

## Photosynthetic production of hydrogen from *Chlamydomonas*

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Photobiological production of H<sub>2</sub> by eukaryotic algae is of interest because it holds the promise of generating a renewable fuel from abundantly available light and water (Gfeller and Gibbs, 1984; Benemann, 1996). Photosystem II utilize the energy of sunlight in photosynthesis to extract electrons from water molecules. Electrons released upon the oxidation of water are transported to the Fe-S protein ferredoxin on the reducing side of photosystem I. The hydrogenase in the stroma of the algal chloroplast accepts electrons from reduced ferredoxin and donates them to two protons to generate one H<sub>2</sub> molecule:

The O<sub>2</sub> evolution on the oxidizing side of photosystem II and H<sub>2</sub> production on the reducing side of PSI, having a ratio of H<sub>2</sub>:O<sub>2</sub> = 2:1 has not yet been achieved as the reversible hydrogenase is sensitive to O<sub>2</sub>-inactivation (Ghirardi et al, 1997). In the absence of functional PSII, the production of H<sub>2</sub> requires the presence and operation of PSI. Upon deletions of photosystem I core subunits *Chlamydomonas* mutants lacked CO<sub>2</sub> fixation, H<sub>2</sub> photoevolution, and photoautotrophic growth.

Continuous purging of H<sub>2</sub>-producing cultures with inert gases has allowed for the sustained production of H<sub>2</sub> (Reeves and Greenbaum, 1985), such purging is expensive and impractical for large-scale mass cultures of algae. The use of exogenous reductants such as sodium dithionite, create irreversible conditions that may lead to cell death. An alternative approach to photoproducing H<sub>2</sub> is based on the concept of indirect biophotolysis, in which metabolite accumulation acts as an intermediary step between photosynthetic H<sub>2</sub>O oxidation and H<sub>2</sub> production (Melis et al., 2000). In this approach, the two reactions, O<sub>2</sub> evolution and H<sub>2</sub> production, are spatially and/or temporally separated from each other (Benemann, 1996).

After 48 h of S deprivation, a sealed *C. reinhardtii* culture became anaerobic in the light, due to higher rate of respiration than photosynthesis which was measure by O<sub>2</sub> electrode. Under anaerobiosis in presence of light *C. reinhardtii* cells produced H<sub>2</sub>. One-liter culture of algae at a cell density of about 7 X10<sup>7</sup> cells/mL was incubated in S-deprived medium under continuous illumination (800 μmol m<sup>-2</sup> s<sup>-1</sup>). The flask was sealed 48 h after S deprivation when cells attained anaerobic condition. The rate of H<sub>2</sub> gas accumulation was constant at approximately 1 mL h<sup>-1</sup> for 48 h. Gas chromatographic analysis revealed that the composition of gases in the headspace of the culture bottle at 48 h was about 78% (v/v) H<sub>2</sub>, 2% (v/v) CO<sub>2</sub>, with the remainder being N<sub>2</sub>. Small amounts of O<sub>2</sub> was also observed.

The results demonstrate that by suppressing O<sub>2</sub> evolution by S-starvation, H<sub>2</sub> production could be increased. The H<sub>2</sub> production process is light dependent and utilizes the chlororespiratory and reversible hydrogenase pathways under anaerobic conditions.

The present work and the work of Melis et al., 2000 show that it is possible to produce and accumulate significant volumes of H<sub>2</sub> gas using photosynthetic organisms. This H<sub>2</sub> production mechanism may serve as the basis for further research and development efforts that could generate renewable alternative source of energy.

### References

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