Chapter 14

Mass Production of Microalgae at Optimal Photosynthetic Rates

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Additional information is available at the end of the chapter

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1. Introduction

Microalgae have been studied in the laboratory and in mass outdoor cultures for more than a century and our initial understanding of photosynthesis became unravelled in the laboratories of Otto Warburg. The breakthrough in his laboratories came when he started using *Chlorella* as model organism [1]. As Grobbelaar [2] pointed out, applied phycology and the mass production of microalgae, became a reality in the 1940's. Since then, microalgae have been grown for a variety of potential applications, such as the production of lipids for energy using flue-gasses, anti-microbial substances, cheap proteins for human nutrition and the production of various bio-chemicals. At present the focus is on bioenergy [3], however, their only real success has been in wastewater treatment. A major frustration for microalgal biotechnologists has been the realization of much lower yields than what is potentially possible from laboratory measurements. The inability to operate photo-bioreactors including raceway ponds, at maximal photosynthetic efficiencies, impacts directly on the economies of scale. Because of this many large-scale projects have not delivered what was predicted and many investors have lost their investments. Richmond's [4] observation that "Microalga culture however is yet very far from supplying any basic human needs.." is as true today as then and he concluded that "the major reason for this stems from the failure to develop production systems which utilize solar energy efficiently". A consequence of low yields is high production costs rendering this technology only suited for exclusive high-priced products.

With the above in mind one can pose the question "whether this technology is a mere dream for cheap mass production of biomass or whether it is only suited for high valued components?" A container is required for the growth of microalgae and to date a distinction is made between open pond systems and photo-bioreactors. Grobbelaar [5] argued that open pond systems where microalgae are grown at high densities are in fact also photo-bioreactors.

However, photo-bioreactors are generally considered to be systems in which the culture has no or minimal contact with the atmosphere and they can be of a variety of designs, such as tubes, plates, coils, bags, etc. [6]. Open ponds, on the other hand, have a large area that is in contact with the atmosphere, but they can be enclosed in e.g. plastic covered greenhouse tunnels. Richmond [4] stated that the major weaknesses of open ponds are the absence of temperature control and the long light-path of about 15 cm. The latter results in large culture volumes and consequently low cell mass densities. The question of temperature control is debatable since the temperature fluctuations will be less in large culture volumes, compared to short light-path cultures with small areal volumes.

Grobbelaar [5] analysed the factors governing microalgal growth in "open" and "closed" systems and concluded that the culture depth (optical-depth/light-path) is the single most important factor that determines microalgal growth and photo-bioreactor productivity. Here we analyse the various variables that could impact on microalgal photosynthesis, especially in large commercial scale production systems, with the aim to develop high yielding micro-algal production systems that could be scaled-up. Results generated in small high density laboratory cultures have little value when mega ton production plants are required and it is generally agreed that open raceway ponds would be the means of large commercial outdoor cultivation. For this reason, this paper will focus mainly on open raceway production systems for the intensive production of microalgal biomass.

2. Open raceway ponds

A number of microalgal species have successfully been grown in open raceway ponds, such as *Chlorella*, *Scenedesmus*, *Spirulina*, *Heamatococcus* and *Nannochloropsis*. Commercial outdoor cultivation is mostly restricted to warm tropical and sub-tropical areas, preferably with low rainfall and cloud cover. Detailed construction details are not readily available because of commercial sensitivity, however, basic information can be found for open raceway systems in e.g. [7-9], centre pivot circular ponds have been used in Japan and Taiwan [10], and sloping cascade ponds in the Czech Republic [11]. Raceway ponds are by far the industrial choice, followed by horizontally stacked tubular systems.

The basic components of open raceway ponds are an oval basin with a central island around which the cultures are stirred. The basins could be excavated or constructed above ground. An impermeable PVC liner is commonly used to seal the ponds. However, open raceway ponds have been constructed using concrete and cement, fibre-glass and even epoxy-coated concrete. Determining factors that dictate the materials used are costs and the requirements of the specific application. For example *Spirulina* and *Dunaliella* are grown in high saline growth media that corrodes concrete over time. Another important consideration is the potential toxicity of the materials used, either for the microalgae as such or the quality of the products produced. Since the basins need to be cleaned from time to time, the materials used should be rigid enough to withstand some abrasive actions and repairs should be simple.

Culture depth varies from 10 to 50 cm with 15 cm as the most common [7]. Culture depth (optical depth/light-path) is an important factor because it influences pond construction, biomass density, harvesting costs and pond operation. Culture depths of <15 cm becomes an engineering challenge, especially when pond areas are > 500 m^2. Any variances can influence the areal density and light penetration, as well as flow and mixing (see below). The deeper the ponds the more dilute the algal suspension becomes because of the larger culture volumes and consequently the higher the harvesting costs. Larger culture volumes imply handling and moving large quantities of liquid, which in itself becomes an engineering challenge.

Various devices have been proposed to circulate and mix the cultures, such as low shear force pumps, air-lifts and paddle wheels [12]. Paddle wheels have become the industrial standard and although various designs and concepts have been used the basic requirement is to circulate the culture and not to lift or aerate it [13]. Flow velocities of 15 to 35 cm s^{-1} are common and Borowitzka [9] calculated that a 2 kW motor is sufficient to produce speeds of 30 cm s^{-1} in a 1000 m^2 raceway pond.

In the design of open ponds, a deeper portion is used as a sump for harvesting and cleaning. Also high photosynthetic rates require the addition of CO_2 and depending upon the size of the ponds, carbonation should be applied through pH-controlled sparging at various points to maintain a pre-determined pH range [14].

3. Growing microalgae in open outdoor raceway ponds

Growing microalgae in large outdoor open raceway ponds is very different to growing them under controlled optimal conditions in the laboratory and according to Grobbelaar [5], the questions applied phycologists need to resolve are:

1. How to improve the capture of light energy, uptake of nutrients and CO_2?
2. The role of excreted metabolites and auto-inhibition?
3. The differences/advantages/disadvantages between ''open'' and ''closed'' photo-bioreactors, if any?
4. The requirements of the specific application and the resources at hand.

Growing microalgae at their optimum temperature, light intensity, nutrient levels and CO_2 will result in high yields, but the growth rates may be low. The aim for applied phycologists, therefore, is to improve the rates or to realize the highest yields in the shortest possible time [5]. When growing algae the numbers of variables that can be controlled are limited. These are:

1. Culture depth or optical cross section. In open ponds light is attenuated with depth (Fig. 1), while the light attenuation and distribution become more complicated in closed vertically placed and transparent tube reactors.

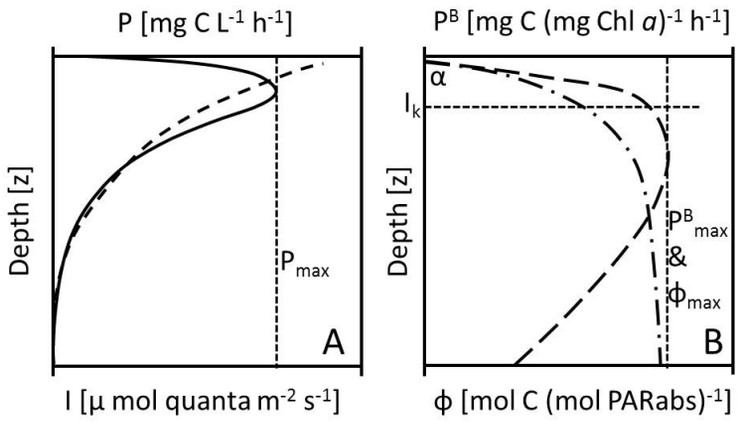

Figure 1. Productivity (solid line) and light attenuation (small dashed) depth profiles (A) in a microalgal culture showing photo-inhibition at the surface, maximum production (P_{max}) and a decrease in production with depth. The productivity profile is normalized with the chlorophyll *a* (Chl *a*) content (P^B) to give the Chl *a* specific productivity (long dashed line in B) and this gives the quantum efficiency (Φ) when normalized with the light intensity (dot dash line in B). Also shown in B is I_k the transition light intensity from light limited to light saturated growth and the photosynthetic efficiency (α) at light limited photosynthesis.

2. Mixing and the resultant turbulence. This differs markedly between open and closed systems, where much higher rates of turbulence can be achieved in closed tubular reactors, while laminar flow is often a problem in open raceway ponds.
3. Supply of nutrients and CO_2, and the prevention of deficit zones.
4. The biomass concentration or areal density. This determines the in-culture light climate, where a higher biomass will attenuate light energy more and *vice versa*. This also determines the light acclimated state of the microalgae.
5. The culture operation being either batch, semi-continuous or continuous.
6. Temperature within limits as well as the dissolved O_2 build-up in closed reactors.

4. Light

The production of mega-tons of biomass implicitly means that natural sunlight must be the source of light. Ironically only about 20 to 25 % of photosynthetically active radiation (PAR) from the sun saturates photosynthesis. Furthermore, the action spectrum of photosynthesis has a peak in the blue and red light regions [15], meaning that the green wavelengths have little photosynthetic value. In mass algal cultures light energy and its capture through photosynthesis is complex and it is governed by;

1. The intensity of the light.
2. The quality of the light and the selective absorption of specific wavelengths by the microalgal biomass.

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3. The angle of the light impinging on the culture surface.
4. The condition of the culture surface, e.g. being smooth or rippled.
5. The optical density of the culture (assuming that only algal mass attenuates light energy).
6. The movement of individual cells through a light gradient caused by light attenuation in an optically dense culture (turbulence).
7. The light acclimated state of the culture.

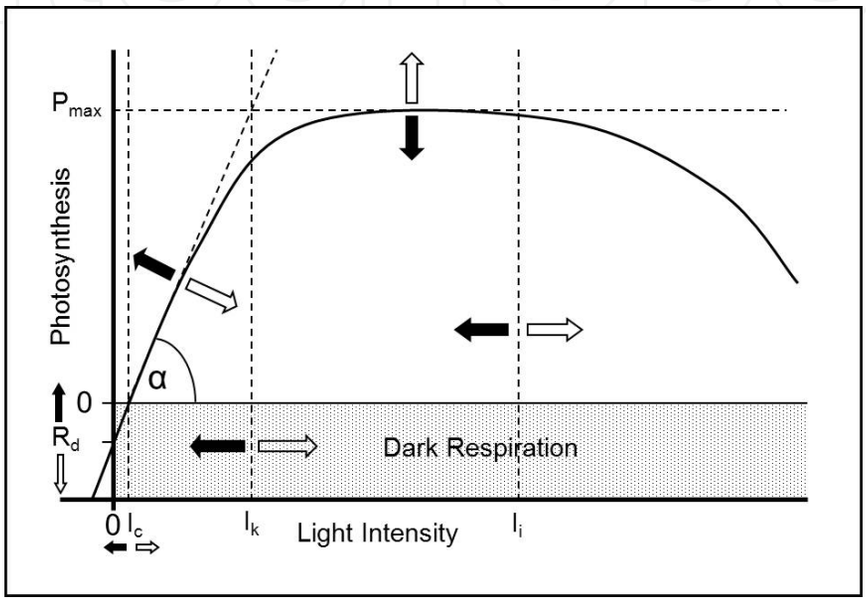

Figure 2. The photosynthetic irradiance (P/I) response of microalgae, where the open arrows indicate the high light acclimated response direction and the filled arrows the low light acclimated direction (figure after [15]). R_d is dark respiration P_{max} the maximum photosynthetic rate, I_c the maintenance light energy, α the maximum photosynthetic efficiency, I_k the transition light intensity from light limited to light saturated photosynthesis, and I_i the light intensity above which photosynthesis is photo-inhibited.

The photosynthetic versus irradiance response curve (P/I) also applies to microalgae and it has been used extensively to describe the response of algae to light energy. Three distinct regions are discernible; i.e. 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 photo-inhibition in which photosynthetic rates decreases with an increase in irradiance. In the light limited region the rate of photon absorption is correlated with the absorption of light energy and thus the rate of electron transport from water to CO_2, with the liberation of O_2. This initial slope or alpha (α) [16] when normalized with chlorophyll *a* or biomass is the maximum quantum efficiency of photosynthesis. The transition between light limited and light saturated photosynthesis could be gradual or abrupt [17], implying a non-linearity between absorbed light and photosynthetic rates. At light saturation photosynthetic rates reach a maximum (P_{max}, Fig. 1B and 2) irrespective of an increase in light intensity. The transition between light limited and light saturated photosynthesis is donated by I_k and it is defined as:

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

At light saturation, the rate of photon absorption exceeds the rate of electron turnover in photosystem II (PS II), without damaging PSII. However, at even higher irradiancies light-induced depression of photosynthesis occurs commonly referred to as photo-inhibition. Over short term time scales, light induced photo-inactivation of PS II could be viewed as a survival strategy by reducing the number of redundant PS II units. If PS II reaction centres are not repaired through the continuous replacement of the D1 protein, then the damage would become permanent resulting in PS II inactivation [18]. Thus the light-dependent inactivation may be reversible or irreversible. An important finding is that P_{max} was unaffected when PSII reaction centres were reduced by almost 50 % [19]. The reason for this is that the acceptor side of photosynthesis is limited by the capacity of the Calvin-cycle, and the impact of photo-inhibition would only become apparent when the numbers of reaction centres are reduced to such a level that the capacity of the Calvin-cycle cannot be met.

Algae (and for that matter all plants) have developed several mechanisms to cope with changes in the quality and intensity of light. In essence the aim of a plant is to acclimate to the prevailing light climate by ensuring that the light reactions exceed the dark photosynthetic reactions. According to Sukenik et al. [20] the Rubisco levels remained relatively constant under varying light regimes, suggesting that the major regulation occurs in the light reactions, especially of PS II. This could be achieved through modulation of the light-harvesting capacity (various photosynthetic pigments) and/or changes in the number and sizes of PS II reaction centres (light harvesting units).

Photo-acclimation affects all components of the P/I curve (Fig. 2), where the open arrows show the direction of the response due to high light and the solid arrows the response due to low light acclimation [15]. The acclimation can take place in time-scales from seconds to hours, depending on the parameters measured [21]. It is important to realize that the microalgae present in a high density algal culture have acclimated to the average light intensity over the entire optical cross section. Furthermore, the microalgae will acclimate during the production cycle in batch cultures, being high light acclimated soon after inoculation of a new culture, to low-light acclimated at the end of the batch process when the cell density is high [22].

There is little that can be done with the above when the scales of mega-mass microalgal production systems are taken into consideration. It may be possible to maintain the biomass in a low light acclimated state by keeping the biomass concentration high. Such acclimated microalgae have high α, low P_{max} at saturating light intensities and low concentration of auxiliary photosynthetic pigments [22]. However, in small-scale production systems, it is possible to utilize the photo-acclimated state of microalgae to achieve very high areal yields. Grobbelaar and Kurano [23] tested a multi-layered flat plate photo-bioreactor, where the microalgae were acclimated to high light in the first layer facing the light source, followed by a layer where the microalgae became progressively more low light acclimated. In this reactor the properties of high and low light acclimated microalgae could simultaneously be exploited

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and productivities were almost 40 % higher compared to a single layer flat plate reactor of similar optical cross section.

5. Light/dark cycles

Grobbelaar [14] proposed that the key to high productivities lies in turbulence induced short L/D cycles. The three major effects of mixing are:

1. Prevention of the cells settling to the bottom of the pond. Should this happen, so-called "dead zones" occur where the accumulated of organic material leads to anaerobic decomposition and the release of unwanted substances and metabolites.

2. To prevent the formation of nutritional and gaseous gradients. Over and above the obvious lowering of oxygen super saturation, mixing would decrease the boundary layer around the cells [23-24]. This would increase the mass transfer rates between the cells and the culture medium for both nutrient uptake and exudation of metabolites. Grobbelaar [26] went on to show that a synergistic enhancement of productivity takes place between decreasing the boundary layer and L/D cycles, with increasing turbulence (see next point).

3. To move the cells through an optically dense gradient, with variations in the quantity and quality of light energy. In outdoor mass cultures the algae are subjected to the dynamic natural environment, with its variations in the quantity and quality of the light, both diurnally and seasonally, as well as through the mechanical means of mixing.

Kok [27] showed that photosynthesis is influenced by the intensity of light, L/D fluctuations and the ratio of dark time to light time. He used the term 'flashing light' to indicate the light time and it has often been confused with single turnover flashes. He concluded that the light time should be less than 4 ms in order to achieve 'full' efficiency and that the dark time should be at least ten times as long as the light time. For many decades, the enhancement of photosynthesis in intermittent light was interpreted as some residual light energy that was captured during the light time and then utilised in the dark until the next light flash is received. Today we know that this is not possible, because the time-scales for electron turnover in PSII and PSI range from femto- to milli-seconds [14].

Kok [27] also found that the pattern of intermittent light required, for high yields of *Chlorella* was dependent on turbulence manifested in a flowing culture. The increased production of biomass as a result of increased turbulence was eloquently demonstrated by Laws et al. [28] who placed aerofoil type of devices in the channels with flowing cultures. These caused vortices of 0.5–1 Hz at a flow rate of 30 cm/s, which resulted in photosynthetic conversion efficiencies increasing from an average of 3.7 to up to 10%.

Increasing photosynthetic rates and efficiencies in intermittent light has been shown by several authors, e.g. [21, 26] and Grobbelaar [2] found that the increase in specific production rates were consistently exponential with increasing L/D frequencies. The observed increases in productivities with increasing L/D frequencies are directly linked to electron turnover rates in

the electron transport chains of photosynthesis. As the L/D frequencies approach the turnover rates, productivities and photosynthetic efficiencies increase until the L/D frequencies match the turnover rates, where production and photosynthetic efficiencies would be at their highest. These rates are in the millisecond range and in practical terms this should form part of the operational considerations when designing photo-bioreactors.

Grobbelaar [14] showed that the photosynthetic rates increased on average 2.1 times at equal L/D cycles when the frequencies were increased from 0.1 to 10 Hz and that the enhancement of photosynthetic rates only becomes significant at cycles > 0.1 Hz. In practical terms this should be achievable in SLP (short light path) reactors [14]. However, special devices would be needed in open raceway ponds, such as aerofoils [28], rippled floors and sides, and curvilinear end geometries.

6. Photo-inhibition

As discussed above, photo-inhibition is the light-induced depression of photosynthesis when the rate of photon absorption exceeds the rate of electron turnover in PS II. Over the short term, light-induced photo-inactivation of PS II is a survival strategy, whereby the number of redundant PS II units is reduced. Prolonged exposure to high light intensities eventually results in PS II reaction centres not being repaired, through the continuous replacement of the D1 protein. The damage then becomes permanent resulting in PS II inactivation [18]. Whether photo-inhibition occurs in dense mass algal cultures is open for debate, especially in turbulent tubular and plate photo-bioreactors. Congming and Vonshak [29] reported a midday maximum quantum efficiency (Φ_{max}) depression of dark adapted *Spirulina platensis* as a result of reaction centre inactivation, which they ascribed to photo-inhibition. Grobbelaar [30] measured a similar midday depression of Φ_{max} on open outdoor raceway *Spirulina platensis* cultures but concluded that it was as a result of light energy being lost as heat dissipation. This was particularly evident in low density cultures where more than 60 % of the reaction centres became "silent", meaning that they neither reduced Q_A, nor returned their excitation energy to the antenna.

Laminar flow is common in large open raceway ponds and Laws et al. [28] found that they had to space their foil arrays 1.2 m apart, because of the rapid dissipation of mixing in the raceway channels. Since raceway channels can be >100 m, it is reasonable to expect photo-inhibition to be a factor in the surface layers of the cultures. Grobbelaar et al. [31] modelled microalgal productivity in large outdoor raceway ponds. The model was calibrated against two years of data collected from five raceway ponds differing in surface area from 71 – 263 m^2. In a generalized form the model is written as:

$$PROD\left(mg\ (dw)m^{-2}h^{-1}\right) = PRD - RES - INB \tag{2}$$

Where PROD = net production, PRD = productivity, RES = respiration, and INB = photo-inhibition.

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PRD is calculated from inputs of the biomass concentration in the culture, culture temperature and light impinging on the surface of the culture. The equation for PRD is:

$$PRD = \left(A_1 . X_1\left(A_2^T\right)\right).\left(\frac{I_z . I_s\left(A_3^T\right)}{I_z + I_s\left(A_3^T\right)}\right) \quad (3)$$

where $A_1 – A_3$ are constants, X_1 is the biomass concentration in mg (dw) L^{-1}, I_z the irradiance in mol quanta m^{-2} h^{-1} at depth z in meters, I_s the light half saturation constant and T a temperature factor. Equation (3) has temperature/biomass and temperature/light energy terms. A_1 is the light utilization efficiency, A_2 the Q_{10} of photosynthesis and A_3 the Q_{10} for the light half saturation.

The component RES includes all losses due to respiration and exudation of organic compounds from the cells and is calculated from the following equation:

$$RES = X_1\left(\left(\frac{1.5^{T\text{-}0.54)}}{100}\right)\right. \quad (4)$$

Photo-inhibition (INB) was included in the model and the equation was constructed such that it only took new production (PRD) into account, that it increases linearly with an increase in irradiance above a threshold irradiance of 1 mol quanta m^{-2} h^{-1}, and that temperature affected the overall rate. Photo-inhibition was calculated from:

$$INB = PRD\left(\left(\frac{2.5^T}{75}\right).I_z\right) \quad (5)$$

Shown in Fig. 3 are the outcomes (predictions) for the day time interval of 12:00 to 13:00, in a 15 cm culture depth open raceway pond, with a daily irradiance of 60 mol quanta m^{-2} d^{-1}, minimum and maximum temperatures of 10 °C and 30 °C, 12 hour day-light length and culture biomass concentrations of 200 (Fig 3A), 400 (Fig. 3B), 600 (Fig. 3C), and 800 mg (dw) L^{-1} (Fig. 3D). The exponential decrease in irradiance with increasing depth is seen where all the light energy is absorbed at different depths, depending on the biomass concentration and the resultant attenuation of light energy. Grobbelaar et al. [31] defined the condition where all the light energy is absorbed over the depth profile (optical cross section) as the optimal areal density for maximum productivity (Fig. 2 B). Net productivity typically showed photo-inhibition at the surface, where the depth of P_{max} depended on the areal biomass density, ranging from 6 cm at an areal density of 30 g m^{-2} to 2 cm at an areal density of 120 g m^{-2}. Below P_{max} productivity decreased as the light energy was attenuated. The impact of photo-inhibition is seen at optical depths deeper than that at P_{max} when the plots of net production (PROD) are compared to the plots where photo-inhibition is excluded (PRD – RES, the dot dot dash lines in Fig. 3).

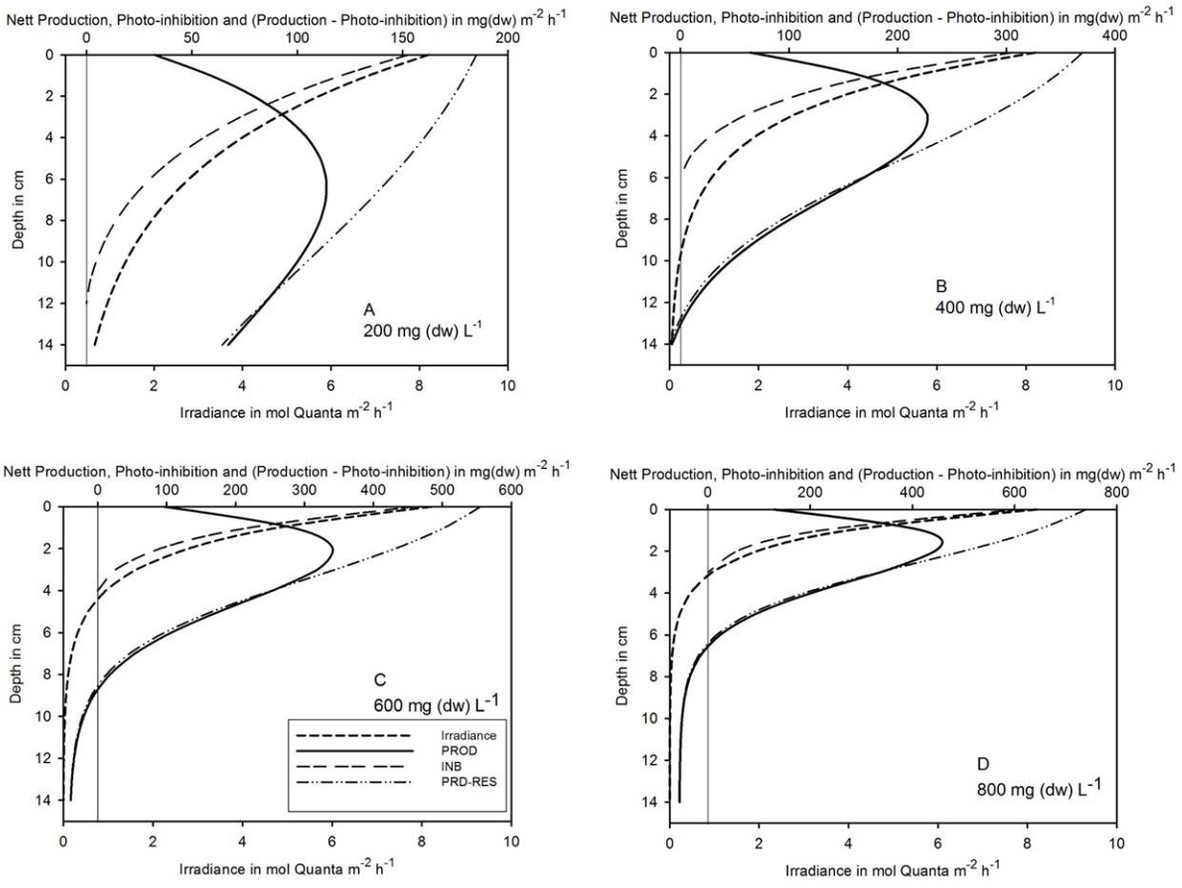

Figure 3. Modelled response of irradiance, PROD, INB and PRD-RES at four areal densities.

The significant impact that photo-inhibition has on the overall productivity is evident in all four examples (Figs. 3A – 3D), when the depth integrated rates are compared (Table 1). Losses due to photo-inhibition are clearly dependent on areal density and light attenuation where it is the lowest at the optimal areal density. At an areal density of 120 g m^{-2} losses due to photo-inhibition can be as high as 66 % (Table 1). Respiratory losses are also dependent on areal density and increases linearly with an increase in areal density. However, as the "dark" zone increases with an increase in areal density a larger percentage of the biomass is lost due to RES (Fig. 3 and Table 1). For example the total RES loss at a biomass concentration of 200 mg L^{-1} is 9.6 %, whereas at 800 mg L^{-1} it is 28 % (Table 1).

With such losses, it is surprising that very little research has gone into eliminating or limiting the losses due to photo-inhibition. Several authors, e.g. [25, 27 and 31], have already reported significant increases in volumetric and areal productivities where mixing was increased. As mentioned above, Laws et al. [28] attributed an increase of up to 10 % in photosynthetic efficiencies in their systems where foils created vortices with rotation rates of about 0.5–1 Hz. However, Grobbelaar et al. [22] showed minimal enhancement at light/dark cycles ≤1 Hz. This

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Biomass in mg (dw) L^{-1}	200	400	600	800
PRD	2167	2719	2860	2966
RES	209	419	628	837
INB	613	665	752	848
PROD	1345	1635	1481	1280
PRD – RES	1958	2300	2232	2128
% loss due to INB	45	40	50	66

Table 1. Predicted depth Integrated results in mg (dw) m^{-2} h^{-1} in four cultures all, 15 cm deep, minimum and maximum temperatures of 10 °C and 30 °C, irradiance from 12:00 to 13:00 of 8.2 mol quanta m^{-2} h^{-1}, and at biomass concentrations of 200, 400, 600 and 800 mg (dw) L^{-1}. The abbreviations are as defined in equations 2 to 5.

was acknowledged by Laws and Berning [33] and they attributed the increased productivity in flumes with foils, rather to the re-suspension of settled cells and breaking down of nutrient gradients. As stated before, Grobbelaar [26] clearly showed how increased turbulence enhanced the exchange rates of nutrients and metabolites between the cells and the growth medium, and this, together with increased light/dark frequencies, synergistically increases productivity and photosynthetic efficiency.

It is thus suggested that mixing microalgal cells in dense cultures other than the obvious re-suspension of the cells are important for:

1. Moving the cells in a light energy attenuating medium resulting in various L/D cycles and patterns.
2. Altering the boundary layers on the cells and thus the uptake and release of substances.
3. Shortening the exposure time allowing above saturating light intensities to be utilized. It is suggested that as the L/D frequencies approach the electron turnover rate full sunlight could be captured through photosynthesis, if the areal density of the cultures are high enough.
4. Ensuring an "average" photo-acclimated state of the entire culture. Grobbelaar et al. [34] clearly demonstrated how the photo-acclimated state of an outdoor culture changes depending on the cell density and how this affects the culture productivity.

7. Conclusions

The success or failure of producing mega-quantities of microalgal biomass will depend on culture systems where high photosynthetic rates are maintained. Although conditions in closed photo-bioreactors (vertical tubular, helical tubular, vertical flat plate or horizontal flat panel) are generally conducive for high photosynthetic rates; fowling, hydrodynamic stress and scale-up limitations [35], essentially rule these kinds of systems out, for mega-scale production microalgal biomass.

It is clear that features of growth reactors that would have to be considered are:

1. the surface/volume (S/V) ratio where the aim should be to operate systems with the highest possible S/V ratio,
2. mixing and not only the devices used, e.g. paddle wheels, airlift or impellers, but critically the prevention of laminar flow,
3. mass transfer rates of nutrients and metabolites, where this is dependent on mixing,
4. the light exposure patterns, especially with the introduction of mixing devices to manifest L/D cycles of > 0.1 Hz and limiting of photo-inhibition losses,
5. nutrient supply and modes of dosing (see [36])
6. the scalability of the systems and potential of automation, and
7. the ease of operation.

Producing the quantities required for, e.g. bioenergy [3] production, would only become a reality when the above factors are systematically researched, analysed and optimized.

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