

# Photobiological production of hydrogen gas as a biofuel

James B McKinlay and Caroline S Harwood

Solar energy can be converted into chemical energy in the form of hydrogen gas using oxygenic and anoxygenic photosynthetic microbes. Laboratory-scale measurements suggest that photobiological hydrogen production rates could yield more energy than current crop-based biofuel productivities. Major challenges, such as inhibitory amounts of oxygen produced during oxygenic photosynthesis and inhibition of H<sub>2</sub>-producing nitrogenase by ammonia, are being overcome through genetic engineering. Further advances are expected as the metabolic and regulatory aspects behind photobiological hydrogen production are revealed. Genetic engineering, coculturing, and bioreactor designs making use of immobilized cells have the potential to increase conversion efficiencies of light energy to H<sub>2</sub> and to decrease the land area needed for photobiological H<sub>2</sub> production.

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## Introduction

In 2006, global energy consumption was 472 quadrillion BTU [1], which is equivalent to enough crude oil (12.9 × 10<sup>12</sup> L) to cover an area the size of Luxembourg, five meters deep. The global energy consumption rate is projected to increase by 44% by 2030 [1]. Rising energy demands have thus far been met by finite fossil fuels, leading to excessive CO<sub>2</sub> emissions that threaten the global processes that sustain human life [2]. Renewable, environmentally sensitive fuels are needed to augment, and ultimately replace, fossil fuels.

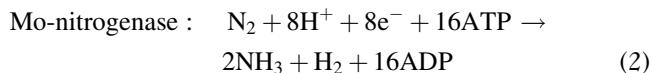
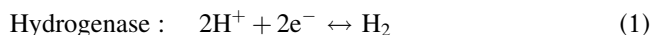
H<sub>2</sub> is a clean-burning alternative to fossil fuels. Its energy content per unit mass is higher than any other conventional fuel, except for nuclear power (<http://hydrogen.pnl.gov>). Widespread H<sub>2</sub> utilization will require technological advances in storage (due to its low energy

content per unit volume), fuel cell electrodes, and sustainable production. Although ~90% of the H<sub>2</sub> we use is currently derived from fossil fuels [3], it can be produced by a variety of sustainable processes including biologically [4].

Light energy can be converted into H<sub>2</sub> chemical energy using photosynthetic microbes that are either oxygenic (O<sub>2</sub>-producing), obtaining electrons from water or anoxygenic, obtaining electrons from organic or inorganic substrates other than water. By convention it is assumed that free protons in water are combined with donated electrons to make H<sub>2</sub>, although in practice protons can come along with electrons that are donated from organic or inorganic substrates. H<sub>2</sub> can also be produced in the dark by fermentative microbes, which is reviewed elsewhere [4,5,6]. Herein we describe the features, advantages, and challenges of photobiological H<sub>2</sub> production, emphasizing the advances within the last two years. Further details on photo-H<sub>2</sub> production are reviewed elsewhere [3,7,8,9,10,11,12].

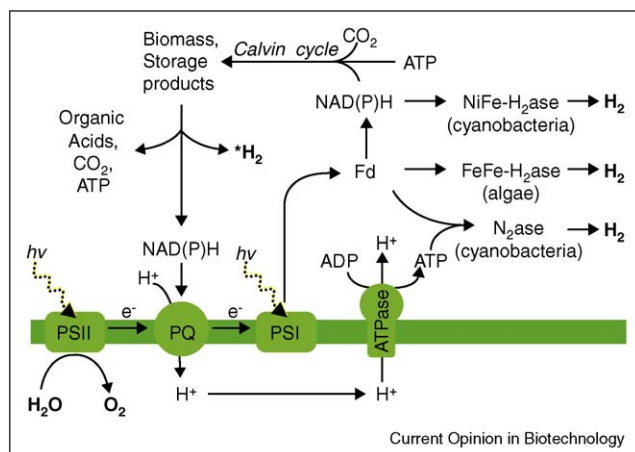
## Oxygenic photo-H<sub>2</sub> production

Algae and cyanobacteria grow by using oxygenic photosynthesis. Electrons are obtained from water using photosystem II, liberating O<sub>2</sub>. Electrons are transferred to photosystem I and eventually used by the Calvin cycle to fix CO<sub>2</sub> into biosynthetic intermediates and storage compounds (Figure 1). In the dark, storage compounds are respired for energy. H<sub>2</sub> is produced via hydrogenase (Eq. (1)) in both algae and cyanobacteria (Figure 1), though many cyanobacteria can also use nitrogenase (Eq. (2)). Fe–Fe hydrogenase, used by algae and some bacteria, has a high specific activity (e.g. 1800 μmol mg protein<sup>−1</sup> min<sup>−1</sup> [13]) whereas Ni–Fe hydrogenase, used by cyanobacteria, has a lower specific activity (e.g. 67 μmol mg protein<sup>−1</sup> min<sup>−1</sup> [14]). Both hydrogenases are reversible and are thus sensitive to product inhibition from H<sub>2</sub> [15].



Oxygenic photo-H<sub>2</sub> production is attractive because, first, water is the electron source, so greenhouse gases are not emitted; second, water is generally available and inexpensive and third, CO<sub>2</sub>, a greenhouse gas, is consumed. However, a central challenge in oxygenic photo-H<sub>2</sub> production is that hydrogenase and nitrogenase are inactivated by O<sub>2</sub>. The inhibitory effects of O<sub>2</sub> have largely prevented the ideal production of H<sub>2</sub> by direct photolysis

Figure 1



H<sub>2</sub> production by cyanobacteria and algae. Water (bold) is oxidized to O<sub>2</sub> by photosystem II (PSII) and electrons are transferred to photosystem I (PSI) via the plastoquinone pool (PQ). Photosystem I transfers electrons to ferredoxin (Fd) which can donate electrons to FeFe-hydrogenase (H<sub>2</sub>ase) in algae or to nitrogenase (N<sub>2</sub>ase) in some cyanobacteria. Fd electrons can also be transferred to NAD(P)<sup>+</sup> by a Fd oxidoreductase. NAD(P)H can donate electrons to NiFe-hydrogenase in cyanobacteria. The above are all mechanisms of direct photolysis. NAD(P)H is also used to fix CO<sub>2</sub> to biosynthetic precursors and storage compounds via the Calvin cycle. Storage compounds are oxidized to lower O<sub>2</sub> concentrations through mitochondrial respiration, allowing H<sub>2</sub> production to proceed. \*Storage compounds can also be fermented to provide electrons for H<sub>2</sub> production (indirect photolysis). In cyanobacteria, NAD(P)H from fermentation can donate electrons directly to NiFe-hydrogenase. In algae, NAD(P)H donates electrons to PSI to be energized for use by Fd-utilizing FeFe-hydrogenase.

(electrons from water are transferred directly from photosynthesis to hydrogenase). There have been attempts to decrease hydrogenase O<sub>2</sub>-sensitivity (reviewed by [4]), but most research has turned to indirect photolysis, where H<sub>2</sub> production is spatially or temporally separated from photosynthesis [7,12]. Spatial separation typically refers to production of H<sub>2</sub> by nitrogenase in specialized cyanobacterial cells called heterocysts, which maintain low O<sub>2</sub> concentrations [7]. Temporal separation often refers to anaerobic fermentation of photosynthetically accumulated storage compounds to H<sub>2</sub> (e.g. induced by sparging with argon) either in the dark or in the light with cells that have impaired O<sub>2</sub>-evolving photosystem II activity. One of the most intensively studied forms of oxygenic photo-H<sub>2</sub> production is to grow the eukaryotic alga *Chlamydomonas reinhardtii* photosynthetically and then switch the cells into a sulfur-limiting medium to initiate H<sub>2</sub> production in light [11]. Sulfur deprivation decreases photosystem II activity, but mitochondrial respiration proceeds at a high rate, thereby maintaining low O<sub>2</sub> concentrations and allowing for H<sub>2</sub> production. Sulfur deprivation typically results in a few days of H<sub>2</sub> production in batch cultures, or for several months using a semi-continuous process [16].

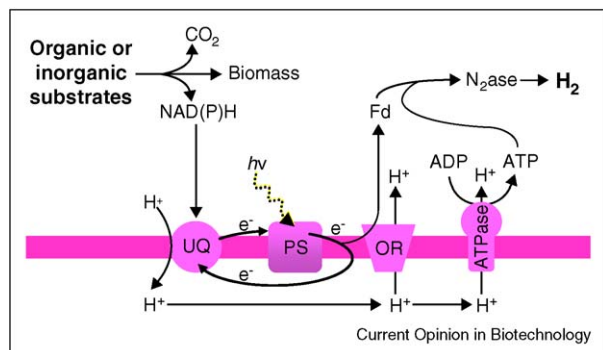
The *C. reinhardtii* transcriptional and metabolic responses to sulfur deprivation have been characterized in batch cultures [17,18]. Shortly after sulfur deprivation, O<sub>2</sub> consumption overtakes O<sub>2</sub> production, starch and triacylglycerides accumulate, photosystem II remodels, protein degradation begins, and the CO<sub>2</sub>-fixing Calvin cycle is downregulated in favor of fermentative metabolism. O<sub>2</sub> is usually depleted within 24 hours and the H<sub>2</sub> production rate increases, degradation of storage compounds begins, and fermentation products accumulate. After 48–70 hours, the H<sub>2</sub> production rate declines to zero, likely due to the accumulation of fermentation products and/or prolonged sulfur deprivation, since ample storage material is left over that could otherwise be used for H<sub>2</sub> production [18]. Accumulation of fermentation products suggests that the algae may produce H<sub>2</sub> to slow the onset of acidosis [18]. A similar transcriptional response was observed for the sulfur-deprived H<sub>2</sub>-producing cyanobacterium, *Synechocystis*, in which genes for photosystem II and the Calvin cycle were repressed but not those involved in O<sub>2</sub> consumption [19].

Metabolic engineering has brought further insights into the physiology of oxygenic photo-H<sub>2</sub> production and has resulted in useful mutants. Although oxidation of starch, triacylglycerides, and protein can contribute to H<sub>2</sub> production, the water-splitting activity of photosystem II alone can drive H<sub>2</sub> production (direct photolysis). A *C. reinhardtii* Rubisco mutant, incapable of Calvin cycle flux and starch accumulation, was recently shown to produce H<sub>2</sub> but not if photosystem II was completely inhibited [20]. This Rubisco mutant had an additional favorable trait of low photosystem II activity, such that it produced H<sub>2</sub> in the presence of sulfur, however at a lower rate than the sulfur-deprived wild type [20,21]. A rapid screening assay was developed to obtain other mutants with low rates of O<sub>2</sub> evolution [21]. Disrupting Calvin cycle flux in one of these mutants resulted in H<sub>2</sub> production in the presence of sulfur at twice the rate of the sulfur-deprived wild type [21]. Unfortunately, without the CO<sub>2</sub>-fixing Calvin cycle *C. reinhardtii* must be grown photoheterotrophically, losing the advantage of CO<sub>2</sub> removal. A genetic switch has therefore been proposed for future use to disrupt Calvin cycle flux after a period of autotrophic growth [20]. Increased H<sub>2</sub> production in the absence of Calvin cycle flux suggests that H<sub>2</sub> production serves to maintain redox balance when the CO<sub>2</sub>-fixing Calvin cycle cannot play this role [21].

### Anoxygenic photo-H<sub>2</sub> production

Anoxygenic photosynthetic bacteria obtain electrons from substrates other than water (Figure 2). Therefore, O<sub>2</sub> is not produced and cannot inhibit H<sub>2</sub>-producing enzymes. The purple nonsulfur bacteria (PNSB) are the most intensively studied anoxygenic phototrophs that produce H<sub>2</sub>. PNSB can acquire electrons from fermentation products found in agricultural and food waste (e.g.

Figure 2

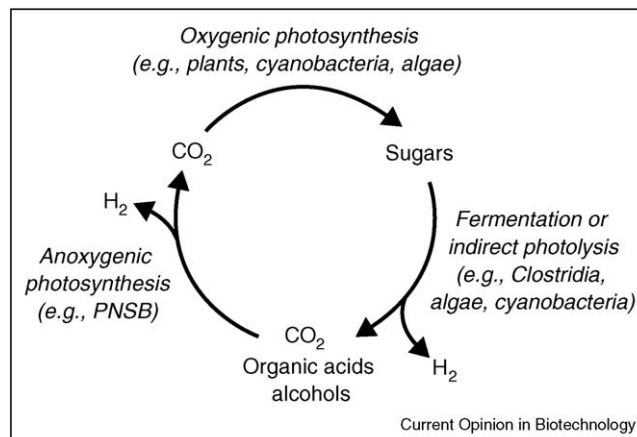


H<sub>2</sub> production by purple nonsulfur bacteria. Organic or inorganic compounds (bold) are oxidized to CO<sub>2</sub> and biomass (for inorganic substrates, CO<sub>2</sub> would be fixed into biomass). Electrons are transferred via ubiquinone (UQ) to the photosystem (PS) where they are energized by light. Electrons are repeatedly energized and cycled through the photosynthetic electron transport chain to produce a proton gradient. Energy from the proton gradient is used to transfer electrons from the photosynthetic electron transport chain to ferredoxin via oxidoreductases (OR). The proton gradient is also used to generate ATP. Ferredoxin and ATP are then used to generate H<sub>2</sub> via nitrogenase (N<sub>2</sub>ase).

acetate and butyrate), and some PNSB can also use sugars. One PNSB, *Rhodospseudomonas palustris* can use aromatic compounds (e.g. lignin monomers). PNSB oxidize organic substrates completely to biomass, H<sub>2</sub>, and CO<sub>2</sub>, and thus near-theoretical maximum H<sub>2</sub> yields (mole H<sub>2</sub> per mole substrate) are possible where nongrowing cells are used as biocatalysts. Since the organic substrates were originally derived from CO<sub>2</sub> fixed by green plants, anoxygenic photo-H<sub>2</sub> production is carbon neutral (Figure 3). Many PNSB can also oxidize inorganic substrates such as S<sub>2</sub>O<sub>3</sub><sup>2-</sup>, H<sub>2</sub>S, or Fe<sup>2+</sup> to obtain electrons for H<sub>2</sub> production [22,23].

PNSB produce H<sub>2</sub> via nitrogenase, which is better known for converting N<sub>2</sub> to NH<sub>3</sub>. H<sub>2</sub> is an obligate product of the nitrogenase reaction (Eq. (3), Figure 2). In the absence of N<sub>2</sub>, nitrogenase acts as an ATP-powered hydrogenase, producing H<sub>2</sub> exclusively, without inhibitory feedback (Eq. (4)). The ATP requirement is not a problem since a single electron can be repeatedly energized through cyclic photophosphorylation to maintain a H<sup>+</sup> gradient and thereby ATP levels. H<sub>2</sub> production via nitrogenase has a specific activity an order of magnitude lower than Ni-Fe hydrogenase (e.g. 1.3 and 2.4 μmol mg protein<sup>-1</sup> min<sup>-1</sup> for Mo-nitrogenase and Fe-nitrogenase, respectively [24]). Even so, *in vivo* H<sub>2</sub> production rates by nitrogenase-utilizing PNSB are comparable with those by hydrogenase-utilizing oxygenic phototrophs (Table 1). Uptake hydrogenase usually accompanies nitrogenase to reuse H<sub>2</sub> produced during N<sub>2</sub>-fixation [15]. This hydrogenase needs to be eliminated for H<sub>2</sub> to accumulate, stressing the importance of working with bacteria for

Figure 3



Carbon cycle of H<sub>2</sub>-producing systems. CO<sub>2</sub> is fixed by oxygenic phototrophs for biosynthesis (e.g. sugars and biopolymers). Sugars can be fermented to H<sub>2</sub>, CO<sub>2</sub>, and organic acids by fermentative bacteria or by indirect photolysis. Organic acids are further oxidized to CO<sub>2</sub> and used for biosynthesis by anoxygenic purple nonsulfur bacteria. CO<sub>2</sub> generated during fermentation and anoxygenic photosynthesis is fixed by oxygenic phototrophs, completing the cycle.

which there are genetic tools. However, there are screening assays for H<sub>2</sub> production that are suitable for use with random mutagenesis [12,25]. H<sub>2</sub>-producing hydrogenases in PNSB are rare. *Rhodospirillum rubrum* and *R. palustris* BisB18 have a Ni-Fe hydrogenase that couples H<sub>2</sub> production to CO or formate oxidation [26,27]. Although this hydrogenase may normally be used during nonphotosynthetic metabolism, expressing *R. rubrum* formate hydrogen-lyase in *Rhodobacter sphaeroides* increased the photoheterotrophic H<sub>2</sub> yield on glucose twofold [28].

Mo-nitrogenase is the most common and the most efficient nitrogenase for converting N<sub>2</sub> to NH<sub>3</sub> (Eq. (3)). It is also found in all nitrogen-fixing bacteria and is thus the most studied. Since NH<sub>3</sub> production from N<sub>2</sub> compromises H<sub>2</sub> yields, nitrogenase-based H<sub>2</sub> production has been suggested for use in future Martian habitats where atmospheric N<sub>2</sub> and O<sub>2</sub> levels are low [29]. There are many possibilities to improve H<sub>2</sub> production in an N<sub>2</sub>-rich atmosphere, beyond the most common practice of sparging cultures with argon. A single amino acid change in the Mo-nitrogenase of the nonphotosynthetic bacterium *Azotobacter vinelandii* caused the enzyme to divert ~80% of the electrons to H<sub>2</sub> [30]. Preventing homocitrate synthesis can result in citrate, instead of homocitrate, incorporation into the catalytic center of nitrogenase, favoring H<sub>2</sub> production in an N<sub>2</sub> atmosphere [31]. The 'alternative' V-nitrogenase (Eq. (5)) and Fe-nitrogenase (Eq. (6)) naturally favor H<sub>2</sub> production, having a H<sub>2</sub> to NH<sub>3</sub> ratio 3-fold and 9-fold higher than the Mo-nitrogenase, respectively. Currently, *R. palustris* CGA009 is the only photosynthetic bacterium known to possess all three

Table 1

Comparison of potential photobiological H<sub>2</sub> production rates with existing biofuel production rates

Organism	Fuel	Gross productivity (equiv L of gasoline ha <sup>-1</sup> day <sup>-1</sup> ) <sup>a</sup>	Reference
Soybean	BioDiesel	1	[58]
Corn	Ethanol	6	[59]
Switchgrass	Ethanol	7	[59]
Oil palm	BioDiesel	16	[60]
<i>Arthrospira maxima</i> <sup>b,c</sup>	H <sub>2</sub>	19	[61]
Growing <i>Rhodospseudomonas palustris</i> <sup>b,d</sup>	H <sub>2</sub>	23	[36*]
Immobilized nongrowing <i>Rhodospseudomonas palustris</i> <sup>b,d</sup>	H <sub>2</sub>	29	[46*]
<i>Chlamydomonas reinhardtii</i> <sup>b,d</sup>	H <sub>2</sub>	99	[21*]
Microalgae (30% oil by DCW) <sup>b,e</sup>	BioDiesel	129	[60]
Microalgae (70% oil by DCW) <sup>b,e</sup>	BioDiesel	300	[60]
<i>In vitro</i> <i>Thermosynechococcus elongatus</i> photosystem I <sup>b,d,f</sup>	H <sub>2</sub>	300	[42]

<sup>a</sup> Energy costs for feedstocks, harvesting, processing, transportation, reactor maintenance, etc., are ignored. Assumes that those rates for small laboratory volumes can be scaled up and maintained. Calculations from literature values are in the [supplementary materials](#).

<sup>b</sup> Assumes volume of 1 ha by 10 cm deep (1,000,000 L).

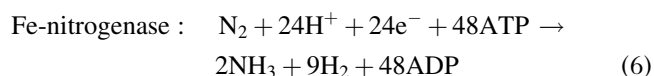
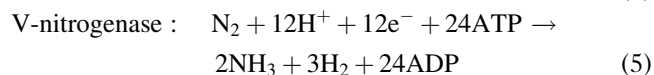
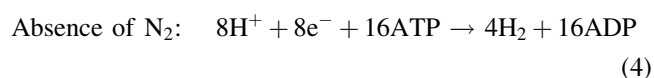
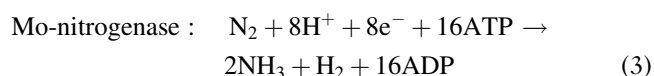
<sup>c</sup> Dark anaerobic H<sub>2</sub> production values. Assumes 24 hours per day operation.

<sup>d</sup> Assumes operation during 12 hours of illumination per day.

<sup>e</sup> On the basis of values obtained from 716,585 L photobioreactors. DCW, Dry cell weight.

<sup>f</sup> Uses platinum coupled to purified photosystem I and cytochrome c<sub>6</sub> from *T. elongatus* to produce H<sub>2</sub> at 55°C using light energy and electrons from ascorbate.

nitrogenases [32]. Several PNSB and the green sulfur bacterium *Chloroherpeton thalassium* possess the Fe-nitrogenase and the oxygenic cyanobacterium *Anabaena variabilis* possesses the V-nitrogenase (Table S1). Applying alternative nitrogenases for H<sub>2</sub> production would benefit from a better understanding of their regulation, of which relatively little is currently known. In *Rhodobacter capsulatus*, Fe-nitrogenase is repressed by Mo, and its expression relies on many of the same regulatory proteins that are used for Mo-nitrogenase (reviewed by [33]). In *R. rubrum* [34], and *R. palustris* [32] Mo does not repress alternative nitrogenases. Microarray analysis of *R. palustris* suggests that alternative nitrogenase expression is linked to severe nitrogen starvation [32].



Fixing N<sub>2</sub> is energetically expensive. Thus, nitrogenase is rapidly repressed by a network of transcriptional and post-translational mechanisms in response to nitrogen compounds, such as NH<sub>3</sub> [35]. For many PNSB, this repression can be bypassed by using glutamate as the nitrogen source [8]. However, since NH<sub>3</sub> is present in waste streams that would serve as feedstock for H<sub>2</sub> production, this repression presents a serious hurdle.

This hurdle has been overcome for Mo-nitrogenase in several bacteria by using a selective pressure in which H<sub>2</sub> production was required for growth to obtain mutants that produce H<sub>2</sub> in the presence of NH<sub>3</sub> [36\*,37]. Remarkably, the entire regulatory network that represses nitrogenase activity in *R. palustris* was bypassed by a single amino acid change in the transcriptional activator of nitrogenase, NifA [36\*]. In *R. rubrum*, *nifA* mutations alone could not achieve constitutive H<sub>2</sub> production, as enzymes that post-translationally inactivate nitrogenase also had to be disrupted [38]. The repressive effects of NH<sub>3</sub> were overcome in *R. capsulatus* by deleting the regulatory P<sub>II</sub> proteins that convey the cellular nitrogen status to the nitrogenase regulatory network [33]. Unfortunately, this strategy did not result in constitutive Fe-nitrogenase activity, indicating an unknown difference in its regulation [33].

H<sub>2</sub> yields by growing PNSB are relatively low (<15% of the theoretical maximum during growth on acetate) as the vast majority of the electrons from organic or inorganic feedstocks are used for biosynthesis. Thus, one should consider uses for the resulting biomass (e.g. as a fertilizer or as a feed for aquaculture [39]). Any increase in H<sub>2</sub> yield must come at the expense of biomass yield. In this respect, nongrowing PNSB (usually nitrogen-starved) are attractive as H<sub>2</sub> yields approaching 80% of the theoretical maximum can be achieved (reviewed by [40]). Reports in the literature of similarly high yields from growing cells should raise questions of mass balance. Fortunately, maintenance of a H<sup>+</sup> gradient and ATP levels by cyclic photophosphorylation allows PNSB to remain active in a nongrowing state for long periods of time, on the order of weeks [8,41].



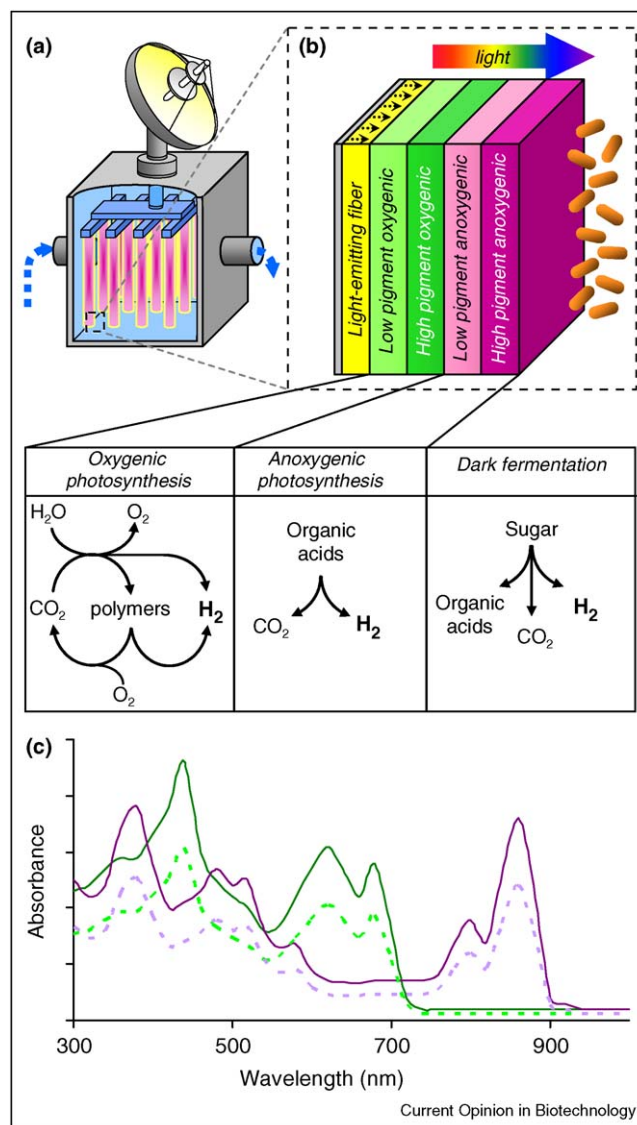
### Common challenges in scaling up

Photobiological  $H_2$  production rates would surpass current crop-based biofuel productivities if laboratory-scale values could be scaled linearly and maintained (Table 1). Although photobiological  $H_2$  production is estimated to generate less energy than biodiesel from algae (Table 1), costs associated with processing and purifying usable biodiesel from algal biomass are greater than those associated with the capture of  $H_2$  as a direct metabolic product. *In vitro*  $H_2$  production rates using purified photosystem components can also be impressive (Table 1), however this technology currently uses a platinum catalyst and ascorbate (vitamin C) as a sacrificial electron donor [42]. Of course, linear scaling of values is an idealized situation. Scaling up any photobiological process is not trivial. Light intensity can decrease exponentially with depth as cells nearest the light source shade those behind [3]. Scaling up photobiological  $H_2$  production must be both effective and economical, as it must compete with renewable forms of electricity production (e.g. wind turbines and photovoltaics) that can make  $H_2$  from water by electrolysis [4\*].

There are many biological strategies to increase the photosynthetic efficiency of  $H_2$  production in a photobioreactor. One of the more economical strategies is to use bacteria with low pigmentation in high light intensity situations. Photosynthetic microbes tend to produce large amounts pigment-protein antennae complexes that are advantageous for capturing light in their natural light-limited habitats. However, these excessive antennae can waste 60–80% of the photons in a photobioreactor [43], resulting in low conversion efficiencies of light energy to  $H_2$  (usually less than 1–2% [7\*,44]). Random mutagenesis has generated low-pigment mutants of *C. reinhardtii* (reviewed by [43]) and *R. sphaeroides* [45]. The *R. sphaeroides* mutant was cultured in front of the wild type in a two-compartment, flat plate reactor, resulting in a 1.4-fold increase in light to  $H_2$  conversion efficiency (to 2.2%) compared to the wild type used in both compartments [45].

An issue that we have experienced but that has not received much attention is that long-term culturing can select for biofilms, making exposing interior cells to light by mixing ineffective. However, biofilms could be spatially arranged (e.g. as naturally or artificially immobilized cells) to maximize photosynthetic efficiency. Many phototrophs have been immobilized including *R. palustris* in flexible latex films [46\*], *Synechocystis* encapsulated in silica sol gel [47], and natural inhabitation of a glass fiber matrix by *C. reinhardtii* [48]. Differently pigmented strains and microbes that use different light wavelengths (e.g. algae (300–700 nm) and PNSB (300–1000 nm)) could be immobilized in overlapping layers to make the best use of the electromagnetic spectrum (Figure 4) [49–51]. Other combinations of bacterial strains could also prove useful. *R. sphaeroides* was used with

Figure 4



Schematic of  $H_2$  production by immobilized cocultures in an advanced photobioreactor. (a) Sunlight is collected and channeled through light-conducting material to light emitting fibers which deliver selected wavelengths of light to immobilized  $H_2$ -producing cells with different light absorption properties. This array is immersed in wastewater effluent containing free-floating  $H_2$ -producing fermentative microbes that convert carbohydrates, proteins and other polymers and their monomers to organic acids that serve as electron donors for anoxygenic  $H_2$ -producing phototrophs. (b) The light emitting fibers are coated with four different immobilized strains of oxygenic  $H_2$ -producing phototrophs (green) and anoxygenic  $H_2$ -producing phototrophs (purple), arranged to maximize photosynthetic efficiency. Key metabolic features of each microbe are shown. Note that the products of some microbes serve as substrates for others. (c) The absorption spectra for wild type (solid lines) and low-pigment strains (dotted lines) of oxygenic (green) and anoxygenic (purple) microbes immobilized in layers on the light emitting surfaces are complementary to maximize the use of the full light spectrum. The spectra are loosely based on those reported for *Anabaena variabilis* and *Rhodobacter sphaeroides* [51].

*Halobacterium salinarum* (which uses bacteriorhodopsin to pump protons when illuminated) resulting in a higher H<sub>2</sub> production rate and light conversion efficiency, presumably due to the increased availability of protons from bacteriorhodopsin activity [52]. Fermentative H<sub>2</sub>-producers could be combined with PNSB to convert residual fermentative organic acids to CO<sub>2</sub> and H<sub>2</sub>, thereby maximizing the H<sub>2</sub> yield from hemicellulosic feedstock [50,53,54] (Figures 3 and 4). Spent photosynthetic biocatalyst could also be recycled into organic acids and H<sub>2</sub> by fermentative microbes (Figure 3) [50].

Scaling of photosynthetic H<sub>2</sub> production effectively will also rely heavily on innovative bioreactor designs that increase the illuminated surface area to volume ratio (reviewed elsewhere [55\*,56]). Common designs include, first, flat plate reactors, where gas (e.g. CO<sub>2</sub>) is often bubbled for mixing, second, cylindrical reactors with a hollow center where light intensity would otherwise be poor, and third, tubular reactors, in which microbes are circulated through long distances of transparent tube [55\*]. One exciting prospect is to design a bioreactor in which selected wavelengths of light are delivered to layers of cells that have different light absorption maxima [55\*] (Figure 4). Although this setup is currently too expensive for practical use, it would significantly decrease the bioreactor footprint because the illuminated surface area inside the reactor can be much larger than the surface area of the light collector [55\*].

## Conclusions

Fossil fuels were derived from ancient photosynthetic microbes at a low conversion efficiency of plant carbon to fuel (<0.01%), such that the planet's current photosynthetic capacity would need over 400 years to fix enough carbon for a year's worth of fossil fuel [57]. In contributing to the energy crisis relief, photobiological H<sub>2</sub> production does not have the same luxury of time. For photobiological H<sub>2</sub> production to be practical and economically viable, biocatalysts must be long-lasting and make the most efficient use of electron and light sources. Advances in these areas are being made through genetic engineering with increasing use of systems biology approaches to understand the processes being engineered. There is ample opportunity to study and optimize productive cocultures in concert with new bioreactor designs and operations. Although scaling up photobiological processes in an economical manner is a major challenge, current laboratory-scale H<sub>2</sub> production values are encouraging.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.copbio.2010.02.012.

## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

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