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Short communication

Photosynthesis basic principles to optimize growth of microalgae cultures outdoors

[Fotosíntesis: Principios básicos para la optimización del crecimiento en cultivos de microalgas en condiciones exteriores]

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Abstract

Microalgae can play an important role in modern bioeconomy since they can be used as source of some important building blocks such as amino acids, poly-unsaturated fatty acids, pigments, vitamins, and antioxidants. To date, the number of species being cultured on a large scale for commercial purposes is still small, the most used being *Arthrospira platensis* (Spirulina) and *Chlorella* spp., produced mainly for use as nutritional supplements, and *Dunaliella* and *Haematococcus* as source of carotenoids.

The production of sufficient amounts of biomass is a prerequisite for meeting large-scale demand and to reach economic viability. To date, in industrial scale microalgal production systems, light conversion efficiency ranges between 1-2% on solar basis, far short of the theoretical maximum of about 10%, owing to energy loss at all the stages of the process. Table 1 shows a summary of the expected energy losses of total incident solar radiation during the biomass production process.

Resumen

Las microalgas pueden desempeñar un papel importante en la bioeconomía moderna, ya que pueden utilizarse como fuente de algunos componentes básicos importantes, como aminoácidos, ácidos grasos poliinsaturados, pigmentos, vitaminas y antioxidantes. A la fecha, el número de especies que se cultivan a gran escala con fines comerciales es todavía pequeño, siendo las más utilizadas *Arthrospira platensis* (Spirulina) y *Chlorella* spp., producidas principalmente para su uso como suplementos nutricionales, y *Dunaliella* y *Haematococcus* como fuente de carotenoides.

La producción de cantidades suficientes de biomasa es un requisito previo para satisfacer la demanda a gran escala y alcanzar la viabilidad económica. A la fecha, en los sistemas de producción de microalgas a escala industrial, la eficiencia de conversión de la luz oscila entre el 1-2% en base solar, muy lejos del máximo teórico de alrededor del 10%, debido a la pérdida de energía en todas las etapas del proceso. La Tabla 1 muestra un resumen de las pérdidas energéticas previstas de la radiación solar incidente total durante el proceso de producción de biomasa.

Table 1. Summary of expected the energy losses of total incident solar radiation energy in outdoor microalgal cultures.

Process	**Energy radiation losses (%)**	**Remaining energy (%)**
Total solar radiation	-	100
Reflection/scattering	10	90
Radiation outside PAR	55	41
Loss of useful absorbed PAR energy at 680 nm (PSII) and 700 nm (PSI) due to non-photochemical processes	20	32.8
Conversion energy to biomass	71.3	9.4
Photosynthesis saturation and photoinhibition	80	1.88
Cell maintenance in cultures subjected to diurnal cycle	10	1.7
Actual light conversion efficiency under solar light		1.7

It is estimated that about 10% of the photosynthetically active radiation is reflected by the cultures. Microalgae, when grown under high light, also synthesize some protective pigments (xanthophyll cycle pigments, in green algae) that dissipate excess light as heat (Masojidek *et al.*, 1999). This photosynthetically inactive light absorption, to the extent it occurs, further lowers photosynthetic conversion efficiency.

Furthermore, photons above 700 nm and below 400 nm are not utilized by microalgae pigments (as well as by higher plants), and thus about 55% of incident solar light is unavailable to drive photosynthesis. The sum of losses by reflection and more importantly the amount of light out of the photosynthetically active radiation (PAR) range, reduces the available light for photosynthesis to about 41%.

The energy required to drive a charge separation event in PSII is approximately 176 KJ/mol (i.e., equal to the energy of a 680-nm wavelength photon), and 171 KJ/mol (i.e., the energy content of a photon at 700 nm) for PSI. If we assume that the mean energy content of photons in the 400-700 nm range is about 218 KJ/mol, then the loss of energy between absorption and charge-separation in the two photosystems will be approximately:

{[218-(171+176)/2]/218}x100;

this means that approximately 20.4% of the incident solar energy is irretrievably lost as heat in the process, because of the relaxation of higher excited states of chlorophyll to the first excited singlet state (Zhu *et al.*, 2008).

For light conversion to biomass, considering that 1 mol of hexose yields 2808 KJ mol^{-1}, and that a minimum of 9.4 mol of quanta are required to release 1 mole of O_2,

(2808 KJ mol^{-1}/(173.5 KJ mol^{-1} x 6 (9.4 mol quanta) x 100 = 28.7 %

(i.e. an energy loss of 71.3%),

then consequently the theoretical maximum light conversion efficiency for biomass production is about 9.4% of solar light. The calculated value of the light conversion efficiency can significantly change according to the biomass composition.

For example, biomass rich in carbohydrates there is a good agreement between theoretical and real quantum requirement, while for biomass rich in lipids or in proteins, the quantum requirement can increase by a factor of 2 (up to 24 photons/CO_2) (Wilhelm and Torsten, 2011). On the other hand, the theoretical light conversion efficiency (LCE) for H_2 production, attainable by direct bio-photolysis, is about 13.4% of incident solar light. This theoretical maximum conversion efficiency is significantly higher than the theoretical limit for biomass or biodiesel production, but lower than present commercial single-junction (single photosystem) silicon solar cell modules, which is typically 18± 2% and photo-electrochemical water-splitting devices 15% (Green *et al*, 2010).

These differences are only apparent and are explained by the fact that the efficiency of the process depends on the number of steps necessary to produce a certain compound (*i.e.*, the more steps required in the biomass case, the lower the efficiency of the process). For example, the annual averaged efficiency for solar water splitting by PV-driven electrolysis can drop to 10–11%, if the cascade of energy loss to produce H_2 is considered.

Measurements of the effective quantum yield of PSII using chlorophyll fluorescence, carried out at midday in summer usually shows a strong reduction indicating that up to 90% of the light energy is lost via non photochemical process, mainly heat. This drops the light conversion efficiency usually attainable in large scale to less than 2%. Finally, although at much lower extent, the night biomass loss occurring during the night must be considered, involving dissipation of energy through the respiration usually at the expenses of carbohydrates (or other stored products) synthesized during the light period (Torzillo *et al*., 1991). Bearing this information in mind, it is possible to estimate the amount and of biomass attainable in a precise location with known light energy available, provided that neither technological aspects (such as mixing, cell concentration), nor environmental constraints (such as temperature, pH, O_2) are limiting.

For example, with an average mean solar energy of 18 MJ m^{-2} day^{-1}, and a cultivation period of 210 days, with a light conversion efficiency of 1.7%, the energy content of biomass being 20 KJ g^{-1}, the corresponding biomass yield will be about 32 t ha^{-1} $year^{-1}$. The corresponding mean yield per square meter of reactor will be about 15 g m^{-2} day^{-1} (mean over the cultivation period of 210 days). Yet, if the theoretical efficiency could be reached, then the mean biomass per square meter would raise to about 83 g m^{-2} day^{-1}.

Unfortunately, neither with laboratory cultures nor outdoor ones, an efficiency of 9.4% has ever been attained. Continuous cultures of *Synechocystis* reached an efficiency of 12.5% on PAR, corresponding to 5.6% on solar basis (Touloupakis *et al*., 2015). It must be pointed out that the first calculations of photosynthetic efficiency coming from laboratory experiments where microalgae were usually exposed to light irradiance within the PAR gave results that must not be confused with those obtained in sunlight (i.e., full light spectra) which are 55% lower.

As can be argued from Table 1, the greatest energy losses are due to the conversion of solar energy into biomass (about 71%) and to light saturation of photosynthesis (80%).

Microalgae, like C4 plants, usually have a well-functioning xanthophyll cycle which is necessary for the development of the non-photochemical quenching (NPQ) mechanism and can efficiently dissipate excess of light (Demmig-Adams, *et al*., 2014; Masojidek *et al.,* 1999; Masojidek *et al.,* 2004).

Microalgae – unlike higher plants in which the amplitude of NPQ is proportional to the light intensity at which they are exposed, strongly upregulate their ability to switch on NPQ even under moderate light irradiance, and this causes a remarkable reduction in the light conversion efficiency (Bonente *et al.,* 2012), and in the ability to dissipate photons absorbed by the light-harvesting complexes under excess light. Obviously, while little can be done to improve the light conversion into biomass, a strong enhancement of yield could be attained if the problem of light saturation could be reduced.

To attenuate the effects of this physical constraint that actually acts as a strong drawback in algal biotechnology, some strategies have been proposed: 1) fast mixing in order to induce rapid light/dark cycles within the culture depth of dense cultures; 2) use of an optimal concentration; and more recently, 3) use of PBR designs in which it is possible to dilute light over a larger surface of the culture (Wijffels and Barbosa, 2010); and 4) use of organisms with small antenna size.

All these strategies aim at improving light conversion efficiency by enhancing the number of cells having access to light and reducing as much as possible the time the cells spend in the dark. Therefore, the optimization of the reactor design cannot exclude an appropriate fluid dynamics study.

Conclusions

A better understand of the mechanisms that regulate light utilization/dissipation may shed light on the potential ways of optimizing productivity and product quality. However, these studies are made complicate by the fact that outdoors very often environmental factors act in synergism between them. Therefore, pure laboratory study, on commercial microalgal species, although very important, must be considered carefully and always confirmed outdoors. On the other hand, in order to better understand the physiological basis of stress phenomena occurring outdoors, it is necessary to return to the laboratory where the effect of environmental factors can be studied separately, under well definite conditions. The critical factor which limits the productivity of the cultures is the low efficiency of light conversion. Indeed, although photosynthesis has been optimized over three billion years of evolution, it remains inefficient at converting solar energy into chemical energy and biomass. Microalgae with truncated antenna size may substantially increase the biomass productivity. This approach represents a synthetic variation of the natural photo adaptive- mechanism of microalgae in which pigments re-organize in response to high light. Unfortunately, mutants generated by random UV or chemical mutagenesis are likely to induce multiple mutations, and among them an increased respiration rate of the cells, which reduces the benefit of the reduced light absorption of the truncated antenna size cells. Therefore, in these studies, an important parameter that need to be addressed, other than the lower chlorophyll per cell and reduced PSII antenna size, is the photosynthesis to respiration ratio which should not significantly affected.

The progress in algal biotechnology could be strongly accelerated if basic research on microalgae were carried out taking into account the new necessities pointed out by applied research, and vice versa: in other words, biologists should understand more about bioengineering and engineers should understand more about biology.

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