the fact that the vertical slab chamber could effectively eliminate the cell settling problem without damaging the healthy cells. The maximum cell concentration achieved in the PBRb configuration was $1.2 \cdot 10^9$ cells/mL (or 5% v/v), which was about 7% of the concentration of the closely packed cells.

VI. NUTRIENT LIMITATIONS AND METABOLITE SECRETION AT HIGH CELL DENSITIES

Introduction

High-density photoautotrophic algal cultures were exclusively established by enclosed forms of PBRs such as tubular air-lift types. Most of the PBRs described were able to support the algal cultures up to 5 to 6 g (dry cell weight)/L. A few PBRs were more successful by constructing special lighting systems and certain forms of nutrient supplies (10-17 g/L).

The efficient design of bioreactor systems relies on the important principle of balancing the physical rate processes with the biological dynamics that take place. In PBRs the physical rate processes are light delivery, gas mass transfer, and fluid flow and mixing. The biological rate processes are metabolism, photosynthesis, growth, and the effects of secondary metabolism. We have reported the performance of a high-density LED-based PBR. The next step is to outline the rates at which nutrients have to be supplied and the waste products removed in order to maintain a viable high density culture in stationary phase.

Materials and Methods

Strain

Chlorella kessleri (UTEX 398, formerly known as *C. vulgaris*) obtained from The Culture Collection of Algae at UTEX (Austin, TX) were cultured in N-8 medium (initial pH of 6.2). The seed culture was prepared by 250 mL flasks with 100 mL working volume in an illuminated shake incubator (Model G-27, New Brunswick Scientific Co. Inc., Edison, NJ). Inoculum density to the PBRs was about $1 \cdot 10^6$ cell/mL.

Culture Conditions

On-line replenishment of medium was performed by perfusion using hollow fiber cartridges (model BR170, UniSyn Technologies, San Diego, CA). Perfusion rates used were 0.15 mL/min and 0.30 mL/min (3 and 6 times the working volume a day, respectively). Two other PBRs were run parallel to compare the performance increase by continuous perfusion; a PBR with batch mode and another PBR with off-line medium exchange after the cell concentration reached $4 \cdot 10^7$ cell/mL (centrifuged down the culture, resuspended to fresh medium, and returned to the PBR). A peristaltic pump recirculated the culture medium at a rate of 100 mL/min to keep the cells in suspension.

Pre-mixed gas with the composition of 10% CO₂ and balanced with N₂ was introduced through the gas analyzer and refreshed whenever either O₂ or CO₂ concentration had changed more than 1% from the pre-mixed concentration. A gas recycle rate of 100 mL/min through the internal sparger was found to be enough for the required gas transfer rate and sparging also helped mixing as bubbles rose in a random motion inside the PBRs.

Measurements of Culture

A gas analyzer with high sensitivity was used to measure the oxygen production rate (OPR). A Micro-Oxymax gas analyzer (Columbus Instruments, Columbus, OH) was connected to the PBR system through a 500 mL gas container and the entire gas phase was hermetically sealed to obtain the desired sensitivity. The gas was recirculated continuously by a separate peristaltic pump and the gas composition in the head space of each PBR was measured at 1 h intervals using a multiplexer. A longer interval made the gas concentration difference larger between the readings, which enabled the detection of small changes in gas phase composition. However, obtaining high sensitivity by this method inevitably introduced some fluctuation in the gas readings since any changes in pressure or volume of gas phase by sampling and perfusion affected the OPR calculation.

The cell concentration was determined by Coulter Counter model ZM with a Channelizer model C256 (both from Coulter Electronics,

Inc., Hialeah, FL) after proper dilution with isotonic diluent (Fisher Scientific, Pittsburgh, PA). Since the average cell size was highly variable (about 100 fL/cell while the cells are actively growing and about 30 fL/cell at stationary phase), the specific volumetric growth rate (based on total cell volume) was used to calculate the doubling time.

The chlorophyll content of the cells was quantified by using a spectrophotometer (UV-160, Shimadzu, Japan) after diluting to proper concentrations following methanol extraction.

Results and Discussion

Chlorella, inoculated at $1 \cdot 10^6$ cells/mL, was grown to $3 \cdot 10^8$ cells/mL (about 1% v/v) in batch mode (m in Fig. 6.1). The culture did not grow after this cell concentration was reached. When medium components were supplied by either continuous perfusion or off-line exchange, the same PBR reached a final algal biomass concentration that was an order of magnitude higher than the maximum concentration obtained in batch mode (o, Δ , and $\diamond$ in Fig. 6.1). The final cell concentrations in a PBR with an off-line medium exchange rate of 1 volume fresh medium per volume reactor per day (1 VVD) was over 7.7% v/v ($2.2 \cdot 10^9$ cells/mL) at 490 h (Fig. 6.1, denoted by Δ in all Figs.) while the PBR with perfusion rates of 3 VVD supported cell densities up to 8.2% or $2.9 \cdot 10^9$ cells/mL at the end of the run (Fig. 6.1, represented by o). The highest final cell concentration was achieved in the PBR with the highest medium perfusion rate (6 VVD). At 20 days after the inoculation, more than 9.4% v/v of total biomass or $4 \cdot 10^9$ cells/mL was recorded in the PBR system with 6 VVD perfusion (Fig. 6.1 denoted by $\diamond$ in all Figs.). The culture seemed to be affected by another limitation other than substrate or biological limitations, since the linear growth rate began to level off after the culture reached 8% v/v (data not shown). Furthermore, all the cultures in the PBRs with *in situ* medium supply reached the cell concentration of $1 \cdot 10^9$ cells/mL (about 3% v/v) in 5 days. From this observation, it was apparent that certain forms of medium supply and waste removal were critical for *Chlorella* to overcome the limiting cell density in batch mode.

Figure 6.1. Comparison of growth curves in PBRs with different medium supply/removal rate. Growth curves are shown in the units of biomass due to highly variable average cell size. The four PBRs used here were: one batch mode PBR; one PBR with off-line medium exchange (whose medium change by centrifuge and resuspension started after the cell concentration reached $4 \cdot 10^7$ cells/mL); two PBRs with continuous perfusion with rates of 0.15 mL/min (3 times the total reactor volume a day) and 0.30 mL/min.

Despite the similar growth patterns in all *in situ* medium-supplied PBRs, there was a significant difference in the quality of the cultures. Chlorophyll content per cell revealed that the medium supply rate of 1 VVD could not maintain a healthy cell population (Fig. 6.2). Average chlorophyll content in the PBR with 1 VVD was about half of the other two perfused PBRs. Medium exchange with concentrated medium (3X) at 443 h to this 1 VVD PBR doubled the per cell chlorophyll content temporarily the next day (from 0.25 fmol/cell to 0.45 fmol/cell). This confirmed that some medium components were

depleted after the culture reached $1 \cdot 10^9$ cells/mL. Per cell chlorophyll content in batch mode dropped significantly just after the cell concentration reached the plateau at about 100 h, which suggested that the culture was not viable. The average cell volume during the logarithmic growth was 3-4 times larger than during stationary phase. Therefore the per cell chlorophyll content varied between the two growth phases but chlorophyll content per biomass was constant throughout the culture.

Figure 6.2. The time profiles of chlorophyll content per cell. The symbols used are as in Figure 6.1: batch, semi-batch with 1 VVD medium exchange, and continuous perfusion with 3 VVD and 6 VVD. The initial high chlorophyll content was due to the higher average cell volume of actively growing cells.

The results obtained from OPR measurement were consistent with these findings. *Chlorella* in the batch mode PBR stopped producing oxygen after the culture reached $1 \cdot 10^8$ cells/mL (thick dotted line in Fig. 6.3). No chlorophyll content, no oxygen production, and no

oxygen consumption clearly suggested that the algae in the batch mode PBR were not viable after 100 h. OPR from the other PBRs were stable for a long period. The peak OPR of the PBR with a 6 VVD perfusion rate (thick solid line) was over 15 mmol/L culture/h. All the PBRs with medium supply could sustain high OPRs for extended periods. Oxygen production of the PBR with 3 VVD perfusion (thin dotted line) was between 8 and 5 mmol/L culture/hr for 400 hrs and that of the PBR with 6 VVD was between 10 to 6 mmol/L culture/hr for 300 hrs. However, frequent draining and refilling of the culture (to achieve 1 VVD medium exchange by centrifugation followed by resuspension) caused a large fluctuation in the OPR reading (thin solid line). These results suggest that an optimized photobioreactor can produce oxygen stably over extended periods of time.

Figure 6.3. The measurement of oxygen production rate. The oxygen production in the PBR with batch mode is shown by the thick dotted line. The thin solid and thin dotted lines

are from the PBRs with 1 VVD and 3 VVD medium exchange rate, respectively. The thick solid line represents the PBR with 6 VVD medium exchange.

Taken together, the medium capacity of N-8 for *Chlorella* culture was about $1 \cdot 10^8$ cells/mL. This concentration could be easily confirmed by the stoichiometric calculations based on the chemical composition of *Chlorella* and the amount of each component of N-8 medium. However, previous results on the effects of osmolarity and the possible accumulation of potentially harmful cell wastes give a significant advantage to algal cultures with perfusion or on-line ultrafiltration over the batch mode cultures with highly concentrated medium.

The data presented here demonstrates that PBR technology may be significantly improved. Advances will most likely come through the improved understanding of the underlying biological dynamics. A proper balancing of biological and physical factors will allow high-density algal cultures in enclosed PBRs to be economically feasible for the production of high-value algal-derived products.

Conclusion

The prototype photobioreactor system was operated in a continuous perfusion mode to achieve high-density photoautotrophic algal cultures by continuously supplying medium components while removing cellular metabolic wastes. This PBR was illuminated by GaAlAs LEDs emitting at 680 nm located internally for direct illumination. When the PBR operated in perfusion mode with a perfusion rate of 6 reactor volumes a day, the LED-based perfusion PBR could support ultra high-density algal cultures up to the cell concentration of $4 \cdot 10^9$ cells/mL and the total cell volume fraction of 9.4% v/v (about 25 g dry weight/L). The highest oxygen production rate was about 13 to 15 mmol/L culture/h.

VII. KINETIC MODEL FOR PHOTOSYNTHETIC MICROORGANISMS

Introduction

Photosynthetic microorganisms have been playing a significant role in the material cycle in lake and marine ecosystems during the past millennia. Their activities have not gained much attention from engineering and science at large, except that microbiologists, biochemists, ecologists, etc. have been revealing various characteristics of photosynthetic microorganisms; for instance, biochemists have been interested in the mechanism of light energy conversion to chemical energy, especially in the study of enzymes and biochemical pathways for the photochemical reaction.

It was in the late 40s and early 50s that microbiologists and plant physiologists, in cooperation with chemical engineers, began to study mass outdoor cultivation of the green alga, *Chlorella*. The main interest then was to clarify the algal growth kinetics that are affected by light intensity, temperature, pH of culture medium, etc., and to resolve the problem of how to design and operate the outdoor plant in order to maximize the algal product yield.

Basic Concept

Light

Beer-Lambert's law is usually used to estimate light intensity within a culture medium; in fact, a photobioreactor (PBR) is conventionally designed from the law on the attenuation of light energy along the depth of a liquid column. It is important to reassess here the effect of light-scattering caused by the presence of photosynthetic microorganisms on the light-intensity distribution within the PBR. Even though experimental methods to estimate light energy scattered by the cells are available, the approach only by

experimentation is not always satisfactory from the viewpoint of designing PBR. For complex mathematical descriptions and modeling of light scattering and attenuation, please refer the following references: Aiba 1982, Cornet *et. al.* 1992a, and Cornet *et. al.* 1992b.

Quantum Requirement

It is well known that pathways through which electrons are transferred ultimately to reach NADP resulting from the stimulus of a photon in algae do not exist in photosynthetic bacteria. Photosystem II in algae that deals with the oxidation of water to gain a proton and an electron and to release oxygen is absent from bacteria.

Taking into account the difference in electron transfer mechanisms between algae and photosynthetic bacteria, the two-step light reaction scheme of photosynthesis in algae will be discussed here. That is, the light energy of one photon, $h\nu$, (where h is Planck's constant, ν is frequency of light or c/λ , c is velocity of light in vacuum space, and λ is wavelength) is doubled to lead to one e^- transported to NADP. According to the Calvin cycle, two molecules of NADPH must be oxidized to yield one molecule of CO_2 that has been reduced photosynthetically. Then the quantum requirement defined by the number of photons required to reduce one molecule of CO_2 (or the reciprocal, which is called the quantum yield) is eight ($= 2 \times 2 \times 2$), independent of wavelength of light (quantum yield of 0.125), since 2 e^- must participate in yielding NADPH from $\text{NADP}^+ + 2 e^- + \text{H}^+$ [Aiba 1982]

The classical work [Warburg and Negelein 1923] using *Chlorella* claimed that the quantum requirement (θ) of 4 remains unchanged irrespective of the wavelength of light ($\lambda = 436 - 660 \text{ nm}$). The quantum requirement of four is questionable for *Chlorella*, but no definitive answer to this question has been presented. Emerson and Lewis observed that the quantum requirement of *Chlorella* depends on the wavelength of light used and claimed θ value as 16 ($\lambda = 480 \text{ nm}$) to 11 ($\lambda = 680 \text{ nm}$) per molecule of CO_2 reduced.

Effect of CO₂ and O₂ on Photosynthesis

Ever since the discovery of photorespiration in a leaf of hybrid tobacco by Dekker [Dekker 1955]--who observed a rapid deceleration of CO₂ evolution immediately following extinction of light that has irradiated the leaf--several workers have studied the existence or non-existence of photorespiration in microalgae. In fact, photorespiration in microalgae seems to have been confused with the adverse effect of O₂ on photosynthesis in algae (Warburg Effect). The argument is significant from the point of view that the algal growth could have been controlled by establishing a growth environment to minimize photorespiration, if the photorespiration were to play an appreciable role in CO₂ reduction in the photosynthetic mechanism of these algal cells. This phenomena is closely related to the competitive inhibition of Ribulose-1,5-bisphosphate (RuBP) carboxylase and oxygenase by O₂ and or CO₂ -- the key and bi-functional enzyme at the threshold of CO₂ fixation in photosynthesis.

The following equation will be used to describe the effects of CO₂ and/or O₂ on photosynthesis; this equation accounts for competitive inhibition enzyme kinetics to assess the relative magnitude of oxygenation reaction of RuBP carboxylase.

$$1 - \frac{v}{v_{\max}} = 1 - \frac{C_{\text{CO}_2}}{C_{\text{CO}_2} - K_m \cdot (1 + C_{\text{O}_2} / K_i)}$$

provided:

v = rate of carboxylation, M·s⁻¹

$v_{\max}$ = maximum rate of carboxylation, M·s⁻¹

C_{CO_2} = CO₂ concentration in liquid, M

C_{O_2} = O₂ concentration in liquid, M

K_m = Michaelis constant with respect to CO₂, M

K_i = equilibrium constant between enzyme (RuBP carboxylase) and inhibitor (O₂), M

Kinetic Modeling

It is evident that an accurate determination of the light energy absorbed by a dilute suspension of photosynthetic microorganisms

minimizing the shading effect, is a prerequisite for the discussion of the efficiency of light energy conversion to biomass. Experimental procedures for this determination will be described below, prior to the display of growth yield data on photosynthetic microorganisms.

Algal Response Curves

The response of algal growth rates to variations in the three main environmental factors--nutrients, light intensity and temperature--can be quantified by examining the shapes of the response curves to each of the parameters with the other two held constant. As shown Figure 7.1a, when temperature and light intensity are held constant $\mu = f(S)$, where S is a particular limiting nutrient and all other nutrients are supplied in excess. The shape of this curve has been characterized by a rectangular hyperbola for many limiting nutrient situations. Other mathematical relationships have been considered [Goldman 1977]. The most important feature of all these curves is that they have the same general shape--they are all first order at low nutrient levels ($\mu = \gamma_S S$) and zeroth order at high nutrient values ($\mu = \hat{\mu}$)--but diverge somewhat from each other in the transition phase from first to a zeroth order behavior. The value of S for which the extended line from the first order portion of the curve intersects with the horizontal asymptote describing $\hat{\mu}$ is K_S . For a rectangular hyperbola, K_S is the half-saturation coefficient, or the nutrient concentration at which $\mu = 0.5 \cdot \hat{\mu}$.

For the situation in which light intensity is variable, but nutrients and temperature are kept constant, a similar response of growth rate to light occurs, $\mu = f(I)$, where I is light intensity (Fig. 7.1b). The actual shape of the curve has been discussed elsewhere [Goldman 1979], but it has the common feature with $\mu = f(S)$ curve (Fig. 7.1a) of being first order at low light intensity ($\mu = \gamma_L I$), and zero order at high light intensity ($\mu = \hat{\mu}$). The parameter I_K has the same physical meaning as does K_S . Another important feature of this curve is the value of light intensity (I_S) for which saturation occurs ($\mu = \hat{\mu}$). Depending on the shape of the curve, the relationship between I_K and I_S can be estimated. For example, the form of the equation describing a rectangular hyperbola shape in Figure 7.1b is:

$$\mu = \hat{\mu} \cdot \frac{I}{I_K + I}$$

Because $\hat{\mu}$ is reached only at infinite I ($I_S \rightarrow \infty$), a practical estimate of the ratio $I_S : I_K$ can be made with the approximation that I_S occurs at $\mu = 0.95 \cdot \hat{\mu}$. Then rearranging and solving the above equation gives $I_S : I_K = 19$. Hence, in this case I_S is substantially greater than I_K .

Figure 7.1. General relationships between algal growth rate and environmental parameters: (a) limiting nutrients; (b) light intensity; (c) temperature; (d) light intensity for varying temperature.

The influence of temperature on algal growth rates was found empirically from many laboratory studies that Arrhenius-type relationship between $\hat{\mu}$ and T could be demonstrated [Goldman 1979]. For example,

$$\hat{\mu} = (1.8 \times 10^{10}) \rho^{-6842/T'}$$

in which T' is absolute temperature. This type of equation generally appeared to be applicable over much of the temperature range that algal growth can be measured (10° to 40°C); but for particular species, depending on its thermal response characteristics, only a portion of curve was found to describe the experimental data. Thus,

in Figure 7.1c, only between T_1 and T_3 is the response of $\ln \hat{\mu}$ to T' linear.

A family of curves would then describe the relationships in Figs 7.1a and 7.1b were temperature no longer constant. For algal mass cultures, all nutrients would be supplied in excess, so that the effects of both temperature (Fig. 7.1c) and light intensity on growth rate would be as shown by the curves in Fig. 7.1d.

Photosynthetic Efficiency

To quantitatively describe the photochemical conversion of radiant energy in algal mass cultures, Shelef [Shelef 1968], following the basic concepts of van Oorschot [van Oorschot 1955], wrote the following energy balance:

$$P = \frac{E_T}{J} (I_o - I_d) - D_r$$

in which E_T is the overall efficiency of photosynthesis, I_o and I_d are respectively the visible radiant energy incident to the surface and at depth d in a culture in units of energy per surface area per time, D_r is the overall decay term, in units of g dry weight per area per time.

Assuming 10 quanta are required to reduce one mole of CO_2 (or $\theta = 10$) and that the heat content of algae is 120 kcal/mol, the thermodynamic efficiency $E_q(\lambda)'$ of photosynthesis for a given wave length of light and $E_q(\lambda)$, the weighted thermodynamic efficiency are:

$$E_q(\lambda)' = 0.000412 \cdot \lambda$$

$$E_q(\lambda) = Q_\lambda \cdot E_q(\lambda)'$$

where Q_λ is the relative quantum requirement or $\theta \theta_{\max}^{-1}$ (in which $\theta_{\max}$ is the maximum quantum requirement attained throughout the visible spectrum). For red light (670-680 nm), the region for maximum chlorophyll a absorption, Q_λ for most algal species is about 1.0, whereas the average $E_q(\lambda)'$ is 0.23, the effect of Q_λ reduces the average $E_q(\lambda)$ to 0.20.

The second consideration in establishing the overall photosynthetic conversion efficiency, E_T , is the determination of the light utilization efficiency E_s . The earliest attempt to quantitatively

account for the effect of light saturation was by Burlew [Burlew 1953] who presented the equation:

$$E_S = \frac{I_s}{I_0} \left(\ln \frac{I_0}{I_s} + 1 \right)$$

The above equation was derived from the basic relationship:

$$E_S = \frac{\text{total applied light} - \text{unutilizable light}}{\text{total applied light}}$$

This relationship was formulated from (1) light energy is dissipated in a vertical water column containing a concentration X of algae by Beer-Lambert's Law:

$$\frac{dI}{dt} = -\alpha X I_d \text{ or } I_d = I_0 \exp(-\alpha X d)$$

in which α is the extinction coefficient (and is wavelength dependent) and I_d is the light energy remaining at depth d , and (2) that light energy greater than I_s is not utilized.

Algal Specific Growth Rate and Productivity

To make the model simpler, while reflecting all the factors discussed above, one can use the following relationship of $\mu = f(I)$ for the three basic saturation type curves (light inhibition effects were not considered):

1. First order - zero order

$$\mu = E_q, \text{ if } I_0 \leq I_s = I_k$$

$$\mu = \hat{\mu}, \text{ if } I_d > I_s = I_k$$

2. Exponential

$$\mu = \hat{\mu} \cdot [1 - \exp(I_d I_k)^{-1}]$$

3. Rectangular hyperbola

$$\mu = \hat{\mu} \frac{I_d}{I_k + I_d}$$

Then, using these relationships, and considering light dissipation through depth d , the final integrated relationships for productivity, P , as a function of incident light intensity I_o were:

1. First order - zero order

$$P = \frac{E_q}{J} \left[I_k \left(\ln \frac{I_o}{I_k} + 1 \right) - I_o \exp(-\alpha X d) \right]$$

2. Exponential

$$P = \frac{E_q}{J} I_k \left[\alpha X d + E_i \left(\frac{I_o}{I_k} \right) - E_i \left(\frac{I_o}{I_k} \exp(-\alpha X d) \right) \right]$$

in which E_i represents exponential integrals that can be determined from standard E_i tables.

3. Rectangular hyperbola

$$P = \frac{E_q}{J} I_k \ln \left[\frac{I_k + I_o}{I_k + I_o \exp(-\alpha X d)} \right]$$

The results of these equations are plotted in Figure 7.2 (please refer to the figure legends for further details).

Figure 7.2. General productivity curves: (a) productivity, P , as a function of algal biomass concentration, X ; (b) Productivity, P , as a function of net specific growth rate, μ_N ; (c) biomass concentration, X , as a function of net specific growth rate, μ_N ; (d) optimum net specific growth rate, μ'_N , as a function of incident total light intensity, I'_0 , for varying depths, d .

Conclusion

A major challenge to design large-scale cultures to achieve maximum potential yields consistently as a function of available light intensity exists. To accomplish this goal, consideration must be given not only to the independent variables controlling light utilization efficiency (flow rate and depth), but also to many other factors affecting algal growth (mixing, nutrient supply, pH, etc.). Few data are available on this subject and clearly, much future research on the bioengineering aspects of mass culturing is warranted.

Probably little can be done to improve the actual efficiency of photosynthesis in algal cultures, as has been achieved over the years in conventional land-based agriculture. The uses of large-scale algal cultures may be restricted to solving specific environmental problems (such as water pollution and water renovation). The models here showed that the efficiency of these processes is directly proportional to the biomass of algae grown and the upper limit in

yield is 30-40 g dry weight $\text{m}^{-2} \text{ day}^{-1}$ is clearly an intrinsic parameter of photosynthesis and will probably never be improved upon very much in large-scale cultures. It is this yield constraint that must be considered in determining the feasibility of any mass culture venture.

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