Beilstein-Institut
Functional Nanoscience
May 17th – 21st, 2010, Bozen, Italy

Reflections on Energy Conversion in Biological and Biomimetic Systems

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Received: 3rd September 2010 / Published: 13th June 2011

Abstract

In principle any form of energy (light, electrical, potential, chemical, kinetic energy, etc.) can be converted into any other, and a large part of biochemistry is concerned with the mechanisms of transduction. Despite this, misleading statements such as "glucose phosphorylation is coupled to ATP hydrolysis" appear even in modern books that appear in general to be based on a thorough understanding of thermodynamics. In reality, harnessing the chemical energy contained in an ATP molecule to drive metabolism involves no hydrolysis at all, and it is exactly because there is no hydrolysis that the process can work. At a grosser level, many authors still write as if production of mechanical work in organisms – from the packaging motor of bacteriophage to the muscles of large animals – operated in much the same way as industrial motors, i. e. that they release chemical energy as heat, which is then converted into work by the sort of pressure-volume effects discussed in elementary thermodynamics courses, but living motors – including not only muscles but also such examples as the DNA packaging motor of bacteriophage Φ29 – are not heat engines. ATP hydrolysis is, of course, a net effect but the heat that is produced is lost: it cannot be converted into work because organisms have no known mechanisms for transforming heat into pressure-volume work, at least, not on a significant scale. Unfortunately, elementary courses tend to

concentrate on the thermodynamics of gases to such an extent that the irrelevance of pressure-volume work to biochemistry is completely lost, and the Gibbs energy, for example, is seen as having something to do with heat, even though its main role in isothermal systems is as a device for expressing equilibrium constants on a logarithmic scale. Thorough understanding of these concepts will be essential for the successful development of new biotechnologies. The case of biohydrogen as a fuel is discussed.

Introduction

All biological processes depend on the management of energy, and all energy except, in recent years, energy from nuclear power plants, arrives to the earth in the form of sunlight. Photosynthetic organisms convert sunlight directly into chemical energy, and some organisms convert chemical energy directly into light. Animals and other motile organisms transform chemical energy into movement, and kinetic and potential energy are easily interconvertible with negligible losses.

Most industrial processes, however, remain primitive by comparison. Transformations between potential energy, kinetic energy and electrical energy can be made directly, and with high efficiency, but other transformations are either very inefficient or possible only on a small scale, or both. Energy harnessing on an industrial scale normally involves heat production by combustion of fuels, followed by conversion of the heat into work, chemical energy or electrical power, and the inevitable energy losses are large. A heat engine working as a reversible Carnot cycle over the widest temperature range practical for a modern car, say from about 25 °C (298 K) up to about 120 °C (393 K) would convert about 24% of the heat consumed into work. In reality, of course, a real engine cannot operate under reversible conditions, and the practical efficiency of the best heat engines is more like 10%.

By comparison, a running animal such as a cheetah can convert the chemical energy of ATP into kinetic energy with about 50% efficiency. Of course, the ATP needs to be produced from food and oxygen by metabolic processes that involve additional losses, so the overall efficiency of conversion of food and oxygen into kinetic energy is around 20%. That is no less true of the estimation of the efficiency of a heat engine, for which the fuel likewise needs to be mined and refined before it can be used, so the estimate of a maximum efficiency of 10% is also over-optimistic.

These considerations have led to great and increasing interest in the possibility of mimicking the efficiency of green plants for converting sunlight into chemical energy, and the efficiency of animals for converting chemical energy into motion. The thermodynamic inefficiency of heat engines is, moreover, just one of the serious problems with current industrial processes. The most readily available fuels today are hydrocarbons, and problems of supply are already

evident, for example, in the need to drill for oil in ever deeper water, and will only get worse. In addition, combustion of hydrocarbons produces not only water, which is largely harmless, but also CO_2, the cause of increasingly severe effects on the temperature of the atmosphere. So we need not only processes of greater efficiency, but also ones that do not produce any more CO_2 than they consume.

Common Errors in Thermodynamic Analysis

Development of alternative fuels, such as H_2, and processing them with high energy efficiency, requires a proper understanding of some central thermodynamic concepts, which may appear elementary but are often forgotten. In this section, therefore, we discuss some problems that occur often in the literature of biochemistry, and make it difficult to make a proper appreciation of the thermodynamic constraints that need to be understood for improving the efficiency of processes for harnessing energy.

Measurement of entropies of reaction or of activation

Textbooks, even ones that are in many respects among the best available, commonly present the determination of an entropy of reaction from a van 't Hoff plot (the logarithm of an equilibrium constant plotted as a function of the reciprocal absolute temperature) as something easy and straightforward to do, because at superficial examination the required entropy value follows easily from the intercept on the ordinate axis. The problem, however, is that the required intercept is typically far away from the range of experimental observations, so a very long extrapolation is needed, as pointed out many years ago [2]. The difficulty does not go away if one uses computer fitting rather than a graph: it just becomes less obvious that there is a problem, and hence easier to deceive oneself. It is not a special problem of the van 't Hoff plot: essentially the same applies to the determination of an entropy of activation from an Arrhenius plot of the logarithm of a rate constant against the reciprocal absolute temperature [3 – 5].

To take a specific example, Figure 4.11 of [6] illustrates a van 't Hoff plot with an unlabelled scale of reciprocal temperature, but one that implicitly starts at zero, because the intercept on the vertical axis is labelled as $\Delta S^0/R$, in which case the observations span an impressive 15-fold range of absolute temperature. However, that is obviously impossible, at least for a biological process: if we assume that the higher temperature was about 85 °C (358 K), a typical temperature for a thermophilic organism, the lower temperature must have been – 250 °C (24 K); if, on the other hand, the lower temperature was – 10 °C (263 K), a typical temperature for a psychrophilic organism, the higher must have been 3700 °C (4000 K). Neither of these interpretations is plausible, so we must suppose that the figure did not represent a real or feasible experiment, but a purely imaginary one.

If the ordinate axis in a van 't Hoff or Arrhenius plot is drawn at the true zero on the $1/T$ axis it becomes evident that estimation of the intercept in any real experiment implies an extrapolation that is typically of the order of $10-15$ times the range of the observations, and therefore cannot be done accurately. For example, in a study of the ribosome featured on the cover of the *Proceedings of the National Academy of Sciences* [7] the $T\Delta S^{\ddagger}$ for peptide bond formation was reported to be accurate to ±10% but as this required an extrapolation of 13 times the range of observations in an Arrhenius plot, it appears unduly optimistic.[1]

The only reasonable conclusions to be drawn are as follows:

1. The entropy of reaction cannot be obtained with usable precision from a van 't Hoff plot;
2. The entropy of activation cannot be obtained with usable precision from an Arrhenius plot.

If these parameters are needed they must be determined in some other way, such as from precise calorimetric measurements.

Metabolic "efficiency"

The notion of metabolic efficiency is less frequently encountered in modern textbooks and papers in biochemistry than it once was, but it is still found more frequently than it ought to be. Statements such as

> *Dividing the $\Delta G^{0\prime}$ of ATP formation by that of lactate formation indicates that homolactic fermentation is 31% "efficient", that is, 31% of the free energy released by this process under standard biochemical conditions is sequestered in the form of ATP.* [8]

have by no means disappeared from the literature. A related but perhaps less objectionable sort of statement that remains common is

> *Glucose phosphorylation is coupled to ATP hydrolysis.* [6]

The problem with this last statement is that the process concerned is the reaction between glucose and ATP to produce glucose 6-phosphate and ADP, which does not involve hydrolysis: no water participates in the overall reaction (contrast this with the ATP hydrolysis used to drive the DNA packaging motor described in *Energy Transduction in Living Organisms*, see below). This may be justified as useful shorthand for calculating Gibbs energies of

[1] By chance this paper was published the same week as a previous Beilstein Symposium at which this topic was being discussed [5].

reaction: the net Gibbs energy for glucose phosphorylation can certainly be calculated as the difference between the Gibbs energies of hydrolysis of glucose and of ATP, and as long as it is limited to that no harm is done. The trouble comes when a legitimate difference in Gibbs energies is taken to sanction a completely illegitimate ratio of Gibbs energies. Atkinson [9] pointed out long ago that a statement such as the first one quoted is ''false in every part'', but his warning has yet to be generally heeded.

The confusion arises from two sources, the introduction of an irrelevant species, water, and the arbitrary definition of the standard state as 1 mol/l. The calculation of ''efficiency'' can be done just as easily with a different irrelevant species, such as fructose, or with a different choice of standard state for removing the units from the calculation of the logarithms of concentrations, and in either of these cases the calculated ''efficiency'' of a process is quite different. With fructose instead of water as the irrelevant species, for example, the ''efficiency'' of the hexokinase reaction is changed from 45% to – 14%; taking the standard state of all species as 0.1 μmol/l instead of 1 mol/l it would be 76% [10]. In effect, with suitable choices of irrelevant species and standard states one can obtain any ''efficiency'' one wishes.[2]

It is important to avoid this sort of confusion, because consideration of thermodynamic efficiency has been an essential part of the design of machines since the time of Carnot, and will certainly be essential for developing better ways of harnessing energy in the future, but it needs to be done properly.

Energy Transduction in Living Organisms

As we have noted, animals can convert chemical energy into kinetic or potential energy with efficiency at least as high as any machine. This capacity is not confined to the animal kingdom, and a striking example of an extremely small motor is provided by the DNA packaging motor of the bacteriophage Φ29, which converts the energy stored in ATP molecules directly into pressure-volume work, achieving a pressure of the order of 50 atmospheres (about ten times the pressure in a bottle of champagne). The way in which this is achieved has been studied in detail in single-molecule studies [11]. It involves a high degree of coordination between the subunits of the ring ATPase present in the bacteriophage. Homomeric ring ATPases are found in all forms of life and are involved in many processes such as chromosome segregation, protein unfolding and ATP synthesis. In the bacteriophage it is used to load the double-stranded DNA genome into the viral shell. A high precision

[2] One of us presented these ideas at a meeting in 1982 [10], after which the distinguished expert in network thermodynamics Jörg Stucki commented that although he thought they were correct, he also doubted whether anyone would make such absurd calculations. A year later, at another meeting, he reported that he had checked the literature and had found that such calculations are, in fact, quite common.

single-molecule assay involving dual-beam "optical tweezers", have shown that the five-member motor packages the DNA in 10-base-pair bursts, each consisting of four individual 2.5-bp steps corresponding to the hydrolysis of a single ATP. They thus provide a direct measurement of a single enzymatic cycle by this ATPase, revealing an unexpected form of coordination between the subunits. The non-integral step size detected is unprecedented, and raises intriguing mechanistic questions about ATP hydrolysis within rings, and the interactions of the ring with DNA.

Note that in this engine the DNA packaging is driven by ATP hydrolysis, in contrast to the sort of reactions discussed in *Metabolic "efficiency"* (see above), in which water is not a substrate. Nonetheless, it still contrasts with the heat engines used in industry, because the heat produced is simply lost, and is not used to generate pressure-volume work. Although the same principles of thermodynamics apply both to the steam engines studied by Carnot and to energy transduction in organisms, the analogy must not be taken too far, because heat engines do not exist (as far as we know) in organisms.

Photodecomposition of Water

The photodecomposition of water by photosynthesis has several characteristics that make it very attractive for the future development of electrical power: it depends only on sunlight as energy source, which is available in vast amounts, far more than any realistic requirement for human activities; it does not produce CO_2 but instead consumes it; it is thermodynamically efficient, as nearly all the light of the appropriate wavelength falling on a leaf is absorbed, and about 50% of the light absorbed is converted into chemical energy [1]. On the other hand, it is far more complicated than a typical industrial process, involving many steps and many catalysts. The subsequent combustion of the glucose produced is likewise complicated, with numerous steps and catalysts. Glucose is thermodynamically very unstable in the presence of O_2, but it is kinetically very stable. In solid form it can survive being kept in dry air for years. In solution it is slowly converted to CO_2 and water, but because of bacterial contamination, not through any direct effect of the water and O_2. In brief, oxidation of glucose can only proceed in mild conditions in the presence of a catalyst.

The need for many steps – each with its own catalyst – is a consequence of chemical constraints in the form of a need to pass through chemically feasible intermediate metabolites, and the need to extract the energy in small packages. Analysis of other metabolic pathways [12] indicates that although pathways shorter than those that exist in living organisms may be feasible, they typically suffer from the need to pass through thermodynamically unfavourable steps.

At the level of small-scale research, photocells are beginning to approach the performance of photosynthesis, the best multijunction concentrators now being reported by the U.S. National Renewable Energy Laboratory [13] to have a thermodynamic efficiency greater than

40%. This is very encouraging, but photocells cannot solve all transportation needs without passing through a chemical step: aeroplanes cannot convert electrical power directly into motion, because they need a transportable energy source. Private cars of the future may perhaps be able to obtain electrical power directly from an external supply as present-day trains do, but at present they require rechargeable chemical stores, whether batteries or fuel cells. In any case, it is hard to imagine that aeroplanes will ever escape the need for chemical stores.

H_2 as Fuel

General considerations

Great efforts are being devoted at present to find ways of escaping from our current reliance on fossil fuels and the production of CO_2, and to develop new sources of power. One of these is H_2, which has several advantages over hydrocarbons as a potential fuel:

1. It can in principle be produced by the photodecomposition of water driven by sunlight, available in unlimited quantities.
2. It can also be produced by electrolysis of water, ideally *in situ* at a hydroelectric power plant: this primary source of energy is not unlimited, but it is renewable.
3. The raw material, water, is abundantly available.
4. Neither the production nor the use of H_2 implies production of CO_2.

However, it also has some associated problems:

1. Much more efficient processes than those available today will be required, not only for production of H_2 by photodecomposition or electrolysis of water, but also for conversion of H_2 into work, in fuel cells. (Heat engines are already available, of course, but no major improvements in thermodynamic efficiency can be expected.)
2. H_2 is much more difficult to store safely than hydrocarbons, which are themselves responsible for many accidents.
3. H_2 leaks through pores in containers that can be considered perfectly hermetic for other fluids.
4. Efficient production and harnessing of H_2 depends on catalysts.

Some of these problems will certainly be solved in future years, and we can expect that the current dependence on heat engines and CO_2 production will be overcome with cyclic processes in which energy from sunlight, wind and hydrostatic potential is used to generate

H_2 that is subsequently oxidized to water with the generation of work. Before this can be achieved, however, a major obstacle will need to be removed: catalysts that can be used on an industrial scale will be required.

Fuel cells

In fuel cells based on H_2 the fuel is oxidized at the anode side, and the electrons that are released by the oxidation reaction are driven through an outer electrical circuit, thus generating electrical current. The electrons reach the cathode, where they combine with an oxidant (typically O_2) and protons to a product (typically water). Enzymatic fuel cells employ enzymes instead of noble metals to catalyse the reaction. Fuel cells or biofuel cells thus present similar problems to those of photodecomposition or electrolysis of water. Use of biocatalysts in fuel cells provides, however, several advantages over catalysis by noble metals. Biocatalysts are abundantly available, in contrast to the limited availability of transition-metal catalysts. The substrate specificity diminishes reactant cross-over, which in theory would allow membraneless fuel cells, and biofuel cells can function at neutral pH and moderate temperatures. Catalysts for the oxygenation are available in the form of the enzyme bilirubin oxidase, but the first step is much more problematic, because although hydrogenases are available from H_2-producing organisms, the most abundant ones are sensitive to O_2.

H_2-producing bacteria occur mainly in anaerobic environments. For example, *Desulfovibrio fructosovorans*, a mesophilic sulphate-reducing bacterium, contains both Fe and NiFe enzymes, both of which are extremely sensitive to O_2 [14]. Nonetheless, there are also some microorganisms found in aerobic environments, such as the pollution tolerant bacteria *Ralstonia metallidurans* and *Ralstonia eutropha* [15], *Escherichia coli* [16] and the hyperthermophilic bacterium *Aquifex aeolicus* [17]. This last grows at 85 °C, and is thus particularly attractive for potential industrial use.

Catalysts

Useful catalysts for production of H_2 driven by sunlight etc., or for conversion of H_2 into electrical power, both of these on an industrial scale, must satisfy three criteria:

1. It must be available in quantity at a modest price: Pt, currently costing almost 50 Euros per gram, is clearly excluded.
2. It must be robust enough to withstand long-term use in industrial conditions: this eliminates many enzymes. Resistance to O_2 may not be absolutely essential, but it is certainly highly desirable.

3. It must be energy-efficient, with negligible losses due to hysteresis. Pt is very efficient in this sense, whereas more readily available metals, such as Co and Ni, are much less so.
4. It must be efficiently immobilized at the electrode surface. This is often a bottleneck, especially for enzymes that need specific electrode modification for establishing electrical contact (in other words, nature did not evolve enzymes for bioelectroanalytical applications).

Unfortunately the best metallic catalysts, Pt as a general catalyst, and Pd as a catalyst especially adapted to reactions of H_2, are among the six or so least abundant elements in the earth's crust, each with an abundance of about 10^{-10} atoms per atom of Si [18]. They clearly cannot form the basis of a large-scale industrial process, either now or in the future. Lighter metals from the same groups of the periodic table, such Co and Ni, catalyse similar reactions, but much less efficiently and with much greater energy losses.

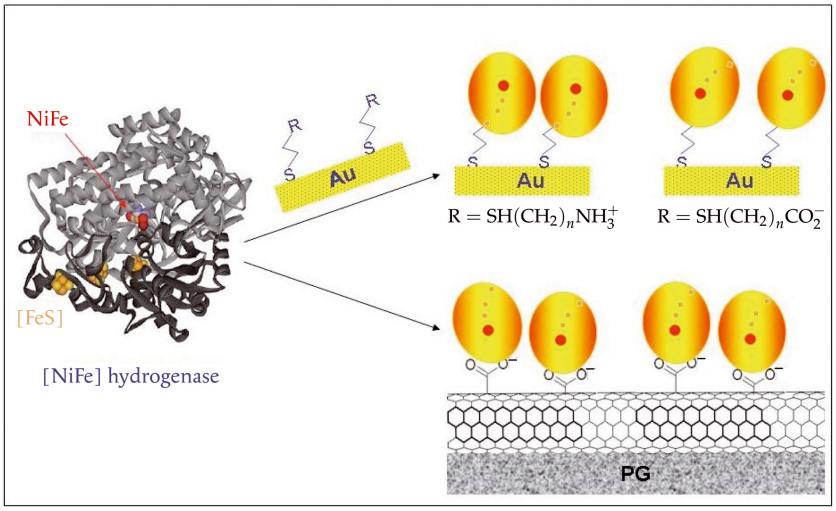

Figure 1. Orientation of *Desulfovibrio fructosovorans* NiFe hydrogenase at self-assembled monolayer gold electrodes or pyrolytic graphite (PG) electrode modified by a carbon nanotube network. Due to the dipole moment of the enzyme and the acidic environment of the surface FeS cluster, switching of the enzyme is achieved by varying the charge of the interface. The electron transfer process consequently shifts from a direct to a mediated electrical communication.

On the other hand they are far more abundant, at more than 10^{-5} atoms per atom of Si, and far less expensive, in the range 15 – 30 Euros per kg. The question therefore arises as to whether biomimetic catalysts, in which metal ions are complexed with organic ligands designed to resemble the active sites of suitable enzymes, might combine the energy efficiency of Pt and some enzymes with a much greater stability than is possible with natural enzymes. Fontecave and collaborators have made substantial progress towards this goal with

Ni-based carbon nanotube coated electrodes that exhibit high catalytic activity for H_2 evolution in aqueous solutions, and also high current densities for H_2 oxidation, though at large overpotentials [19]. This is a promising approach, and even if biomimetic catalysts are not ready to be used on an industrial scale today there is a realistic possibility that they will become so.

Immobilization of hydrogenase

A different approach is to improve hydrogenase immobilization onto structurally modified electrodes. In recent years remarkable successes have been obtained in different fields that contribute to the efficient electrical communication between the enzyme and the electrode. These successes relate to enzyme stability, co-immobilization of the enzyme with a redox mediator, control of the enzyme orientation and increase in the enzyme density, as now outlined:

1. Enzyme stability has been improved by immobilizing a membrane-bound hyperthermophilic hydrogenase inserted into liposomes. A five-fold increase in the enzymatic efficiency is observed, mainly due to the reconstitution of a physiological like environment [20].
2. Co-entrapment of a NiFe mesophilic hydrogenase with a redox mediator (either methyl viologen or the hydrogenase physiological partner) in clay films deposited at the surface of an electrode shows satisfactory catalytic efficiencies both for H_2 evolution and uptake [21].
3. The modification of the electrode so that a redox relay at the protein periphery is located at a tunnel distance from the electrode surface allows efficient H_2 oxidation with no need of a redox mediator (Fig. 1). This situation is encountered through chemical modification of self-assembled-monolayer gold electrodes to match the enzyme surface close to the redox relay [22]. A switching in space has been demonstrated for the O_2-sensitive NiFe hydrogenase from *Desulfovibrio fructosovorans*, which has a high dipole moment and a group of negatively charged residues around a FeS cluster located at the surface of the enzyme.
4. The size of the hydrogenase results in a low density of catalytic centres at a smooth electrode surface. The development of 3D architectures provides new basis for efficient H_2 oxidation. Both long-term stability and catalytic efficiency are improved by modification of the electrode by carbon nanotube deposits. Further physical [22] or chemical [23] modification of the nanotubes has been found to allow the control of the orientation of the hydrogenase on each nanotube, and thus the electron transfer process. The association of carbon nanotube films developing large surface areas and numerous anchoring sites with the capability of the hyperthermophilic hydrogenase from *Aquifex aeolicus* to oxidize H_2 at elevated temperatures (up to 85°C) yields current densities as illustrated in

Figure 2. Values up to 1 mA/cm^2 have been obtained. Encapsulation of the hydrogenase in a carbon nanotube network prior to immobilization on the electrode surface permits stabilization of the catalytic signal with time. H_2 oxidation occurs even in the presence of O_2 and CO [24]. Work is now in progress towards lowering the cost of hydrogenase production, and using a matrix other than toxic carbon nanotubes.

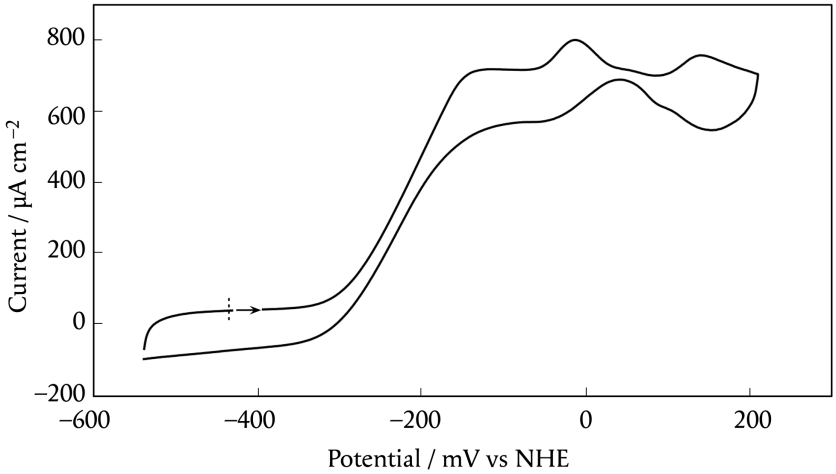

Figure 2. Cyclic voltammogram recorded at a pyrolytic graphite electrode modified with an amino-functionalized carbon nanotube film, under H_2 at 60 °C. The voltammogram shows the catalytic oxidation of H_2 by the hyperthermophilic hydrogenase from *Aquifex aeolicus* encapsulated in the carbon nanotube network. The dashed vertical line indicates the potential of the H^+/H^2 couple under the experimental conditions (HEPES buffer, pH 7.2, 10 mV s^{-1}), and the arrow indicates the direction of the scan.

Perspectives

It will be clear from these examples that a H_2-based economy is not yet ready to replace the existing dependence on hydrocarbons. The use of chemical processes that 9 mimic enzyme action is promising, but the performance in aqueous solution needs to be improved. On the other hand, isolated enzymes present problems of stability and O_2 sensitivity, though attempts are in progress to overcome this difficulty [25]. A third possibility is to harness the H_2-generating capacity of living organisms, but it should never be forgotten that all of these organisms have been endowed by billions of years of evolution with regulatory mechanisms that work for their own good, not for the good of humanity, so the realistic potential of this approach is far from being established. Many attempts to use microorganisms in biotechnological processes have failed completely, or have at best been very disappointing, because the regulatory mechanisms have proved to be very effective at resisting efforts to force

metabolic flux towards commercially valuable metabolites that are not valuable for the microbe itself. Suppressing the regulation is possible, but it results in an unhealthy microbe, so although it may work to some degree [26] in the short-term it cannot be the basis for an industrial process, and the biomimetic strategy seems more promising.

At present we are studying the possibility of using consortia of microbes, in which the different partners produce metabolites needed by the others, and the overall effect is to generate H_2 from cheap raw materials, such as domestic waste [27].

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