Photophysiology and Energetics of Light-Driven H_2 Production by *Cyanothece sp.* ATCC 51142

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<u>Project Goals:</u> The PNNL Biofuels Scientific Focus Area (BSFA) conducts fundamental research of microbial photoautotrophs with specific emphasis on photosynthetic energy conversion, reductant channeling, and central carbon metabolism. Subsequent to that are the mechanisms of reductant generation and partitioning in cyanobacteria and how relevant pathways are affected by external environmental factors and macronutrient concentrations. We specifically explore the conditions and mechanisms whereby reducing equivalents are funneled to H_2 as well as the physiological and biochemical bases for light-driven proton reduction. Generally, H_2 evolution in cyanobacteria is catalyzed by hydrogenase and/or nitrogenase enzymes and is inhibited by the presence of O_2 or other competing electron acceptors such as CO_2 and N_2. In *Cyanothece* 51142, however, H_2 production occurs simultaneously with photosynthetic release of O_2 under continuous light conditions as a direct result of nitrogenase activity in the absence of exogenous nitrogen and carbon sources. We hypothesize that, under these conditions, sustained and uninterrupted H_2 production depends upon electrons derived from photocatalytic water splitting.

Cyanothece sp. ATCC 51142 is a unicellular cyanobacterium which can tolerate substantial amounts of O_2 during the operation of its dinitrogenase enzyme, despite the strong inhibitory effect of O_2 upon the catalytic mechanism of this enzyme. As the dinitrogenase enzyme has an auxiliary function to reduce protons to H_2 as well as its primary role in N_2 fixation, the organism is capable of producing H_2 gas in the presence of O_2. Nevertheless, *Cyanothece* 51142 is well known for its dynamic physiology which can temporally separate its dinitrogenase activity from the O_2-evolving process of oxygenic photosynthesis. As H_2 is an attractive candidate as a clean renewable fuel, it has been a scientific imperative to elucidate strategies whereby the catalysis of H_2 production can be driven efficiently by the energy in sunlight. Here we show, for the first time, the continuous production of H_2 by a unicellular photosynthetic organism with concomitant evolution of O_2.

Under continuous illumination with a feedback-controllable dual-wavelength light-enclosure, steady-state chemostat cultures of *Cyanothece* 51142 were maintained under NH_4^+-limitation in a custom photobioreactor. Despite the high expression of proteins and transcripts for the dinitrogenase system, no H_2 production could be detected during chemostat growth. Instead, the production of H_2 was shown to be easily induced by these cells upon interruption of the steady-state growth. When transferred from the NH_4-limited chemostat to sealed anaerobic tubes incubated under constant illumination, H_2 production was induced after an initial 12 h phase of O_2 accumulation. The dynamic pattern of alternating H_2 and O_2 evolution showed a periodicity of 24 h, reminiscent to previous descriptions of the temporal separation of N_2 fixation and photosynthesis. However, the

level of each gas that accumulated did not significantly decrease between each production phase, and thus it became of interest to monitor the fine-scale dynamics of this process.

By halting the supply of NH_4^+ to the photobioreactor, chemostat growth was interrupted, and again H_2production activity was induced. However, facilitated by the high time-resolution instrumentation of the reactor, in-situ measurements enabled the striking observation that H_2 production can occur concomitantly with photosynthetic O_2 evolution in this unicellular organism. Within 1 h following the halt of NH_4^+ supply, H_2 production appeared with a rate which accelerated steadily over the next 24 h, reaching a maximal productivity of 400 μmol H_2/mg Chl/h. Within 6 h of the treatment, the rate of O_2 evolution had declined to a steady rate of 100 μmol O_2/mg Chl/h. However, following the peak of maximal H_2 production rates, the rate of net O_2 production rose, and the rates for both gases began to oscillate every 12 h, displaying an inverse correlation. However, throughout 100 h of continuous illumination, both H_2 and O_2 production activities maintained a positive net evolution rate, eventually dampening toward stable rates of 125 and 90 μmol/mg Chl/h, respectively. Additionally, dynamic changes in CO_2 evolution were observed, positively correlated with the rising and falling of the H_2 production rate.

By additionally halting the CO_2 supply during the chemostat interruption, *Cyanothece* 51142 induced a H_2 production activity which now displayed a *positive correlation* with changes in O_2 evolution rates, no longer following a 24 h periodicity. The light-dependence of the process was demonstrated by brief periods of darkness within the photobioreactor, leading to an immediate cessation of H_2 production, as well as by performance of in-situ light-saturation curves which showed dose dependence for both gases. Interestingly, the PS II-specific inhibitor DCMU was only capable of suppressing H_2 production in the long-term (hours as opposed to minutes). The yield of H_2 was partially diminished by the presence of increasing amounts of O_2, N_2, or CO_2, suggesting that other electron sinks may compete with the proton-reducing function of nitrogenase when they are available.

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