PHOTOSYNTHETIC MICROORGANISMS AND VALUABLE PRODUCTS

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Summary

Photosynthetic microorganisms, such as microalgae and cyanobacteria, can be used to produce high-value pharmacological or nutraceutical products, biomass for human or animal consumption, and for liquid or gas waste treatment. All these applications are based on the use of solar radiation and the photosynthetic machinery to convert inorganic forms of carbon, nitrogen, phosphorous, etc., into organic matter in solar photobioreactors, which must fulfill microorganism requirements, especially solar

energy, at a minimum cost and with maximum efficiency. This chapter analyzes the main factors in the photosynthetic yield of microorganisms and optimized photobioreactor design and operation. Finally, industrial applications are reviewed.

1. Introduction

Microalgae are defined as a type of photosynthetic protest able to perform oxygenic photosynthesis, that contain chlorophyll *a* and other photosynthetic pigments. Both cyanobacteria with a prokaryotic cell structure, and microalgae, with a eukaryotic cell structure are usually included in this category. There are more than 30,000 different species catalogued, although only 50 of them have been studied in depth in the laboratory, and less than 10 are exploited commercially, thus constituting a practically unexplored source of revenues.

These microorganisms are mainly photoautotrophs, although they may also grow under mixotrophic or heterotrophic conditions. When illuminated they get the energy for their metabolism from light, use water as the terminal electron source, carbon dioxide and other oxidized inorganic compounds as biomass substrates, and release oxygen.

Mass production of microalgae began in Germany in the middle of the last century, when diatoms were cultured in the laboratory to produce lipids by nitrogen starvation. At the same time, studies on the growth of the green algae, *Chlorella*, a microalgae characterized by its fast growth rate under high irradiance, were performed at the Carnegie Institution in Washington D.C. These experiments were published in the first book in this field, entitled "Algal Culture from Laboratory to Pilot Plant" (Burlew, 1953).

Analogous studies were also performed in Japan, at the Tokugawa Institute in Tokyo, where production of proteins from *Chlorella* cultures was studied in the laboratory and at pilot scale. The yield of these systems was much higher than traditional cultures (Tamiya, 1957). Since the seventies, the *Chlorella* microalga has been commercialized for nutritional and dietetic uses in Japan and Taiwan. Today, Taiwan, where six companies produce this microalga for human consumption, is the first global producer of *Chlorella*, with more than 50% of the world market.

Microalgae have traditionally been produced as a primary feed in aquaculture for fish larvae during the first stage of growth. In juvenile fish, microalgae can be replaced by cereals or fish meal, although as this is detrimental to their health, a mixture of microalgae and other materials is used. Adult fish are fed with cereals and fish oils as an economical substitute for microalgae. On the other hand, shellfish must be fed microalgae constantly. Moreover, not all microalgae are suitable for feed, and appropriate strains must be selected.

In addition to aquaculture, microalgae also have an enormous potential in the field of human nutrition and health, as they produce a wide variety of active functional compounds, such as natural antioxidants, pigments, carotenoids, and others, which are purified as pharmaceuticals for humans and animals. Some of these compounds are anticarcinogenic or antimutagenic, while others have been demonstrated to stimulate the

immune system response, and lower blood pressure and cholesterol. Microalgae are the first producers of long-chain polyunsaturated fatty acids, such as docosahexaenoic acid (DHA) or eicosapentaenoic acid (EPA).

Commercial eicosapentaenoic acid and its derivates are effective in the treatment and prevention of coronary diseases, cholesterol, some types of cancer, and some mental disorders.

Finally, the use of microalgae in waste treatment represents a promising technology. Microalgae have been used for a long time in wastewater treatment along with bacteria, yeast and fungi in activated sludges and oxidation channel-tank technologies. However, new processes based on selective microalgae are now being developed.

The cyanobacteria *Spirulina platensis* is being used for the treatment of pork prunes and olive oil waste. Moreover, several strains of microalgae and cyanobacteria are being evaluated in the United States, Canada, Japan, Spain, and elsewhere, for their use in the mitigation of greenhouse gas emissions.

There are three main tasks in any industrial microalgal application.First, selection of the appropriate strain, and its improvement; in this context, genetic techniques which can increase productivity over that of wild strains are of great interest. Second, culturing conditions, such as light availability, nutrient saturation, pH, temperature, fluid-dynamic conditions, etc., must be optimized for the best yield.

Finally, the culture systems in which solar energy is converted to chemical energy must be designed to operate at a minimum cost, thus maximizing the overall productivity of the system.

2. Factors Affecting Photosynthetic Microorganisms

2.1. Light and Photosynthesis Rate

The most important factor in the growth and productivity of photosynthetic microorganism cultures is light availability. The photosynthesis rate of microalgae is a direct function of the irradiance to which the cells are exposed. Irradiance is defined as the total amount of radiation reaching a point from all directions in space, at every wavelength.

However, photosynthetic microorganisms can only make use of the 400 to 700 nm range, the photosynthetically active radiation (PAR). Therefore, in the field of microalgae, irradiance is usually taken as the total amount of photosynthetically active radiation reaching a point from all directions in space.

In microalgal cultures, photosynthesis-irradiance response curves have a hyperbolic shape, analogous to those for growth of yeast or bacteria versus substrate concentration, although there are some differences (Figure 1).

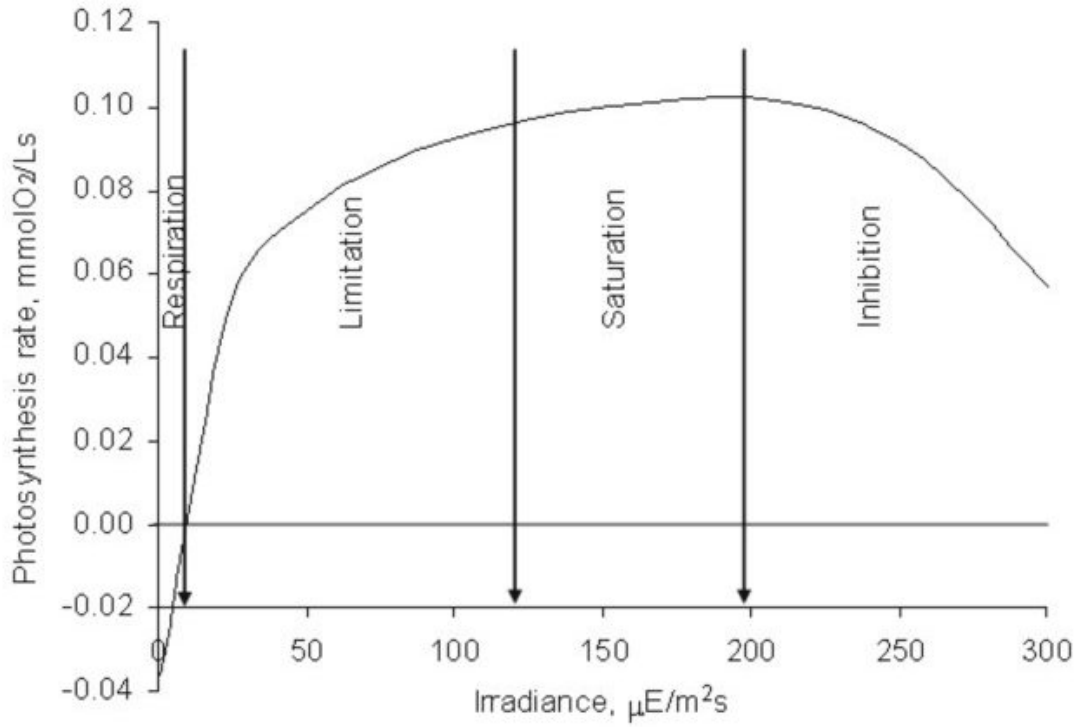

Figure 1. Photosynthesis-irradiance response curve in microalgae.

A minimum irradiance, called compensation irradiance, I_c, is necessary to activate photosynthesis. Below this value, oxygen consumption by respiration overcomes oxygen evolution by photosynthesis. There is also saturation irradiance, I_s, above which the photosynthesis rate is saturated. At very high irradiances, the photosynthetic apparatus can be damaged and the photosynthesis rate decreases. The culture is then said to be photoinhibited. This irradiance is called the inhibition irradiance, I_i. For most microalgae, photosynthesis is saturated at about 30% of the total terrestrial solar radiation. Some microalgal species grow best at irradiance values as low as 50 μE/m^{2}s, but irradiances over 100 μE/m^{2}s are usually necessary for most species. Photoinhibition appears at irradiances over 1000 μE/m^{2}s in most strains, although some sensitive strains are photoinhibited at irradiances no higher than 200 μE/m^{2}s. The influence of light on the growth of any particular strain must be studied in each case.

Many P-I relationships have been found empirically for specific cases (e.g., optically dilute culture, specific algae). From these relationships, different growth models have been proposed, most of them disregarding the photoinhibition effect (Eqs. (1, 2)).

$$\mu = \frac{\mu_{\max} I}{I_k + I} \tag{1}$$

$$\mu = \mu_{\max} \cdot \left(1 - \exp\left[-\frac{I}{I_k}\right]\right) \tag{2}$$

Where μ is the specific growth rate, μ_{max} is the maximum specific growth rate, and I_k is the irradiance constant or irradiance necessary for half the maximum growth rate under specific culture conditions. To take photoinhibition into account, some other equations have also been proposed (Eq. (3, 4)), in which I_i is the photoinhibition irradiance.

$$\mu = \mu_{max} \cdot \frac{I}{I_s} \exp\left[-\frac{I}{I_i} \right] \tag{3}$$

$$\mu = \frac{\mu_{max} I}{I_k + I + \frac{I^2}{I_i}} \tag{4}$$

Otherwise, most of the available *P-I* models lack generality, apparently because they are purely empirical, and do not consider the underlying biochemistry of photosynthesis. The kinetic constants of such empirical models are difficult to link to biological cell phenomena. Often, the empirical models fail to describe the well-known photoadaptive response of photosynthesis. Photoadaptive processes can dramatically modify the growth–irradiance relationship (Zonneveld, 1998).

Another important phenomenon that is generally disregarded in *P-I* models is photoinhibition, a decrease in the photosynthesis rate that occurs when the irradiance exceeds a certain value. Photoinhibition is associated with partial deactivation of key components of the photosynthetic apparatus. To complicate matters, the various physiological responses to varying intensities of light can be interactive. For example, cells adapted to low irradiance are more prone to photoinhibition when transferred to intense light. In response to the many limitations of the fixed-parameter empirical (or "static") *P-I* models, more realistic "dynamic" models of photosynthesis have been developed.

Dynamic models typically break photosynthesis down into single steps, including at least one photochemical energy-collection step and a metabolic consumption step. Differential equations are used to model these steps. Additional steps can be included to account for adaptive responses (Eilers and Peeters, 1988; Zonneveld, 1998). Although dynamic photosynthesis models available do consider photoinhibition and photoadaptation, none of these models are sufficiently general. Existing models do not simultaneously account for photoadaptive responses (photoacclimation), photoinhibition, and the well-documented phenomenon known as the "flashing light effect" (Terry, 1986).

As demonstrated by the flashing light effect, illumination of the microalgal culture need not be continuous for cell growth; growth can be promoted quite efficiently by intermittent or "flashing" light of the same intensity as the continuous light. The flashing light effect is of considerable importance in designing photobioreactors for algal culture, because commercially viable culture systems must operate at high cell densities and, therefore, a photobioreactor necessarily contains an illuminated outer

zone and a darker core. The movement of fluid between the illuminated zone and the dark interior unavoidably subjects the cells to fluctuating illumination.

2.2. Average Irradiance

Even when incident radiation is constant, the irradiance within a culture varies as a function of position. Cells nearer the light-receiving surface are exposed to higher irradiance than cells elsewhere in the vessel. Cells closer to the light source shade those further away, so productivity varies with position and time. Mean irradiance may be defined as the average of local irradiance values inside a culture. The average irradiance, I_{av}, is the amount of light received by a single cell moving randomly inside the culture (Molina et al., 1994).

In a cell-free system, average irradiance is independent of the state of mixing. When cells are distributed homogeneously under given conditions, the average irradiance is again the same for all cells; however, average irradiance is not a sufficient criterion of culture performance, because it considers only the total length of dark and light periods, and not the switching frequency.

Ignoring cell dynamics for the moment, the average irradiance inside the culture depends on the following factors: the external irradiance on the surface of the reactor, I_o; reactor geometry; cell concentration and morphology; cell pigmentation and its absorption characteristics.

An additional complicating factor, generally specific to outdoor cultures, is the cyclic changes in irradiance to which they are subject. There are at least two cycles with substantially different timing: (i) a relatively long daily cycle; and (ii) an even longer seasonal cycle.

A third cycle is due to fluid movement between different illumination zones inside the photobioreactor. Cycles (i) and (ii) affect only incident radiation on the photobioreactor surface, but beyond that factor these cycles are unlikely to have any other impact on culture kinetics. Cycle (ii) is much longer than the cell residence time in the continuous-culture photobioreactor.

Due to the diurnal cycle, a culture is light-limited at dawn and dusk; however, during the midday-peak light period, when peak light may exceed 2000 $\mu E/m^2s$, which is several times higher than saturation, the culture may be photoinhibited. When external irradiance varies over time, average irradiance is determined by time-averaging over short intervals.

Average illumination estimation methods consist of: (i) estimating the total incident photosynthetically active radiation on the photobioreactor surface; (ii) determining the radiation at any depth inside the culture by the Beer–Lambert law, as a function of cell concentration and cell pigment light absorption characteristics; and (iii) integrating local values over the total culture volume. While there are rigorous solutions for specific geometries and photobioreactors, a simplified model has also been proposed:

$$I_{av} = \frac{I_0}{K_a pC_b}\left(1 - \exp\left(K_a pC_b\right)\right) \tag{5}$$

where K_a is the biomass extinction coefficient, p is the optical light path and C_b is the culture biomass concentration. In continuous microalgal cultures as commonly practiced, biomass productivity, P_b, is a function of cell concentration, C_b, in the effluent and the dilution rate, D, defined as the flow rate of liquid entering the reactor, Q to the reactor volume, V, ratio:

$$D = \frac{Q}{V} \tag{6}$$

$$P_b = DC_b \tag{7}$$

In steady state, the dilution rate is equal to the specific growth rate, μ, which is governed by the amount of light, the rate controlling factor. The dependence of μ on the average irradiance has been expressed in various equations.

One of the most widely used is the one proposed by Molina et al., (1994). Studies suggest that growth models that express μ in terms of the average irradiance raised to a power greater than one fit experimental observations best.

$$\mu = \frac{\mu_{max} I_{av}^n}{I_k^n + {I_{av}}^n} \tag{8}$$

However, this equation does not consider the existence of photoinhibition, especially in outdoor cultures when the irradiance on the reactor surface is over 2000 μE/m^{2}s. The following equation has been proposed to include it (Acién et al., 1998).

$$\mu = \frac{\mu_{max} I_{av}^{\left(b+\frac{c}{I_0}\right)}}{I_k\left(1+\left(\frac{I_0}{I_i}\right)^a\right)^{\left(b+\frac{c}{I_0}\right)} + I_{av}^{\left(b+\frac{c}{I_0}\right)}} \tag{9}$$

Where a, b, and c are empirical parameters, and I_k and I_i are the irradiance constant and photoinhibition irradiance, respectively. This equation accounts for photoinhibition and the fact that dependence of μ on average irradiance varies with incident irradiance, I_0.

This equation was derived from outdoor cultures of *Phaeodactylum tricornutum* UTEX 640 (Acién et al., 1998). Simulations using this model fit experimental values from two years of continuous operation (Figure 2).

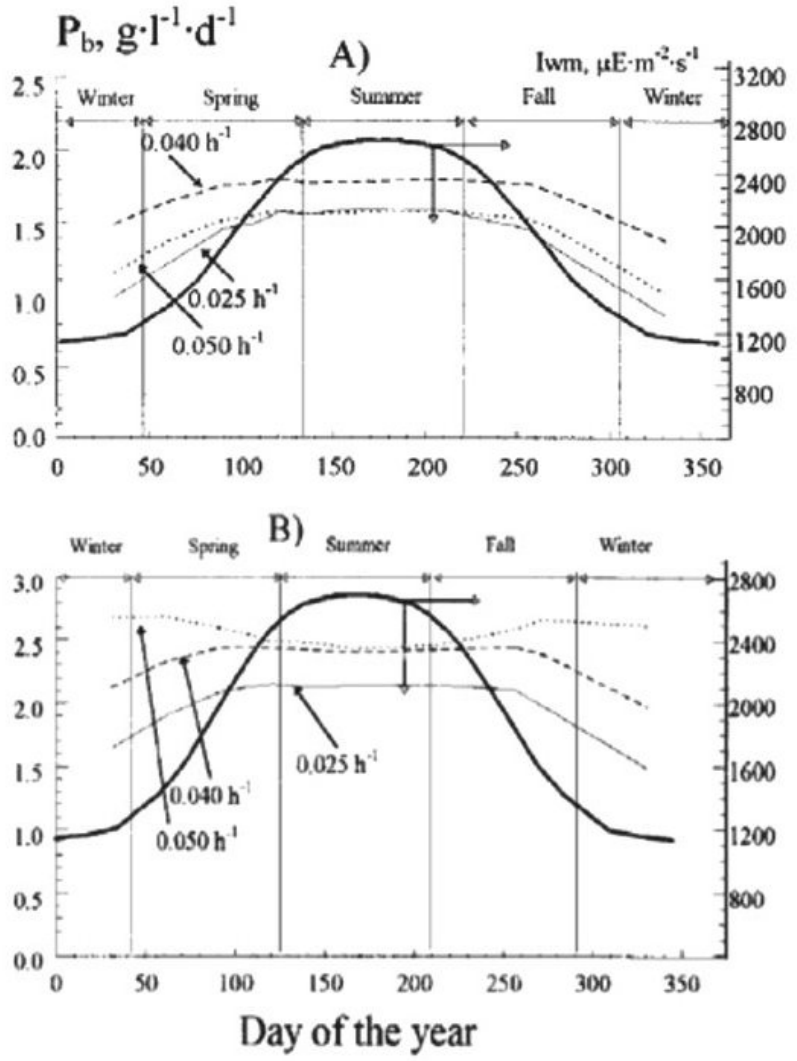

Figure 2. Variation of biomass productivity throughout the year for chemostat cultures of P. tricornutum UTEX 640 in tubular photobioreactors. Data estimated from solar radiation models and the proposed growth model (Eq. (9)). A) Tubular photobioreactor with a 0.06 m tube diameter, B) Tubular photobioreactor with a 0.03 m tube diameter. Solid line is the mean daily photosynthetic irradiance on the reactor surface (Acién et al., 1998).

To summarize, the biomass productivity of cultures is a function of the average irradiance the cells are exposed to in them, and their yield is determined by the existence of photolimitation-photoinhibition. Apart from that, for maximum yield, the photoreactor must be operated to provide adequate culture conditions (nutrients, temperature, pH, etc.). Mixing must also be sufficient to enhance the light regime, but excessive high-power agitation could damage the cells.

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Biographical Sketches

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