PERSPECTIVE

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Photosynthesis: a blueprint for solar energy capture and biohydrogen production technologies†

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Solar energy capture, conversion into chemical energy and biopolymers by photoautotrophic organisms, is the basis for almost all life on Earth. A broad range of organisms have developed complex molecular machinery for the efficient conversion of sunlight to chemical energy over the past 3 billion years, which to the present day has not been matched by any man-made technologies. Chlorophyll photochemistry within photosystem II (PSII) drives the water-splitting reaction efficiently at room temperature, in contrast with the thermal dissociation reaction that requires a temperature of *ca.* 1550 K. The successful elucidation of the high-resolution structure of PSII, and in particular the structure of its Mn_4Ca cluster (K. N. Ferreira, T. M. Iverson, K. Maghlaoui, J. Barber and S. Iwata, *Science*, 2004, 303, 1831–1838, ref. 1) provides an invaluable blueprint for designing solar powered biotechnologies for the future. This knowledge, combined with new molecular

† Dedicated to Professor James Barber on the occasion of his 65th birthday.

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genetic tools, fully sequenced genomes, and an ever increasing knowledge base of physiological processes of oxygenic phototrophs has inspired scientists from many countries to develop new biotechnological strategies to produce renewable CO_2-neutral energy from sunlight. This review focuses particularly on the potential of use of cyanobacteria and microalgae for biohydrogen production. Specifically this article reviews the predicted size of the global energy market and the constraints of global warming upon it, before detailing the complex set of biochemical pathways that underlie the photosynthetic process and how they could be modified for improved biohydrogen production.

Section I. Overview of energy market and greenhouse gas limits

Overview

The development of zero-CO_2 emission fuels is one of the greatest energy challenges facing our society. There are two main reasons for this urgency. The first is the rapid depletion of oil reserves, which requires the development of replacement fuels and infrastructure on the decades to a century time horizon. Second, future fuels will increasingly have to be free of CO_2 emissions, as fossil fuel combustion causes anthropogenic CO_2 emissions, that exacerbate global warming. The constraints of global warming clearly indicate that the implementation of clean fuel technologies must take place much more quickly. The non-CO_2 emitting energy options currently considered to be most viable, include nuclear power, coal-fired power stations coupled to anticipated CO_2 sequestration systems, and renewable energy sources such as solar, geothermal, wind and hydroelectric. Of these, only renewable energy sources can sustain long term supplies and energy security (millennia) owing to their borderless distribution. The promise of clean energy by nuclear fusion remains inaccessible. Of the renewable resources, incident solar energy is by far the largest (178 000 TW year^{-1})[2] and capable of supplying 13 500 times the total global energy demand (13 TW year^{-1} in 2000). However, solar energy is diffuse and solar energy capture technologies relatively expensive.

This perspective aims to provide a broad framework to assess the constraints and opportunities for the development of future biotechnologies for solar energy capture and conversion, based on the natural blueprint of photosynthesis. As we cannot do justice to each of these areas in the available space, the reader is pointed to key papers and reviews. The first section of the article outlines the predicted size of the global energy market and the constraints put upon it by the effects of global warming. The second section covers the complex set of interacting biochemical pathways that drive the photosynthetic process. At many points in these pathways there is an inbuilt flexibility in the system that confers adaptability to environmental conditions, often at the cost of efficiency. The third section highlights key points of interest for improved photon conversion efficiency (PCE), particularly for the production of biohydrogen. In terms of biological systems we have focused specifically on microalgae and cyanobacteria, as the environmental constraints upon photosynthetic efficiency, can be controlled more effectively in bioreactors than in fields of higher plants. Furthermore, large scale microbial culturing is already a huge industry involved in the production of vitamins, pigments and food supplements. Each of these areas of research has or is likely to benefit from the tireless work of Jim Barber on the elucidation of the structure of photosystem II, to whom this article is dedicated.

Global energy market in the 21st century—a brief overview

Although an extensive analysis of the global energy market is a highly complex field, beyond the scope of this review, it is important at least to establish "ballpark" figures for its predicted size during the 21st century, to highlight the potential for and constraints upon developing solar powered biotechnologies.

Recent "business as usual" global energy consumption models (*i.e.* 2–3% economic growth, 1% improvement in energy intensity (W yr \$$^{-1}$) per year[3]), predict that global energy demand will rise from 13 TW in 2000 to a level of the order of 46 TW in 2100. Uncertainties in the final predicted value of 46 TW by 2100 include the rate of population growth, the actual economic growth and the rate of improvement in energy intensity.[3] Additional uncertainties of particular note include the growth rates of the emerging economies of China and India that are exerting a strong upward pressure on energy demand. To meet this level of energy demand will be a major challenge. It is compounded not only by extensive predictions that peak oil production is likely to fall into the narrow time frame of 2007–2038,[4] but by more recent data suggesting that oil production levels may already be close to their peak.[5] This latter prediction is consistent with the rapid increase in crude oil prices, observed over the past year. International Energy Agency figures[6] suggest that in addition to the ~540 TW supply of oil that is reported to remain, coal and natural gas could supply an additional ~1000 TW and ~580 TW, respectively. Furthermore, nuclear energy and as yet largely untapped methyl hydrate reserves are also candidates to contribute to future energy markets provided major technological breakthroughs are realized. Sub-marine gas hydrate deposits are estimated to be in the range (1–5) × 10^{15} m^3, a proportion of which are thought to be concentrated enough to allow economically viable recovery.[7] However given the constraints of global warming, there appear to be two main ecologically responsible routes for their use. The first is to couple their combustion with carbon sequestration, which will raise the cost of energy production.[8,9] Furthermore carbon sequestration processes are far from proven, present potential ecological hazards[8,10] and are only suitable for large scale power stations as they require expensive CO_2 purification and storage systems. The second, and in the long term preferable option, would be to conserve fossil fuel resources for chemical and C-based material production. That said it is clear that any solar biotechnologies will have to be cost competitive with nuclear energy, fossil fuel combustion CO_2 sequestration and existing clean energy technologies. Currently solar electricity prices in the USA are around 30 cents kWh^{-1}, which is 2–5 times greater than average residential electricity tariffs,[11] without adjustment for environmental externalities associated with fossil and nuclear fuels.

The constraints of global warming

Since 1850 atmospheric CO_2 levels, which were stable between 200 and 280 ppm for the previous 400 000 years,[12] have risen sharply to 370 ppm.[13] Although the increased atmospheric CO_2 level is now widely accepted as a major contributor to global warming, its potential effects are only beginning to be understood. Recent high profile reports for example indicate that atmospheric CO_2 levels of 450 ppm are likely to result in severe and probably irreversible coral reef damage.[14] At levels of 550 ppm, the melting of the West Antarctic ice sheet, 4–6 m sea level rises[14] and the extinction of 24% of plant and animal species are predicted.[15] A level of 650 ppm has been predicted to result in disrupted thermohaline circulation (*e.g.* switching off the Gulf Stream), major local climate changes[14] and the extinction of 35% of plant and animal species.[15] More recent global climate change models[16] suggest that the effects may be even more pronounced than previously predicted[14,17,18] emphasizing the importance of stabilizing CO_2 levels as close to 450 ppm as possible and preferably below. Currently however it appears highly unlikely that CO_2 levels will be kept even below this target, due to our high current CO_2 emission levels and the long residence time of CO_2 in the atmosphere. Hoffert and colleagues have for example calculated that to achieve a

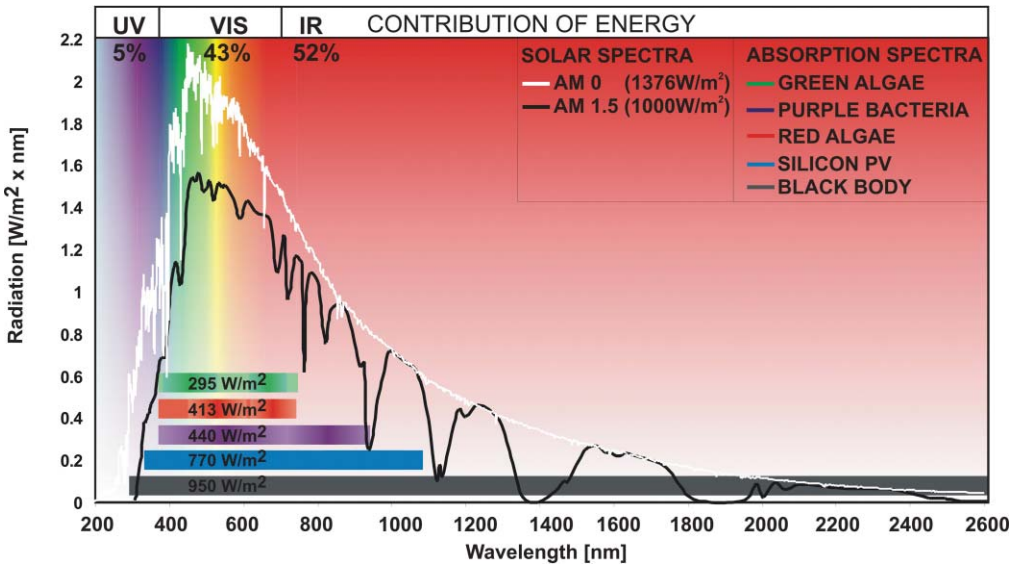

Fig. 1 Solar energy distribution and capture. The AM0 and AM1.5 solar irradiation spectra show the solar energy distribution outside of the Earth's atmosphere (white line) and at the Earth's surface (black line), respectively. At the Earth's surface (AM1.5; for more information see: http://rredc.nrel.gov/solar/standards/am1.5) approximately 5% of the energy is in the ultraviolet (300–400 nm), 43% in the visible (400–700 nm) and 52% in the infrared range. A very small proportion of energy is in the 2600–4000 nm range (not shown). The coloured bars show the absorption range of green plants, purple bacteria, red algae and typical silicon PV (photovoltaic) panels, as these are the most prevalent light harvesting systems. Not all wavelengths are absorbed equally well by photosynthetic organism or silicon PV panels. The absorption peak maxima (where available), are depicted by darker shading in each coloured band. The energy contained in the 300–350 nm and 1000–4045 nm regions are only captured by black absorbers and correspond to 1.37% and 26.31% of the total energy in each spectrum, respectively.

stabilization of atmospheric CO_2 levels at a level of 450 ppm, would require the use of 11 TW CO_2-emission-free fuel by 2025.[3] In other words, if Hoffert's predictions are correct we are faced with the challenge of installing systems capable of producing energy free of CO_2 emissions at a level almost equivalent to the total current global energy demand in 2000 (13 TW) in 20 years time. Based on this information it is clear that an abundant clean fuel alternative is needed urgently.

The potential of solar energy

Solar energy is widely accepted as being overwhelmingly the most abundant (178 000 TW $year^{-1}$) and accessible renewable energy source available.[2] In contrast to geothermal, wind and hydroelectric energy, solar energy is relatively evenly distributed and easily accessible to small, large, low-tech and high-tech systems. Even in countries with relatively low irradiation intensity it is still high enough to make solar energy systems profitable. The main challenges associated with the use of solar energy are the development of more cost-effective systems with improved photon conversion efficiency and the ability to convert captured energy into chemical energy (fuel) such as hydrogen. In the light of advances made in hydrogen fuel cell development, the latter is becoming even more important.

How big do solar collectors have to be?

The solar energy spectrum emitted by the sun is described by the air mass 0 spectrum (AM0, Fig. 1) and differs from the solar energy reaching the Earth's surface (AM1.5, Fig. 1), the latter being largely reduced by atmospheric absorption losses. At the Earth's surface solar irradiation maximally yields 1000 W m^{-2}.[19] This value is however usually lower due to less than optimal climatic conditions. Typical average values are ~300 W m^{-2} for temperate (*e.g.* Europe and parts of the US) and ~650 W m^{-2} for desert conditions (*e.g.* Sahara desert).[20] Using these values the areas required to capture a given amount of solar energy can be calculated on the basis of the level of irradiance and the efficiency of the system (Fig. 2). For example, existing commercial solar cells (12% efficiency rating as opposed to maximum solar cells efficiency, *i.e.* 30–41%[21]) can be calculated to supply the entire global energy demand of 13 TW (at high irradiance) using an area of (634 × 634 km). Although at first this seems large it is only 4.4% of the Sahara desert (9 065 000 km^2), and based on existing technology.

The main reason why such large scale systems have not been installed is likely to be that the costs are still higher than those of using fossil fuels and economic and military costs for foreign access. The next challenge is therefore two-fold. First to develop cheaper/more efficient solar energy capture systems; second to increase competition within the energy market by including the costs of CO_2 induced environmental damage as well as the cost of decommissioning nuclear power plants and dealing with the associated radioactive waste. Photosynthetic organisms offer answers to this problem as the photochemistry of the initial light-to-charge conversion reaction alone (*i.e.* not including fluorescence and heat losses in the antenna and the inefficiencies of CO_2 fixation), has an efficiency of ~50%.‡ However under maximal solar irradiance the overall photon conversion efficiency (*i.e.* energy in photons stored as chemical energy) is much lower, due to natural losses adopted by the organisms for photoprotection and the required thermodynamic inefficiencies of a highly flexible network of competing reactions, which are the subject of the next section.

Section II. Energy conversion-efficiency constraints on photosynthesis

The reactions of photosynthesis

Overview. To provide a scientific basis for subsequent discussions of how to improve the photon conversion efficiencies of photosynthetic organisms for biohydrogen production, this section will describe the complex interplay and regulation of the reactions of photosynthesis and respiration (Fig. 3). The fixation of atmospheric CO_2 and its storage as carbohydrate was shown more than 50 years ago to include two phases. The first phase consists of a set of reactions that together form the photosynthetic electron transport chain and which produce ATP

‡ For a 1.78 eV photon (700 nm) photosynthetic organisms driving the reaction: $\frac{1}{4}$PSII–plastoquinone + H^+ (stroma) + $\frac{1}{2}H_2O \rightarrow \frac{1}{4}O_2$ + H^+ (lumen) + $\frac{1}{4}$PSII–plastoquinol; $\Delta E = -0.887$ V for a $\Delta pH = 3$ gradient across the thylakoid membrane.

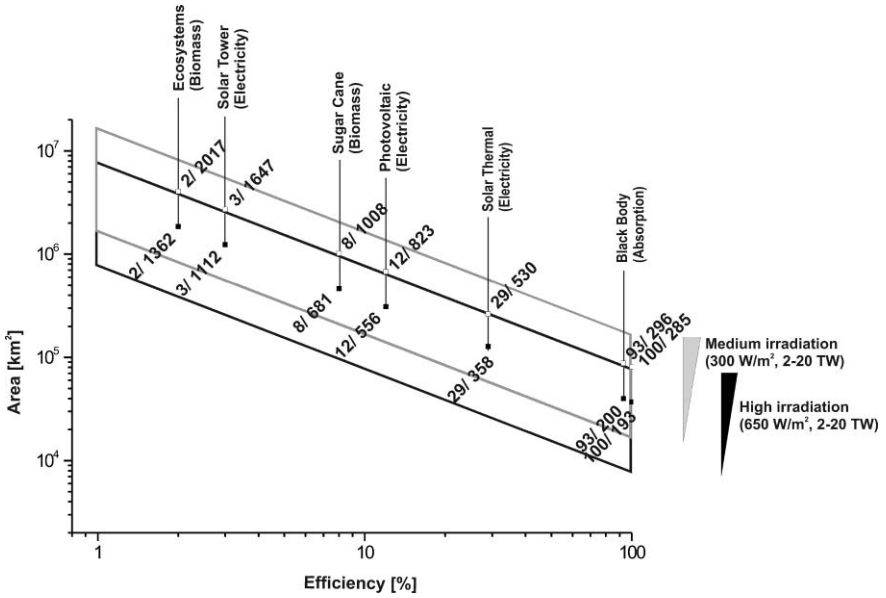

Fig. 2 Area requirement for renewable energy capture. Efficiency *vs.* area conversion graph for a range of solar energy capture systems. The areas required for the annual capture of 2–20 TW using the main solar energy capture systems are shown for medium (~300 W m^{-2}, white points) and high irradiance (~650 W m^{-2}, black points). The numbers marking each point (*e.g.* 100/193) are the percentage efficiencies (100%) of the system and the length (km) of one side of the square area required to capture 10 TW (*e.g.* a 193 × 193 km area). The regions outlined in grey and black lines are required for the annual capture of 2–20 TW under high and medium irradiance.

and NAD(P)H. This phase is followed by a second phase, which is not immediately coupled to the processes in the thylakoid membrane. In this set of reactions the generated ATP and NAD(P)H are used in the photosynthetic reduction cycle to convert CO_2 into carbohydrates, which can subsequently be stored as starch or other biopolymers (chemical energy store).[22]

Location of the protein complexes forming the photosynthetic electron transport chain. The photosynthetic electron transport chain which is driven by photosystem II, the cytochrome b_6f ($Cytb_6f$) complex and photosystem I (PSI), takes place in the thylakoid membrane of the chloroplast. PSII is located in the grana (stacked thylakoid membranes) and PSI in the stromal lamellae (unstacked thylakoid membranes). In contrast, the $Cytb_6f$ complex has been localized both in granal and stromal thylakoid membranes while ATP synthase is more typically located in the stroma lamellae.[23] Photosystems I and II each have extensive and environmentally adaptive antenna systems consisting of light harvesting complex (LHC) proteins which capture the energy required to drive the two photochemical reactions (Fig. 3). One reason for the spatial separation of PSII (grana) and PSI (stroma), may be to regulate and optimize the rate of PSII and PSI activities during linear and cyclic electron transport. The kinetics of PSI are much faster than those of PSII. By preventing the antenna systems of PSII and PSI from coming into close contact the uncontrolled flow of excitation energy from the antenna system of PSII to the reaction center of PSI, is likely prevented.[23] In this context it is interesting to note that in higher plants and green algae, photosystems I and II are not only spatially separate but often organize into complex 2D and 3D arrays.[24]

The photosynthetic electron transport chain. PSII catalyses the first step of the photosynthetic electron (e^-) transport chain. It is powered by the excitation energy captured by its antenna system, and upon excitation generates a chlorophyll radical cation (P_{680}^+) and reduces a plastoquinone molecule (Q_A). Oxidized P_{680}^+ is the strongest oxidant known to occur in biology and through a series of redox active components including a Mn_4Ca-cluster (water-oxidizing complex, WOC) catalyses the water oxidation reaction of the electron transport chain (Fig. 3). The PSII-WOC is located towards the luminal surface of the thylakoid membrane where protons from water oxidation are concentrated against an electrochemical potential.[1]

During linear electron transport, e^- derived from water are passed along the photosynthetic electron transport chain (Fig. 3—points 1 to 3) *via* plastoquinone (PQ), the $Cytb_6f$ complex, plastocyanin (PC) or cytochrome c_6, photosystem I and ferredoxin (Fd) before being used to reduce $NADP^+$ (Fig. 3—point 3). H^+ released into the thylakoid lumen by PSII and the PQ/PQH_2 cycle, generate a H^+ gradient, which drives ATP production *via* ATP synthase.[25] Linear electron transport results in a relatively high NADPH : ATP production ratio. Fast kinetics and favorable thermodynamics make the forward coupled e^-/H^+ transfer processes largely irreversible in dark adapted phototrophs. But at high light flux the formation of large ΔpH gradients and populations of reduced PQH_2 and Fd pools, leads to efficiency losses due to charge recombination.

For the optimal conversion of light energy into chemical energy, light harvesting, photosynthetic energy transfer and ATPase activity are coordinated and fine tuned in response to environmental parameters. The photoautotrophic cell can adjust the ratio of ATP/NAD(P)H production by switching from linear to cyclic electron transport around PSI (Fig. 3—point 4). Under these conditions the proton gradient is increased for enhanced ATP production, while the reduction of $NAD(P)^+$ is markedly decreased. Consequently, a switch to cyclic electron transfer automatically reduces the direct photosynthetic efficiency of light energy conversion but maintains high rates of ATP production inside the chloroplast when required.[26] As will be discussed later cyclic e^- transport can limit the rate of hydrogenase-dependent photobiological hydrogen production by consumption of protons from the stroma.[27]

Antenna systems. Different organisms have developed a variety of antenna complexes for capturing and funnelling absorbed photons to the photosynthetic core complexes. Cyanobacteria use phycobilisomes as their dominant light capturing complexes,[28] but when experiencing iron deficiency, they can also build up an alternative, ring-like antenna consisting of iron-stress induced chlorophyll-binding protein (IsiA) (typically 12–35 copies) that encircle central PSI complexes.[29,30] In contrast to cyanobacteria, each of the two photosystems of green algae

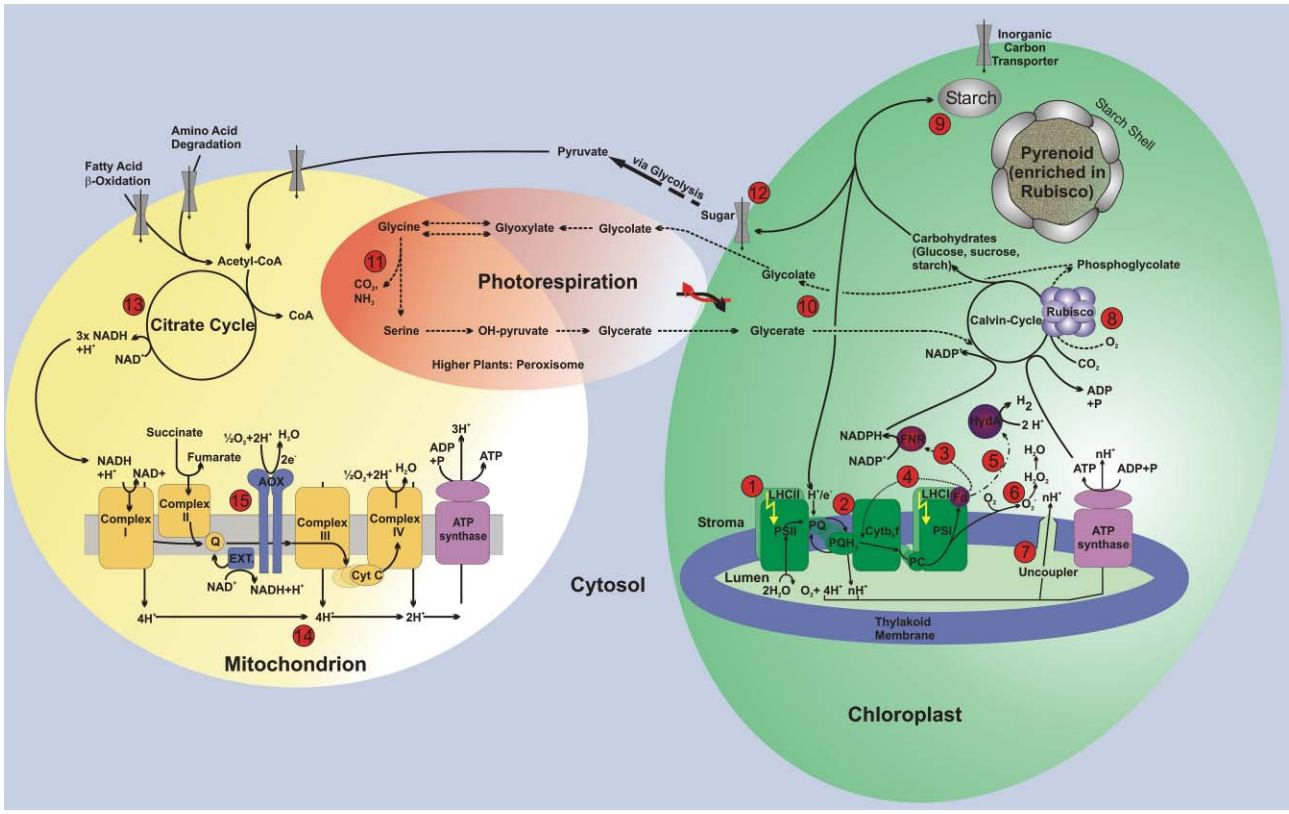

Fig. 3 Biochemical pathways related to photon conversion efficiency. The processes of photosynthesis, C-fixation and metabolism take place in chloroplasts, cytosol, mitochondria and peroxisomes (higher plants). The so called light reactions of photosynthesis which take place in the chloroplast thylakoid membrane are driven by light captured by the antenna proteins (LHCI/LHCII) bound to PSI and PSII (1), respectively. Under conditions supporting linear electron transport, electrons derived from H_2O by PSII are passed along the photosynthetic electron transport chain (2) *via* plastoquinone (PQ), the cytochrome b_6f ($Cytb_6f$) complex , plastocyanin (PC), photosystem I (PSI) and ferredoxin (Fd), before being used for the production of NADPH (3) by ferredoxin–$NADP^+$ oxidoreductase (FNR). Simultaneously, H^+ are released into the thylakoid lumen by PSII and the PQ/PQH_2 cycle to generate a gradient which drives ATP production *via* ATP synthase. Electrons from the electron transport chain can also drive cyclic electron transport around PSI (4), reduce H^+ to H_2 *via* hydrogenases (5) or reduce O_2 to H_2O *via* the Mehler reaction (6). Chemical uncouplers (7) can be used to break down the H^+ gradient and release H^+ into the chloroplast stroma. The ATP and NADPH generated by the above processes are consumed, during CO_2 fixation, in the photosynthetic reduction cycle. Rubisco activity (8) is responsible for incorporation of CO_2 into RuBP, which is subsequently used to generate C_3 and C_6 sugars and ultimately starch (9). In some photosynthetic organisms (*e.g.* algae), highly concentrated rubisco is found in a dense structure (pyrenoid). Rubisco also catalyses an oxygenation reaction in the first step of photorespiration (10–11). Photorespiratory reactions take place in chloroplasts/mitochondria and peroxisomes (in higher plants) and metabolize 2-phosphoglycolate to regenerate RuBP which is fed back into the photosynthetic reduction cycle. Starch (9) can be converted to cytosolic sugar molecules that can subsequently be fed into glycolysis (12) to produce pyruvate. Pyruvate is used to fuel the citrate cycle (13) in the mitochondria, thereby generating the reducing equivalents that drive oxidative electron transport (14) in the inner mitochondrial membrane, *via* complexes I–IV, ubiquinone (Q) and Cytc. The oxidative electron transport process generates a H^+ gradient which drives ATP production *via* ATP synthase (F_oF_1 ATP synthase). Alternative oxidase (AOX) (15) can oxidize ubiquinol (QH_2) to reduce oxygen directly to H_2O. This has the effect of diminishing the H^+ gradient and ATP synthesis.

and plants is coupled to specialised LHC proteins, LHCI (predominantly associated with PSI) and LHCII (predominantly associated with PSII). Depending on their relative positions with respect to the PSII dimer, PSII antenna proteins can be subdivided into peripheral and inner antenna proteins. Some of the peripheral LHCII proteins form trimers and are found in high copy numbers per PSII dimer and are therefore called major LHCII proteins, in contrast to the less abundant, monomeric minor LHCII proteins. Intensive structural studies revealed that PSII and LHCII proteins are associated in approximately rectangular supercomplexes,[31,32] which are thought to represent a native organizational form and can aggregate to even larger megacomplexes within thylakoid membranes.[23]

Light capture is the first step of photosynthesis. It is therefore not surprising that the amount and composition of LHC proteins strongly depend upon the prevailing light environment. This flexibility is highlighted by the fact that LHCs are encoded by a large gene family. In *Chlamydomonas* at least 20 nuclear genes encode peripheral antenna proteins alone,[33] some of which undergo additional post-translational modifications.[34,35] Because light is required for photosynthesis, but is also damaging in excess (resulting in particular in production of toxic-oxygen species) its input into the photosynthetic reaction centers must be finely controlled[36] (see fluorescence, xanthophyll cycle and state transition sections). The physiological flexibility of the antenna complexes is further emphasized by protein expression and degradation studies. LHC proteins are reported to be controlled at all stages of protein expression examined so far. One important principle which can be derived is that LHC levels increase under conditions of low light and decrease under conditions of excess (*e.g.* constant light and decreased temperature,[37] low CO_2,[38] or high light[39,40–42]). The redox state of the PQ pool has been shown to be an important sensor for LHC antenna size regulation[39] but there also seems to be a redox independent pathway.[38]

Variations in the LHC antenna composition and size are reflected by chlorophyll (Chl) *a* fluorescence levels. Consequently steady state fluorescence is a good parameter to measure photosynthetic light energy transfer efficiency (high steady state fluorescence proportional to low photosynthetic efficiency and *vice versa*). PSII-related fluorescence depends upon a wide range of non-photochemical and photochemical fluorescence

quenching events which include the xanthophyll cycle, luminal pH changes, LHC state transitions and LHCII antenna size regulation.[43–46]

Xanthophyll cycle. In higher plants and green algae the xanthophyll cycle is thought to be activated under high light conditions to protect the reaction centre of PSII against damage. The xanthophyll cycle is involved in the dissipation of excess excitation energy as heat by a process that is often referred to as pH or energy dependent quenching of chlorophyll fluorescence (qE).[46,47]

Prior to photoinhibition, high light conditions drive the water splitting reaction of PSII, and with it a reduction of luminal pH. The low luminal pH activates violaxanthin de-epoxidase which catalyses the rapid conversion of the carotenoid violaxanthin, *via* the intermediate antheraxanthin to zeaxanthin. Recent gene dosing studies have shown that thermal dissipation is proportional to the level of expression of the zeaxanthin binding subunit PsbS.[48,49] PsbS also has a luminal pH sensing capacity linked with one of four highly conserved pairs of glutamates (E122 and E226) at its lumenally exposed surface. The protonation of these glutamates is thought to induce qE, *via* the xanthophyll cycle.

PsbS is reported to be part of the PSII complex but was not present in PSII–LHCII supercomplexes.[50] This suggests that it is perhaps more closely associated with the peripheral antenna system of PSII.[51] In algae the xanthophyll cycle may be associated with a protein other than PsbS. Indeed algae are also thought to rely on additional dissipation mechanisms that work alongside the xanthophyll cycle.[52] Engineering mutants with a reduced level of qE may improve photon conversion efficiency under controlled environmental conditions (*e.g.* algal bioreactors). In this respect the identification of mutants able to maintain a high PQ : PQH_2 ratio may lead to reduced levels of xanthophyll cycle activity. Furthermore engineering solutions aimed at controlling light levels could contribute to limiting heat losses.

State transition process. Environmental changes such as light intensity and light quality influence the energy state of PSII and indeed the redox active components of the whole photosynthetic electron transport chain. Its redox state plays an important role in light induced adaptation processes. For example a chemically reduced PQ-pool induces several redox-controlled mechanisms by specific phosphorylation of PSII and LHCII proteins.[53,54] The phosphorylation of specific Thr residues of LHCII proteins induces the so called state transitions.[55,56] These result in the translocation of subunits of the LHCII complex from PSII to PSI.[44,57,58] This short-term light adaptation mechanism enables the plant to regulate and control the energy distribution between the two photosystems.[44,57–59]

State transitions seem to be particularly important for green algae like *Chlamydomonas reinhardtii*, where up to 90% of the antenna is reported to move between PSII and PSI.[26] General features of state transitions in higher plants and green algae are the supply of light energy to PSII under low light conditions (transition to state 1) and the reduction of the PSII antenna size under increasing light conditions to avoid the formation of reactive oxygen species (transition to state 2). However, the high capacity of *C. reinhardtii* to perform these state transitions appears to have an additional reason. State transitions mediate a switch from linear (state 1) to cyclic (state 2) e^- transfer around PSI. The importance of this process lies in the fact that increasing the rate of cyclic e^- transport maintains a proton gradient across the thylakoid membrane, allowing an increase of chloroplast ATP synthesis on demand (*e.g.* in the absence of mitochondrial oxidative phosphorylation). Switching off cyclic electron transport in *C. reinhardtii* can play an important role in increasing the rate of photosynthetic hydrogen production as it competes for e^- from PSI with at least one of the two hydrogenases (HydA1 and HydA2), both of which are expressed under anaerobic sulfur depleted conditions.[60,61,122]

The photosynthetic reduction cycle. The photosynthetic reduction cycle (Fig. 3—point 8) is the fundamental process thought to be used by all photosynthetic organisms to fix CO_2, from primitive algae through to higher plants. The process uses ATP and NAD(P)H generated by the light reactions. In C_4 and CAM plants it is coupled to ancillary processes that aid CO_2 fixation, but the fundamental photosynthetic reduction cycle reactions remain the same.[22]

The photosynthetic reduction cycle can be divided into three main steps that involve carboxylation, reduction and substrate (ribulose-1,5-bisphosphate (RuBP)) regeneration. The first step at which CO_2 enters the cycle to react with RuBP is catalysed by ribulose bisphosphate carboxylase/oxygenase (rubisco). The importance of rubisco is hard to overstate as essentially all carbon found in living organisms on Earth was once fixed by this enzyme from atmospheric CO_2. Furthermore it is the most abundant protein on Earth, constituting some 30% of total proteins in most leaves.[62] This is partly because of the central role that it plays, but also because it has a very low catalytic carboxylase performance, using as little as 2–3 RuBP per second.[63] As its name suggests rubisco has two catalytic functions; it functions as a carboxylase as part of the photosynthetic reduction cycle, and under aerobic conditions as an oxygenase as part of photorespiration. O_2 and CO_2 compete for the same catalytic site, so that the efficiency of CO_2 fixation can be impaired in certain aerobic environments. For example, although the specificity of the enzyme is higher for CO_2 (*e.g.* tobacco (higher plant) 82 times, *Griffithsia monilis* (red alga) 167 times, *Rhodospirillum rubrum* (purple non-sulfur bacteria) 12 times),[64] the molecular ratio of O_2/CO_2 is about 540 : 1 in air and 24 : 1 in air saturated water at 25 °C. As will be discussed later, this lower ratio makes it a factor to consider in particular for the optimization of photosynthesis in microbial culture.

In the first step of the photosynthetic reduction cycle, rubisco catalyses the formation of two 3-phosphoglycerate (3-PGA) molecules from RuBP, CO_2 and H_2O. The forward reaction is strongly favoured by the negative change in free energy of the process. In the second step, an ATP/NADPH dependent reduction phase, these carboxylic acids are reduced to form 2 molecules of glyceraldehyde-3-phosphate, by the action of phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase. In a third step, consisting of a series of reactions a proportion of glyceraldehyde-3-phosphate is converted back to RuBP required to allow the photosynthetic reduction cycle to continue.[22]

Starch synthesis and breakdown. Storage of polysaccharides *via* an ADP glucose-based pathway, is a distinct feature of the photosynthetic eukaryotic cell. In contrast to bacterial glycogen metabolism, starch metabolism in plants and green algae involves multiple forms of enzyme activities. The entry of glucose into the plastid is largely dependent on the exogenous supply of ATP, essential for the activity of a hexose–phosphate translocator in the chloroplast membrane.[65] The major steps in starch synthesis in the chloroplast are the conversion of glucose-6-phosphate to glucose-1-phosphate followed by the formation of ADP–glucose, the elongation of $(glycosyl)_{n+1}$ and the formation of branched polymers *via* glucan α-1,6 branch points.

Functional genomic studies have identified a number of mutants with defective enzymes involved in starch metabolism. Such studies report that starch synthases and in particular branching and debranching enzymes are involved in regulating the rate of starch synthesis.[66]

The ability of plants to produce highly crystalline and osmotically inert carbohydrates as starch (Fig. 3—point 9) enables them to build up a fuel supply to maintain metabolic processes as required. For example, green algae like *C. reinhardtii* can use

their starch stores to fuel photophosphorylation and hydrogen production (Fig. 3—point 5) under anaerobic conditions when oxidative phosphorylation is inhibited, and PSII activity is perturbed.[67] The importance of the large starch supply for this process under anaerobic conditions was emphasized by the finding that disrupting an isoamylase gene causes a rapid decline in chloroplast hydrogen evolution capacity.[68]

Photorespiration. The carboxylation of RuBP as part of the photosynthetic reduction cycle is accompanied by the competing oxygenation reaction of the same substrate during the first step of photorespiration.[69] This oxygenation reaction (Fig. 3—point 8) leads to the formation of one molecule of 3-PGA and one molecule of 2-phosphoglycolate instead of two molecules of 3-PGA.[70] 2-Phosphoglycolate is then recycled *via* the photorespiration pathway which results in the uptake of oxygen and release of CO_2 in the light (Fig. 3—point 11).

Rubisco is located in the chloroplast, where the first steps of photorespiration (oxygenation of RuBP and hydrolysis of 2-phosphoglycolate) take place. The product, glycolate, is subsequently exported from the chloroplast in exchange for glycerate by integral membrane translocators. In higher plants, glycolate next enters a peroxisome and becomes oxygenated to form glyoxylate which is then converted to glycine by transamination. Glycine is imported into mitochondria where, in a complex reaction cascade, 2 molecules of glycine are used to form one molecule of serine. In the course of the oxidative decarboxylation of glycine, CO_2 is released (Fig. 3—point 11). Serine then leaves the mitochondrion and enters a peroxisome where it is converted to hydroxypyruvate. Hydroxypyruvate is then reduced to glycerate and is translocated from the peroxisome to the chloroplast stroma before being phosphorylated to 3-PGA.[22]

The microalga *Chlamydomonas* has no peroxisomes, with the consequence that glycolate oxidation and glycine synthesis take place either in mitochondria or the chloroplast, while oxidative decarboxylation of glycine and glycerate production is thought to take place in mitochondria.[71] Furthermore, under high photorespiratory conditions the cells simply excrete glycolate instead of converting it to 3-PGA.

The physiological importance of photorespiration is still a matter of debate. Oxidation of RuBP seems to be an unavoidable side reaction connected to the catalytic center of rubisco. During the early stages of the evolution of photosynthesis (~3 billion years ago) atmospheric O_2 levels were low and consequently the oxygenase activity of rubisco relatively unimportant. However, as atmospheric O_2 levels rose, competition between photorespiration and the photosynthetic reduction cycle increased with the result that the efficiency of CO_2 fixation was reduced.[63,72] In accordance with this view a lot of photosynthetic organisms have evolved strategies to avoid photorespiration by concentrating CO_2 near the site of its incorporation (*e.g.* by CO_2/HCO_3^- pumps, C_4 or CAM metabolism). However, beneficial effects of the photorespiratory pathway as a means to dissipate excess redox equivalents (thereby protecting against photodamage) are also thought to be possible.[73]

Mitochondrial respiration. Mitochondrial respiration uses the stored chemical energy (in part *via* the citrate cycle, Fig. 3—point 13) to generate ATP. Mitochondrial ATP is needed to drive cellular reactions, particularly in the dark. However mitochondrial respiration also serves an important role in the light by maintaining an appropriate redox balance within the chloroplast *via* O_2 and diffusible metabolites.[74] Through the use of metabolite shuttles, within the inner envelope membrane of the chloroplast, reducing equivalents produced by photosynthetic electron transport activity (*e.g.* malate, ornithine and dihydroxyacetone phosphate) can be exported. These are subsequently consumed within the mitochondrion through the mitochondrial electron transport chain (Fig. 3—point 14) and *via* the alternative oxidase system (AOX, Fig. 3—point 15) that bypasses ubiquinone reduction.[75] Several studies of mitochondrial respiration have provided evidence that it plays a significant role in the regulation of light reactions in the chloroplast.[67,76–78] For example, mutants with a reduced mitochondrial capacity to oxidize reductants in the chloroplast were shown to have an over-oxidised cytosol and an over-reduced chloroplast stroma. This imbalance in cellular redox homeostasis enhances redox pressure inside the chloroplast (*e.g.* low PQ : PQH_2 ratio, Fig. 3—point 2) and consequently the risk of photoinhibition induced by the production of reactive oxygen species. In addition perturbations of mitorespiration can also inhibit photorespiration,[79] which again can result in sensitivity to photoinhibition.

In summary, mitochondrial respiration directly influences photosynthetic energy efficiency parameters in the chloroplast. Manipulation of AOX (Fig. 3—point 15) and cytochrome oxidase activities (Fig. 3, complex IV) may be used to reduce cellular oxygen concentration under aerobic conditions in the light, down to a level where oxygen sensitive pathways in the chloroplast, such as hydrogen evolution (Fig. 3—point 5), are activated (see Section III). On the other hand, down-regulation of oxidative phosphorylation rates may also help to increase starch accumulation in the chloroplast which then can be used under anaerobic conditions to fuel hydrogen production.[78]

Section III. Improving biohydrogen production

In its most basic sense photosynthesis can be considered to be the biological blueprint for the conversion of solar energy to chemical energy (fuel). Consequently photosynthesis is also central to the production of biofuels such as biohydrogen, bioethanol and biodiesel. This is important as ~66% of global energy is used as fuel and because almost all other solar technologies (*e.g.* photovoltaics and solar thermal) only convert solar energy to heat or electricity. Given the need to develop clean fuels for the future, biofuels are likely to become increasingly important. In this article we focus on the factors affecting the development of improved biohydrogen production systems.

To date, most biofuel production systems are still centered on traditional agriculture and forestry. These land-based systems are adapted to cope with constant environmental changes and stresses (light, temperature, water, nutrients). In contrast, the use of microbial photo-bioreactors for the production of biofuels such as biohydrogen is only beginning to be explored.[80] Although more technically challenging, microbial bioreactors can range in their simplest form from open ponds, stirred with paddle wheels up to more complex Biocoil systems[81] and fully enclosed bioreactors, illuminated by fibre optic type light sources.[82,83] Furthermore tubular photo-bioreactor systems have already been used for long-term outdoor hydrogen production using the cyanobacterium *Anabaena variabilis*.[84] Increased complexity is accompanied by increased reactor costs but has the advantage of providing more control over factors such as light, temperature, water and nutrients which is advantageous in terms of improved photon conversion efficiency. In addition, due to the increased ability to control environmental parameters, such bioreactors can be located in low value land, freeing up valuable arable land for food production. Central to the development of light driven hydrogen bioreactors, is not only a detailed understanding of the biochemistry underlying the process, but also the options available to control these reactions for optimal H_2 production efficiency.

Biodiversity of H_2 production

Towards the end of the 1930s Gaffron discovered that under certain conditions unicellular green algae are able to produce hydrogen during illumination.[85,86] A hydrogenase was found to be responsible for this reaction. Since then hydrogenases and indeed H_2 production have been found to be ubiquitous throughout the prokaryotic and eukaryotic kingdoms.[87] These hydrogenases can be divided into Ni–Fe, Fe and [FeS]-cluster

free Hmd types which exhibit a range of catalytic activities and O_2 sensitivities.[88,89] Improved biohydrogen production rates will clearly benefit from both the selection of a suitable phototroph and the engineering of its biochemical pathways.

The majority of microorganisms currently studied for hydrogen photoproduction were selected because of ease of cultivation (which is often consistent with slow growth rates), or as genetic model systems for studying metabolism or photosynthesis. Consequently these few organisms were not selected for optimal H_2 production. Prior studies from a relatively limited number of microbes indicate a great range in O_2 sensitivity and rates of H_2 photoproduction, differing by over two orders of magnitude.[90] There is a great untapped potential for discovery of new organisms possessing more efficient active hydrogenases that exhibit higher specific rates, greater O_2 tolerance, control of catalytic activity by reversible cofactor binding, and/or inducible control of gene expression levels.

Cyanobacteria comprise a large and diverse group of oxygenic photoautotrophic prokaryotes (at least 1500 species currently known) that are capable of growth in an enormous range of habitats, which vary in pH (3–11), temperature (−10 to 73 °C), and nutrients. Members of all five major taxonomic groups of cyanobacteria have been shown to contain hydrogenase genes; however, few hydrogenases have been biochemically characterized, and many widely divergent orders have been neglected completely.[91] Known hydrogen metabolism in cyanobacteria can directly involve three enzymes: nitrogenases, which produce H_2 concomitant with N_2 fixation; a membrane-bound uptake hydrogenase, which is co-expressed with nitrogenase under N_2-fixing conditions and which re-oxidizes the H_2 evolved by nitrogenase and delivers the electrons to photosynthetic and respiratory electron transport chains; and the bidirectional hydrogenase, which catalyzes both uptake and evolution of H_2. The latter two enzymes are both [NiFe]-hydrogenases in cyanobacteria.[91–94]

Physiological studies of cyanobacteria have identified over 15 H_2-producing strains, distributed among several families.[90,91] These include 3 filamentous heterocystous strains (N_2-fixing), 4 filamentous non-heterocystous strains, 4 unicellular strains and a unique marine strain containing Chl *d*. In the diazotrophic strains, H_2 is produced principally under N_2-fixing conditions by nitrogenase. Some non-heterocystous strains use temporal separation of carbon fixation/photosynthesis and nitrogen fixation/respiration to achieve the anaerobic (or microoxic) conditions needed for nitrogenase activity. However, in at least 9 strains, hydrogenase has been implicated in the initial light-stimulated burst of H_2 production in anaerobically adapted cultures grown in nitrate-containing media.[90] In one of these strains with low chlorophyll levels, *Oscillatoria* sp. Miami BG7, initial rates of light-driven H_2 evolution are 10–15-fold higher than in the others,[95] implying a significantly higher upper theoretical limit if conditions for activity-stabilization can be found or created.

The H_2-metabolizing pathways in eukaryotes are more diverse than in prokaryotes, with extremely wide variations in H_2 photoproduction capacity. Although many eukaryotic algae contain hydrogenases, the capacity for H_2 photoproduction has been found in species from only 30 genera of green algae (represented by at least 1050 species), two species of yellow–green algae, and one diatom.[90] Algal hydrogenases, like the *C. reinhardtii* [Fe]-hydrogenase HydA, are located in the chloroplast and can reach specific activities of up to ~1000 units (mg protein)$^{-1}$.[96,97] Such enzyme activities are reported to be 100-fold higher than those of cyanobacterial hydrogenases[98] and highlight the potential usefulness of green algae for the development of solar powered hydrogen production systems. In spite of their high activity, hydrogenases of green algae, which lack nitrogenase and their associated protection systems, are hypersensitive to O_2 inactivation. This limits their current utility for H_2 production, but also provides a strategy for controlling hydrogen production in the case of the accidental release of a genetically modified organism.

Pathways for biosolar H_2 production

Maximal H_2 photoproduction is limited by photon to H_2 conversion efficiencies. Three pathways have been observed for light-induced H_2 production from water in natural photosynthetic microbes. Fig. 4 illustrates two of these pathways and the competing processes that may occur within a prokaryote cell or algal chloroplast.[90] Studies are most detailed for *C. reinhardtii*, and pilot scale culturing studies on mutants are in progress at three institutions in the US (NREL, UC Berkeley, ORNL, see ref. 99).

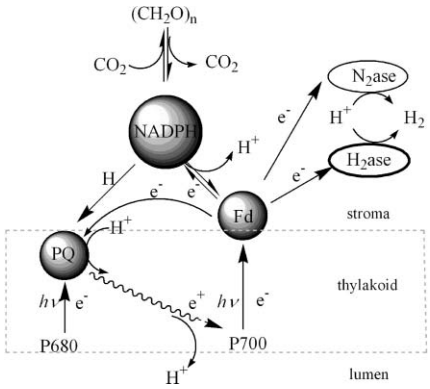

Fig. 4 Electron transport to nitrogenase and hydrogenase in photosynthetic microorganisms. A common pathway occurs in cyanobacteria (to nitrogenase) and green algae hydrogenase. Sites of ATP synthesis and hydrolysis are shown by wavy lines with upward and downward arrows, respectively. Light-driven reactions are denoted by *hv*. Adapted from ref. 90.

The first pathway, given by the overall stoichiometry of eqn (1), requires the two photosystems and complete electron transport chain to bring electrons from water to ferredoxin (Fd), the physiological electron donor to hydrogenase.

$$H_2O \rightarrow H_2 + \tfrac{1}{2}O_2 \qquad (1)$$

This pathway requires 4 quanta per H_2 evolved and usually occurs concurrently with O_2 evolution in a single temporal stage with no gas separation. The pathway occurs in some cyanobacteria, but is inefficient and occurs appreciably in green algae only under conditions of sulfur deprivation, which significantly reduces PSII activity.[100] If this pathway was able to operate at full PSII capacity, it would require an O_2-tolerant hydrogenase, a promoter that enables gene expression in the presence of elevated O_2 levels, and a hydrogenase assembly process that is O_2-insensitive.

The second pathway for H_2 formation involves electron and proton flow to the plastoquinone pool, from fermentation and oxidative carbon metabolism of photosynthetically stored carbon, mediated by a dehydrogenase, through PSI into ferredoxin and then to hydrogenase. The stoichiometry of this pathway is given in eqn (2a) and (2b).

$$H_2O + CO_2 \xrightarrow{light} [CH_2O]{-}\text{biomass} \qquad (2a)$$

$$\text{dark} + \text{anaerobiosis} \xrightarrow{-\text{oxygen}} \text{hydrogenase induction;}$$

$$\xrightarrow{light} H_2 + CO_2 \qquad (2b)$$

and requires 6 quanta/H_2 (assuming all electrons are ultimately derived from water). This pathway is most efficient in two temporally resolved stages, a photosynthetic growth stage followed by an anaerobic stage in which hydrogenase expression is induced and PSI uses light to pump "fixed" electrons derived from stored

reductants *via* the PQ pool to the potential needed to reduce ferredoxin, while the protons are pumped into the thylakoid lumen for use in ATP synthesis. The anaerobic stage requires O_2 to be substantially depleted (cyanobacteria) or virtually eliminated (green algae). The temporal separation of the H_2- and O_2-evolving steps is a major advantage for gas separation and safety.

Natural mechanisms used to lower intracellular O_2 are: consumption by elevated respiration, chemical reduction of O_2 by PSI (the Mehler reaction; Fig. 3—point 6) and temporary, reversible inactivation of PSII O_2 evolution activity. Suppression of O_2-induced inactivation of hydrogenase by attenuation of PSII activity is being implemented by a collaborative team of scientists working with funding from the DOE Hydrogen Program.[100–102] They have used sulfur deprivation of green algae to suppress the biosynthetic repair of an essential PSII subunit that is damaged during photoinhibition[103] and requires replacement for recovery of O_2 evolution. Current rates of continuous H_2 photoproduction reported by the NREL team using the S-depletion strategy yield around 2.2 ml H_2 h^{-1} l^{-1} culture,[101] while rates of 3.5 ml H_2 h^{-1} l^{-1} have been achieved using the *C. reinhardtii* mutant *Stm6*.[61] This strategy produces H_2 using both pathways, one and two. DCMU inhibition of PSII suggests that approximately 80% of the H_2 is reportedly being produced from water. The projected H_2 evolution rates expected using *C. reinhardtii* mutants that have truncated Chl antenna size[104] and collapsed proton gradient in the chloroplast (J. Lee and E. Greenbaum, ORNL unpublished) are in the range 70–100 ml h^{-1} l^{-1} culture (0.3–0.4 mmol h^{-1} l^{-1}) (M. Seibert, personal communication). This corresponds to about 5–10% of the maximum photosynthetic rate based on the light-saturated O_2 evolution rate to CO_2, or about 1–1.5% of the incident solar intensity into H_2 production. If this efficiency goal can be attained, it will be a major milestone in biosolar research.

Despite the importance of this development, H_2 production capacity is limited to the fact that permanent media repletion is necessary and anaerobiosis has to be lifted for some time to allow cell recovery. Ideally, a commercial H_2 production system would be based upon a continuous process in which PSII and HydA function simultaneously to drive H_2 production from water such as described in ref. 105.

The third pathway to produce H_2 from water, eqn (3), is much like the second pathway, but utilizes nitrogenase in cyanobacteria. It is more energy intensive as it relies on the availability of a source of electrons and protons and energy from ATP, which are all derived from photosynthesis. This process requires 28 quanta for ATP synthesis based on the ATP requirement for nitrogenase and 14 quanta for electrons (7 NADPH) per H_2 molecule produced. Proton reduction in eqn (3c) is obligatorily coupled to N_2 reduction in eqn (3b) in nitrogenases. This calculation uses the fact that the yield of H_2 is roughly one quarter that of NH_3 in N_2-saturated samples. However, nitrogenase catalyzes proton reduction in the absence of N_2 using less ATP/H_2, eqn (3c)

$$H_2O \rightarrow \tfrac{1}{2}\, O_2 + 2H^+ + 2e^- \text{ [as NADPH + ATP]} \qquad (3a)$$

$$N_2 + 6H^+ + 6e^- \text{ (12ATP)} \rightarrow 2NH_3 \text{ (12ADP + Pi)} \qquad (3b)$$

$$2H^+ + 2e^- \text{ (4ATP)} \rightarrow H_2 \text{ (4ADP + Pi)} \qquad (3c)$$

The most efficient use of photons is accomplished in the first pathway, eqn (1), but results in the co-evolution of H_2 and O_2 gases which are technically challenging to separate, economically unfeasible and a safety hazard as mixture. The third pathway eqn (3) requires the largest number of photons, occurs in a minority of cells (non-vegetative) and thus is viewed as economically impractical. The favored pathway is therefore currently the indirect two stage pathway outlined in eqn (2).

The quantum efficiency of photosynthesis

Photosynthetic organisms have a number of mechanisms by which they adapt to the varying environmental conditions they face every day and which impacts their quantum efficiency and utility for biohydrogen production.

For example, green algae have evolved genetic strategies to assemble large light harvesting antenna complexes (LHCI and LHCII, Fig. 3—point 1) that give them a competitive advantage in terms of light capture. However the downside of this strategy is that under high irradiance, up to 80% of the absorbed photons can be wasted,[104] limiting their use for solar powered biohydrogen production. Energy is dissipated from the antenna system by fluorescence, or as heat (see xanthophyll cycle). In contrast to the antenna systems, the efficiencies of the light reactions are high (typically 95% of visible absorbed photons are converted in open reaction centers to separated charges at low irradiance). The maximum energy conversion efficiency into chemical energy of the primary photoproducts is ~50% using red photons. A different story emerges when one calculates the amount of captured solar energy that is actually stored as chemical energy in the form of carbohydrate (eqn (4)).

$$CO_2 + H_2O \rightarrow CH_2O + O_2 \qquad (4)$$

9–10 photons are required for this reaction (and other cellular processes that draw upon the light reactions for energy (ΔpH and $\Delta\Psi$) and reducing power PQH_2 and NADPH), under the most favourable conditions, with low irradiation flux. Using red light of 680 nm wavelength it can be calculated that an energy input of 1760 kJ is required per mole of O_2 produced. This is approximately four times more than the standard free energy change of eqn (4) (+467 kJ mol^{-1}), and equates to a maximum efficiency of 27% of the absorbed light at this peak wavelength.[22] The 27% efficiency quoted for 700 nm reduces by 700/400 for 400 nm illumination, to 15%. The mean optimal efficiency between 400 nm and 700 nm thus reduces to *ca.* 21% of the absorbed light. This is equal to a conversion efficiency of 9% of the total solar spectrum, assuming only the spectrum between 400–700 nm is used, furthermore, this is comparable to most energy conversion systems (*e.g.* commercial multi-junction Silicon photovoltaic cells which are rated at 12%, but absorb above 1.1 eV from the visible through to the near infrared region). Much of this energy is subsequently used to support cellular processes with only a fraction being stored as biomass. Typically conversion efficiencies to biomass in the leaf are of the order of 5% under aerobic conditions.[22] By reducing extracellular O_2 concentrations to inhibit photorespiration, quantum efficiencies can be raised from ~5% to ~10%.[22] In subsequent sections the mechanisms involved in further energy loss are discussed as the light flux as well as the competition for charge transfer between pathways increases, starting from the point of light capture in the antenna systems of PSI and PSII.

The main photochemical challenges to achieving efficient and sustainable production of biohydrogen from water by oxygenic phototrophs can be expressed as a product of three types of independent terms involving light energy transfer from the antenna, photochemistry in the reaction centers, and competition for electrons from ferredoxin and protons in the stroma. Eqn (5) defines the quantum yield of hydrogenase-dependent biosolar H_2 production, in terms of the incident solar spectrum, at wavelengths that are absorbed by, and result in, photochemical turnover of the photosystems. Similar expressions define the quantum yield for the other biosolar chemical energy yields, but are not presented.

$$QY(H_2) = \Lambda_i\,(1 - \Lambda_{NPQ})\Phi_1\Phi_2\,(1 - X_C)\,(1 - X_A)\,(1 - X_N)(1 - X_O)(1 - \Omega_2) \qquad (5)$$

The following factors determine the magnitude of the quantum yield $QY(H_2)$.

(1) A_i is the fraction of the incident solar spectrum in the active interval ($\sim$380 nm $< \lambda <$ 740 nm) that is absorbed by the photosynthetic apparatus and available for photochemistry. This spectral range accounts for about 43% of the incident solar energy at the Earth's surface (AM1.5, see Section I).

(2) A_{NPQ} is the fraction of absorbed light in the antenna complex that is lost due to non-photochemical quenching (NPQ), *e.g.*, conversion of excited states in the antenna into heat (see xanthophyll cycle). NPQ essentially acts as a light switch by turning off energy transfer into the reaction centers, thereby preventing photochemistry that could result in oxidative damage to PSII in particular.

(3) The photochemical quantum yield of primary charge separation in both PSI (Φ_1) and PSII (Φ_2) reaction centers. Losses in the reaction centers such as charge recombination (leading to heat) or fluorescence need to be minimized at all solar fluxes, including both daily and seasonal variations.

(4) Ω_2 is the fraction of PSII centers that become photoinactivated by visible and UV solar irradiation by the processes of photoinhibition.[106–108] This process has at least two different mechanisms. Turnover photoinhibition occurs during aberrant photochemistry within PSII centers that typically lack a functional Mn_4-cluster. Also UV-induced photoinhibition can occur when the Mn_4-cluster directly absorbs light.

(5) X is the branching fraction of electron flux from ferredoxin into competing pathways: CO_2 fixation and photorespiration (X_C), cyclic electron flow around PSI resulting in ATP generation (X_A), PSI-dependent O_2 reduction, the Mehler reaction (X_O), and possibly dinitrogen fixation if a nitrogen source for growth is unavailable (X_N). The remaining fractional flux of electrons into ferredoxin is available for direct (forward) hydrogenase-dependent biosolar H_2 production. The flux into carbon is not lost. Redirecting hydrogen atoms stored in biomass into H_2 production *via* alternate pathways involving dehydrogenases that equilibrate with the plastoquinone pool or with ferredoxin represents a significant source of H_2 production capacity (eqn (2)).

The light intensity dependence of most of the terms in eqn (5) is complex and light adaptation by the organisms produce differences that change with age of the culture and the physical geometry and flow properties of the reactor. We can expect that strategies that redirect the electron and proton fluxes produced both by water oxidation and by oxidation of stored carbon (such as glycogen) into hydrogenase-dependent H_2 production will improve $QY(H_2)$. However, these fluxes must be coordinated to permit temporal separation of H_2 and O_2 gases (for economic feasibility). They must also be reversible to ensure that the flux changes can be balanced with the physiological needs of the organism for energy, repair and growth. In this light we discuss possible bioengineering strategies for improving biosolar H_2 yield.

Approaches to improve biosolar efficiency

$QY(H_2)$ increases to a maximum as a function of the solar intensity and decreases at higher intensities owing to several of the factors in eqn (5). Peak yield occurs at relatively low solar fluxes (*ca.* 50 W m^{-2}) for most native strains of microalgae and cyanobacteria during stationary phase growth.[109] This intensity is about 10-fold lower than peak solar intensities at sea level (400–600 W m^{-2}). The additional light is either wasted as heat, fluorescence, reflected or causes aberrant photochemistry leading to photoinhibition of PSII. These inefficiencies create major limitations with multiple opportunities for improvement. The best efficiencies reported to date for sustainable (multiple days) photobiological hydrogen production from water and cellular glycogen stored during autotrophic photosynthesis by cyanobacteria[95,110–113] and microalgae[114–116] fall in the range of 1–20% of the maximum CO_2-fixation rates in terms of the photosynthetically absorbed spectrum of light, *e.g.* did not exceed 2–3 mmol (g Chl)$^{-1}$ h^{-1}. Short term initial rates are much higher and reach values of 100–300 mmol H_2 (g Chl)$^{-1}$ h^{-1}, approaching the rate of hydrogenase activity *in vitro*. These values are roughly halved if one normalizes to the total solar spectrum, since the incident solar wavelengths with energy above the water splitting threshold ($E_0 = 1.23$ V *vs.* standard hydrogen electrode potential) comprises about 44% of the total solar output.

Specific areas for the improvements of photobiological H_2 production are highlighted in the following points.

(1) Antenna size. In microalgae and cyanobacteria, the achievable photosynthetic productivity and light utilization efficiency are the single most important factors in the determination of utility for biohydrogen production, metabolic products and arising products as hydrogen, oils and ethanol. The use of natural populations of immobilized microbial cultures which adhere to a surface or are embedded in alginate beads enables spatial adaptation to the light intensity gradient through the sample and thus natural antenna size adjustment will tend to optimize photosynthesis (the "leaf effect").[117] This contrasts with stirred reactors that create more homogenous cultures in which light saturation occurs at lower intensities. The trade-off between lower biomass accumulation for exolithic microbes that grow on surfaces, *versus* more optically dense cultures that grow in liquid media needs to be evaluated.

Improved hydrogen production yield may be achieved by engineering a reduced antenna size.[118] In the case of the microalgae *C. reinhardtii*, genetic deletion of a gene encoding a factor that controls the expression of the LHC antenna system has increased the light saturation threshold.[104] A reduction of 4–5 fold in cross section is theoretically possible by complete deletion of the LHC antenna system which better matches the solar light saturation. Additional improvement is also possible owing to reduction in non-photochemical quenching in the antenna which further reduces loss from heat and fluorescence.[42] Smaller antenna systems would be expected to allow better light penetration into the bioreactor, increasing the overall process efficiency. A third benefit might under certain situations be reduced O_2 evolution, resulting in down regulation of photorespiration, an increase in the efficiency of the carboxylase activity of rubisco and an increase in the hydrogenase activity. Comparative studies of dark and light adapted algae (large and small antennae, respectively) have shown that the photon conversion efficiency of the system could be increased by a factor of $\sim$3.[42]

An additional area of improvement on an engineering level would be the development of gasification systems (*e.g.* to produce H_2) with improved efficiency. Using biomass in a conventional way (wood, sugar cane, agriculture waste products, *etc.*) as a gasification source has been studied extensively to evaluate costs and efficiency.[119] However systems are less developed for the gasification of wet algal biomass and are likely to have a lower efficiency.[120]

(2) Oxygen sensitivity of hydrogenases. The high oxygen sensitivity of algal hydrogenases is one of the major limiting factors for a successful permanent sunlight-driven hydrogen production. Several approaches have been pursued, though with limited success to date, to engineer an O_2-insensitive hydrogenase in *C. reinhardtii.* An alternative approach to avoid hydrogenase inhibition during active photosynthesis would include the reduction of intracellular oxygen concentration during light cultivation. This could be achieved either by optimizing the photon conversion efficiency rates under lower light conditions (which would automatically reduce oxygen evolution rates) and/or by increasing respiratory activities in the light. The latter feature is realized in some cyanobacteria derived from alkaline soda lakes. These cyanobacteria have high photosynthetic growth rates, store reduced carbon in high yield, and require a high ATP production rate to maintain osmotic balance.[121] A third approach is to use phototrophs for which

O_2 production by the water-oxidizing complex can be reversibly inactivated by removal of the small molecule cofactor, carbonate. To date, no reports of this approach have been described.

(3) Hydrogenase expression. The reversible hydrogenase content in anaerobically adapted algal cells is estimated as being less than 1% of the soluble protein,[97] which corresponds to a stoichiometry of one hydrogenase per >10 000 Chl, or one hydrogenase per >10 PSI units. Therefore, increasing the amount of hydrogenase and/or its synthesis rate in chosen organisms may lead to substantially improved, longer-term H_2 production rates.[90]

(4) High H_2 production mutants. An alternative strategy to improve biosolar hydrogen production is the systematic forward genetic screening for mutants with an increased ability to produce high rates of H_2. This can be done by identifying strains with defects in specific metabolic pathways that are relevant for hydrogen production rates such as starch accumulation[68] or improved e^-/H^+ supply for the hydrogenase.[122] The rate of e^- supply to the hydrogenase HydA in *C. reinhardtii* is closely coupled to the photon conversion efficiency rates of the photosynthetic electron transport chain. Improvements of photosynthetic efficiency include the inhibition of cyclic electron flow. A blockage of cyclic electron transfer around PSI in algae avoids e^- competition with hydrogenase.[122] This is the case in mutants with a perturbed state 1 to state 2 transition. *C. reinhardtii* is the ideal candidate to identify such mutants. Its suitability for large scale screening in forward genetic approaches on agar plates was already shown,[123] the genome is fully sequenced and molecular tools for further cloning strategies are established. By following this forward genetics strategy, we succeeded in identifying the *C. reinhardtii* strain *Stm6* with a hydrogen evolution capacity 5 (long term) to 13 (short term, flash induced) times higher than wild-type.[61,122] *Stm6* cells are inhibited in cyclic electron flow under anaerobic conditions and show a perturbed ability to use their mitochondria to consume cellular reducing equivalent in the light.[78,122] Our current experiments yield 500 ml H_2 per litre algal culture over the course of ~10 days.

(5) Quantum efficiency. Species or strains that have no or minimal PSII back reactions, such as achieved by microbes with large PQ pool capacity and strong proton buffer capacity of the thylakoid lumen, have a larger Φ_2 (effectively have larger capacitors for electron and proton charge storage). Φ_2 is directly proportional to the ratio of PSII variable to total fluorescence emission (Fv/Fm).[124] This ratio when measured in dark-adapted organisms typically decreases in the series: plants > microalgae > cyanobacteria, although with substantial overlap and many unique individual species across the oxygenic phototrophs. The Fv/Fm series parallels the evolutionary sequence of these organisms and reflects several possible sources for improving the efficiency. An important measure of practical utility is the photochemical quantum efficiency for PSII charge separation attainable at full solar flux. At full solar flux Fv/Fm decreases relative to its dark adapted level, owing to losses from increased probability of charge recombination in PSII as the proton/electron circuits back up. Phototrophs reduce electron constipation at the PQ pool by biosynthesis of variable amounts of PQ molecules and by biosynthesis of a 2–6-fold stoichiometric excess of PSI centers relative to PSII centers, thereby using several PSI centers to draw electrons from the PQ pool. Some strains of cyanobacteria have 3–5-fold larger PQ pool capacity than higher plants or algae (typically 6–8 PQ/PQH_2 molecules per electron transport chain).[125] Nevertheless, oxidation of the primary plastoquinone electron acceptor Q_A by the PQ pool (mediated by Q_B), is typically the rate-limiting step in most phototrophs.[124]

As an example, the upper limiting rate of turnover of the PSII water-oxidizing complex in intact cells of a few cyanobacteria and algae has been determined recently.[125] The results show that alkalophilic cyanobacteria that grow at high carbonate concentrations exhibit the highest dark adapted Fv/Fm ratio (more typical of microalgae) and can split water at the fastest rates yet recorded for any oxygenic phototroph (5-fold higher turnover rate at full solar flux). The origin of this kinetic advantage is unknown, but may be due to efficient removal of protons by (bi)carbonate and the presence of a larger plastoquinone pool than found in other oxygenic phototrophs. Clearly, choosing phototrophs that produce the largest Fv/Fm under ambient solar flux conditions is an important criterion in selecting candidates for optimal H_2 production. Should proton release during water oxidation by PSII-WOC become rate-limiting, it may be possible to improve the kinetics by modifications or exchange of the extrinsic PSII subunits found in plants and microalgae (psb-O, -P, and -Q) *versus* cyanobacteria (psb-O, -U and -V). The comparative kinetic data for PSII turnover rates at maximum solar flux are not known in general to our knowledge, and so this possibility remains untested.

(6) Photoinhibition. Some cyanobacteria exposed to full sunlight contain non-photosynthetic photoprotective pigments (scytonemins found in the extracellular polysaccharide sheath) which are known to suppress UV-induced photoinhibition.[126] Selection of cyanobacteria having such photoprotective pigments should benefit the stability of cultures used for biosolar energy production in some locations.

(7) Organelles and auto-reduction. A greater degree of protection against intracellular reductants is afforded the PSII-WOC by compartmentation within internal organelles such as the chloroplast (none in cyanobacteria). Cyanobacterial PSII-WOC centers are more susceptible to environmental and internal reductants owing to the fewer compartments. Many uncharged small molecule reductants are known which penetrate the various membrane layers into the lumen where they release Mn^{2+} by reduction of the Mn_4-cluster. The susceptibility to disassembly of the Mn_4-cluster by exposure to H_2 gas has not been studied to our knowledge, but is potentially a limiting factor for the cogeneration of O_2 and H_2 gases. For this reason and overriding cost issues for gas separation and safety, temporal separation of the O_2 evolution and H_2 evolution stages is mandatory.

(8) Mn_4Ca-cluster assembly[127]. All oxygenic phototrophs use the same class of water-oxidizing enzymes to achieve the oxidation of water. No consequential variation of the catalytic inorganic core of this enzyme, $Mn_4Ca_1O_xCl_{1-2}(HCO_3)_y$, has been identified to date among the dozen or so available examples, nor the active site region of the protein residues that make up its binding site (primarily *psbA* gene product in >100 published sequences). The Mn_4Ca-core disassembles during extended dark periods in some cyanobacteria (*Gloeobacter violaceus*, *etc.*) and requires low light flux to reconstitute *in vivo* (photoactivation). If this process is blocked by insufficient access to Mn^{2+}, subsequent illumination leads to rapid photoinhibition. By ensuring adequate availability of intracellular Mn^{2+}, a proper Mn^{2+}/Ca^{2+} ratio (1/500) and adequate internal supply of (bi)carbonate, PSII turnover-photoinhibition can be suppressed. The availability of internal stores of (bi)carbonate is a key factor that accelerates the kinetics and increases the yield of the photo-assembly of the Mn_4-cluster from apo-WOC-PSII[128] and in maintaining a stable cluster once assembled in the holo-enzyme.[129]

Conclusion

Photosynthesis is probably still an undervalued blueprint for future solar energy capture and conversion technologies. There appear to be three main reasons for this. First, fossil fuels are still in relatively cheap supply. Second, there is a common

misconception that the process of photosynthesis is inefficient. Third, we are only beginning to realise what the current use rate of fossil fuels and their depletion mean for the future, both in terms of lifestyle (upon their depletion) and ecological stability. In fact the effects of fossil fuel combustion (*e.g.* global warming) may still be underestimated. On the day of completion of this article a new report[18] suggested that up until 1990 we were in part shielded from the extremes of global warming by aerosol and particulate pollutants that result in "global dimming", and that a more rapid increase in global warming might well be possible. Such findings together with many others[3,14,16,17] collectively suggest that CO_2 levels should be kept as close to 450 ppm or preferably below to maintain ecological stability. If this prediction is correct we may be faced with the challenge of installing systems capable of producing energy free of CO_2 emissions at a level approximately equivalent to the total current global energy demand (13 TW $year^{-1}$) within the next 20 years.[3] This is clearly a very short timescale and likely due to the fact that current generations are the first to understand the effects of global warming and perhaps the last who can do anything meaningful about it in terms of ecological protection.

That said, photosynthesis can perhaps provide the blueprint for a solution to the problem. Solar energy is in abundant supply, providing 13 500 times the energy that we currently use globally. To put this abundance into the context of our current global energy needs, it is of note that by covering an area equivalent to ~5% of the Sahara desert our entire current energy needs could be met with existing commercial systems rated at 12% efficiency. However, solar energy is diffuse and solar energy capture technologies are still relatively expensive, making efficiency improvement and the development of market ready systems important areas of research.

Photosynthesis has a particular advantage compared to almost all other solar technologies, by directly synthesizing high energy molecules (fuels), instead of electricity. The importance of this lies in the fact that currently approximately two thirds of our energy is used in the form of fuel and one third as electricity.

This review has attempted to summarize a very wide range of information related to solar powered energy capture and conversion to chemical energy (fuel) by photosynthesis. Section I shows that the need to develop clean fuels for the future is of critical importance for a sustainable future and that solar energy conversion technologies have enormous potential to harvest the huge solar energy resource available to us. Section II shows that the common misconception that the photosynthetic process is intrinsically inefficient is far from true. On the contrary, the photochemistry underpinning the process is highly efficient (~50%). The lower efficiencies observed at the level of biomass production (*e.g.* ~1–8%) are in many cases due to necessity of phototrophic organisms to adapt to a wide range of environmental conditions (light, CO_2, temperature, nutrient). It is therefore likely that the most major gains in efficiency can be anticipated when the need for environment adaptation is reduced, both by editing out the genetic flexibility of natural phototrophs and by optimization of design of algal bioreactors. A quantitative model for how the natural photosynthetic pathways contribute to energy flow and storage is presented, with examples of where genetic engineering and natural selection have been used to alter the yield of biosolar H_2 production.

Three main areas for improvement have been identified, realized or proposed. First, engineering a reduced antenna size to suppress fluorescence and heat dissipation that can result in the loss of ~80% of the captured light energy, and shift the photosynthesis light saturation curve to better match the incident solar intensity. Second, choosing or engineering phototrophs with a large plastoquinone (PQ) pool (to slow the PSII back reaction, to accelerate the electron flux to PSI, and to oxidize reducing equivalents stored during CO_2 fixation). Third, redirecting the flux of electrons through PSI and ferredoxin into hydrogenase by downregulating of competing pathways.

In summary, given the complex interplay between the biochemical pathways involved in the storage of light energy as biohydrogen much remains to be discovered. In our opinion the development of biological and bio-inspired systems for solar energy capture and conversion could play a major role in supplying environmentally clean fuels, provided adequate resources are applied to the significant remaining technological challenges.

Abbreviations

AM: air mass spectrum; AOX: alternative oxidase; Chl: chlorophyll; Cyt: cytochrome; e^-: electron; Fd: ferredoxin; FNR: ferredoxin–$NADP^+$ oxidoreductase; HydA: hydrogenase; LHC: light harvesting complex; NPQ: non-photochemical quenching; PC: plastocyanin; PCE: photon conversion efficiency; 3-PGA: 3-phosphoglycerate; Pi: phosphate; PS: photosystem; Q: quinone; qE: energy dependent quenching of chlorophyll fluorescence; rubisco: ribulose bisphosphate carboxylase/oxygenase; RuBP: ribulose-1,5-bisphosphate; TW: terawatt; WOC: water-oxidizing complex.

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