Hydrogen from Sun and water!

Hydrogen generation from just light and water: is this too good to be true? Research workers, at CEA in particular, are striving to mimic what some microorganisms do naturally. Their dream is one of processes involving genetically optimized photosynthetic microorganisms, and subsequently, in the longer term, of biomimetic photocatalysts.

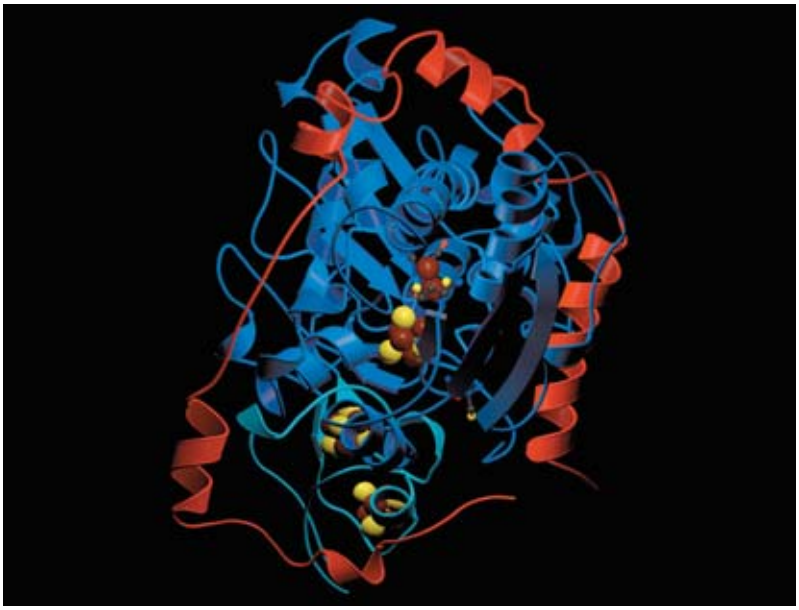
Many microorganisms naturally evolve **hydrogen**, using the **reduction** capability the living cell generates to effect an exceedingly simple reaction: two reducer **electrons**, joined to two **protons**, yield a molecule of hydrogen. Were that reduction potential obtainable at low cost, this process could be used to generate hydrogen on an industrial basis. One of the cheapest energy sources on our planet is sunlight. Indeed, the main source of energy for the **biosphere** is **photosynthesis**.

If biogeneration of hydrogen could be efficiently coupled to a photosynthetic process, hydrogen could be made from just light and water. This may seem too good to be realistic, however that is precisely what some microorganisms do naturally: research workers, at CEA in particular, are thus striving to understand, optimize and copy these processes. This work could result in new hydrogen production processes. In the medium term, this could take the form of extensive cultivation of genetically optimized microorganisms, and, in the long term, the bringing into use of artificial **catalysts** designed according to **biomimetic** principles.

Photosynthesis: a green chemistry, source of energy for the living world

Plants, algae and some **bacteria** use photosynthesis to convert solar energy into chemical energy, a contribution indispensable to the living world. The photophysical part of this process involves absorption of visible light by a green pigment, **chlorophyll**, whose molecules are carried by membrane proteins, and are arrayed in such a manner as to capture the greatest possible number of solar **photons.** Which further enables them to transfer the energy captured, passing from molecule to molecule, to special chlorophylls, exhibiting properties that are altered by a specific protein environment (known as a reaction enter). These reaction-center chlorophylls are host to a charge separation, yielding pairs of radicals of opposite electric charges, which are highly reactive chemically. These radicals are involved in reactions yielding high-energy compounds, indispensable for the unfolding of the complex processes required for cell life.

Life on Earth is based on the chemistry of carbon, and is strictly dependent on the contribution of carbonaceous molecules. The main source of carbon for the biosphere is **carbon dioxide** (CO_2, also known as carbon gas) which, if it is to be integrated into these molecules, must be reduced. This reduction consists in bringing **electrons** and **protons** to the CO^2. Photosynthesis provides these reducer electrons. This leaves positive "holes" carried by the chlorophylls at the reaction centers, which must be compensated for by an outside source of electrons for the process to function. Some photosynthetic organisms solved that problem very early on in the history of life, through a trick that literally changed the face of the world: they took out electrons from water.



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Molecular structure of an iron hydrogenase, one of the two major family of enzymes having the ability to catalyze hydrogen generation.

The release of photosynthetic oxygen and its consequences for the planet

A side effect of the extraction of electrons from water by photosynthetic organisms was the release of molecular oxygen into the environment. Now oxygen is a highly reactive element. When it appeared in the terrestrial environment, it almost certainly acted as a poison to virtually all living species. For those very few that managed to survive and adapt, however, the presence of oxygen brought the ability to use respiration, i.e. to make use of an energy uptake process from foodstuffs more efficient than those thus far relied on by the living world.

This higher efficiency exhibited by respiration enabled emergence of more complex life forms, in particular of multicellular organisms. The increased oxygen content in the atmosphere further allowed the ozone layer to form, this shielding life on Earth from the highly deleterious effects of the Sun's UV radiation. Photosynthetic organisms were thus directly responsible for creating, and sustaining right up to the present, the environmental conditions that allowed development of a complex form of life on Earth. Their own success arose from the fact they required, for their own purposes, little else than light, CO_2, and water: they were thus able to colonize the entire planet.

The energy contribution of photosynthesis to human development, from the present to tomorrow

Photosynthesis remains, in the 21st century, the main source of energy for humankind. Our food essentially comes from agriculture, for which the source of energy is photosynthesis. **Fossil fuels** were all formed initially from biological material, produced mainly through photosynthesis. **Biomass**, which is generated by photosynthesis, continues to be one major source of renewable energy. Photosynthetic organisms have had a long time over which to optimize the chemistry involved in these processes, and this optimization had but one driving force: survival of these species. Were we fully to understand the chemical and physical mechanisms of photosynthesis, we could design artificial photocatalytic systems to mimic them. Rather than recreate new plants, we might devise other kinds of chemistry, of direct relevance to some of our needs. One notion that is currently holding the imagination of many research workers is to use the high-reduction-potential electrons yielded by photochemistry, rather than for incorporation of CO_2 into organic molecules, to reduce protons, and generate molecular hydrogen (H_2). While hydrogen is not of much use to photosynthetic organisms, it is of great interest to humankind as an energy source. If the electrons used in this process were to be extracted from water, as in photosynthesis, we could avail ourselves of an ideal method of energy generation: solar energy yielding hydrogen from water! In fact, this process does exist, in certain photosynthetic organisms.

Green microbes for solar-driven hydrogen photogeneration

Many microorganisms feature an **enzyme** that catalyzes hydrogen generation through reduction of protons. Some of these are photosynthetic organisms, and solar energy drives their hydrogen production. The problem is that this hydrogen-evolving enzyme is destroyed or inhibited by oxygen, while water oxidation yields molecular oxygen. The two processes must thus be kept apart. Some hydrogen-evolving photosynthetic bacteria do use sources of electrons other than water, however these sources, being more readily oxidized than water, result in lower overall efficiency as regards hydrogen generation. Some algae, and some **cyanobacteria** do carry out both water oxidation and hydrogen evolution, however the two processes take place in two distinct cell compartments, or occur at different points in the life cycle. A green alga of this type has aroused much interest: this grows using normal photosynthesis (**photooxidation** of water, fixation of CO_2, and evolution of O_2), however, by altering its growth medium, its water-oxidation capacity can be made to fall, to a point where its oxygen output is equal to its own consumption through respiration (see Box). In such conditions, a culture of this alga will consume all the oxygen in the medium, and then switch on synthesis of its hydrogen-evolving enzyme. This enzyme makes use of the strongly reducing electrons provided by the photosynthetic apparatus to bind protons and evolve hydrogen. By switching between "oxygen-yielding" and "hydrogen-yielding" growth phases, hydrogen can actually be generated from water and light. This system, which is functional, though complex to operate, has prompted a twofold research thrust: investigation of microorganism cultures, and work on artificial systems taking their cue from the biological process.

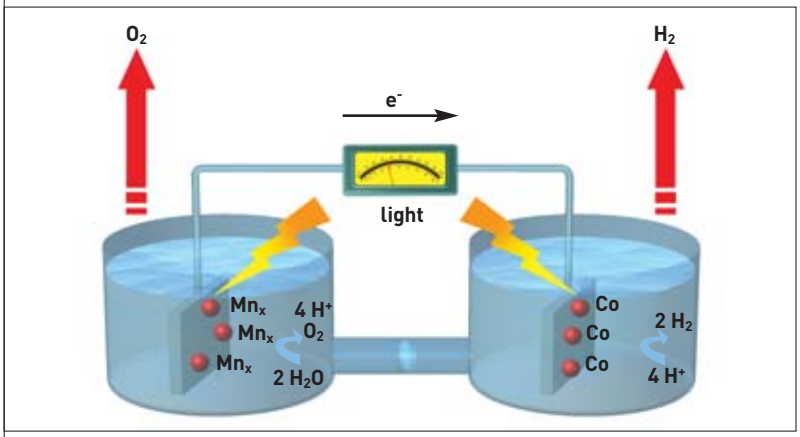
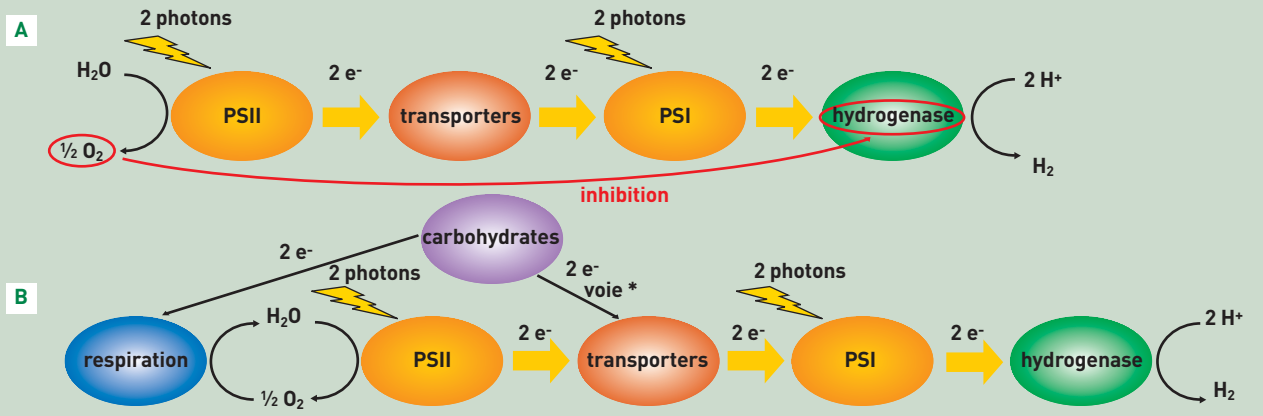


Figure.
A biomimetic dream: a photoelectrochemical cell for the photolysis of water. A manganese- (Mn) and ruthenium- (red dot) based photocatalyst oxidizes water, this being accompanied by release of oxygen, while the second, cobalt- (Co) and ruthenium-based photocatalyst reduces protons into hydrogen. Cell layout takes its cue from that of the reactions taking place in green algae. Synthesis of both types of photocatalyst stands as the goal of many teams around the world, including the team set up by CEA and its partners.

French and European research work

Within CEA's Life Sciences Division, several teams are actively involved in a number of research directions concerned with solar biohydrogen, at Cadarache, Grenoble, and Saclay. At Cadarache, specialists in algal electron transfer are intent on understanding the mechanisms of hydrogen photoevolution, to develop more efficient algal strains. They are working in connection with other groups, in France and abroad, seeking to optimize hydrogen production in pilot facilities. At Saclay, the aim is to gain understanding of the molecular detail of the electron-transfer reactions resulting in hydrogen generation and water oxidation. Such information is used to further the effort to obtain, through genetic means, improved hydrogen-evolving algal or bacterial strains. It also provides crucial functional details as to catalytic reactions. Research workers at Saclay have strong working links with teams of chemists who, taking up a biomimetic approach, are devising new catalysts. Characterization of the properties of such artificial photosystems is carried out at Saclay. One of these teams is the inorganic chemistry group at the Molecular and Materials Chemistry Institute (Institut de chimie moléculaire et des matériaux) at Orsay University nearby, a correspondent research laboratory (LRC: *laboratoire de recherche correspondant*) of CEA, with respect to projects of this type. Such programs are also benefiting from the participation, since 2004, of Professor Thomas Moore, from Arizona State University, a world leading authority on artificial photosynthesis, and recipient of an International "Blaise Pascal" Research Chair. At Grenoble, a number of CEA teams are concentrating on investigation of the structures and functional mechanisms of the hydrogen-evolving enzymes themselves. They are seeking, in particular, to lower, through molecular engineering, sensitivity of these enzymes to oxygen. Other

How to optimize hydrogen production in a green alga



In green algae, such as *Chlamydomonas reinhardtii,* generation of hydrogen (H_2) is carried out in anaerobic conditions by an iron hydrogenase using electrons provided by the photosynthetic chain. This process is limited by hydrogenase sensitivity towards oxygen (O_2) evolved at the level of photosystem II (PSII) (see Figure A). A strategy allowing to get around this difficulty consists in decoupling O_2 evolution phases from H_2 evolution phases. For that purpose, the algae are initially placed in conditions favorable to carbohydrate accumulation, these forming an internal reserve of reducing power. In a second stage, PSII activity is inhibited, and H_2 evolution is carried out by taking up these reserves again (see Figure B).
Physiological, genetic and molecular investigations are under way to optimize the various critical steps in the process. Their goal? To find molecular tools in order to control PSII expression and activity, which must be active during the phase of reserve accumulation, and inhibited during H_2 evolution; to identify determining factors as regards assimilation and mobilization of carbonaceous reserves, through investigation of starch-metabolism mutants; and, finally, to identify the limiting steps as regards electron transfers during the H_2 evolving phase: since this is to take place in a bioreactor, it must be as efficient as possible, to minimize costs. Research efforts are being specially focused on the pathway marked * in the Figure, which presents the twin benefits of not being restricted by residual PSII activity or by respiration, and of exhibiting high quantum efficiency (2 photons/H_2, rather than 4). It has been shown at CEA/Cadarache that this pathway involves NADH dehydrogenase (NDH) activity, which research workers are presently seeking to enhance.

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research workers at Grenoble are developing biomimetic complexes with the ability to effect reduction of protons into hydrogen. This ensemble of investigations on biohydrogen is coordinated under the aegis of a cross-cutting program by CEA's Life Sciences Division. The research efforts at Saclay and Orsay have been supported for some years by the European Union, as part of a network on natural and artificial photosynthesis. Under the aegis of the **6th Framework Program**, a joint initiative by these French teams, together with Swedish teams, has enabled a multinational program to be set up, with the title Solar-H: Hydrogen from Sun and Water, this being approved in 2004 as a NEST (New and Emerging Science and Technology) program.

Photocatalysis and photovoltaics

For solar biohydrogen evolution from microorganisms, the main goal is to select species and develop strains that convert photosynthetic energy into hydrogen, rather than biomass. Screening for the highest-performance species and strains is currently being carried out. Modern genetic methods will be invaluable, to engineer strains that are better performers than natural ones: hydrogen evolution by photosynthetic organisms probably not having been subjected to strong selective pressure over the ages, there is indeed room for much improvement in this respect.
In the field of biomimetic approaches, our understanding of natural systems will have to be refined further, so that target structures may be obtained, for new oxidation catalysts. Whether it be for hydrogen generation or water oxidation, we still need to achieve a deeper understanding of the functional mechanisms of the natural enzymes, and of the reactions involved, at the level required to allow their chemical modeling. Design and development of structures enabling photo-induced charge separations are now well in hand. Such model structures still require to undergo further evolution, if they are to be efficiently coupled to new models of catalytic sites. This area leads on to that of chemical photoelectric systems (see *Hydrogen production by water photolysis?*), and future advances, in particular as regards new materials to be used as matrices for charge-separation structures, will serve both for the development of novel photocatalysts, and that of new photovoltaic components.

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