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Increasing algal photosynthetic productivity by integrating ecophysiology with systems biology

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Oxygenic photosynthesis is the process by which plants, algae, and cyanobacteria convert sunlight and CO_2 into chemical energy and biomass. Previously published estimates suggest that algal photosynthesis is, at best, able to convert approximately 5–7% of incident light energy to biomass and there is opportunity for improvement. Recent analyses of *in situ* photophysiology in mass cultures of algae and cyanobacteria show that cultivation methods can have detrimental effects on a cell's photophysiology – reinforcing the need to understand the complex responses of cell biology to a highly variable environment. A systems-based approach to understanding the stresses and efficiencies associated with light-energy harvesting, CO_2 fixation, and carbon partitioning will be necessary to make major headway toward improving photosynthetic yields.

Oxygenic photosynthesis is inefficient

Algae and cyanobacteria could represent the next generation of biofuels and provide a major source of sustainable transportation fuel to reduce society's dependence on fossil fuels [1,2]. There are several technological hurdles to overcome before the process can become commercially viable, including the essential improvement of the photosynthetic process [3]. Previously published exercises have suggested that algal photosynthesis is, at its best in controlled culture conditions, able to convert approximately 5–7% of incident light energy to biomass [4]. Pushing photosynthetic efficiencies in mass culture toward this level will drastically reduce the land area and associated infrastructure required to produce biofuel [5].

Our understanding of algal photosynthesis has reached a critical knowledge base. We know the major players in light capture and carbon fixation, as well as how loss terms associated with them can be physiologically quantified. What is unclear is how algae and cyanobacteria integrate rapid, irregular changes in their light and CO_2 environment into the regular day/night rhythms associated with growth in any reasonable production environment. Understanding the systems biology associated with the stresses experienced in a photobioreactor (including light, CO_2, O_2, heat, and nutrient stress) will be essential for pushing algal photosynthetic yields up to their theoretical maximum of 12% [4].

Photosynthetic rates saturate at a fraction of full sunlight (~10–20%, depending on species and environmental conditions). In full sunlight, processes such as non-photochemical quenching of light energy (NPQ) and alternative electron transport (AET) reduce the probability of damage from photo-oxidative stress (Box 1) but also reduce the overall efficiency of algal photosynthesis. Inevitably, there is damage to the photosynthetic apparatus despite the aforementioned protective mechanisms. The reduction in overall rates of carbon fixation, electron transport, or the ability to convert light energy to chemical energy associated with these processes is termed photoinhibition. Reducing the impact of photoinhibition represents a major target for increasing overall efficiency. However, we know little about the role of these processes, or any other cellular processes for that matter, in mass culture.

In the first part of this Opinion, I give an overview of what is known about the physiology of photosynthesis in mass cultures of algae and show that photoinhibition is a significant issue in these systems. This is followed by a summary of advances in systems biology as applied to photosynthesis in algae and cyanobacteria. I propose that the integration of these two approaches will allow the field to better identify and quantify the inefficiencies associated with the large-scale autotrophic cultivation of algae and cyanobacteria.

The ecophysiology of dense algal growth

Dense culturing is required to reach high aerial yields of algal biomass, which effectively reduces light penetration into the culture to only a few centimeters [6]. The cells perceive this as a net light-limited scenario and photoacclimate. Photoacclimation involves an increase in the cellular concentration of pigment–protein antenna complexes, photosystems, or both to capture more light and vice versa. This further reduces light penetration into the cell suspension. It also increases the probability of overwhelming the light-harvesting reactions in full sunlight, increasing the loss of energy as NPQ, AET (Box 1), or photoinhibition. Reducing the impact of this phenomenon has been an active area of research. Strains engineered to have constitutively small antennae have higher

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Box 1. Light-energy harvesting and associated loss terms

Only a portion of the light spectrum is able to drive the photosynthetic reactions [400–700 nm; photosynthetically active radiation (PAR)] and any unused portion is immediately lost. Most of the light is absorbed by pigment–protein antenna complexes and this energy is transferred to the photosystems. In Photosystem II (PSII) the energy of a red photon (regardless of the photon energy absorbed) drives charge separation and liberated electrons are subsequently donated to quinones. The electron that is removed from PSII is replaced by the sequential stepwise oxidation of a water molecule, yielding molecular oxygen. The electron passes through an electron-transport chain, eventually replacing an electron in photosystem I (PSI) that has become oxidized from an independent photon-absorption event. The electron lost from PSI is ultimately used to reduce $NADP^+$ to NADPH. Protons are liberated from water during water oxidation and are also pumped across the thylakoid membrane during electron transport. This creates a proton motive force driving the formation of ATP. Light energy that is absorbed in excess of downstream demand for ATP and NADPH is dissipated by several different pathways. NPQ is a process that toggles the light-harvesting antennae from a state of light absorption and transfer to the reaction centers, to a state of absorption and the dissipation of light energy as heat. Up to 80% of absorbed sunlight is thought to be lost through this process [9]. The process is under physiological and genetic control and the proteins involved vary between algae and cyanobacteria [43]. There is also considerable diversity in the pigments and proteins that constitute the antenna complexes in algae and cyanobacteria. AET is a process where electrons are removed from the electron-transport chain and used to reduce oxygen to create water. This process helps to oxidize an over-reduced electron-transport chain and can consume up to 49% of the total electron flow in excess light conditions in some species [44].

photosynthetic efficiency at high light intensities and, in some cases, higher biomass productivity [7–9].

Dense culturing also creates a complex light environment for the photoautotrophic organism: cells are rapidly moved from an excess-light environment into near-complete darkness. Cellular physiology under these conditions has not been well studied and the dynamics of mixing, the depth of the culture, and cell density will all vary between different industrial-scale scenarios. For instance, the conditions experienced in a well-sparged, thin (5 cm) tube photobioreactor will differ from that of a 1-acre or larger-sized raceway pond mixed by paddlewheels [10–12]. A successful engineering strategy to increase photosynthetic yields must anticipate the cellular response to these conditions and for that we need more observations of the photosynthetic physiology in industrially relevant conditions.

In situ physiology

There have been relatively few published studies investigating the physiology of photosynthesis in algal mass culture. This is probably due to the financial constraints of constructing and operating large-scale algal aquaculture facilities. However, the data reported thus far are beginning to provide insights into the physiology associated with industrial scenarios.

Torzillo *et al.* [13] found that reducing the biomass of the diatom *Phaeodactylum* to 0.3 from 0.6 g/l in a 4.85-cm tubular photobioreactor reduced the photochemical efficiency of photosystem II (PSII) and electron-transport rates before solar peak irradiance, presumably reducing overall carbon-fixation rates. Cells grown at the lower density in small ponds (10 cm depth) had little inhibition of photosynthesis and the highest aerial productivity. These results suggest that more exposure to full sunlight reduced the overall efficiency of the process. All cultures had some degree of NPQ capacity, although less than has been observed in algae grown in constant light in the laboratory. In a separate study, *Chlorella* and *Scenedesmus* grown in 50-l tubular photobioreactors also showed NPQ capacity along with inhibition of PSII [14].

Cultures of the cyanobacterium *Arthrospira* (*Spirulina*) also show reduced inhibition of PSII activity when grown at higher densities [0.5 versus 0.3 optical density (OD)], when incident sunlight was shaded by 25% or with more rapid mixing [15]. Photosynthetic rate tracked insolation in the shaded culture compared with the uncovered culture, which clearly had 4 h of photoinhibition around noon.

Nannochloropsis grown in flat-panel bioreactors behaved as though acclimated to high light, while those grown in highly mixed small ponds appeared to be acclimated to low light. Both showed photoinhibition just after peak irradiance and rapidly relaxing NPQ [16]. Interestingly, the cells grown in flat panels experienced higher temperatures and this led to severe inhibition of PSII activity and slowed recovery of the maximal rates of photosynthesis [17]. This suggests that we should also consider the effects of other abiotic stresses on photosynthesis.

The examples above show us, perhaps unsurprisingly, that there is clear variation between species and that there is variation between culture conditions. The observations that photochemical efficiency (or productivity) is increased by reducing incident light or increasing culture density are also intriguing, considering that this goes against the common view that more light penetration into the culture will increase productivity.

The choice of cultivation method also affects the delivery and removal of gases within the culture. Dense cultures of algae or cyanobacteria will quickly deplete CO_2 from the medium due to their rapid photosynthetic rates and increase ambient O_2, increasing the probability of photorespiration (Box 2). This is likely to have some effect on the

Box 2. CO_2 fixation and associated loss terms

The NADPH and ATP generated from light-energy harvesting (often referred to as the 'light reactions') are used to drive the CBB cycle. Ribulose bisphosphate carboxylase/oxygenase (RuBisCO) catalyzes the essential step of CO_2 fixation, with the remainder of the cycle consuming NADPH and ATP to regenerate the substrate for RuBisCO. RuBisCO has several inefficiencies. It is a torpid enzyme, capable of only a few turnovers per second, and it can also use oxygen as a substrate, creating 2-phosphoglycerate (photorespiration). Photorespiration is an energetically expensive process, involving metabolite transport between organelles (in the case of algae) and the loss of fixed CO_2 and NH_3. Algae and cyanobacteria have evolved many different mechanisms to reduce the impact of photorespiration. Carbon-concentrating mechanisms (CCMs) favorably increase the CO_2:O_2 ratio near RuBisCO. CCMs can range from active pumping of inorganic carbon species into the cells (bicarbonate transporters) to a C_4-type mechanism that captures CO_2 in an organic acid that is then transported to, and decarboxylated at, the location of RuBisCO [18,45]. There are energetic costs associated with CCMs. Despite the presence of these mechanisms, photorespiration may account for a significant fraction of RuBisCO activity in laboratory culture (up to 28%) [46].