DOE/PC/93212--T6
1

DOE Grant #: DE-FG22-93PC93212

BIOLOGICAL DETERMINANTS OF PHOTOBIOREACTOR DESIGN

7th Quarterly Report: Period - April 1, 1995 - June 30, 1995

PI:

Prof. Bernhard O. Palsson
G.G. Brown Associate Professor
Department of Chemical Engineering
University of Michigan
Ann Arbor, MI 48109

Abstract:

The photosynthetic conversion of light energy into algal biomass in large-scale cultures is controlled by the availability of light, the photosynthetic machinery of algae, nutrients, temperature and the design characteristics of the culture system. For the situation in which light is made the growth rate limiting factor, there is an upper limit in the light conversion efficiency of a large-scale culture, which translates to a maximum potential yield of 30-40 g dry weight m⁻² day⁻¹ under ideal light conditions. The development of large-scale mass cultures involves many considerations, but the two major design parameters for optimizing yields at a particular time are the flow rate throughout the culture and the depth of the culture.

MASTER

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED
a

RECEIVED
USDOE/PETC
95 AUG 10 AM 10:19
ACQUISITION & ASSISTANCE DIV.

DISCLAIMER

**Portions of this document may be illegible
in electronic image products. Images are
produced from the best available original
document.**

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, make any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

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 a PBR. For complex mathematical descriptions and modeling of light scattering and attenuation, please refer to 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 have 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 definite 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 CO_2 and O_2 on Photosynthesis

Ever since the discovery of photorespiration in a leaf of hybrid tobacco by Dekker [Dekker 1955], who observed a rapid deceleration of CO_2 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 O_2 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 CO_2 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 O_2 and or CO_2 - the key and bi-functional enzyme at the threshold of CO_2 fixation in photosynthesis.

The following equation will be used to describe the effects of CO_2 and/or O_2 on photosynthesis; this equation accounts for competitive inhibition of enzyme kinetics to assess the relative magnitude of oxygenation reaction of RuBP carboxylase.

$$1 - \frac{v}{v_{\max}} = 1 - \frac{C_{CO_2}}{C_{CO_2} - K_m \cdot (1 + C_{O_2} / K_i)}$$

provided:

v = rate of carboxylation, $M \cdot s^{-1}$

$v_{\max}$ = maximum rate of carboxylation, $M \cdot s^{-1}$

C_{CO_2} = CO_2 concentration in liquid, M

C_{O_2} = O_2 concentration in liquid, M

K_m = Michaelis constant with respect to CO_2 , M

K_i = equilibrium constant between enzyme (RuBP carboxylase) and inhibitor (O_2), 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 in Figure 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 reaction. 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. 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. 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 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 .

The influence of temperature on algal growth rates was found empirically from many laboratory studies that an 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 the absolute temperature. This kind of equation generally appeared to be applicable over much of the temperature range that algal growth can be measured ($10^\circ - 40^\circ\text{C}$); but for a particular species, depending on its thermal response characteristics, only a portion of the curve was found to describe the experimental data. Thus in Figure 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. 1a and 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. 1c) and light intensity on growth rate would be as shown by the curves in Fig. 1d.

Figure 1. General relationships between algal growth rate and environmental parameters: (a) limiting nutrients; (b) light intensity; (c) temperature; (d) light intensity for varying temperature.

Photosynthetic Efficiency

To quantitatively describe the photochemical conversion of radiant energy in algal mass cultures, [Shelef 1968], following the basic concepts of [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 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 1953] who presented the equation:

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

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:

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

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

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

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

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 2. Please refer to the figure legends for details.

Figure 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_o' , 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.

Reference

- Aiba, S., *Adv. Biochem. Eng.*, **23**: 85-156, 1982
- Burlew, J.S. (Ed.), *Algal Culture From Laboratory to Pilot Plant*, Carnegie Institute, Washington, DC, 1953
- Cornet, J.F. and Dussap, C.G. and Dubertret, G., *Biotechnol. Bioeng.*, **38**: 817-825, 1992
- Cornet, J.F. and Dussap, C.G. and Cluzel, P. and Dubertret, G., *Biotechnol. Bioeng.*, **38**: 826-834, 1992
- Dekker, J.P., *Plant Physiol.*, **30**:82, 1955
- Emerson, R. and Lewis, C.M., *Am. J. Bot.*, **30**(3):165-178, 1943
- Goldman, J.C., *J. Phycol.*, **13**:251-258, 1977
- Goldman, J.C., *Water Res.*, **13**:119-136, 1979
- Shelef, G., Oswald, W.J. and Golueke, C.G., *SERL Report*, 68-4, UC Berkeley, 1968
- Van Oorschot, J.L.P., *Med. Van. Lund. Wang. Nederland*, **55**:225-277, 1955
- Warburg, O. And Negelein, E., *Z. Physik. Chem.*, **106**:191, 1923