Auto-flotation of heterocyst enables the efficient production of renewable energy in cyanobacteria

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Utilizing cyanobacteria as a bioenergy resource is difficult due to the cost and energy consuming harvests of microalgal biomass. In this study, an auto-floating system was developed by increasing the photobiological H2 production in the heterocysts of filamentous cyanobacteria. An amount of 1.0μM of diuron, which inhibited O2 production in cyanobacteria, resulted in a high rate of H2 production in heterocysts. The auto-floating process recovered 91.71% of the accumulated microalgal biomass from the liquid media. Quantification analysis revealed that 0.72–1.10 μmol H2 per mg dry weight microalgal biomass was necessary to create this auto-floating system. Further bio-conversion by using anaerobic digestion converted the harvested microalgal biomass into biogas. Through this novel coupled system of photobiological H2 production and anaerobic digestion, a high level of light energy conversion efficiency from solar energy to bioenergy was attained with the values of 3.79% ± 0.76.

The conversion of solar energy into clean chemical energy has attracted much attention in recent years, due to the fossil fuel crisis and the emission of greenhouse gases1,2. Photobiological H2 production via cyanobacteria is an important strategy for converting solar energy into renewable and sustainable energy3–5; however, a major obstacle in the production of renewable bioenergy from cyanobacteria is its relatively low light energy conversion efficiency (LCE). The theoretical maximal LCE of photosynthesis in cyanobacteria is reported to be approximately 11%6. However, the LCE for renewable energy production has always been relatively low because most of the converted solar energy is stored as biomass6,7. For example, the LCE for microalgal H2 is 0.042% and 0.075% for Anabaena sp. strain PCC 7120 (henceforth referred to as Anabaena 7120) and Chlamydomonas reinhardtii respectively8–10. In these microalgal cells, the electron pathway for hydrogen production is strictly oxygen sensitive, and most of the electrons from water photolysis were utilized to assimilate CO2 and produce biomass6,7. Converting the light energy stored in microalgal biomass into renewable energy is a promising strategy for a new source of bioenergy.

Unfortunately, biomass harvest is still a technical bottleneck for microalgae-driven solar energy conversion11,12. Many strategies have been attempted for the efficient harvest of the biomass, such as centrifugation, filtration, and flocculation13,14. But each one of these harvesting methods are also energy-intensive and expensive14. Bio-flocculation has been utilized successfully in recent years to obtain microalgae sedimentation15, but the recovery of sentimental cells is still a time consuming and costly process. Dissolved air flotation (DAF) is considered to be the primary technology of choice for harvesting of microalgal biomass due to its high efficiency and relatively lower energy consumption compare to centrifugation16–18. However, some of the chemical coagulants used in DAF created additional problems, such as excess cost and pollution to the environment19,20. Furthermore, the operational cost of the DAF unit is still high because of the enormous energy required to achieve the necessary air compression of 390 kPa (56 psig)21,22. Therefore, an auto-floating system without the need of coagulants and compressed air will be a promising strategy for an economical and environmental friendly method to harvest microalgae.

Two key features are essential for an auto-floating system. One required element is the ability to auto-flocculate and sediment, and the other is the ability of the microalgae to produce an adequate amount of gas to enable floatation. The heterocyst-containing N2-fixing cyanobacterium Anabaena 7120 is a photoautotrophic microalga that assimilates CO2 from the atmosphere in order to grow. This microalga has a marked flocculation activity attributed to its filamentous cellular structure and excretion of mucilage23,24. Using sunlight as its only source of energy, Anabaena can generate H2 gas under anaerobic conditions25,26. Photobiological H2 production of
Anabaena 7120 occurs both in vegetative cells and heterocysts. Research has shown that this photobiological activity is mainly catalysed by nitrogenase in the heterocysts.

In this study, auto-flotation of Anabaena 7120 was induced by stimulating photobiological H₂ production though inhibition of photosynthetic O₂ evolution. The relationship between the amount of H₂ production and biomass weight was determined by quantification analysis. Following the auto-floating harvest process, an anaerobic digestion of the separated cellular biomass was performed to convert its stored solar energy to H₂ and CH₄, contributing to a high total LCE value for clean energy production.

**Results**

Anabaena 7120 rapidly self-flocculated in standing culture. We investigated the self-flocculation ability of Anabaena 7120 and compared it with two other representative photo-H₂ producing microalgae, the green alga Chlamydomonas reinhardtii and the cyanobacterium Synechocystis sp. PCC 6803. As clearly shown in figure 1, unlike the unicellular microalgae Chlamydomonas and Synechocystis, the algal biomass of the Anabaena culture aggregated and flocculated rapidly. The culture of Anabaena 7120 settled completely in just 2 hours.

H₂ production was significantly enhanced in DCMU-treated samples. H₂ gas can be used as a clean and renewable energy, and also act as an effective force to provide buoyancy for flotation of the microagal biomass. Anabaena 7120 can produce H₂ gas under N₂-starved conditions by using solar light as the energy resource. Previous reports have shown that O₂ is the major inhibitor in the production of photosynthetic H₂ in N₂-fixing cyanobacteria. Anaerobic conditions induce H₂ production within Anabaena 7120 but the amount of H₂ produced under these conditions was quite low. As shown in table 1, the highest rate obtained by our laboratory was 1.26 µmol of H₂ per mg of chlorophyll a per hour (per mg Chl a.h). Moreover, as shown in Supplementary Fig. S1a, O₂ gas (red line) and H₂ gas (blue line) were produced simultaneously in the culture. These results corresponded to previous research results presented in Anabaena cylindrica. Although both O₂ and H₂ evolved in Anabaena 7120, O₂ is only produced in vegetative cells. The increase of O₂ content (see Supplementary Fig. S1a) revealed the active photochemical function of photosystem II (PSII). As shown in Supplementary Fig. S1b, O₂ evolution activity of PSII was quite high in the early stage of H₂ production (0–12 h).

To prevent O₂ evolution, diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCMU), an obligate inhibitor of PSII was added to Anabaena 7120 cultures (Fig. 2a). The results showed that the O₂ evolution in the Anabaena cells was inhibited by 60% of the original level with the addition of 0.05 µM DCMU, and a 100% suppression was achieved when treated with 1.0 µM DCMU. To investigate the effects of 1.0 µM DCMU on the photochemical properties of PSII, the effective photochemical quantum yield of PSII in dark-adapted samples (Fig. 2b) and the electron transport rate of PSII (ETR II) (Fig. 2c) were also analyzed. These data revealed that the photochemical activity of the PSII reaction center was almost completely suppressed and that electron transfer in PSII was significantly blocked from 26.25 ± 0.65 to 1.25 ± 0.15 µmol electrons m⁻²s⁻¹. Therefore, a 1.0 µM concentration of DCMU efficiently interrupted photosynthetic O₂ evolution in the Anabaena 7120 cells.

To determine the photobiological H₂ production in Anabaena 7120 treated with 1.0 µM DCMU, the treated cells were grown with illumination at 100 µE m⁻² s⁻¹. As shown in figure 3, the H₂ production increased drastically in DCMU-treated cultures and maintained a relative high level for more than 120 hours under continues illumination. Hydrogen production in untreated and treated cells is presented in table 1. DCMU-treated cultures accumulated H₂ about 200 folds more and around 10 times faster. These results demonstrated that under illumination, Anabaena 7120 cells produced a high rate of H₂ in the absence of O₂.

### Table 1 | The rates and accumulation of H₂ production in untreated cells and DCMU treated cells of Anabaena 7120

| Sample                   | Accumulated H₂ (µmol) | Maximal rate (µmol/mg Chl.a/h) |
|--------------------------|-----------------------|---------------------------------|
| Untreated cells          | 0.42 ± 0.03           | 1.26                            |
| DCMU treated cells       | 77.41 ± 7.36          | 11.58                           |

¹ The data is the average value of triplicate biological texts of sample tubes containing 40 mL new Anabaena 7120 cell cultures (A₇30 = 0.8–1.0), and all samples were illuminated for 120 hours to produce H₂.
Microalgal biomass automatically floated with high level of H₂.

From figure 1, *Anabaena* 7120 self-flocculated rapidly in standing culture. The flocculated microalgal biomass cluster treated with DCMU was observed floating within 12 hours (Fig. 4a). The relationship between H₂ accumulation and the amount of floating biomass were investigated (Fig. 4b). Under our experimental conditions, 0.72–1.10 μmol of H₂ per mg of dry weight microalgal biomass was necessary for the biomass to float. Thus, auto-flotation occurred with a relatively high level of H₂. Additional evidence was provided by the observation that the auto-floating process was disrupted when H₂ production ceased under dark or anaerobic conditions (Fig. 4c). Together, these results demonstrated that auto-floating of the biomass was a process that depended on a high level of hydrogen. The floating microalgae biomass was harvested at an efficiency of 91.71% ± 1.22.

Heterocyst was required for high level of H₂ production and self-floating. In untreated *Anabaena* 7120 cells, heterocysts are the major contributors to photobiological H₂ production, however their contribution is unclear in DCMU-treated cultures. Because heterocysts differentiation in *Anabaena* 7120 is dependent on nitrogen starvation, the H₂ production of 1.0 μM DCMU-treated cultures under BG-II (−N) and BG-II (+N) media conditions were evaluated and compared (Fig. 5). Forty mL *Anabaena* 7120 cultures in the logarithmic growth phase were treated with DCMU. During a 120-hour period, H₂ accumulation in DCMU-treated cultures grown in BG-II (−N) containing heterocysts increased significantly from 0.42 μmol to 77 μmol (Fig. 5a). In contrast, DCMU-treated cultures grown in BG-II (+N) without heterocysts accumulated only baseline volume of H₂ gas (0.08 μmol in untreated cells and 0.06 μmol in DCMU-treated cells) shown in figure 5b. To determine whether the
For most species of microalgae, much of the solar energy consumed during photosynthesis is converted and stored as cellular biomass. Thus, only a fraction of the converted solar energy is available for photobiological H₂ production by microalgae. H₂ and CH₄ gas produced in microalgal anaerobic digestion is a type of bioenergy that can be readily used. Sulphur-deprived *Chlamydomonas* and nitrogen-deprived *Cyanothece*, as unicellular microalgae, have shown high levels of photobiological H₂ production under the proper conditions. However, the separation and harvest of the biomass from these species of microalgae is quite difficult and expensive due to the low specific gravity of the individual cells keeping the cells suspended in culture. Furthermore, the LCE for H₂ production in most species of cyanobacteria is relatively low, i.e., below 1%. Compared with unicellular microalgae, flocculation is more likely to occur naturally in *Anabaena* 7120 cultures due to its filamentous cell structure and excretion of mucilage. Figure 1 revealed that a standing culture of *Anabaena* 7120 flocculated and settled automatically in a very short time period (approximately 60 min). This fast self-flocculation activity provides a significant advantage in the creation of an auto-floating system for the efficient and economic harvest of microalgal biomass. According to the requirements of an auto-floating system, as mentioned above, microalgae floated if an adequate amount of H₂ gas was also produced.

In this study, a high level of photobiological H₂ production in heterocysts was stimulated and sustained by the inhibition of O₂ production in the microalgal cells. Previous research had shown that any trace of O₂ ceased the anaerobiosis induced nitrogenase activity. Even though heterocysts already have low O₂ concentration, the strict anaerobic intracellular condition resulting from DCMU treatment was essential. The same conclusion was drawn in an *Anabaena* strain in which nitrogenase activity within the anaerobic heterocysts was suppressed significantly by exposing to a trace amount of O₂. Argon gas and DCMU induced strict anaerobiosis is the most likely explanation for the high level of photo-H₂ production in *Anabaena* 7120. We also investigated the possible reason for such a stimulated long stage of H₂ production in 1.0 μM DCMU treated cells. As the major carbohydrate storage in N₂-fixing cyanobacteria, glycogen can provide nitrogenase energy and reducing power for N₂ fixation and H₂ production. As shown in Supplementary Fig. S3a, the accumulated glycogen was consumed in DCMU treated *Anabaena* 7120 cells during the H₂ production period. In addition, the increase in nitrogenase (NifH) expression suggested that more electrons and reductants from glycogen were transferred to nitrogenase for proton reduction and H₂ gas evolving (see Supplementary Fig. S3b).

As a PSII photochemical inhibitor, DCMU addition is an effective strategy to inhibit O₂ evolution and improve the long stage H₂ production in *Anabaena* cultures used in this study. However, DCMU utilization may bring some environmental concerns as well. Metal ion inducible gene expression systems, such as Cu and Ni, could also be chosen as a possible alternative strategy for repression of PSII activity and increase photoautotrophic H₂ production in microalgae. The protein such as NaC2 which is required for stable expression of the core polypeptide of PSII could be a possible target. Beside the metal ions, some environmental factors (light intensity, O₂ level and CO₂ concentration) inducible gene systems also had been reported in microalgae, which would eliminate the use of environmentally hazardous inducers.

As shown, *Anabaena* 7120 cells auto-flocculated rapidly and the high level of H₂ gas evolution from the heterocysts induced flotation of the flocculated microalgal biomass. Harvesting efficiency of over 90% was achieved by using this method. This auto-floating system of the filamentous cyanobacterium provided an economical and environmental friendly strategy for microalgal biomass harvests. Moreover, some auto-flocculating microalgae have been utilized successfully to sediment un-flocculating microalgae. The former...
research revealed that the sedimentation rate of the un-flocculating microalgae was increased considerably when mixed with auto-flocculating microalgae, leading to a higher recovery percentage. Referring to the recent bio-flocculation research of Salim et al.15, such an auto-floating system has the potential to be widely used for harvesting of the un-flocculating microalgae, such as the unicellular microalga *Chlorella*.

The LCE for photosynthesis can reach a theoretical maximum of 11% in cyanobacteria, while the LCE for direct photobiological H\(_2\) production is always less than 0.1% in *Anabaena* 7120. By the auto-floating harvest strategy, the further conversion of microalgal biomass into clean bioenergy can become a promising bioenergy resource with a relatively high LCE. The bioenergy obtained by this auto-floating system can be calculated in two steps: the H\(_2\) gas energy produced during the first stage and the biogas produced in the second stage (Table 2).

During the first stage, photobiological H\(_2\) was produced from the heterocysts. At the second stage, the microalgal biomass was separated from the auto-floating system and further converted by anaerobic digestion into clean energy as H\(_2\) and CH\(_4\). The auto-floating process combines effective algal photobiological H\(_2\) production and residual microalgal biomass digestion for the production of clean energy. The LCE of this couple system for biofuel production from solar energy was obtained with an average value of 3.79%. Moreover, it avoids costly energy-consuming processes, such as centrifugation and filtration, to harvest the microalgal biomass.44 The LCE for direct photobiological H\(_2\) production increased to an average value of 3.79%.

Photobiological H\(_2\) production in cyanobacteria can be further increased through genetic engineering or by optimization of growth factors.8,29,45 In addition, strategies to improve clean energy production from the accumulated microalgal biomass continued to be
explored. Based on the auto-floating process described here, commercial applications can be developed for the future production of clean energy from microalgae.

**Methods**

**Species and growth conditions.** Filamentous cyanobacteria *Anabaena* 7120 (FACHB-418), *Anabaena variabilis* (FACHB-319) and *Anabaena cylindrica* (FACHB-1038) were purchased from the Institute of Hydrobiology, Chinese Academy of Sciences. The *nifHDK* operon promoter mutant AMC-100 was provided by Professor Jame W. Golden. The unicellular cyanobacterium *Synechocystis* 6803 and green alga *Chlamydomonas reinhardtii* CC503 were kind gifts from Professor Quanxi Wang. Microalgal cells were cultured at 30°C in BG-II medium (with or without nitrogen) and buffered with Tris-HCl (5 mM, pH 8.0). The liquid culture was bubbled with 2–3% (v/v) CO₂ in air under continuous illumination by fluorescent lamps (30 μE m⁻² s⁻¹).

**Determination of cell density and chlorophyll a content.** The cell density was calculated using light absorption at 730 nm (A₇₃₀) in a UV-Vis mini-1240 spectrophotometer (SHIMADZU, Kyoto, Japan) with a 3 mL cuvette and an optical path of 1 cm. Chlorophyll *a* (Chl *a*) content was measured as previously described.

In summary, *Anabaena* 7120 cells (1.5 mL) were collected by centrifugation and washed twice with 1 mL of fresh BG-II medium. The supernatant was discarded after centrifugation; 1.5 mL of methyl alcohol was added to the cell pellet; and the mixture was incubated at 4°C for 6–14 h. The supernatant was then collected by centrifugation and absorbance at 665 nm (A₆₆₅) was measured. The concentration of Chl *a* (μg mL⁻¹) was calculated as 13.9 × A₆₆₅⁻¹.

**Sample preparation for photobiological H₂ production.** *Anabaena* 7120 cells were cultured in 0.5 L BG-II medium for 5 days (A₇₃₀ = 0.8–1.0) and were transferred to 60 mL glass tubes (leaving 20 mL of head space). DCMU was added to the cultures for a final concentration of 1.0 μM. The tubes were sparged with Ar gas.

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**Figure 5** | Morphological and physiological investigation of the relationship between heterocyst development and photobiological H₂ production in *Anabaena* 7120 cells. Cells in the logarithmic growth phase were used for the assay. (a1–a3) A confocal microscopy image of the *Anabaena* 7120 cells grown in BG-II (–N) medium showed normal development of heterocysts. (b1–b3) The confocal microscopy image shows *Anabaena* 7120 cells grown in BG-II (+N) medium without heterocysts. The cells were immobilised with 1% (w/v) agar in BG-II (–N) medium before observation and graphic analysis. A number of heterocysts (white arrows) were observed at the frequencies between 1/10 to 1/20 (heterocyst/vegetative cell). a1, b1–a bright field micrograph of the cells; a2, b2–an auto fluorescence (exited at 488 nm) micrograph of the cells; a3, b3–a combined micrograph of the cells. (a4) Physiological analysis of the photobiological H₂ accumulation in *Anabaena* 7120 cells with normal heterocysts. (b4) Physiological analysis of the photobiological H₂ accumulation in *Anabaena* 7120 cells without heterocysts.
Biogas production. After the *Anabaena* 7120 microalgal biomass finished producing photobiological H2, it was collected and transferred to the biogas incubator and monitoring system for sludge-based anaerobically digested biogas production. Substrate fermentation was conducted in batch tests as follows: 100 mL biogas batch fermenters were filled with 5.0 g of anaerobic sludge from a mesophilic anaerobic digester in the Gaobeidian Sewage Treatment Plant in Beijing, China. The substrate was equivalent to 9.0 g of wet microalgal biomass per test. It was loaded and the fermenters were then sealed with rubber septa and incubated in a 37 °C water bath for dynamic monitoring of biogases production. The incubation and monitoring process were performed in the Automatic Methane Potential Test System (AMPTS) II.

**Calculation of light energy conversion efficiency (LCE).** The LCE of the whole conversion process consists of two steps: direct photobiological H2 production and the anaerobic digestion of microalgal biomass for biogas production. The LCE of step 1 was determined based on the supplied light energy during the H2-producing phase; the LCE of step 2 was based on the supplied light energy during the cell growth of the biomass accumulation phase. The efficiency of each step was calculated according to the following equation:

\[
\text{LCE} = \frac{\text{Energy of H}_2 \text{ production}}{\text{Energy of light input}}
\]

Figure 7 | Biogas production from the microalgal biomass substrate after photobiological H2 production. (a) The detected total biogas volume of the blank sample and the sample with algal biomass as a substrate. (b) The gas composition (H2 and CH4) analysis during the anaerobic digestion stage. Error bars indicate the s.d. values that were calculated from the average of triplicate experiments.
In full expression the equation is in form:

\[
\eta_{\text{H}_2 + \text{CH}_4} \times 100 = \frac{\text{E}_{\text{H}_2 + \text{CH}_4}}{\text{E}_A}
\]

\[
= 100 \left(\frac{\Delta G_{\text{H}_2}}{\text{R} \times \text{H}_2} + \frac{\Delta G_{\text{CH}_4}}{\text{R} \times \text{CH}_4}\right) / \text{(E_A)}
\]

where \(\Delta G_{\text{H}_2}\) and \(\Delta G_{\text{CH}_4}\) are the standard Gibbs energy for the energy storage reaction (1,237 mW mol\(^{-1}\) at 297 K) and \(\text{CH}_4\) (890 J mol\(^{-1}\) at 297 K) respectively. \(\text{R}\) and \(\text{E}_A\) are the amounts of accumulated \(\text{H}_2\) and \(\text{CH}_4\) gas respectively. \(\text{E}_A\) is light energy radiation, and \(A\) is the illumination area.

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### Table 2: The LCE calculation of the two stages and the whole process

| Stage     | light energy absorption (J) | energy production (J) | LCE (%) |
|-----------|-----------------------------|-----------------------|---------|
| Algae growth | 79619.48                    | -                     | -       |
| Photobiological | 2948.87                     | 18.36 ± 1.75          | 0.62 ± 0.06 |
| H\(_2\) production | -                       | 3112.77 ± 631.47    | 3.79 ± 0.76 |
| Anaerobic dark digestion | -                         | 82568.35             |         |

Conversion efficiency % = (Energy content of \(\text{H}_2\) and \(\text{CH}_4\)) / (Absorption of light energy) × 100 (1)

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22. Annimede, Y., Troeger, B. W. & Reed, L. W. Mutual Flocculation of Algae and Clay: Evidence and Implications. Science 216, 63–65 (1982).

23. Walsby, A. E. Mucilage secretion and the movements of blue-green algae. Protoplasma 65, 223–238 (1968).

24. Benemann, J. R. & Weare, N. M. Hydrogen Production by Nitrogen-Fixing *Anabaena cylindrica* Cultures. Science 184, 174–175 (1974).

25. Tsygankov, A. A., Serebryakova, L. T., Rao, K. K. & Hall, D. O. Acetylene reduction and hydrogen photoproduction by wild-type and mutant strains of *Anabaena* at different CO\(_2\) and CO\(_3\) concentrations. FEBS Microbiol. Lett. 167, 13–17 (1998).

26. Dutta, D., De, D., Chaudhuri, S. & Bhattacharya, S. Hydrogen production by cyanobacteria. Microb. Cell Fact. 4, 36 (2005).

27. Petersen, R. B. & Burris, R. H. Hydrogen metabolism in isolated heterocysts of *Anabaena* 7120. Arch. Microbiol. 116, 125–132 (1978).

28. Houchins, J. P. & Burris, R. H. Occurrence and localization of two distinct hydrogenases in the heterocystous cyanobacterium *Anabaena* sp. strain 7120. J. Bacteriol. 146, 209–214 (1981).

29. Masukawa, H., Mochimaru, H. & Sakurai, H. Disruption of the uptake hydrogenase gene, but not of the bidirectional hydrogenase gene, leads to enhanced photobiological hydrogen production by the nitrogen-fixing cyanobacterium *Anabaena* sp. PCC 7120. Appl. Microbiol. Biotechnol. 58, 618–624 (2002).

30. Fay, P. Oxygen relations of nitrogen fixation in cyanobacteria. Microbiol. Rev. 56, 340–373 (1992).

31. Tamagnini, P. et al. Hydrogenases and Hydrogen Metabolism of Cyanobacteria. Microbiol. Mol. Biol. Rev. 66, 1–20 (2002).

32. Weissman, J. C. & Benemann, J. R. Hydrogen production by nitrogen-starved cultures of *Anabaena cylindrica*. Appl. Environ. Microbiol. 33, 123–131 (1977).

33. Ghirardi, M. L. & Hurd, M. Hydrogenase and Hydrogen photoproduction in *OxygCUS* photosynthetic Organisms. Annu. Rev. Plant Biol. 58, 71–91 (2007).

34. Golden, J. W., Whorff, L. L. & Wiest, D. R. Independent regulation of nifHDK operon transcription and DNA rearrangement during heterocyst differentiation in the cyanobacterium *Anabaena* sp. strain PCC 7120. J. Bacteriol. 173, 707–7105 (1991).

35. Melis, A., Zhang, L., Foretist, M., Ghirardi, M. L. & Seibert, M. Sustained Photobiological Hydrogen Gas Production upon Reversible Inactivation of Oxygen Evolution in the Green Algae *Chlamydomonas reinhardtii*. Plant Physiol. 122, 127–136 (2000).

36. Bandypadhyay, A., Stickle, J., Min, H., Sherman, L. A. & Pakrasi, H. B. High rates of photobiological H\(_2\) production by a cyanobacterium under aerobic conditions. Nat. Commun. 1, 139 (2010).

37. Craggs, R. J., McAuley, P. J. & Smith, V. J. Wastewater nutrient removal by marine microalgae grown on a corrugated raceway. Water Res. 31, 1701–1707 (1997).

38. Rippka, R. & Stanier, R. Y. The Effects of anaerobiosis on nitrogenase synthesis and heterocyst development by nostocacean cyanobacteria. J. Gen. Microbiol. 105, 83–94 (1978).

39. Suzyrczyki, R., Cournac, L., Peltier, G. & Roach, J. D. Potential for hydrogen production with inductable chloroplast gene expression in *Chlamydomonas*. P. Natl. Acad. Sci. USA 104, 17548–17553 (2007).

40. Hihara, Y., Kamei, A., Kanehisa, M., Kaplan, A. & Ikeuchi, M. DNA Microarray Analysis of Cyanobacterial Gene Expression during Acclimation to High Light. Plant Cell 13, 793–806 (2001).

41. Murata, N., Takahashi, S., Nishiyama, Y. & Allakhverdiev, S. I. Photoinhibition of photosystem II under environmental stress. BBA-Bioenergetics 1767, 414–421 (2007).

42. Sicoa, C. L., Ho, F. M., Salmeni, T., Styring, S. & Aro, E. M. Transcription of a “silent” cyanobacterial psA gene is induced by microaerobic conditions. BBA-Bioenergetics 1787, 105–112 (2009).

43. Hanawa, Y., Watanabe, M., Karatsu, Y., Fukuzawa, H. & Shiroya, I. Induction of a high-CO\(_2\)-inducible, periplasmic protein, H43, and its application as a high-CO\(_2\)-responsive marker for study of the high-CO\(_2\)-sensing mechanism in *Chlamydomonas reinhardtii*. Plant Cell Physiol. 48, 299–309 (2007).

44. Greenwell, J. R. Soc. Interface 7, 703–726 (2010).

45. Burrows, E. H., Chaplin, F. W. R. & Elly, R. L. Optimization of media nutrient composition for increased photofermentative hydrogen production by *Synechocystis sp.* PCC 6803. Int. J. Hydrogen Energy 33, 6092–6099 (2008).

46. Yen, H. W. & Brune, D. E. Anaerobic co-digestion of algal sludge and waste paper to produce methane. Bioresour. Technol. 98, 130–134 (2007).

47. Allen, M. M. Simple conditions for growth of unicellular bulle-green algae on agar plates. J. Phycol. 41, 1–4 (2005).

48. Ma, W., Shi, D., Wang, Q., Wei, L. & Chen, H. Exogenous expression of the wheat chloroplastic fructose-1,6-bisphosphate gene enhances photosynthesis in the transgenic cyanobacterium *Anabaena* PCC 7120. J. Appl. Physiol. 17, 273–280 (2005).

49. Arnon, D. I. Copper enzymes in isolated chloroplasts polyphenoloxidase in Beta Vulgaris. Plant Physiol. 24, 1–15 (1949).

50. Wang, L. et al. Treatment with moderate concentrations of NaHSO\(_3\) enhances photobiological H\(_2\) production in the cyanobacterium *Anabaena* sp. strain PCC 7120. Int. J. Hydrogen Energy 35, 12777–12783 (2010).
51. Lehner, J. et al. The morphogene AmiC2 is pivotal for multicellular development in the cyanobacterium *Nostoc punctiforme*. *Mol. Microbiol.* 79, 1655–1669 (2011).

52. Sukenik, A., Kaplan-Levy, R. N., Welch, J. M. & Post, A. F. Massive multiplication of genome and ribosomes in dormant cells (akinetes) of *Aphanizomenon ovalisporum* (Cyanobacteria). *ISME J.* 6, 670–679 (2012).

53. Mi, H., Endo, T., Ogawa, T. & Asada, K. Thylakoid Membrane-Bound, NADPH-Specific Pyridine Nucleotide Dehydrogenase Complex Mediates Cyclic Electron Transport in the Cyanobacterium *Synechocystis* sp. PCC 6803. *Plant and Cell Physiol.* 36, 661–668 (1995).

54. Schreiber, U., Schliwa, U. & Bilger, W. Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynth. Res.* 10, 51–62 (1986).

55. Schreiber, U., Endo, T., Mi, H. & Asada, K. Quenching Analysis of Chlorophyll Fluorescence by the Saturation Pulse Method: Particular Aspects Relating to the Study of Eukaryotic Algae and Cyanobacteria. *Plant Cell Physiol.* 36, 873–882 (1995).

56. Campbell, D., Hurry, V., Clarke, A. K., Gustafsson, P. & Oquist, G. Chlorophyll Fluorescence Analysis of Cyanobacterial Photosynthesis and Acclimation. *Microbiol. Mol. Biol. Rev.* 62, 667–683 (1998).

57. Kuhl, M., Chen, M., Ralph, P. J., Schreiber, U. & Larkum, A. W. D. Ecology: A niche for cyanobacteria containing chlorophyll d. *Nature* 433, 820–820 (2005).

58. Schreiber, U., Quayle, P., Schmidt, S., Escher, B. I. & Mueller, J. F. Methodology and evaluation of a highly sensitive algae toxicity test based on multiwell chlorophyll fluorescence imaging. *Biosens. and Bioelectron.* 22, 2554–2563 (2007).

59. Liu, J., Bukatin, V. E. & Tsygankov, A. A. Light energy conversion into H2 by *Anabaena variabilis* mutant PK84 dense cultures exposed to nitrogen limitations. *Int. J. Hydrogen Energy* 31, 1591–1596 (2006).

60. Yoon, J. H., Shin, J. H., Kim, M. S., Sim, S. J. & Park, T. H. Evaluation of conversion efficiency of light to hydrogen energy by *Anabaena variabilis*. *Int. J. Hydrogen Energy* 31, 721–727 (2006).

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**Author contributions**

M.C. performed the experiments with input from C.L. and J.L. M.C., J.L., L.Z. and S.C. designed the experiments, analysed the data and wrote the paper. S.L. and J.W. provided the overall guidance on study design, execution and wrote the paper.

**Additional information**

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