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Factors governing algal growth in photobioreactors: the "open" versus "closed" debate

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Abstract Until recently, most large commercial scale microalgal production systems employed open systems. However, several large-scale closed systems have now been built and, for the first time, actual comparisons can be made. There are major operational differences between open and closed photobioreactors and, consequently, the growth physiology of the microalgae is different between the two systems. Several of the factors governing growth can, within certain boundaries, be manipulated while others are specific to the cultivation system. Crucial factors are the optical depth, turbulence, light acclimated state of the organism, nutrient availability and metabolite accumulation. In the final analyses, systems are used for specific purposes and each will determine which system is the most suitable, since there is no universal all-purpose photobioreactor.

Keywords Growth physiology · Light acclimation · Microalgae · Optical depth · Photobioreactor · Turbulence

Introduction

Several definitions describe the meaning of bioreactor and most contain concepts such as "a device in which organisms are grown for the production of substances or for the conversion of biogenic wastes". A simple definition states that a bioreactor is a container in which living organisms carry out biological reactions. In none of the definitions is it stated that it must be a closed container or that it may not be in contact with the atmosphere. Yet applied phycologists have generally distinguished between open ponds for growing microalgae and photobioreactors where the latter implies that the light does not impinge directly on the culture's surface and that there is no direct contact between the culture and the atmosphere (Tredici 2004). This narrow definition of a photobioreactor is open to debate, because open systems are extensively used in algal biotechnology, including the raceway, sloping and cascade systems. Biomass concentrations >20 g (dw) l^{-1} are common in ultra thin-layered cascade systems (Grobbelaar 1995) and are among the highest attained in large-scale algal production systems.

There are, nevertheless, major differences between "open" and "closed" systems for the mass production of microalgae. These are shown in Table 1 together with some of the advantages and disadvantages. Here, we analyze and consider the factors governing microalgal growth in these two different systems, while possible advantages of the one over the other will be discussed.

Requirements for growing microalgae

Microalgae require an energy source, either light energy for autotrophic growth or an organic compound for heterotrophic growth. Between these two extremes there are several intermediate trophic routes (Grobbelaar 2004). Growth in a photobioreactor implies autotrophic growth and for this they require plant growth nutrients, especially the macro- and micronutrients, and in certain instances, vitamins and hormones. Microalgae also require a carbon source and, for autotrophic growth, this is either dissolved CO_2 or HCO_3^-. The temperature and pH should be in the toleration range of the cultured microalga. Furthermore, a habitat is needed being either moist

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Table 1 Advantages and disadvantages of open and closed algal cultivation plants (modified from Pulz 2001)

Parameter	Open ponds (raceway ponds)	Closed systems (PBR systems)
Contamination risk	High	Low
Water losses	High	Low
CO2-losses	High	Almost none
Reproducibility of production	Variable but consistent over time	Possible within certain tolerances
Process control	Complicated	Less complicated
Standardization	Difficult	Possible
Weather dependence	High	Less because protected
Maintenance	Easy	Difficult
Construction costs	Low	High
Biomass concentrations at harvesting	Low[a]	High
Overheating problems	Low	High
Super dissolved oxygen concentrations	Low	High

[a] Very high in thin-layer sloping systems

soil, a moist surface or an aquatic environment. If these basic requirements are present, then microalgae will grow.

However, they may grow extremely slowly, such as in caves or on coral reefs, and will be of no interest to applied phycology. Even apparent blooms that develop in natural waters have slow growth rates and reaching 1 g (dw) m^{-2} day^{-1} would be considered exceptionally high. Such rates are also only sustained for very short periods of time.

Requirements for achieving and maintaining high growth rates

All organisms have a minimum, optimal and maximum requirement for the variables mentioned in the previous section. Growing an alga at its optimal temperature, light intensity, nutrient concentrations and CO_2 levels will significantly increase the yield, but the rates will be low. In fact, increased rates are what algal biotechnologists strive for and not yields (yield defined as the biomass produced from a given set of resources). The object, therefore, is to realize high yields in the shortest possible time (high rates), i.e. high volumetric and areal production rates. The questions applied phycologists face are:

1. Howcan the capture of light energy, uptake of nutrients and CO_2 be improved?
2. Do other factors such as metabolites play a role?
3. What are the differences between "open" and "closed" photobioreactors, if any?
4. Does the application govern the type of photobioreactor that should be used?

Light

Light energy penetrating a culture surface can either be absorbed or scattered. Light energy can be absorbed by photosynthesizing microalgal cells, particulate material, water, and colored or chemical substances. In the absence of particulate materials and in optical depths of less than 250 mm, absorption due to water, colored or chemical substances would be minimal and the available light energy would be available to drive photosynthesis. Scattering in an algal culture simply means that the light path is redirected until it is absorbed (in dense algal cultures, this means by an algal cell). Thus, in shallow algal cultures, the light energy that crosses the air culture interface is available to drive photosynthesis.

The photosynthetic versus irradiance response curve (P/I) has been used extensively to describe the response of algae to light intensities (e.g., Grobbelaar 2006). Three distinct regions are discernable: an initial light-limited region at low light intensities where photosynthetic rates increase with increasing irradiance, a light-saturated region where photosynthetic rates are independent on irradiance, and a region of photoinhibition in which photosynthetic rates decreases with an increase in irradiance. In the light limited region, the rate of photon absorption is correlated with the rate of electron transport from water to CO_2, with the liberation of O_2. This initial slope is usually denoted by the symbol α and, when normalized with chlorophyll *a* or biomass, it represents the maximum quantum yield of photosynthesis.

The transition between light-limited and light-saturated photosynthesis could be gradual or abrupt (Leverenz 1987), implying a non-linearity between absorbed light and photosynthetic rates. At light saturation, the rate of photon absorption exceeds the rate of electron turnover in PS II and photosynthetic rates reach a maximum (P_{max}) without changing even though the light intensity increases. This transition between light-limited and light-saturated photosynthesis is denoted by I_k and it is defined as:

$$I_k = \frac{P_{\max}}{\alpha} \quad (1)$$

Algae (and plants) have developed several mechanisms to cope with changes in the quality and intensity of light. In essence, the aim of the plant is to balance the light and dark photosynthetic reactions. The regulation of photosynthesis appears to be in the light reactions and this is possible through modulation of the light-harvesting capacity and/or changes in the number of PS II reaction centers. Furthermore, the quantity of pigments per cell and the accessory pigments vary

considerably between low and high light-acclimated cells. The time scales of these changes could be from seconds to hours depending on the parameter measured (Grobbelaar 2006).

The net effect of responding to different light intensities and being able to acclimate to specific conditions means that algae can utilize any available light energy efficiently and thus ensure their survival. Acclimation strategies include changing the photosynthetic efficiency, rate and onset light intensities of saturating photosynthesis, light intensity at the onset of photoinhibition and the rate of dark respiration (Grobbelaar 2006). Thus, in any given situation, algae will have specific photosynthetic characteristics which are governed by the average light climate to which they are exposed.

The next complication is the fact that the light field is ever changing such that individual cells are subject to L/D (light/dark) variations, the dynamics of which are controlled by:

1. Culture depth or optical cross-section, where the deeper the system the longer the time-scales and vice versa.
2. Mixing induced turbulence, where through various means and techniques the L/D time-scales and patterns can be altered.
3. Biomass concentration and areal density where this determines light attenuation and thus the ratio of exposure to either light or dark. Below the optimal areal density all the cells are exposed to light, depending on their position in the optically dense medium, and they experience light intensity fluctuations. At the optimum areal density they are subject to equal L/D fluctuations if they are moved from the illuminated side to the deepest point. Above the optimal areal density a portion of the culture is in the dark and, depending on the areal density, different L/D ratios manifest themselves, with the implication that the cells move in and out of the photic volume (Grobbelaar 2006).

Because light is attenuated exponentially the L/D fluctuations should ideally be sinusoidal, but because the movement is not uniform the exposure is chaotic.

The enhancement of photosynthesis by "flashing light" (both the rate and the efficiency) has been known for many years (Kok 1953), and increased productivities have been demonstrated in mass algal cultures when mixing was improved (e.g., Richmond and Vonshak 1978; Laws et al. 1983). Enhancement of photosynthesis in L/D environments depends on a number of conditions, and Grobbelaar et al. (1996) showed that this only becomes important at L/D cycles of less than 1 Hz. They concluded that:

1. Photosynthetic rates increased exponentially with increasing light/dark frequencies,
2. A longer dark period in relation to the light period can further increase photosynthetic efficiencies, but not vice versa, and
3. Algae do not acclimate to specific L/D frequencies, but they become low light-acclimated at long L/D frequencies and high light-acclimated at short L/D frequencies.

A further important consideration is the rate of dark respiration, acknowledging that high rates of productivity are possible only with concurrent high rates of dark respiration. The immediate light history determines the rate of dark respiration, being higher in algae following exposure to high intensities (high light-acclimated), becoming less as the intensity of the pre-illumination decreases as well as the time in the dark (low light-acclimated algae) (Grobbelaar and Soeder 1985).

Nutrients

The supply of nutrients is often considered relatively simple and easily controllable in mass algal cultures. This is open to debate and the supply of nutrients to the cell may by more complicated than what it is generally considered to be.

One can, therefore, pose the question whether it is possible to satisfy the nutritional requirements of fast growing microalgae in mass algal cultures (see also the discussion on turbulence)? This is furthermore complicated by the fact that different microalgae will respond differently to nutrient supply concentrations depending on their quota flexibility (Grobbelaar 2004). The lower the half saturation constant (K_s), the higher will be the growth rates at low nutrient concentrations, and vice versa. Also, when an alga can lower its half saturation constant through acclimation, it will have a very high growth rate at low nutrient supply rates. N and P typically have low half saturation constants, but it is high for dissolved inorganic carbon.

Both the Monod and Droop models state that the growth rate of an organism may be limited by just a single resource (Droop 1974). In practice, however, co- and multiple limitations (stresses) are possible. The optimal supply of N, P and C is crucial for high growth rates, and this optimum is the ratio at which a transition from one nutrient limitation to another occurs (thus, both could be limiting at this transition) or where the cellular ratio of the resources required is such that the resource is not in short supply relative to another (Rhee and Gotham 1980). From quota measurements for ratios of Q_N:Q_P, it can be shown that, at higher growth rates, pro rata more N is required and vice versa (Grobbelaar 2004). This is important in mass algal cultures especially where the goal is to achieve and maintain maximal productivities.

Turbulence

The major differences between "open" and "closed" photobioreactors are the degree of turbulence achieved in the

cultures and oxygen accumulation in closed cultures (open thin-layer sloping ponds are excluded from this generalization). Since the mass transfer rates between the nutrient solution and the algal cells, as well as the transfer of metabolites (e.g., oxygen) from the cells to the growth medium, are affected by turbulence, this would have a direct influence on growth rates. Grobbelaar (1994) clearly showed that the boundary layer that directly determines the mass transfer rates depends on turbulence being larger at low turbulences and vice versa. The thinner the boundary layer the more readily nutrients are taken up and the faster metabolites (e.g., oxygen) are transported away from the cells. Turbulence essentially prevents the formation of nutritional and gaseous gradients. Over and above the obvious lowering of super oxygen saturation concentrations, mixing would decrease the boundary layer around the cells (Grobbelaar 1989, 1991). This would increase the mass transfer rates between the cells and the culture medium for both nutrient uptake and exudation of metabolites.

Turbulence also moves the cells through an optically dense gradient, with variations in the quantity and quality of light energy (see above), and Grobbelaar (1994) showed that there is a synergistic relationship between nutrient uptake, release of metabolites, L/D cycles and growth rates. For example, increased turbulence would result in increased exchange rates of nutrients and metabolites between the cells and their growth medium. Together with the increased light/dark frequencies this could increase productivity.

Conclusions

From the above, it is clear that higher turbulences are achievable in short light-path (SPL, <50 mm) photobioreactors and, because of higher mass transfer rates and shorter L/D cycles, they would have definite advantages over medium light-path (MLP, 50–300 mm) systems (Grobbelaar et al. 1996). Thus "closed" systems will have several advantages over "open" systems (only MLP because open systems can also be SLP) such as:

- Higher light utilization efficiencies
- Higher nutrient uptake (nutrient removal)
- Significantly higher volumetric biomass concentrations
- Lower compensation light/dark ratios or respiratory losses
- Less contamination and competition with alien algae
- Less water losses

SLP systems come at a cost and this needs to be part of the overall considerations.

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