Enhanced Phototrophic Biomass Productivity through Supply of Hydrogen Gas

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ABSTRACT: Industrial production of phototrophic microorganisms is often hindered by low productivity due to limited light availability and therefore requires large land areas. This letter demonstrates that supply of hydrogen gas (H₂) increases in phototrophic biomass productivity compared to a culture growing on light only. Experiments were performed growing *Synechocystis* sp. in batch bottles, with and without H₂ in the headspace, which were exposed to light intensities of 70 and 100 μmol/m²/s. At 70 μmol/m²/s with H₂, the average increase in biomass was 96 mg DW/L/d, whereas at 100 μmol/m²/s without H₂, the average increase in biomass was 27 mg DW/L/d. Even at lower light intensity, the addition of H₂ tripled the biomass yield compared to growth under light only. Photoreduction and photosynthesis occurred simultaneously, as both H₂ consumption and O₂ production were measured during biomass growth. Photoreduction used 1.85 mmol of H₂ to produce 1.0 mmol of biomass, while photosynthesis produced 1.95 mmol of biomass. After transferring the culture to the dark, growth ceased, also in the presence of H₂, showing that both light and H₂ were needed for growth. A renewable H₂ supply for higher biomass productivity is attractive since the combined efficiency of photovoltaics and electrolysis exceeds the photosynthetic efficiency.

1. INTRODUCTION

In the last decades, phototrophic microorganisms (e.g., cyanobacteria and microalgae) have gained attention for their role in a more sustainable and biobased society.¹ Due to their vast diversity, they can have a multitude of applications like, for example, biofuels, food, feed, and chemicals. Economically viable production is, however, in most cases still limited by the low productivity of large-scale outdoor systems, leading to large required land area.²,³ Light availability depends on the geographical location and is further affected by the variation of solar irradiance in day–night cycles and seasons. Therefore, the key to economic application is to increase the productivity of phototrophic microorganisms and to make the process less dependent on variations in solar irradiance. One way to achieve this would be to supplement the energy available from sunlight with energy in the form of hydrogen gas (H₂). H₂ is considered to be the clean energy carrier of the future.⁴ It is predicted that H₂ can be produced by water electrolysis with renewable electrical energy as input (i.e., wind and solar) at a cost of €1.0 kg/H₂ by 2030.⁵ Besides an increase in productivity, the supply of H₂ would lead to a more simplified production system as only gaseous substrates are consumed. New biomass or specific biomolecules can be built from water, through electrolysis, into H₂ and carbon dioxide (CO₂).

Biological H₂ production from sunlight and water by phototrophs has been studied in detail.⁶,⁷ All known cyanobacterial H₂ production pathways are presumed to be mediated by the enzyme hydrogenase.⁸ Under anaerobic conditions, interestingly, microalgae and cyanobacteria can express bidirectional hydrogenases.⁷,⁹ With these bidirectional hydrogenases, they can, in addition to producing H₂, also consume H₂ using a metabolic pathway, called photoreduction, which is similar to photosynthesis. The photoreduction...
pathway uses energy in the form of both H₂ and light to reduce CO₂ and therefore requires less light than photosynthesis based on light alone. The theoretical light requirement for photosynthesis is 8–10 photons per molecule CO₂ converted. The number of photons per CO₂ when H₂ is used in addition to light depends on the mechanism used for ATP generation.

Photosynthesis:

\[
\text{H}_2\text{O} + \text{CO}_2 \xrightarrow{\lambda} \text{CH}_2\text{O} + \text{O}_2
\]

Photoreduction:

\[
2\text{H}_2 + \text{CO}_2 \xrightarrow{\lambda} \text{CH}_2\text{O} + \text{H}_2\text{O}
\]

where \(\lambda\) is the required light, CO₂ the supplied CO₂, CH₂O the formed biomass, H₂ the supplemented hydrogen, and O₂ the produced oxygen.

Photoreduction enables high growth rates, since H₂ is an additional energy source to the available light. An additional advantage of using H₂ is that the gas can be distributed evenly through the entire bioreactor, enabling higher biomass density, throughput the entire bioreactor, enabling higher biomass density, and O₂ the surface exposed to the light source. So far, the supply of H₂ has not been studied to increase phototrophic biomass productivity.

To show the effect of H₂ supply, growth experiments with *Synechocystis* sp. were performed in batch bottles, with and without H₂ in the headspace, exposed to a light intensity of 70 and 100 \(\mu\text{mol}/\text{m}^2/\text{s}\), and results show that the addition of H₂ indeed leads to an increase in productivity when compared to phototrophic growth on light alone.

2. MATERIALS AND METHODS

2.1. Experimental Design and Strategy. Experiments were performed in batch in Schott Duran bottles of 500 mL. These bottles were filled with 50 mL of medium, and the headspace was flushed with a gas mixture suitable for the specific experiment (Table 1). After inoculation, the biomass concentration was 40 mg/L. The bottles were placed in a temperature-controlled cabinet (35 °C) on an orbital shaker (120 rpm). Light was provided by an LED panel (40 W; 3600 lm; 4000 K) placed horizontally above the bottles. All experiments were performed in triplicate. Due to the cap on the bottles, actual light intensities in the Scott Duran bottles were slightly lower, and small variations in light intensity occurred due to the positioning of the bottles under the LED light. These differences in light intensity could not be quantified as the light intensity inside the bottles could not be determined. However, triplicate experiments were always performed at different positions, making sure the trends in the presented results are not due to these differences in light intensity.

Four experiments were performed to study the effect of H₂ supply on biomass productivity of cyanobacteria. The batch experiments were finished when CO₂ was nearly depleted (15–20 days, depending on biomass accumulation).

2.2. Cultures, Media, and Headspace. Photoautotrophic cultures of *Synechocystis* sp. (PCC 6803) were acquired from the Pasteur Culture Collection (Paris, France). *Synechocystis* sp. was used in this study as it is a model organism to represent cyanobacteria and well described in literature; its genetics also have been studied in detail. Furthermore, *Synechocystis* has the capacity to both consume and produce H₂.

The cultures were maintained in 500 mL of liquid in Erlenmeyer flasks closed with porous stoppers. The flasks were kept in an orbital shaker (120 rpm) under a LED light at a light intensity of 100 \(\mu\text{mol}/\text{m}^2/\text{s}\) at 30 °C.

Modified BG11 medium17 was used to grow and maintain the cultures. The medium used contained (in mM): CaCl₂, 2H₂O, 24.5; MgSO₄·7H₂O, 30.4; EDTA, 10.3; FeCl₃·6H₂O, 4.44; K₂HPO₄, 23.0; Na₂SO₄, 35.7; and NH₄Cl, 17.6; trace elements (in \(\mu\text{M}\)): H₂BO₃, 0.46; MnCl₂·4H₂O, 9.15; ZnSO₄·7H₂O, 77.2; Na₂MoO₄·2H₂O, 1.61; CuSO₄·5H₂O, 31.6; and CoCl₂·6H₂O, 16.8. For the experiments, the medium was adapted by adding 54 mL of sodium bicarbonate (NaHCO₃). The medium was sterilized via filtration by using 0.22 \(\mu\text{m}\) filters (VWR International, Amsterdam, The Netherlands). The initial CO₂ concentration in the headspace was set at 31 vol % to maintain a pH of 7.8 in the medium for favorable growth conditions, based on a gas to liquid ratio of 1:10 (10% liquid phase, 90% gas phase). Three mass flow controllers (ELFLOW SELECT F-201CV, Bronkhorst HIGH-TECH B.V., NL) were used to mix the gases according to the desired composition (Table 1).

2.3. Measurements and Analysis. At regular time intervals (24 or 48 h), 2 mL of liquid sample and 5 mL of gas sample were taken. The samples were analyzed for pH, optical density, ammonium and carbon contents, and gas composition. The liquid and gas sampling volumes were directly compensated by readdition of the same volumes of a fresh medium and gas mixture.

Optical density at 440, 480, 620, 680, 720, and 750 nm was measured using a Victor3 1420 Multilabel Counter (Perkin-Elmer, Groningen, The Netherlands). The optical density measurements were used to calculate the biomass dry weight (DW) based on a previously established calibration. The ratio between the optical densities at different wavelengths was used to verify that the culture was not contaminated.

Ammonium content was analyzed using a Metrohm Compact IC Flex 930 with a cation column (Metrosep C 4-150/4.0) equipped with a conductivity detector (Metrohm Nederland BV, Schiedam, The Netherlands) with a limit of detection of 0.1 mg/L. Carbon content was analyzed using a TOC analyzer (TOC-L in combination with ASI-L; Shimadzu, s-Hertogenbosch, The Netherlands) with a limit of detection of 1 mg/L. Gas composition (H₂, O₂, N₂, CO₂, and CH₄) was analyzed using a dual-channel Varian CP4900 microgas chromatograph (Varian, Middelburg, The Netherlands) with a limit of detection of 0.1% v/v for CO₂, CH₄, H₂, 0.75% v/v for O₂, and 1.5% v/v N₂. The used equipment is calibrated regularly by qualified personnel as suggested by the suppliers. All measurements have been performed in the linear range of detection.

Table 1. Experimental Design to Demonstrate the Effect of H₂ Supplementation on Productivity of Cyanobacteria*

| Experiment | Headspace | Light intensity (\(\mu\text{mol}/\text{m}^2/\text{s}\)) | Operation |
|------------|-----------|---------------------------------|-----------|
| 1          | Nitrogen  | 70                              | Continuous light |
| 2          | Nitrogen  | 100                             | Continuous light |
| 3          | Hydrogen  | 70                              | Continuous light |
| 4          | Hydrogen  | 70                              | Only light during first 6 days |

*Each experiment was performed in triplicate.*
The amount of CO2 used for photoreduction was calculated from the consumed amount of H2 and the total amount of biomass using a biomass composition of \( \text{CH}_{1.84}\text{O}_{0.4}\text{N}_{0.18} \). The remaining CO2 consumption was assumed to be used by photosynthesis.

3. RESULTS AND DISCUSSION

3.1. Hydrogen Supply Leads to Increased Productivity.
Triplicate bottles were inoculated and exposed to low light intensity (70 \( \mu \text{mol/m}^2/\text{s} \)), elevated light intensity (100 \( \mu \text{mol/m}^2/\text{s} \)), and low light intensity (70 \( \mu \text{mol/m}^2/\text{s} \)) with H2 to investigate the effect of H2 supply on the biomass productivity of cyanobacteria. Figure 1 shows the increase in dry weight as a function of time. At 70 \( \mu \text{mol/m}^2/\text{s} \), there was no detectable growth, which was supported by the constant nutrient concentrations. At 70 \( \mu \text{mol/m}^2/\text{s} \), the light intensity was too low for the cyanobacteria to perform photosynthesis. At 100 \( \mu \text{mol/m}^2/\text{s} \), however, after a lag phase of approximately 7 days, the biomass density (dry weight, DW) increased, reaching a maximum of 0.62 g/L at day 20. At 100 \( \mu \text{mol/m}^2/\text{s} \), sufficient light was available to sustain growth, contrary to the operation at 70 \( \mu \text{mol/m}^2/\text{s} \).

The cyanobacteria that were grown with both light and H2 showed a short lag phase since growth was observed from the first measurement point onward (day 2). At 70 \( \mu \text{mol/m}^2/\text{s} \) with a H2 supply, the biomass density increased from 0.04 to 1.15 g/L within 12 days. At 100 \( \mu \text{mol/m}^2/\text{s} \), the biomass density increased from 0.05 to 0.21 g/L within the same 12 days. On average, the increase in biomass at 100 \( \mu \text{mol/m}^2/\text{s} \) was 27 mg DW/L/d with a maximum increase of 85 mg DW/L/d (day 12 to 15), while at 70 \( \mu \text{mol/m}^2/\text{s} \) with a H2 addition, the increase was 96 mg DW/L/d during the first 12 days. The addition of H2 tripled the biomass yield compared to growth at a higher light intensity without H2. The addition of H2 leads to a more rapid growth of phototrophic biomass compared to a supply with light alone. Thus H2 can be used as an additional energy source for photoreduction in cyanobacteria.

It is important to mention that O2 was detected (~30%) in the headspace of all cultures and thus also in the ones growing with supplemented H2. Apparently, the formed oxygen does not affect the activity of the hydrogenases and is not limiting the uptake of H2 by hydrogenases.

A light intensity of 70 \( \mu \text{mol/m}^2/\text{s} \) should have been sufficient to achieve growth. Apparently, the light intensity in the bottle was slightly lower due to the shielding effect of the bottle cap. Also other effects cannot be excluded, such as the fact that an anaerobic starting condition is not favorable for photoautotrophic growth.

The headspace gas composition was analyzed for H2, CO2, and O2 contents throughout these experiments. Figure 2A shows the consumed H2 for the experiment where H2 was added to the headspace. During the first 12 days of operation, there was both H2 uptake and O2 evolution. This means that both photosynthesis and photoreduction occurred. If photoreduction was the only growth mechanism, it is important to mention that O2 was detected (~30%) in the headspace of all cultures and thus also in the ones growing with supplemented H2. Apparently, the formed oxygen does not affect the activity of the hydrogenases and is not limiting the uptake of H2 by hydrogenases.

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3.2. Photosynthesis and Photoreduction Occur Simultaneously. During photoreduction, both H2 and O2 are consumed for biomass production, while during photosynthesis O2 is produced (eqs 1 and 2). During the first 12 days of operation, there was both H2 uptake and O2 evolution. This means that both photosynthesis and photoreduction occurred. If photoreduction was the only growth mechanism,
theoretically, two moles of H\textsubscript{2} are required per mole of CO\textsubscript{2}. Figure 2B shows the share of CO\textsubscript{2} consumption for biomass production through photoreduction and through photosynthesis. The combination of photoreduction and photosynthesis produced 2.95 mmol of biomass in 12 days. Photoreduction used 1.85 mmol of H\textsubscript{2} to produce 1.0 mmol of biomass, while photosynthesis produced 1.95 mmol of biomass. Since the (molar) CO\textsubscript{2} to H\textsubscript{2} consumption ratio was never 1:2, both photoreduction and photosynthesis pathways were used.

It has been suggested before that the two pathways cannot happen simultaneously,\textsuperscript{11} and though overall we see both pathways occurring, it could be possible that the microorganisms changed between both pathways within the experiments. As this is a population of microorganisms, both processes also might have occurred simultaneously but in different microorganisms.

As during photoreduction O\textsubscript{2} is consumed (eqs 1 and 2), ideally, in an optimized system, photoreduction could consume all photosynthetically produced O\textsubscript{2}. This would create a photobioreactor in which gases are only consumed and not produced. Such a reactor system could be drastically simplified as no explosive mixture of H\textsubscript{2} and O\textsubscript{2} is formed.

3.3. Both Hydrogen Gas and Light Are Needed for Growth. Theoretically, cyanobacteria can take up H\textsubscript{2} and grow without light exposure.\textsuperscript{9} A final experiment was performed to determine if growth on H\textsubscript{2} without light is possible. In this experiment, triplicate reactors with CO\textsubscript{2} and H\textsubscript{2} were first cultivated with light (70 μmol/m\textsuperscript{2}/s), as in the previous experiment. However, after 7 days, these cultures were transferred to the dark. Figure 3 shows that the initial growth curves were comparable to the earlier experiments with light and H\textsubscript{2} during the first 7 days. After transfer into the dark, however, no further growth was observed. This was confirmed by the headspace concentrations of H\textsubscript{2}, O\textsubscript{2}, and CO\textsubscript{2}, which did not change after day 7. These cyanobacteria were thus not able to grow autotrophically on H\textsubscript{2} and CO\textsubscript{2} without light, which is another indication that photoreduction and photosynthesis occurred simultaneously.

3.4. Outlook. This letter demonstrates that H\textsubscript{2} supply results in higher phototrophic biomass productivity compared to light alone. The supplemented H\textsubscript{2} is used as an additional energy source for growth. After transferring the culture with H\textsubscript{2} to the dark, growth stopped, meaning that light was required to perform photoreduction. Future research should focus on the mechanisms involved in photoreduction and the effect of the photosynthetically produced O\textsubscript{2} which, on the one hand, can be toxic to the hydrogenases involved in the photoreduction, while it, on the other hand, is required for biomass production. A combination of photosynthesis and photoreduction was demonstrated already at low light intensity, and the possibility to further enhance the biomass growth rate by increasing the light intensity (>70 μmol/m\textsuperscript{2}/s) should be investigated. So far, it is unclear if H\textsubscript{2} uptake and the possibility to perform photoreduction is a common feature among phototrophic microorganisms. However, all cyanobacteria contain NiFe-hydrogenases which are usually active in the uptake direction and should therefore be able to take up H\textsubscript{2}.\textsuperscript{20,21} These cyanobacterial NiFe-hydrogenases are known to be less oxygen sensitive compared to other types of hydrogenases which is important as photoreduction and photosynthesis, where O\textsubscript{2} is produced, occur simultaneously. Moreover, the photosynthetic and respiratory electron transport chains are located on the same thylakoid membranes making it more easy for photosynthetically evolved O\textsubscript{2} to diffuse to the respiratory oxidases\textsuperscript{22} and, as such, lower the O\textsubscript{2} partial pressure in the vicinity of the hydrogenases.

The production of phototrophic biomass using light and H\textsubscript{2} can be used to build new biomass or specific biomolecules\textsuperscript{14} from only water, CO\textsubscript{2} and some nutrients. In the envisioned process, water is first converted to H\textsubscript{2} through electrolysis, which is then used, together with CO\textsubscript{2}, by the phototrophic microorganisms, together with light, to grow (produce biomass). The photosynthetic efficiency of the phototrophic microorganism is around 4%–5%, while the efficiency of H\textsubscript{2} production through PV panels has exceeded 20%.\textsuperscript{23–25} Therefore, the amount of land area required to produce phototrophic biomass from H\textsubscript{2} and light can be lower than compared to the land area required to produce phototrophic biomass from light only. The addition of H\textsubscript{2} to photobioreactors would lead to a partial decoupling of phototrophic biomass production from available land.\textsuperscript{26–28} It would also be possible to produce biomass in winter, if excess H\textsubscript{2} that is produced and stored in summer can be used. On top of that, the improved productivity would lead to a reduction in water requirement to produce the same amount of biomass. This is especially interesting for areas with high light intensity, which often have a lack of freshwater.

These results show that the productivity of photobioreactors can be improved through the H\textsubscript{2} supply. Already at the nonoptimized conditions in this study, the biomass yield tripled at lower light intensity compared to the biomass yield at higher light intensity. In the future, the supply of H\textsubscript{2} might be an interesting option to boost the productivity of phototrophic biomass for the production of biofuels, food, feed, and chemicals.

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**Figure 3.** Growth of phototrophic culture expressed as dry weight in time under continuous light (70 μmol/m\textsuperscript{2}/s) and after transfer into the dark. Both H\textsubscript{2} and light are required to perform photoreduction.
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Notes
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