I. PRIMARY ENERGIES AND THEIR TRANSFORMATION

Hydrogen, the energy vector of the future

Hydrogen will be the energy star of this new century. Its isotopes too have a part to play: in controlled thermonuclear fusion. Moreover, the thermonuclear fusion that occurs in the very centre of the Sun is at the source of the solar energy which will also more or less modify the energy scene as we know it.

Used for itself, in thermal, thermodynamic and above all photovoltaic form, it may be advantageous for certain applications, as long as its cost is estimated on the basis of energy which is *distributed* at the place of use and not only on the basis of production costs. Plentiful, but difficult to collect due to its diffuse character (a maximum of 1 kW/m^2), its day/night cycles and unpredictable weather events, it is not adapted to high energy intensities. Nonetheless, the CEA devotes a considerable amount of R&D to solar energy, whereas wind energy, a more indirect manifestation of solar energy, has the benefit of skills and know-how developed in field of nuclear research. The other link between solar energy and hydrogen is that solar energy produces hydrogen, indirectly *via* photosynthesis, using by-products of the transformation of the biomass, or more directly, *via* the "digestion" of this biomass by enzymes. However, hydrogen ensures an energy storage function and this will probably make it a complementary energy vector of electricity, hence the CEA's investment in this field. Moreover, it could also be produced *via* water electrolysis, by a non-polluting power-generating source such as nuclear energy (at off-peak times) or solar energy. With oxygen from the air, it will ensure direct conversion into electric power and heat in fuel cells, or may even be used directly as a fuel.

J. Demolin/DIAPHOR

A mountain lake. The hydrogen atom is very widespread on the Earth, in the water of lakes, rivers and oceans, as well as in fossil fuels.

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THE PROMISES OF HYDROGEN

Hydrogen is an energy vector that combines the virtues of electricity and fossil fuels without their disadvantages. It is unique in this, which is why it is worth a major research program such as the one that has just been inaugurated by the CEA. Eventually, it should become widely accepted in combination with new technologies, including that of fuel cells. The major challenge is to provide satisfactory answers to the four main problems: its production, its use as fuel, its storage and its distribution.

Liquid hydrogen plant for Ariane launchers in Kourou, French Guyana. In Europe, 65 billion cubic meters of hydrogen are already produced for chemical and other industries.

Le Square des photographes/AIR LIQUIDE

Although at present we rely on well-controlled energy vectors (electricity and liquid and gas fossil **fuels**), the unavoidable rise in constraints will bring about changes in the very nature of these vectors. In fact, their limits stand out quite clearly. Electricity requires a distribution network that may be vulnerable to bad weather, and this has consequences on the environment (electromagnetic and aesthetic). Nonetheless, its major disadvantage is the impossibility of storing it in large quantities. Fossil fuels are handicapped by the fact that they are growing scarce in the medium term, as well as cause pollution as generators of CO_2, an alarming greenhouse gas cited in the Kyoto agreements (see *Energies of today and tomorrow*).

In order to be competitive, a new vector, aiming to back up electricity, must be able to satisfy several criteria: it must be possible to produce it from different **primary energy** sources, fossil and non-fossil alike, and be easy to transport, store and distribute. Lastly, it must be easy to convert into the final forms in which it is used: mechanical, electrical, chemical, thermal, etc.

The idea of using hydrogen as an energy vector is not new. In 1847, Jules Verne, one of our greatest visionaries, wrote in *The Mysterious Island:* "I think that one day, hydrogen and oxygen will be the inexhaustible sources to provide heat and light." Since then, the idea has made headway and is now accepted by most parties working in the field of energy. The only real question is when the change will come about. The answer will depend on how we deal with the four hydrogen challenges: its production, its use as fuel, its storage and distribution.

However, hydrogen, which we often associate with danger (the *Hindenburg* and *Challenger* accidents, or even thermonuclear weapons), has a number of attractive qualities. As the most common element in the universe, it is light, plentiful and it provides energy: its **energy density** (120 MJ/kg) is high compared to that of, say, petrol (45 MJ/kg). On the other hand, its energy volume density is lower: one litre of petrol is equivalent to 4.6 litres of hydrogen compressed at 700 bars.

It is a non-polluting, non-toxic fuel which, when burned, discharges only water and possibly a small amount of nitrogen oxide (if it is used with air at high temperature). Only its explosiveness, under special conditions (confined environment) causes problems, although these are quite easy to solve. And after all, which energy vector is entirely *risk free*? Among all the existing or imaginable vectors, hydrogen is the safest from the moment that the parameters to be managed have been identified and taken into account. As it is lighter than air and diffuses very quickly, the fact that it does not accumulate reduces the risks. Its transportation (hundreds of kilometres of hydrogen gas pipelines have been operating without the slightest failure for over 70 years), and its use (more than 6 million tonnes per year worldwide) are based on extensive experience, all the more reason for lifting any remaining doubts. In Europe alone, no fewer than 65 billion cubic meters are produced every year

for use in chemical and other industries. It should also be remembered that hydrogen accounted for fifty percent of town gas in the middle of the past century.

Although hydrogen gas (in its molecular form) is all but non-existent in nature, the hydrogen atom is extremely abundant on the earth, with prime examples being the water in lakes, rivers and oceans and, of course, in fossil fuels. At present, 96% of the hydrogen produced and consumed in the world (mainly by chemical industries) comes from the **reforming** of **natural gas**, that is, from fossil energy. As long as this primary energy has to be used in order to satisfy energy needs, there is no point in transforming more of it into hydrogen. In fact, even if conversion efficiency gains (and therefore CO_2 emission gains) are possible through the use of new technologies such as **fuel cells**, these gains will almost be cancelled out by the multiplying effect of the efficiency of reforming fossil fuel into hydrogen. Put bluntly, as long as fossil fuels are indispensable, it seems preferable to maintain current transformation techniques, but gradually, as they are replaced by **renewable energies**, hydrogen will come to predominate, combined with these new technologies. It is absolutely essential to use hydrogen, produced without the greenhouse gas effect in fuel cells thus preserving their advantage in terms of carbon dioxide emission. In fact, a fuel cell using natural gas reforming emits a little more carbon dioxide than the new diesel engines currently being studied. These engines, combined with a battery (**hybrid** cars), offer even more advantages in terms of reducing the greenhouse gas effect.

To be consistent with the use of clean electricity in order to produce hydrogen for fuel purposes, the CEA therefore intends to give pride of place to the direct use of hydrogen in fuel cells, even though **hydrocarbon** reforming may be an intermediate stage.

Three major production approaches

This hydrogen will necessarily be extracted from water, but how will it be produced? Three major production approaches are possible (and can be combined): oxidation of gas or organic products of fossil or plant origin, water **electrolysis**, and direct production from the **biomass** or through the use of bacteria.

The most attractive technique in terms of greenhouse gas emission appears to be that of water electrolysis, provided that it is economically viable and carried out using electric power sources that do not themselves produce greenhouse gases. Nuclear energy satisfies these conditions. Another family of nuclear processes uses heat produced by high-temperature reactors (HTR) supplying calories at around 900 °C, to feed water **cracking** thermochemical and **endothermic** reactions. In this case, efficiency of around 60% seems realistic. If we did have to resort to the use of solar energies, in the strict or in the broader sense of the term, the production method would depend on the form considered. If **photovoltaic** or **wind energy** is used, the energy produced and not consumed directly on the network results in hydrogen through the process of water electrolysis. This hydrogen is then stored *in situ* or injected into a specific network.

In order to use the biomass, two possibilities have been imagined: either the direct production of hydrogen through photobiochemistry (hydrogenase enzymes), or the production of **methane** gas or alcohol, which are then transformed into hydrogen through reforming (see *Producing hydrogen from water and light using micro-organisms* and *Producing fuel* via *the thermochemical transformation of the biomass*).

Production efficiency varies considerably depending on the energy used. Thus, in order to produce 1 kWh of useable hydrogen, it is necessary to use 3.3 kWh of hydraulic energy, 8 kWh of nuclear energy (less with high-temperature nuclear energy), and 20 kWh of solar energy.

Four types of storage

The storage problem arises as of the production stage, whether centralised or decentralised at the distributor to meet consumption modes. This storage can be implemented in four different ways, depending on the volumes in question and the duration. Hydrogen can be transformed into a hydrogenated product (such as an alcohol). It can be liquefied, although the very low temperature necessary (20 K) requires costly thermal insulation and leads to losses of between 0.1% and 1% per day, depending on the size of the reservoir. It can be compressed in a natural reservoir (underground) or in an artificial, stationary or transportable container. In this field, the work in which the CEA is involved has, in particular, given rise to the development of safe hydrogen-storage technologies at 700 bars. Lastly, hydrogen can be absorbed in *ad hoc* materials: **hydrides** and carbon nanostructures (nanotubes) appear to be particularly well-suited for this purpose, but will they be competitive in the future? The introduction, as of 2005, of the first series of industrial fuel cells in the field of transport requires that hydrogen storage conditions be assessed as early on as possible (see *How to store hydrogen safely and efficiently*). The CEA, which is studying the feasibility of the various formulas, is planning to take part in the assessment of adapted infrastructures, in the preparation of standards and, more generally, in the analysis of the safety of facilities using hydrogen as a fuel.

Optimised use

As regards the use of hydrogen itself, this can be done either by known and controlled means, such as heat engines, gas turbines, boilers, etc., subject to certain modifications, or by the use of new technologies, of which the fuel cell – a very good **energy converter** – is the most promising for the production of heat and electricity. Hydrogen could also be used to produce synthetic fuels by hydrogenation of the biomass or, added to natural gas, to increase its calorific value. In recent years, significant progress has been made in the design of fuel cells. The CEA has played an active role in this field; its unwavering objective has been a substantial reduction in the cost of manufacturing the fuel cells, in two preferred systems (see chapter II). In the framework of its hydrogen program, it is also planning to emphasise the optimisation of this fuel for each type of fuel cell, whether mobile or stationary, that of global systems and, in a word, that of the entire hydrogen cycle. ●

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Producing fuel *via* thermochemical transformation of the biomass

At a time when industrial society is beginning to perceive the end of the period of relatively inexpensive oil, and when the collective consciousness is beginning to change in favour of the fight against **the greenhouse effect** (box A, *The greenhouse effect*), the use of the **biomass** and waste as energy sources, and/or the supply of hydrogen, constitutes a particularly attractive alternative and is a major issue for the future of society. The skills and know-how of the French Atomic Energy Commission (CEA) in the fields of **thermohydraulics**, thermochemistry, and the management and optimisation of processes, are decisive advantages for ensuring the development and transfer of the required technologies to industry.

The major issues

The biomass is comprised of all plants which develop at the surface of the Earth. It collects and stores solar energy which can then be restored in the form of **carburants** or **fuels**, with no consequences on the greenhouse effect, through more or less efficient transformations with respect to both energy and economics (figure 1). There are four ways to do this.

The first one, *combustion* (or incineration), mainly produces heat with a limited temperature level, around 650 °C, through the presence in the smoke of nitrogen introduced with the **air** and acid vapours or tars[1] which can be condensed, resulting in corrosion phenomena. Under these conditions, the **cogeneration** of electricity and heat, by means of a vapour cycle, leads to only 30% electricity and 70% heat. Such a back-up source is difficult to turn to account.

The second way is *methanization*, carried out by anaerobic fermentation, that is, decomposition through bacterial action in the absence of air, very damp substances such as algae, animal excrement or household waste. It is thus possible to obtain a gaseous mixture of **methane** (50 to 60%) and **carbon dioxide** (35 to 40%), making its use complicated and limited to *in situ* combustion for the production of heat and electricity.

A third way, *alcoholic fermentation*, is adapted to sacchariferous products, containing sugar such as beets or sugarcane, or starchy products, including starch such as cereals. After hydrolysis and the preparation of a sugar solution, subjected to fermentation, ethanol[2] is obtained through **distillation**. The overall efficiency suffers considerably from the high consumption of energy associated with the cultivation of plants and, above all, by the distillation operation which limits production to approximately 1.4 **kWh** for 1 kWh consumed.

Lastly, *thermochemical transformation*, which leads to the gasification of organic and vegetal substances, is particularly well-suited to the conversion of lignocellulose products[3] such as wood or straw into energy (box 1). This system has the highest energy potential for manufacture fuels, with 2.5 to 3 kWh produced for 1 kWh consumed.

For metropolitan France, the lignocellulose biomass resource could conceivably supply 15% (30 **million tonnes oil equivalent**/year) of the current consumption of **primary energy** (box B, *Chains and energy systems*). This would require the harvesting of logging residues (residual trees) and log-
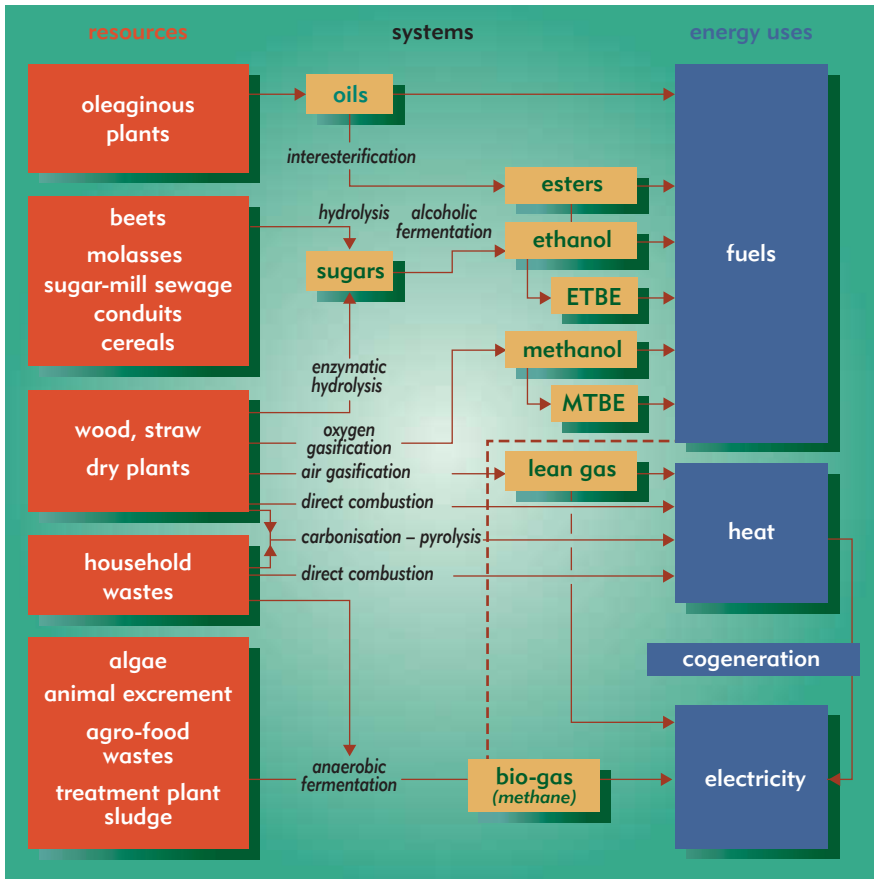

Jolyot/Campagne, Campagne

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The gathering and pressing of straw into bales (top); Vosges forests devastated by the December 1999 storm (bottom). Wood and straw can be used for energy purposes when transformed into a fuel capable of producing electricity and heat at the same time, or into a clean fuel, such as hydrogen or methanol.

Vincent Kessler/SIPA PRESS

Figure 1. *The various systems for making use of energy from the biomass. ETBE: ethyl-tertio-butyl-ether; MTBE: methyl-tertio-butyl-ether.*

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(From Ademe)

ging of coppice forests which, at the present time, have been abandoned and damage the productivity of forests. Moreover, it would be necessary to harvest the agricultural residues (straw) which, for the moment, are eliminated in unnecessarily high proportions, through burial in the ground. Once again, this would involve finding the right way to take advantage of the 15% of farming land which is not used for producing foodstuffs, by planting herbaceous or lignocellulose energy crops, depending on the type of land and climate. Finally, it would be important to improve the sorting quality, in order to better ensure the

conversion of the biomass which, at the end of the cycle, returns in the form of waste products (wood, paper, cardboard, etc.).

This double production issue of clean fuels and the conversion of wastes into energy is a serious challenge for society in the coming decades. This explains why the CEA felt it was necessary to employ its skills and know-how in the search for the most appropriate solutions.

Technological obstacles

Technological obstacles are to be found at different levels (box 1). Moreover, the gasification of the biomass, as well as that of coal, is not a recent possibility. In particular, it was practiced before and during World War II in order to compensate for the shortage of petroleum, oils and lubricants. However, it was quite quickly abandoned as soon as fuels of a **fossil** origin once again became available at a low price. Thus, it is not the feasibility of gasification that needs to be proven, but rather the improvement in its reliability and the progression of its economic profitability by using new bases represented by the end of relatively inexpensive oil, the active fight against the greenhouse effect, and the necessity of creating jobs, especially in the rural environment.

Among the technological challenges open to the CEA's expertise, three appear essential. They determine the operating cost of an

installation, or call its operating reliability into question.

The first one involves the price of the raw material, highly influenced by collection, storage and transport difficulties in agricultural and forest environments. In order to lower these costs, it is possible to relax the constraints on the type and homogeneity of the materials, particularly by agreeing to simultaneously treat "clean" biomass as well as waste that can be cost-effectively eliminated. For this, it is necessary to develop sufficiently flexible technologies and have precise knowledge about the thermal and chemical behaviour of the materials to be transformed.

The second obstacle depends on the efficiency of the transformation, which determines the quantity of material to be used in order to produce a given quantity of energy. Optimising the operation of installations requires experimental work enabling one to establish and qualify a model of the entire process, which will later aid in the design and control of the operation of efficient units. For this purpose, three essential operations must be studied and analysed separately. First of all, **pyrolysis**, whose operating conditions influence the resulting gaseous or solid fractions. Secondly, the treatment, catalytic if possible, of tars and acids contained in the gaseous fraction, with the possibility of accelerating their elimination or eliminating them at lower temperatures, in the presence of carefully-selected reagents. Lastly, the steam gasification of the solid fraction for which the **fluidised bed** technique seems particularly well adapted (box 2).

The third obstacle is the incompatibility between the temperature levels to be reached in order to eliminate the acid compounds and tars, on the one hand, and prevent the ashes from melting on the other. Acid compounds and tars begin to **crack** with the heat at around 1 000 to 1 100 °C only while, as of 900 °C, melting of ashes begin to appear as clinker agglomerates[4] are formed. This means that there is a risk that these materials are held prisoner during the transformation process.

Thus, it is through means of a meticulous, detailed analysis of the various useable materials and numerous thermochemical operations to be carried out, that it will be possible to conceive the adapted technologies and then to optimise and control the gasification plants that will produce the renewable fuels of the future.

(1) The tars, which are oily, viscous and of a brown or black colour, are by-products of the **distillation** or carbonisation of hard coal or wood.
(2) Alcohol (C_2H_5OH) derived from ethane (C_2H_6), a gaseous **hydrocarbon**.
(3) Formed from lignin and cellulose. Lignin is a complex organic substance, the main constituent of wood, which impregnates the cells, fibres and conducting vessels, making them impermeable, inextensible and rigid. Cellulose is a macromolecular product of the carbohydrate group, glucose polymer, the essential and characteristic component of plant cell walls.
(4) A combustion residue, especially of hard coal and wood, resulting from the melting and solidification of mineral salts making up the ashes.

A succession of laborious and controlled operations

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The thermochemical system of **pyrolysis** and gasification of the **biomass** (diagram) includes a series of operations that simultaneously require the transfer of large quantities of heat and the control of the proportion and contact time of the reagents that are present.
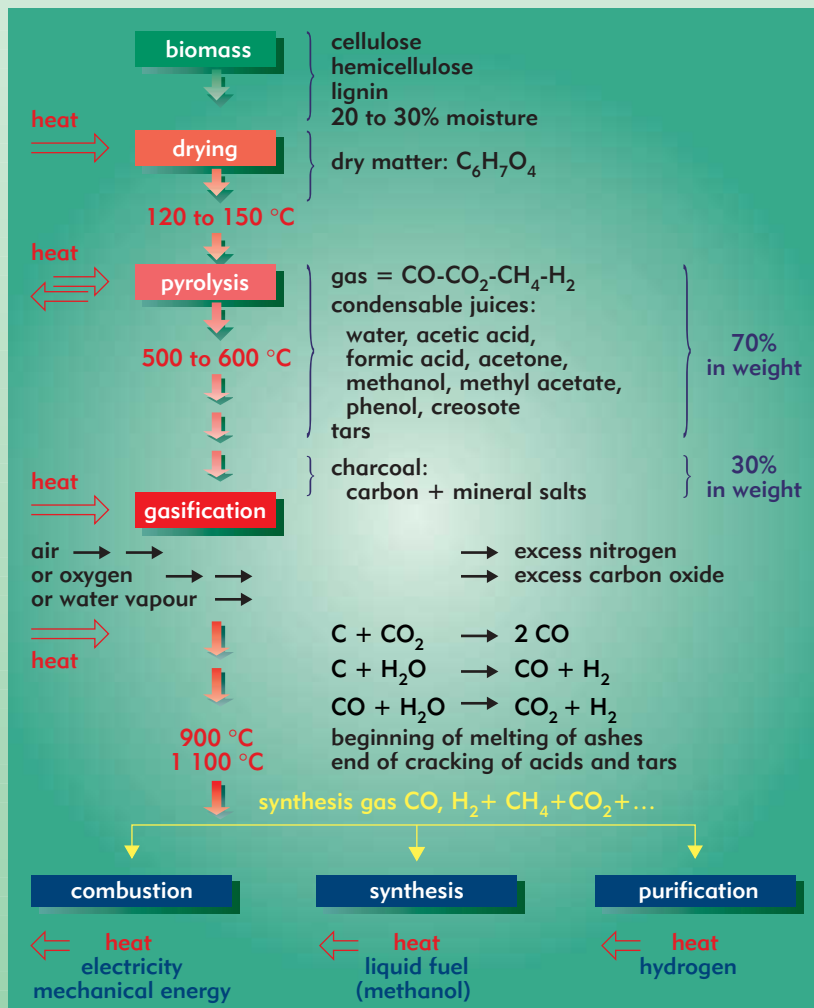
After the drying operation, which is highly **endothermic**, pyrolysis follows and consists in the thermal degradation of products which, at around 600 °C, have lost 70% of their weight and become gaseous, whereas 30% of the original weight remains solid and represents charcoal, comprised mainly of carbon (C). Gasification of the carbon continues up to 900 or 1 000 °C by means of a reagent such as **air**, oxygen (O_2) or water vapour (H_2O), which effects a more or less complete oxidation of the carbon into carbon monoxide (CO), or **carbon dioxide** (CO_2). At this stage, use of air is not very costly, but unfortunately it introduces nitrogen into the end product. Oxygen from air **distillation** is expensive and dangerous. Additionally, it furthers the formation of carbon oxide. With water vapour as a reagent, an additional quantity of hydrogen (H_2) is recovered. Nonetheless, an additional means of heating must be used, for example, through the combustion of carbon or residual purge gases. Obtaining a good-quality, non-corrosive gas requires the elimination of acids and tars[1] resulting from the pyrolysis. For this, it is necessary to heat these products to around 1 000 or 1 100 °C so that they can be thermally degraded (**cracking**), or to carry out a **catalysed** reaction at around 800 to 900 °C to prevent the ashes from melting and clinkers[4] from being formed (between 900 and 1 000 °C), as these have an adverse effect on gasifier operation.

The gas obtained can be burned in an engine or gas turbine in order to produce mechanical or electric power. It may also lead to the synthesis of **hydrocarbons** (**methanol**, dimethyl-ether[5], etc.) which can be used directly as fuels or energy vectors, or else as raw material for the chemical industry. Lastly, it is possible to refine the biomass gas in order to extract hydrogen which, by means of this system, will become the clean and renewable fuel needed by fuel cells.

It is important to note that the same quantity of carbon dioxide is emitted by slow oxidation of wood left in the open air (decomposition), or by rapid oxidation during (good) combustion that generates energy. As this quantity of carbon dioxide is what a plant uses to grow, the effect on the environment and consequences for the greenhouse effect remain neutral.

Diagram

biomass
- cellulose
- hemicellulose
- lignin
- 20 to 30% moisture

heat → drying — dry matter: $C_6H_7O_4$

120 to 150 °C

heat → pyrolysis

500 to 600 °C

gas = CO-CO_2-CH_4-H_2
condensable juices:
  water, acetic acid,
  formic acid, acetone,
  methanol, methyl acetate,
  phenol, creosote
tars
} 70% in weight

charcoal:
  carbon + mineral salts
} 30% in weight

heat → gasification

air →
or oxygen →
or water vapour →

→ excess nitrogen
→ excess carbon oxide

heat

$C + CO_2 \longrightarrow 2\,CO$
$C + H_2O \longrightarrow CO + H_2$
$CO + H_2O \longrightarrow CO_2 + H_2$

900 °C
1 100 °C

beginning of melting of ashes
end of cracking of acids and tars

synthesis gas CO, $H_2 + CH_4 + CO_2 + …$

combustion	synthesis	purification
heat	heat	heat
electricity	liquid fuel	hydrogen
mechanical energy	(methanol)	

Skills and know-how in two main fields

The CEA has skills and know-how as well as experimental resources in the two important fields of thermal treatment of materials and plant or organic waste, and the design, modelling and optimisation of units and industrial processes implementing thermics and thermohydraulics.

The Nuclear Energy Division teams responsible for the fuel cycle have acquired extensive experience in operations involving pyrolysis, incineration and **vitrification** of wastes of all kinds and, on the Marcoule and Cadarache sites, they have the laboratory equipment (furnaces and analysis apparatuses) necessary for characterising the potential products of the future. Since the middle of the year 2000, characterisation campaigns have been conducted in Cadarache. Initially, they involved the pyrolysis of wood, straw or cereal grains, as well as meat-and-bone meal and sludge from urban water treatment plants or else paper and cardboard or other non-recyclable residues. In a second phase, a priority for the year 2001 is the study, after adaptation of the experimental means, of the treatment of pyrolysis gases in order to eliminate tars from them. On the Grenoble site, the teams responsible for the design of nuclear reactors will put their expertise to the task on the analysis and modelling of thermal and thermohydraulic processes in order to improve understanding and knowledge of the basic processes, such as drying and the pyrolysis of materials, and then the steam gasification of carbonated residues.

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Two main goals have been set for the coming years. The first one involves the design and control of the key component represented by the fluidised bed reactor (box 2). The second one, more general, is the modelling of the entire process in order to acquire the right tools both for decision-making as regards the choice of products to gasify, or the means for designing or optimising future facilities. Thus, the CEA will focus its development activities on pyrolysis and gasification operations. Later on, it will be necessary to foresee different ways of transforming or using synthesis gas, either as a cogeneration fuel (50% electricity, 50% heat), or as a source of fuels, in gaseous form such as hydrogen or methane, or in liquid form such as **methanol**, dimethyl-ether[5] or other synthesis **hydrocarbons**.

Throughout the program, cooperative efforts will be established with an expanded network of national or European partners. In the field of basic knowledge, working relations are being set up or are about to be finalized with several public and university research groups. With its extensive experience in the treatment of tars and the implementation of circulating fluidised beds, the LERMAB (Laboratory for research on wood) at the University of Nancy (Meurthe-et-Moselle), and associated with the ENSTIB (National engineering school for wood technologies and industries) in Épinal (Vosges), is already quite active. From the outset, industrialists who are interested in building facilities are invited to contribute to the implementation and development of the programs. This is the case, for example, of Traidec which, in Sainte-Foy-l'Argentière (Rhône), builds pyrolysers for the elimination of industrial or household waste. It is also true of the CNIM (Industrial construction company for the mediterannean) in La Seyne-sur-Mer (Var), which is the largest French constructor of waste incineration units and cogeneration plants using agricultural residues in the overseas territories and departments (DOM-TOM). In order to carry out exploratory work and make the resources available, farmers have grouped together under the auspices of the AGPB (General association of corn producers) and the ITCF (Technical institute of cereals and forage). Lastly, with respect to lignocellulose resources, various departments depending on the ministry of Agriculture and Forests are involved, as are the major sawmills. In order to prepare or develop appli-

(5) Hydrocarbon with a CH_3— O— CH_3 composition has physical properties mid-way between those of butane and propane in the liquid state at 20 °C under a pressure of 5.3 bars.

2

Steam gasification in fluidised bed reactors

In a **fluidised bed** reactor (diagram), steam gasification of solid residues from **pyrolysis**, mainly carbon (C) and 5 to 10% mineral salts, is carried out at around 700 to 800 °C under carefully-controlled temperature homogeneity and reagent concentration conditions. The **endothermic** reaction, from gas to water, between the carbon (black dots on the diagram) and the water vapour H_2O (blue background) produces a mixture of carbon monoxide (CO) and hydrogen (H_2) called synthesis gas. The reaction heat is supplied by a heat-carrying fluid (ochre-coloured dots) put into a counter-current flow between the gasification reactor, where it is dispersed in order to provide homogeneous heat, and a com-

bustion furnace with entrained bed, where it is brought to around 900 °C through combustion with the **air** of residues extracted from the process (dust and solid particles not gasified or unusable purge gases such as, for example, excess carbon monoxide or **methane**). The maximum temperature of 900 °C prevents the ashes from being melted and the formation of clinkers[4] which might otherwise hinder the movements of the particles. The choice as a heat-carrying fluid of a material rich in calcium and magnesium, such as dolomite sand, ensures the right **catalytic** effect on the gasification and tar elimination[1] reactions as these occur in either a shorter time, or at lower temperatures.
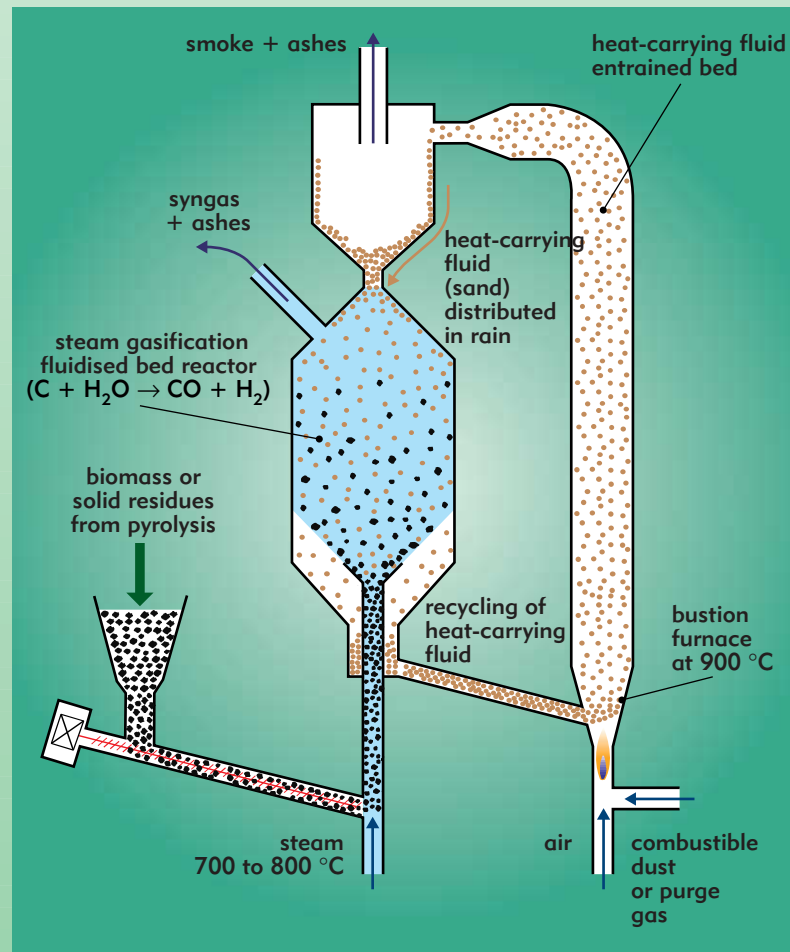
cations, contacts have been established with such groups as EDF-GDF (the French electricity and gas authorities) and Dalkia (Vivendi group). Relations have also been established at international level, particularly with industrialists and academics in Denmark, a coun-

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CEA/Cadarache pyrolysis bank with rotary kiln. The biomass is fed via the tapered hopper located on the right. The solid residues are collected at the lower left. The gaseous residues are removed and treated on the top left. In the background, on the left, is the smoke purification and scrubbing stage.

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CEA

try with a very well thought-out policy in the field of renewable energies.

An abundant source of hydrogen

The biomass, a form of transformed solar energy, is capable of covering a substantial part of the energy consumption needs of a country such as France, on a lasting basis. In addition to the traditional types of thermal or electrical energy, the biomass may one day serve as an abundant source of hydrogen, the clean fuel of the future. Research and development programs have been undertaken with this in mind. The CEA will be making substantial contributions in this area through various multidisciplinary and multinational research groups. ●

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CEA/Grenoble

Producing hydrogen from water and light using microorganisms

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Molecular structures of iron hydrogenases (left) and iron-nickel hydrogenases (right), enzymes which are capable of catalysing the production of hydrogen. The large and small protein subunits, represented in the form of a ribbon, are shown in blue and red, respectively. Each hydrogenase has three iron-sulphur aggregates (red and yellow or red and green spheres) which act as relays for the transportation of electrons to the active site embedded in the centre of the enzyme.
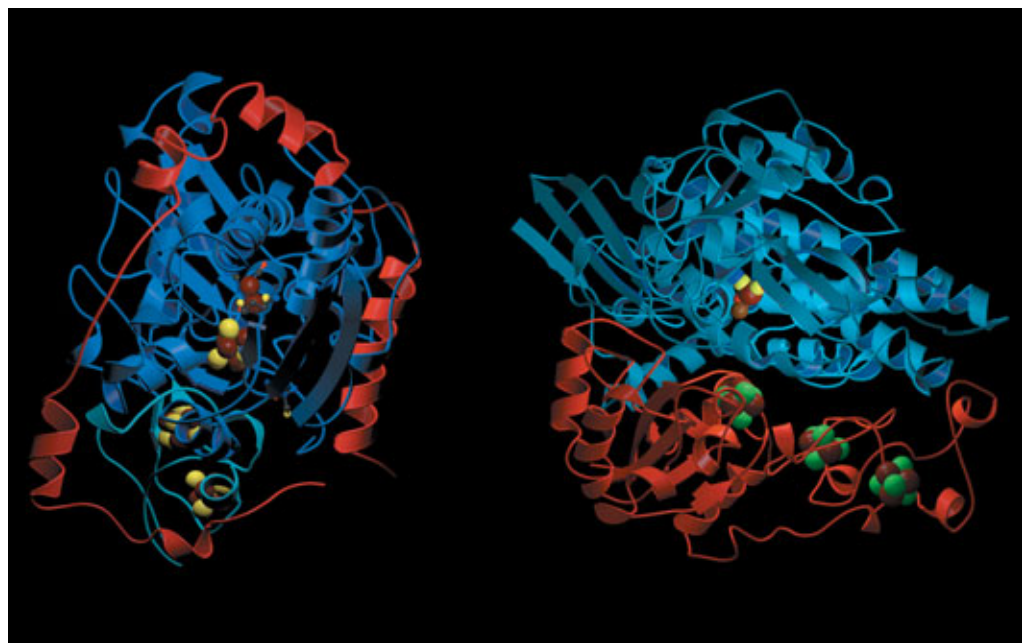
IBS/CEA-CNRS

For the past century, biologists have known that **bacteria** capable of **photosynthesis** and algae can produce gaseous hydrogen under the effect of light (box 1). However, it was only as of the 1970's that, after the initial oil crisis, the biological production of hydrogen was first conceived in the United States. Initially, work was carried out on reconstituted photosynthetic systems (isolated **chloroplasts** combined with the **hydrogenase enzyme** and a **ferredoxin**), on **cyanobacteria** (blue-green algae which effect water **photodissociation**), and on photosynthetic bacteria. The search for new technologies to enable the production and use of clean, renewable energy has recently renewed interest in the biological production of hydrogen, particularly through the capacities of **microalgae**.

Using the same process as plants, microalgae are able to **photooxidise** water molecules into oxygen and into highly-**reducing** species. The reducing power formed this way is used by the photosynthetic organism in order to assimilate **carbon dioxide** in the form of sugar, by means of the Calvin cycle (box 1). Quite simply, the absorption of a photon by **chlorophyll** molecules leads to a separation of charges in photosystems I and II, an operation similar to that of a photovoltaic cell. These photochemical reactions enable the **photolysis** of water into oxygen, with the creation of very highly-reducing species called ferredoxins. Under certain conditions, ferredoxin molecules interact with a hydrogenase, an enzyme which **catalyses** the production of hydrogen from two protons (2 H$^+$) and reducing power. Overall, two hydrogen molecules and one oxygen molecule are obtained from two molecules of water and solar energy.

Two main biological constraints limit the continuous production of hydrogen by microalgae using this process. First of all, the hydrogenase is extremely sensitive to the oxygen created during photodissociation. Additionally, the reducing power formed in photosystem I must be diverted from its natural function, the **reduction** of carbon dioxide into sugar, and deflected to the hydrogenase. Until now, these two obstacles have limited the potential applications of the biological production of hydrogen. Recent progress in molecular biology and structural biology enable one to anticipate new approaches by controlling the expression of the enzymes in question, especially at in the chloroplasts, or by modifying the structure of the hydrogenases to make them insensitive to oxygen, in order to open up new possibilities to operational solutions.

Sensitivity of the hydrogenase with respect to oxygen

The ultimate objective is to prevent the hydrogenase from being inhibited by the oxy-
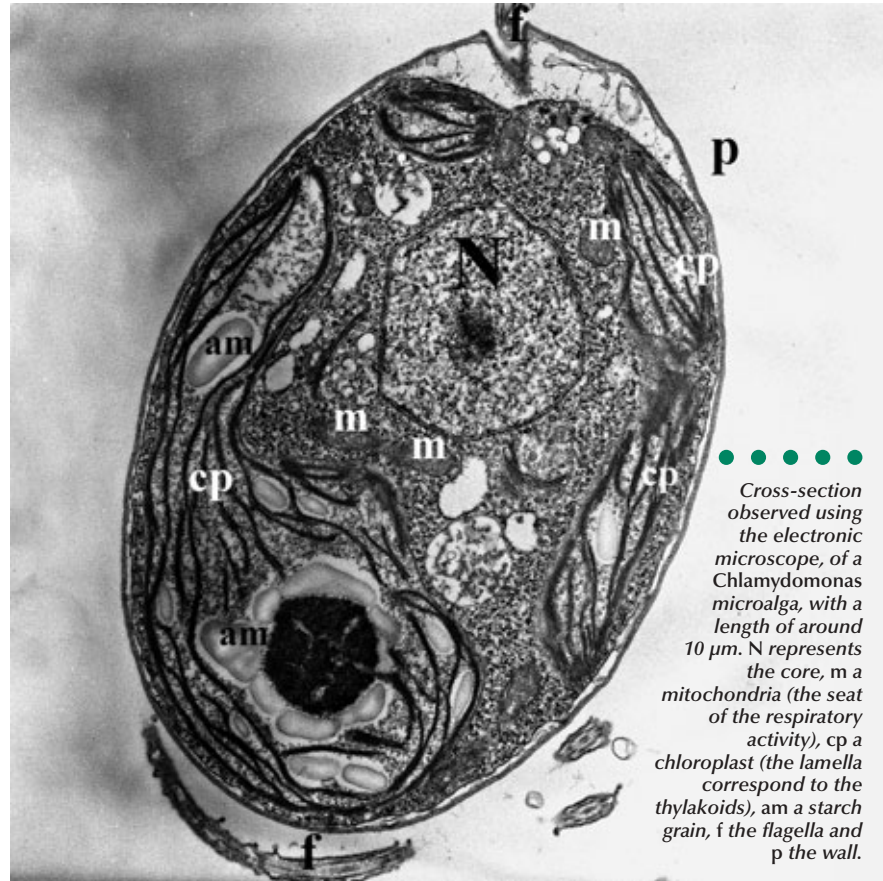
Cross-section observed using the electronic microscope, of a Chlamydomonas microalga, with a length of around 10 μm. N represents the core, m a mitochondria (the seat of the respiratory activity), cp a chloroplast (the lamella correspond to the thylakoids), am a starch grain, f the flagella and p the wall.

Dr Jacqueline Olive/CNRS-INSTITUT JACQUES MONOD

gen. This can be achieved by gradually eliminating the oxygen, as it is produced, by flushing it using a neutral gas such as nitrogen, for example. This approach seems costly and technically complex. Another approach consists of trying to select hydrogenases that are resistant to oxygen. Several unsuccessful attempts have been carried out in the past. In the United States, recent work by Dr. Michael Seibert's team (the *National Renewable Energy Laboratory* in Golden, Colorado) led to the selection of resistant hydrogenases. Nonetheless, the improvement is minor, and the enzyme remains sensitive to relatively low concentrations of oxygen, necessitating substantial flushing.

The molecular structures of two major hydrogenase families have been obtained at the Institut de biologie structurale Jean-Pierre Ebel (Institute of structural biology IBS) in Grenoble, and the layout of their **active site** has been determined (box 2). In-depth knowledge of the structure of this enzyme enables one to anticipate the modification, through **site-specific mutagenesis**, of its active site in order to make it resistant to oxygen and other reaction products such as carbon monoxide. In fact, work carried out at the IBS has also shown that hydrogen reaches the active site embedded in the molecule by hydrophobic canals. By partially blocking these canals, it should be possible to prevent

the oxygen from reaching the active site without blocking access to the hydrogen, which is much smaller.

Directing electrons to the hydrogenase

It has been shown that under conditions in which photosystem II is not active (inhibition, as **mutants** do not have any), the *Chlamydomonas* microalga is capable of producing hydrogen under light from a carbonaceous substrate. These alternative electron transfer mechanisms, grouped together under the name of chlororespiration (box 1), have been studied for several years at the French Atomic Energy Commission (CEA) by the Life Sciences Division on the Cadarache site. A recent way of producing hydrogen, making use of these reaction mechanisms, was recently explored by the Anastosios Melis group (University of Berkeley, United States of America). Nonetheless, the yield obtained (the number of hydrogen molecules produced per absorbed photon) is low, the processes being limited by the low flow-rate of alternative transfers of electrons which are under the control of the small number of enzymes. The identification of these enzymes is currently being pursued. When it is finished, and when the genes have been identified, it will then be possible

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Photosynthesis, chlororespiration and hydrogen production in the *Chlamydomonas* green microalga

During **photosynthesis**, the light energy captured by the **chlorophyll** molecules of the collector **antennas** (LHC) of photosystems I and II (PS I and PS II) is transmitted to the P700 and P680 reaction centres, resulting in a separation of charges. In PS II, the negative charge enables the **reduction** of a primary electron acceptor, the pheophytin[1], and then that of **plastoquinones** (PQ), which are small electron carrier, soluble in the **thylakoid** membrane. The accumulation of 4 positive charges from the donor side of the PS II will lead to the **photodissociation** of water (H_2O) and the production of molecular oxygen (O_2). The reduced plastoquinones $PQ(H)_2$ act as electron donors to the PS I, *via* a small soluble protein containing copper, **plastocyanin** (Pc). The $cytb_6$-cytf complex represents an important intersection of the electron paths. Under the effect of light, the PS I reduces the **ferredoxin** (Fd) and then the **$NADP^+$**, using the FNR protein, to **NADPH**. **ATP** is synthesised from adenosine diphosphate (ADP) and inorganic phosphate (Pi) *via* the trans-membrane electrochemical gradient formed in the light. NADPH and ATP are then used for the reduction of the **carbon dioxide** (CO_2) during a group of metabolic reactions in which the ribulose-diphosphate **enzyme** (RuBP) intervenes. These reactions, known by the name of the *Calvin cycle*, constitute the main path for incorporating carbon (C) in living matter (figure 1). These reactions occur in the **chloroplast**, a specific compartment of plant cells. The electron transfer reactions are confined mainly to the
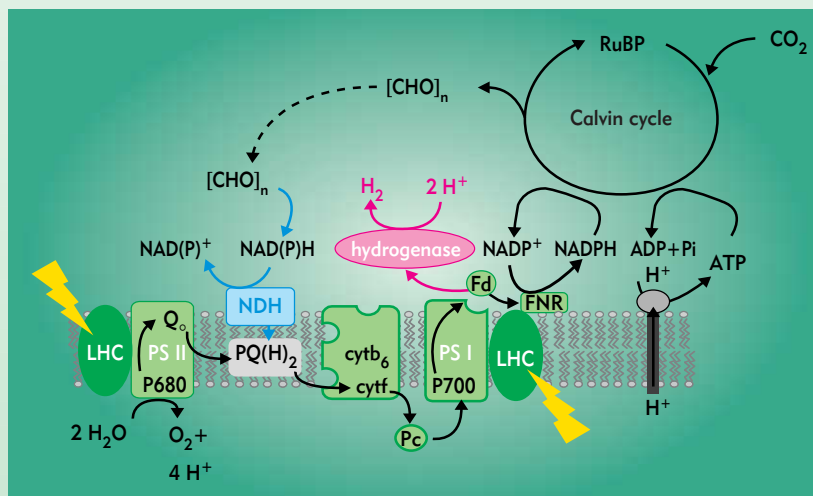

Figure 1. The main electron transfer reactions and the metabolic reactions governing photosynthesis and the production of hydrogen in the chloroplasts of the Chlamydomonas *microalga. Reactions related to the hydrogenase are in pink, and those associated with the chlororespiration are in blue.*

thylakoid membranes, whereas the metabolic reactions occur in the internal liquid medium (or stroma) of the chloroplast.

Certain photosynthetic organisms such as the unicellular green alga *Chlamydomonas* are capable of using the reducing power formed by the PS I to produce molecular hydrogen (H_2). This reaction, **catalysed** by an enzyme – the **hydrogenase** – causes the protons (H^+) to intervene as an electron acceptor substrate. Illuminating suspended algae placed in **anaerobic** conditions enables one to visualise the competition between these two ways of using electrons: the reduction of carbon dioxide and the production of hydrogen. Initially, electrons from the PS II and the PS I are directed

towards the hydrogenase. However, the increase in the concentration of oxygen in the medium, which has an inhibiter effect on the hydrogenase, and the triggering of the Calvin cycle, lead to rapid stoppage of the production of hydrogen (figure 2a).

Over the past years, various other electron transfer methods have been described. In particular, they involve non-photochemical reduction or **oxidation**, that is, not dependent on the action of the PS I and PS II, of the PQ molecules using enzymes such as an NADH dehydrogenase (NDH, see figure 1) or an oxydase quinol. These new methods, which provide evidence of the presence of a respiratory chain in the very centre of the chloroplast, are better known under the name of *chlororespiration*. The existence of these alternative electron transfer paths enables one to cause the production of hydrogen to operate in a prolonged way in anaerobia, under conditions in which the PS II and the Calvin cycle are inhibited, by using carbonaceous reserves $[CHO]_n$ as electron donors to the PQ molecules *via* the NDH (figure 2b). Nonetheless, as these reserves are limited, the alga must restore them during a photosynthetic phase which produces oxygen.
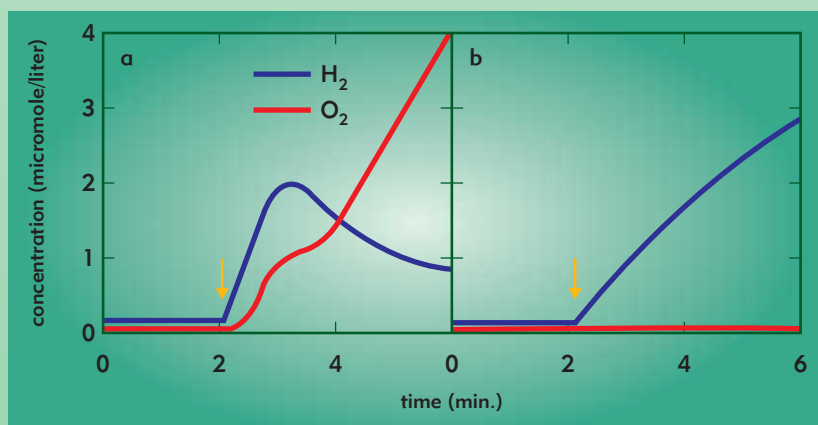

Figure 2. Production of hydrogen during the illumination (vertical arrow) of a suspension of Chlamydomonas *microalgae placed in anaerobic conditions. At a, control sample; at b, sample for which the PS II and Calvin cycle have been inhibited.*

(1) A genuine primary acceptor, discovered well after the role of a molecule of quinone Q_0 as acceptor was brought to light.

The hydrogenases

These small proteins are divided up into two major families: the iron-nickel **hydrogenases** (NiFe), consumers of hydrogen, and the iron-only hydrogenases (Fe), producers of hydrogen. The first molecular structure was obtained at the Institut de biologie structurale Jean-Pierre Ebel (Institute of structural biology IBS, CEA-CNRS) in 1995 for the oxidised and therefore inactive form of isolated NiFe hydrogenase using the sulphate-**reducing bacterium** *Desulfovibrio gigas*. Most surprisingly, it appeared that the **active site** was much more complex than had been foreseen and was deeply embedded in the core of the **enzyme** at around 3 nm (1 nm = 10^{-9} m) from any point on the surface. In cooperation with spectroscopists, it was also shown that the atom of Fe was itself linked to three non-protein diatomic **ligands** identified as one molecule of carbon monoxide and two molecules of cyanide. In this inactive oxidised form,
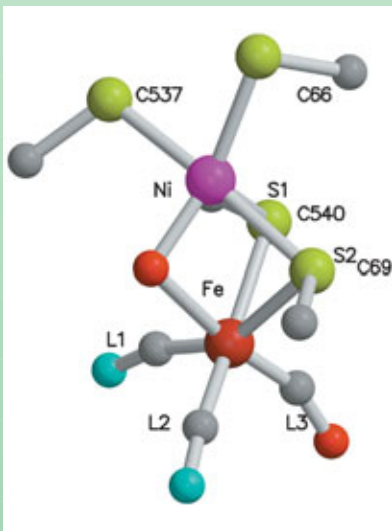
a fourth non-protein ligand, cross-linking atoms of Fe and Ni, has been identified as being an **oxo-species** on the basis of spectroscopic data and electronic density.

A topological analysis of the hydrogenase and a crystallographic study, in which xenon under pressure is diffused in the crystals, reveal the existence of a large hydrophobic cavity which is itself linked to the active site and to the surface of the molecule by canals that are completely or partially hydrophobic. Molecular dynamic calculations suggest that this network could be used exclusively to transfer, or even to store, hydrogen from the active site to the surface, or conversely.

Fe hydrogenases have been studied considerably less. The crystallographic structure of the hydrogenase of the *Desulfovibrio desulfuricans* bacterium was determined at IBS only very recently (1999) at 1.5 Å (1 Å = 10^{-10} m) resolution. The aspects that have been especially investigated are the structure of the active site and the possible hydrogen binding site, as well as the comparison between the Fe hydrogenases and the NiFe hydrogenases. A detailed analysis, conducted in Grenoble, enabled all 6 of the Fe, called "cluster H", to be visualised. This aggregate can be separated into two functional groups: a centre [4Fe4S], typical of **ferredoxins**, and a centre with 2 Fe, showing unprecedented coordination in the history of Biology. The first one most likely constitutes a centre of electron transfers, whereas the second one appears to correspond to the active site itself. The 2 Fe centre is coordinated by several non-protein ligands. According to the chemical environment, infrared spectroscopy data and electronic density, these ligands were identified as being two molecules of carbon monoxide, two molecules of cyanide, a propanedithiolate and a cross-linking carbon monoxide. One of the two Fe ions (Fe2) has an empty coordination site which may be the molecular hydrogen binding site.
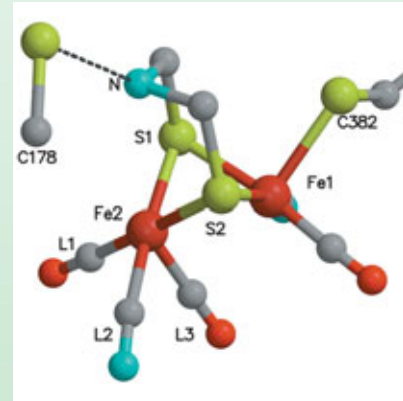
Active site of the Fe hydrogenase isolated from the Desulfovibrio desulfuricans *bacteria. Each of the Fe2 iron atom ligands, L1 and L3, corresponds to a carbon monoxide molecule while L2 corresponds to a cyanide molecule. The same types of ligands are associated with the iron atom Fe1. A small molecule, modelled as a dithiomethylamine (N), cross-links the S1 and S2 sulphur as well as the cysteine C178.*

Active site of the NiFe hydrogenase isolated from the Desulfovibrio fructosovorans *bacteria. Ligands L1 and L2 each correspond to a cyanide molecule, L3 to a carbon monoxide molecule, and ligands C66, C69, C537 and C540 to an amino acid, cysteine. S1 and S2 represent atoms of sulphur.*

This hypothesis is reinforced by the fact that there is a hydrophobic cavity, similar to the one observed with the NiFe hydrogenases, which goes from the surface of the protein to the active site where it points towards the Fe2.

The Fe hydrogenases and NiFe hydrogenases do not have a sequence homology in **amino acids** or substantial structural homologies. However, given that they have the same **catalytic** activity, any analogy between the two enzymes can be considered as important for this function. One of the most remarkable aspects of the active sites of these enzymes is the presence, in the iron coordination sphere, of diatomic ligands such as carbon monoxide and cyanide. This type of coordination privileges the reduced form of the atom of iron. This characteristic of the active site can be linked to the fixing and/or catalysis of the hydrogen. Another significant analogy is the existence of hydrophobic cavities joining the active site to the surface of the protein in the two hydrogenase families.

to place them under the control of strong **promoters** to increase the abundance of the proteins involved, and thus the yield of the associated electron transfers.

Molecular techniques also provide the means to control the photosynthetic reactions which slow down the transfer of electrons to the hydrogenase. One solution would consist of effecting the conditional expression of an essential subunit of photo-

system II placed under the control of a promoter. This would lead to entering into a hydrogen production phase, as and when required, by making full use of the potential of the cells. One of the main subunits of the enzyme responsible for the carbon dioxide fixation could be under the control of the same promoter, which would enable the electrons to be switched to the hydrogenase.

On the left, cultures in a liquid medium in sterile flasks with different strains of Chlamydomonas reinhardtii *to be tested for their photosynthetic characteristics and their hydrogen production capacities. On the right, optical microscope view of a culture of* Chlamydomonas*, a unicellular microalga. The chloroplast (in green) is quite large in relation to the entire cell.*

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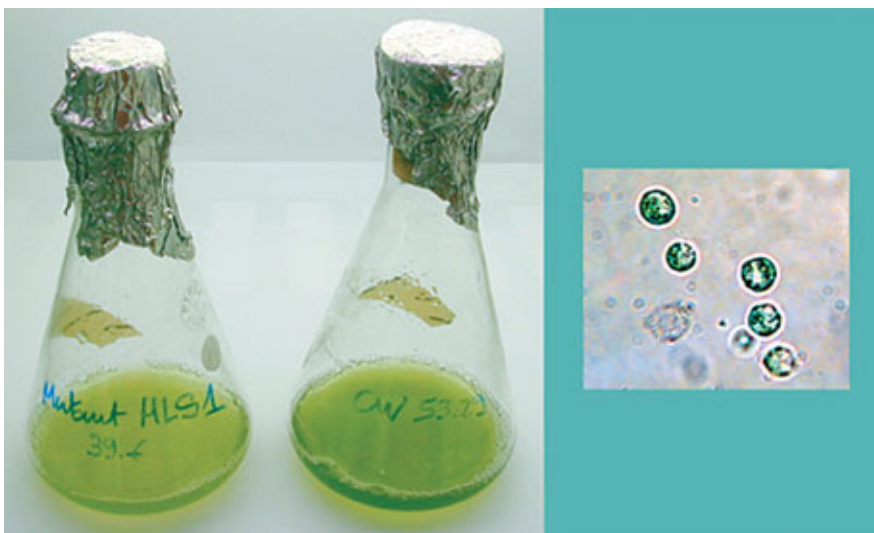
CEA

The biomimetic approach

The production of hydrogen from **biomimetic** purified or artificial biological elements reconstituted in a functional system is also possible. Since the hydrogenases are highly efficient **catalysts**, their catalytic power can be combined with any reaction capable of supplying electrons that are sufficiently reductive to enable the reduction of protons into hydrogen. With a view to biotechnological applications, work on these enzymes has several goals:

• to stabilise the hydrogenases with respect to various denaturing agents, particularly oxygen;

• to understand the catalytic mechanism of the reduction of protons into hydrogen for the purpose of improving or modifying it;

• to understand the assembly of the metal active site of the hydrogenase *in vivo*, which causes several proteins to intervene, some of which remain to be discovered. Reaching this goal will doubtless be a crucial stage in the development of biomimetic catalysts;

• locating and testing reducers, photobiochemical or other, capable of reducing the hydrogenase as well as being economically feasible;

• building synthetic photosystems combined with electrodes imitating water photolysis and the production of reducing power, which would make it possible to obtain the spatial separation of the production of oxygen and the reduction of protons into hydrogen.

These various approaches are being developed at the CEA, on the Grenoble and Saclay sites.
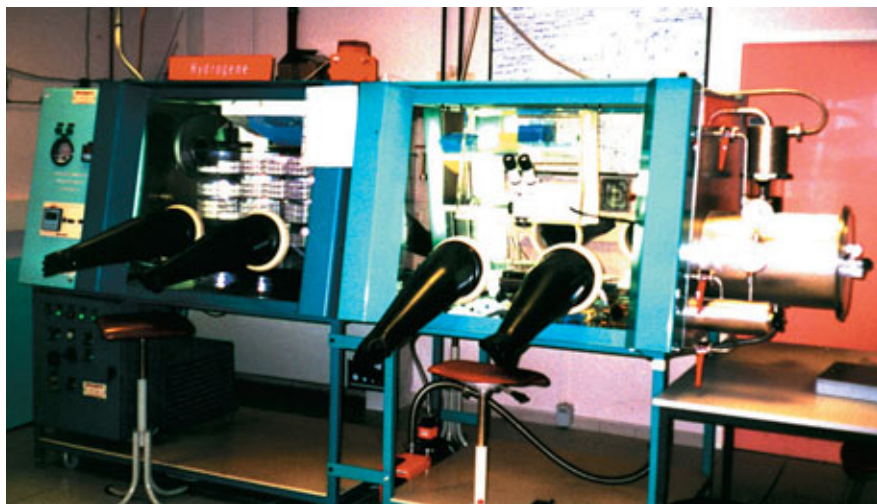
The CEA has the scientific and technical skills and know-how to explore these various areas of research. The teams working in the Life Sciences Division are internationally known for their advanced work in the structural biology of hydrogenases, the photochemistry of photosystems I and II, protein engineering, and the genetic transformation of chloroplasts. It is clear that the proposed projects are upstream studies of possible applications, requiring long-term efforts. Attaining these ambitious goals will require the continuation of work already under way in the fields of biochemistry, molecular engineering, crystallography, spectroscopy, **transgenesis** and chloroplast transformation. •

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Apparatus in which the crystallisation and manipulation of hydrogenase crystals are carried out in a controlled atmosphere (reducing conditions). The crystallisation stage is taking place in the module on the left. On the right, a compound binocular microscope enables visualisation of the formation of the crystals and their manipulation.

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