Sustained H₂ Production Driven by Photosynthetic Water Splitting in a Unicellular Cyanobacterium

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ABSTRACT The relationship between dinitrogenase-driven H₂ production and oxygenic photosynthesis was investigated in a unicellular cyanobacterium, *Cyanothece* sp. ATCC 51142, using a novel custom-built photobioreactor equipped with advanced process control. Continuously illuminated nitrogen-deprived cells evolved H₂ at rates up to 400 μmol · mg Chl⁻¹ · h⁻¹ in parallel with uninterrupted photosynthetic O₂ production. Notably, sustained coproduction of H₂ and O₂ occurred over 100 h in the presence of CO₂, with both gases displaying inverse oscillations which eventually dampened toward stable rates of 125 and 90 μmol · mg Chl⁻¹ · h⁻¹, respectively. Oscillations were not observed when CO₂ was omitted, and instead H₂ and O₂ evolution rates were positively correlated. The sustainability of the process was further supported by stable chlorophyll content, maintenance of baseline protein and carbohydrate levels, and an enhanced capacity for linear electron transport as measured by chlorophyll fluorescence throughout the experiment. *In situ* light saturation analyses of H₂ production displayed a strong dose dependence and lack of O₂ inhibition. Inactivation of photosystem II had substantial long-term effects but did not affect short-term H₂ production, indicating that the process is also supported by photosystem I activity and oxidation of endogenous glycolgen. However, mass balance calculations suggest that carbohydrate consumption in the light may, at best, account for no more than 50% of the reductant required for the corresponding H₂ production over that period. Collectively, our results demonstrate that uninterrupted H₂ production in unicellular cyanobacteria can be fueled by water photolysis without the detrimental effects of O₂ and have important implications for sustainable production of biofuels.

RESEARCH ARTICLE

IMPORTANCE The study provides an important insight into the photophysiology of light-driven H₂ production by the nitrogen-fixing cyanobacterium *Cyanothece* sp. strain ATCC 51142. This work is also of significance for biotechnology, supporting the feasibility of “direct biophotolysis.” The sustainability of the process, highlighted by prolonged gas evolution with no clear sign of significant decay or apparent photodamage, provides a foundation for the future development of an effective, renewable, and economically efficient bio-H₂ production process.
activity in these cells protected by a coordinated downregulation of PSII activity and high O₂ consumption rates (12). Unicellular diazotrophic cyanobacteria, e.g., *Cyanothece* and *Crocosphaera*, carry out N₂ fixation at night at the expense of stored photosynthetic O₂, which has accumulated during the day (13, 14).

The ability to spatially and temporally separate photosynthetic and dinitrogenase activities provides a foundation to exploit N₂-fixing cyanobacteria for H₂ production (15-17). Recent studies of *Cyanothece* sp. ATCC 51142 (here referred to as *Cyanothece* 51142) demonstrated that diazotrophically grown cultures entrained on light-dark cycles are capable of H₂ evolution under constant illumination during the “subjective dark” (18). This process was shown to be driven by dinitrogenase and was thought to rely upon respiration-induced microoxic environments resulting from oxidation of endogenous glycogen. While substantial H₂ production was shown to occur in the light, no mechanistic details linking photosynthetic metabolism and H₂ production in *Cyanothece* 51142 are available. To gain insight into the photophysiology of the process, we utilized a novel custom-built photobioreactor that coupled online gas monitoring and feedback-controlled lighting. To avoid circadian regulation (19), continuous illumination was used to grow low-density cultures in an N-limited chemostat where H₂ production was achieved by interrupting the influx of NH₄⁺. The ability to monitor and control gas and light input into the system afforded high-resolution physiological details, opening a window on the relationship between photosynthetic O₂ evolution and H₂ production in unicellular cyanobacteria. Furthermore, the combination of nutrient-limited chemostat cultivation and feedback-controlled illumination enabled us to sustain long periods of light-driven H₂ production in *Cyanothece* 51142.

**RESULTS**

**H₂ production under continuous light.** When grown under continuous illumination, NH₄⁺-limited chemostat cultures of *Cyanothece* 51142 express high levels of dinitrogenase (20), which is the primary catalyst of H₂ production in diazotrophic cyanobacteria (8). Under these steady-state conditions, *Cyanothece* 51142 evolved O₂ at a rate of 350 μmol · mg Chl⁻¹ · h⁻¹; however, no H₂ was detected. Light-dependent H₂ production was observed only after chemostat-grown cells were transferred into sealed tubes and incubated under continuous light. Notably, after 72 h of continuous illumination with white light under an Ar atmosphere, *Cyanothece* 51142 cells evolved 4.46 ± 0.58 mmol H₂ · mg Chl⁻¹ and 2.65 ± 0.14 mmol O₂ · mg Chl⁻¹, implying PSII activity as well as a possible O₂ tolerance (see Fig. S1 in the supplemental material). In order to observe the relationship between the H₂ and O₂ which had accumulated in sealed tubes, N deprivation was imposed directly on the chemostat culture by transitioning to batch mode via a possible O₂ tolerance (see Fig. S1 in the supplemental material). In order to observe the relationship between the H₂ and O₂ which had accumulated in sealed tubes, N deprivation was imposed directly on the chemostat culture by transitioning to batch mode via illumination with white light under an Ar atmosphere, *Cyanothece* 51142 cells evolved 4.46 ± 0.58 mmol H₂ · mg Chl⁻¹ and 2.65 ± 0.14 mmol O₂ · mg Chl⁻¹, implying PSII activity as well as a possible O₂ tolerance (see Fig. S1 in the supplemental material). In order to observe the relationship between the H₂ and O₂ which had accumulated in sealed tubes, N deprivation was imposed directly on the chemostat culture by transitioning to batch mode via illumination with white light under an Ar atmosphere, *Cyanothece* 51142 cells evolved 4.46 ± 0.58 mmol H₂ · mg Chl⁻¹ and 2.65 ± 0.14 mmol O₂ · mg Chl⁻¹, implying PSII activity as well as a possible O₂ tolerance (see Fig. S1 in the supplemental material).

Gas dynamics by N-deprived *Cyanothece* 51142 cultures. Fine-scale resolution of production rate dynamics was obtained in the LED photobioreactor upon interruption of NH₄⁺ influx, during continuous sparging with 1.3% CO₂-Ar (a) or pure Ar (b).

**Macromolecular dynamics during photobiological H₂ production.** Correlation between H₂ and CO₂ dynamics in the off-gas suggests that the catabolism of cellular carbon reserves may play a role in driving H₂ production. These oscillations eventually dampened, exhibiting median H₂ and O₂ production rates of 125 and 90 μmol · mg Chl⁻¹ · h⁻¹ at the end of the experiment (100 h). Thus, not only did H₂ evolution occur simultaneously with oxygenic photosynthesis, these two processes proceeded concomitantly over a long, sustained period.

The effects of disrupting substrate flows were also investigated by additionally removing CO₂ from the sparge gas when halting the influx of NH₄⁺ (Fig. 1b). Once again, H₂ became detectable shortly after N deprivation, with rates which also steadily increased over the first 24 h. As CO₂ levels in the off-gas quickly diminished, O₂ evolution rates also decreased rapidly, reaching values much lower than those found in the reactor sparged with Ar-CO₂. However, O₂ evolution rates began to recover at 4 h, remarkably increasing in parallel with H₂ evolution. From 17 to 28 h, however, O₂ evolution deviated from the H₂ dynamics, as a transient CO₂ evolution peak was detectable in the off gas. Upon the disappearance of CO₂, the O₂ resumed its mirroring of the H₂ dynamics, which had already begun to decelerate. Although the gas dynamics were dampened by 48 h, a second low-level oscillation of H₂ production occurred from 48 to 90 h, with a maximum at approximately 60 h, corresponding with small but discernible changes in the O₂ rate at these times. No CO₂ in the off-gas was detected throughout this second oscillation. Thus, under conditions where no external electron acceptors are available, phases of H₂ and O₂ evolution were positively correlated.

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role in H₂ evolution by *Cyanothece* 51142. Within the first few hours after interrupting the NH₄⁺ supply, the total carbohydrate levels in both reactors increased, reaching 55 mg · liter⁻¹ in 12 h with Ar-CO₂ and 45 mg · liter⁻¹ in 6 h with Ar (Fig. 2a and b). Thereafter, for both reactors, the carbohydrate content declined for the remainder of the first H₂ evolution cycle and well into the following phase of decelerating H₂ activity. Mass balance calculations for this time period indicate that the theoretical contribution of carbohydrate consumption may, at best, account for 40 and 51% of the electrons necessary for the corresponding H₂ production observed under Ar-only and Ar-CO₂ conditions, respectively (see Fig. S2a and b in the supplemental material). Furthermore, in the reactor sparged only with Ar, the total carbohydrate level remained relatively constant throughout the second half of the experiment, despite the H₂ production, which continued to proceed without interruption (see Fig. S2c in the supplemental material). However, in the Ar-CO₂ reactor, there was a second oscillation from 30 to 60 h, slightly offset from the H₂ dynamics, with carbohydrate accumulation occurring primarily as the H₂ rate accelerated, and carbohydrate consumption preceding the H₂ production peak. Cellular glycogen concentrations at select time points were between 75 and 94% of the total carbohydrates, indicating that the observed dynamics were primarily a reflection of changes in glycogen.

Cessation of NH₄⁺ influx was followed by a decline in total protein in both reactors as H₂ rates accelerated (Fig. 2c and d), followed by a recovery to the levels observed during steady state. From a biotechnological perspective, it is remarkable that prolonged N deprivation did not lead to a catastrophic decrease in protein content over the 100 h of the experiment. However, the accessory pigment phycocyanin had degraded to ~40% of the steady-state level over the first 24 h, with a further decline to ~20% after 50 h (see Fig. S3 in the supplemental material). In contrast, no substantial changes in the level of chlorophyll pigment could be observed.

**Illumination dependence and photophysiology of H₂ production.** In situ rates of O₂ and H₂ production were examined as a function of light intensity and quality (Fig. 3). At irradiances of <50 μmol m⁻² s⁻¹, H₂ production was detectable, whereas net O₂ evolution was extremely low. The H₂ production rate reached saturation at irradiances lower than those at which O₂ evolution saturated and which were below the level of incident irradiance supplied to the bioreactor during feedback control (240 μmol m⁻² s⁻¹). As O₂ evolution increased at higher irradiances, the H₂ production rates maintained the level achieved upon light saturation. Because the photobioreactor was irradiated with discrete LEDs that emitted at 630 or 680 nm, favoring either phycobilin or chlorophyll excitation, it was possible to measure light saturation curves for each pigment independently. At 680-nm ir-
Radiance, the maximum rate of H₂ production was about 20% higher than when both wavelengths were presented, and the initial slope (alpha) was increased, representing enhanced light utilization efficiency of H₂ production (see Table S1 in the supplemental material). In contrast, photosynthetic production of O₂ had a much lower alpha when 680 nm of light was supplied alone, compared to the values with 630 nm alone or with a balanced profile. There does not seem to be an additive effect of supplying both wavelengths; furthermore, the inclusion of 630 nm light may slightly suppress the efficiency of H₂ production.

H₂ production activity was also measured in the presence of the electron transport inhibitors 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and p-benzoquinone (BQ) to investigate the dependence of the process upon photocatalytic H₂O oxidation (Fig. 4a and b). The specific PSII inhibitor, DCMU, completely and immediately inhibited PSII activity at concentrations as low as 1.0 μM (Fig. S4 in the supplemental material). As shown in Fig. 4a, long-term incubation with DCMU for 72 h caused a dramatic, but incomplete, inhibition of H₂ accumulation in the light. Remarkably, short-term (1-h) incubation of H₂-evolving cells did not inhibit H₂ production at any concentration (data not shown). The plastoquinone analog BQ was observed to cause a substantial inhibition of H₂ production after 50 min, with only a modest effect upon O₂ evolution (Fig. 4b).

The capacity for photosynthetic electron transport was further investigated by measuring the variable level of chlorophyll fluorescence originating from PSII, using a pulse amplitude modulated (PAM) fluorometer. The maximum theoretical quantum efficiency of PSII, represented by the proportion of the maximal fluorescence which varies according to PSII activity (Fᵥ/Fₘₐₓ), showed an increase over the first 48 h of H₂ production in both bioreactors (Fig. 5). In the presence of CO₂, the Fᵥ/Fₘₐₓ reached a higher value than that of the CO₂-deprived condition and maintained this high value throughout the second half of the experiment, whereas the Fᵥ/Fₘₐₓ declined during the latter part of the CO₂-deprived experiment. Further information about PSII activity was obtained from rapid light curves using the saturating pulse method to infer the rate of linear electron transport from PSII. Under both reactor conditions, the maximal electron transport rate (ETRₘₐₓ) showed a brief decline over the first few hours of nitrogen deprivation, thereafter rising concurrently with the H₂ production rate. However, in the CO₂-deprived condition, the ETRₘₐₓ peaked at 22 h and subsequently oscillated between about 11 and 17 μmol · m⁻² · s⁻¹, whereas this parameter continued to rise further in the CO₂-supplied condition. It should be noted that, throughout the H₂ production process, neither of these photophysiological parameters decreased far below the starting values observed during steady-state growth, indicating a
robust capacity for photosynthesis during prolonged H₂ production over 100 h.

**DISCUSSION**

Future developments of H₂ as a photosynthetic biofuel depend on understanding how cells catalyze H₂ production concurrent with oxygenic photosynthesis (2). Here, we examined the photophysiological relationship between dinitrogenase-driven H₂ production and photosynthetic O₂ evolution and uncovered physiological conditions in which these processes co-occur. The experimental evidence revealed that metabolic dynamics and coordinated regulation of photosynthesis and dinitrogenase activity in *Cyanothece* 51142 displays oscillating patterns which may or may not be governed by circadian mechanisms or the onset of illumination during diurnal growth. Nonetheless, by avoiding the entrainment of cells to light-dark cycles—as no oscillations were observed during the NH₄⁺-limited chemostat growth—such a mechanism is not necessary to preconditioen the cells for H₂ production. Irrespective of CO₂ supply, continuously illuminated N-deprived *Cyanothece* 51142 cells evolved H₂ at rates which accelerated steadily for 24 h. After 24 h, the H₂-producing cultures did begin to exhibit oscillations; however, only when CO₂ was available did the gas dynamics follow a pattern which resembled the 12-h alternating phases observed with entrained cultures; in stark contrast, there was a positive correlation between H₂ and O₂ evolution in the CO₂-deprived reactor, suggesting that not only was dinitrogenase activity protected from O₂ inactivation but it also alleviated photoinhibition. In fact, the dynamic measurements of biochemical and biophysical characteristics of H₂-producing *Cyanothece* 51142 reveal a lack of prominent photoactivation (Fig. 3), stable chlorophyll levels (see Fig. S3 in the supplemental material), and robust electron transport capacity (Fig. 5). Despite the initial decline of PSII activity upon halting the delivery of NH₄⁺, it appears that linear electron transport may recover and even be enhanced by the achievement of high rates of H₂ production. The correlated rise in O₂ and H₂ evolution rates during the first phase of the CO₂-deprived experiment (Fig. 1b) supports this hypothesis. Because there are few electron sinks under this condition, overreduction of the PQ pool leads to deleterious effects on PSII function (21); however, dinitrogenase-mediated proton reduction may provide relief (22) by consuming reductant and thus preventing PSII closure.

While the observation of concomitant O₂ and H₂ evolution in a well-mixed unicellular system is provocative from a photophysiology standpoint, it has important implications for biofuel production. Our results clearly demonstrate that these two activities proceeded uninterrupted for several days of continuous illumination. Although changes in rates of the two processes were phased, their co-occurrence persisted in the CO₂-supplied bioreactor. As dinitrogenase is primarily responsible for H₂ evolution (18), this research also suggests that the mechanism of aerotolerance has been underappreciated. While there is no reason to speculate that the protein itself is O₂ tolerant (23), the concept of “respiratory protection” (24) seems plausible, reminiscent of the situation in Azotobacter (25) and Trichodesmium (26). In *Cyanothece* 51142, such a mechanism is supported by the inflection in O₂ evolution coinciding with the appearance of CO₂ (Fig. 1a and b) as well as by the decline in carbohydrate content which begins whenever the H₂ rate approaches a maximum (Fig. 2a and b). Electron micrographs of diazotrophically grown *Cyanothece* 51142 had shown a homogenous distribution of immunogold-labeled dinitrogenase, suggesting a lack of physical separation from oxygenic photosynthesis (27); but this neither precludes the existence of intracellular O₂ gradients nor discounts the possibility that the enzyme may not be equally active throughout the cell.

Furthermore, continuous net O₂ evolution indicates that PSII activity was maintained throughout the experiment and raises the question of whether H₂O oxidation may directly contribute to H₂ production. The nearly immediate loss of detectable H₂ within 3 min of a shift to darkness underscores the importance of the photosynthetic light reactions (see Fig. S5 in the supplemental material). Although inactivation of PSII with the inhibitor DCMU had no short-term effect upon H₂ production, a dose-dependent inhibitory effect of DCMU was indeed observed upon prolonged incubation (Fig. 4a), suggesting that the provision of electrons from H₂O by PSII may be necessary to maintain the capacity for H₂ evolution. Furthermore, mass balance calculations indicate that the H₂O-derived electrons which must accompany the observed O₂ evolution are more than sufficient to account for the H₂ yield under Ar-only conditions (see Fig. S2c in the supplemental material).

The lack of any short-term inhibition by DCMU suggests a major role for PSI activity (26). The enhanced quantum efficiency of H₂ production with 680 nm compared to 630 nm light supports this (Fig. 3), especially as it coincided with a decreased efficiency of O₂ evolution, resembling the preferential excitation of PSI by chlorophyll-specific light. The inhibitory effect of BQ upon H₂ but not O₂ production (Fig. 4b) may further support the role of PSI, as this plastoquinone analog is known to oxidize the quinone pool, maintaining PSII function, although the downstream effects on electron transport are unclear. Similarly, important contributions by catabolism of endogenous organic carbon are also suggested by the correlated dynamics of H₂ and CO₂ evolution (Fig. 1a), the declining carbohydrate content which precedes maximal H₂ rates (Fig. 2a and b), and the requirement of exogenous O₂ for H₂ production in the dark (see Fig. S5 in the supplemental material). However, mass balance calculations using the stoichiometric H₂ yield of complete glucose oxidation suggest that carbohydrate consumption in the light may, at best, account for no more than 51% of the reductant required for the corresponding H₂ production (see Fig. S2 in the supplemental material), although this contribution may be much lower due to respiratory O₂ scavenging and/or the thermodynamic constraints of fermentative metabolism. Furthermore, under Ar-only conditions, significant H₂ production is still observed during the latter half of the experiment, when carbohydrate consumption is negligible.

These results imply that *Cyanothece* 51142 has a flexible metabolism in which different mechanisms and electron sources may be utilized for H₂ production (6, 12). The reductant and ATP necessary for H₂ production, as well as for cell maintenance, is likely supplied by linear electron transport through PSII and by light-independent substrate catabolism in the presence of O₂, with offsets in the demand for ATP fulfilled by cyclic electron transport around PSI. Moreover, as cyanobacterial thylakoid membranes have several branch points between photosynthetic and respiratory electron transport components (28), “pseudocyclic” processes involving both PSI and respiratory complexes may also contribute to the generation of reductant and ATP (29), as well as toward the minimization of oxidative photodamage (30). The observed oscillations may indeed involve regulatory phenomena.
among these processes (28) but are likely also intertwined with feedback mechanisms at the level of the electron transport chain (31).

This work has a large significance for biotechnology: for the first time, unicellular phototrophs have been shown to produce H₂ and O₂ concomitantly without interruption for at least 100 h. The high rates of H₂ production attained with this strain of *Cyanothecae* are in agreement with what had been previously cited as the highest yet reported for any oxygenic phototroph (18). Yet here, such substantial H₂ production rates were observable for an extended period, without requiring light-dark entrainment or high cell density. The sustainability of the process is highlighted by a lack of apparent photodamage and the dampening of oscillations toward the end of the CO₂-supplied experiment (Fig. 1a), which maintained significant rates of net H₂ and O₂ evolution with no clear sign of decay. Possibly, a feed-batch process with intermittent N replenishment might permit an improvement of the dampened rate, as was shown in purple bacteria (32). It is likely that the imposition of nitrogen limitation in a photobioreactor equipped with advanced process control was influential to these results, but such operation is more relevant to an industrial setting than most laboratory-scale batch experiments and is quite amenable to optimization. The continuous illumination should not be a practical concern, as indoor 24-h operation of bichromatic LEDs, powered by electricity from advanced photovoltaics and other renewables, can permit large increases in solar utilization efficiency (33).

**MATERIALS AND METHODS**

**Media and cultivation conditions.** *Cyanothecae* 51142 was routinely maintained in modified ASP-2 medium (34), containing 17 mM NH₄Cl, 0.03 mM FeCl₃, and 0.75 mM K₂HPO₄ under continuous white-light illumination (50 μmol m⁻² s⁻¹) and air sparging. Controlled cultivation was carried out in BioFlo 3000 reactors (New Brunswick Scientific, Edison, NJ) using a 7.5-cm-diameter cylindrical borosilicate glass vessel housed within a custom-manufactured aluminum enclosure equipped with LED illuminator chips (Marubeni America Corp., New York, NY) and quantum sensors (LI-COR, Lincoln, NE). LEDs provided 630 and 680 nm of actinic illumination and minimal fluorescence (Fo) after 10 min of incubationorage (seawater, 30°C). In situ dissolved H₂ (dH₂) was measured with a modified O₂ sensor, which was adapted by reversing the polarity, preconditioning for 6 h in proprietary electrolyte (number 9920; Mettler-Toledo), swapping the electrolyte to an HCl/KCl solution (0.1 M each) (40), desensitizing with a certified gas mixture of 15,200 ppm H₂-Ar (American Air Liquide, Houston, TX), and calibrating as 11.53 μM (freshwater, 30°C). Instantaneous net production rates of H₂ and O₂ were obtained using first-order removal coefficients (74.320 and 55.024 h⁻¹, respectively), determined from the slope of the natural logarithm of dH₂ or do₂ removal during brief darkness, averaged across six measurements. Bioreactor off-gas composition was measured by online mass spectrometry (MGA iSCAN, Hamilton Sunstrand). Gas composition of tube headspace was analyzed with a Hewlett-Packard 5890 series II gas chromatograph, using a thermal conductivity detector, Supelco 60/80 Carboxen 1000 column (Sigma-Aldrich, St. Louis, MO), and Ar carrier gas.

**Photophysiological measurements.** Light response curves for O₂ and H₂ evolution were determined in situ within the photobioreactor by interrupting BioLume’s feedback control with a manual routine. Five-minute steps of increasing incident irradiance from each illuminator were implemented sequentially, following 10 min of darkness, and repeated with 630- or 680-nm LEDs independently. The average dO₂ or dH₂ at equilibrium for each step was converted to net production rate as described above and plotted against irradiance. Photosynthetic parameters were obtained by fitting the data to a hyperbolic tangent function (41). Variable chlorophyll fluorescence was measured using PAM fluorometry in a DUAL-PAM-100 system (Walz GmbH, Effeltrich, Germany) with a photodiode detector and RG665 filter (42). Red measuring light (620 nm) at the lowest power was pulsed at 1,000 Hz during the dark and at 10,000 Hz during 635-nm actinic illumination at 98 μmol m⁻² s⁻¹. The maximum theoretical quantum yield of PSII (Fv/Fm) was calculated from maximal fluorescence (Fm) recorded with 15 μM DCMU during actinic illumination and minimal fluorescence (Fₒ) after 10 min of incubation with only far-red (730 nm) light (43). Electron transport rates through PSII were determined with rapid light curves (44) using 20-s steps of incremental actinic light followed by a 200-ms saturating pulse at 1,000 μmol m⁻² s⁻¹ and 5 s of only far-red light.

**SUPPLEMENTAL MATERIAL**

Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.00197-12/-/DCSupplemental.

Figure S1, DOCX file, 0.1 MB.
Figure S2, DOCX file, 0.1 MB.
Figure S3, DOCX file, 0.1 MB.
Figure S4, DOCX file, 0.1 MB.
Figure S5, DOCX file, 0.1 MB.
Table S1, DOCX file, 0.1 MB.

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M.R.M., A.E.K., and A.S.B. conceptualized the research and wrote the manuscript. M.R.M. and L.A.K. planned and performed the experimentation, took samples, performed biochemical analyses, and calculated the data. G.E.P. and A.H. designed the photobioreactor and supervised reactor operations. M.R.M. and E.A.H. designed the dissolved hydrogen probe. E.A.H., G.E.P., J.K.F., and A.S.B. made the initial discovery of H₂ production and defined the chemostat operating parameters. A.S.B., A.E.K., and J.K.F. supervised the project. All authors edited the manuscript.

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