BIOHYDROGEN - THE MICROBIOLOGICAL PRODUCTION OF HYDROGEN FUEL

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Summary

Concerns about climate change and dwindling petroleum reserves are fuelling resurgence in the search for alternative, renewable fuels. Among the possible candidates is hydrogen, and there is ongoing research for its production, storage and utilization. One requirement for a sustainable hydrogen economy is a renewable source of hydrogen fuel. A variety of processes are potentially available, among them microbiological hydrogen production ("biohydrogen"). We describe several different approaches to biohydrogen production. All biohydrogen production systems depend upon the activity

of enzymes, the hydrogenases, which reduce protons to H_2. The various types of hydrogenases are introduced and the diversity of metabolic processes in microbes capable of driving hydrogen evolution are reviewed. Biohydrogen can be made from water, either directly or indirectly, using solar-driven photosynthesis to split water ("biophotolysis"), or, alternatively, through a variety of anaerobic fermentative processes that generate hydrogen from organic substrates, or with hybrid processes that combine both photosynthetic and fermentative processes We discuss these processes and the major barriers to practical applications, such as inefficient utilization of light energy, the high costs of H_2 gas-imperable, transparent photobioreactors, the sensitivity of the hydrogenases to oxygen, and the low yields of hydrogen obtainable by dark fermentations of organic substrates, among others. We conclude that there is yet no actual, demonstrated, mechanism that could plausibly be considered for practical development, and that biohydrogen still requires long-term basic R&D before such any applications could be considered.

1. Introduction

The appetite, we may even call it addiction, of our technological societies and economies for energy delivered by the presently still relatively cheap, if not much longer plentiful, fossil fuels, is threatening our global environment, our economies, and even the very survival of our soceties. A rapid transition to sustainable energy systems is one of the greatest shared challenges facing humanity this century [see also Topic 9 – *Socio-economic strategies for sustainability*]. Reducing demand for energy intensive services, improving the efficiency of necessary energy uses, and development of renewable energy sources, must all combine to defuse the imminent crises of fossil fuel depletion, global warming, and environmental degradation. Research and Development (R&D) of practical, renewable energy sources, including hydrogen-based technologies, is now a major focus of science and technology, suggested to help mankind's transition to an economically and environmentally acceptable energy future. Biohydrogen is one of the many renewable energy technologies being studied.

Biohydrogen is a fuel produced by microbial metabolism, similar to bioethanol or biogas (a CH_4:CO_2 mixture) [see also– *Indfustrial biofuels and biobased products*]. There are two basic types of biohydrogen production processes:

1. Sunlight-driven microbial photosynthetic processes using water or organic substrates; and
2. Dark fermentations by heterotrophic bacteria utilizing starches, sugars and other organic substrates.

These two options differ in the microorganisms used: green microalgae, cyanobacteria and photosynthetic bacteria for the first and fermentative bacteria for second. Both approaches have, thus far, achieved only relatively low efficiencies in transforming the substrates (e.g. sunlight and/or organic substrates) to H_2 fuel. For example, photosynthetic microbes produce H_2 at less than 1% solar energy conversion efficiencies, compared to over 10% for photovoltaic cells, and dark fermentations of starches or sugars recover, at best, only 20-25% of the energy content of these substrates as H_2, compared to well over 90% in the commercial production of bioethanol or

biogas. In addition to these low process efficiencies, present R&D approaches to biohydrogen production do not provide a clear pathway to the development of practical and economically viable technologies.

Here we review the various alternative processes, their theoretical and practical limitations, current research and development efforts, and outline approaches that could at least potentially achieve both the high yields and low costs required for practical applications. We first, briefly, describe the H_2 producing enzymes, the hydrogenases and nitrogenases that can generate H_2 and then the bioreactors, both photobioreactors and fermentation vessels that can be used to contain these processes and recover the H_2 produced. The main focuses are various biohydrogen production processes, summarized in Figure 1.

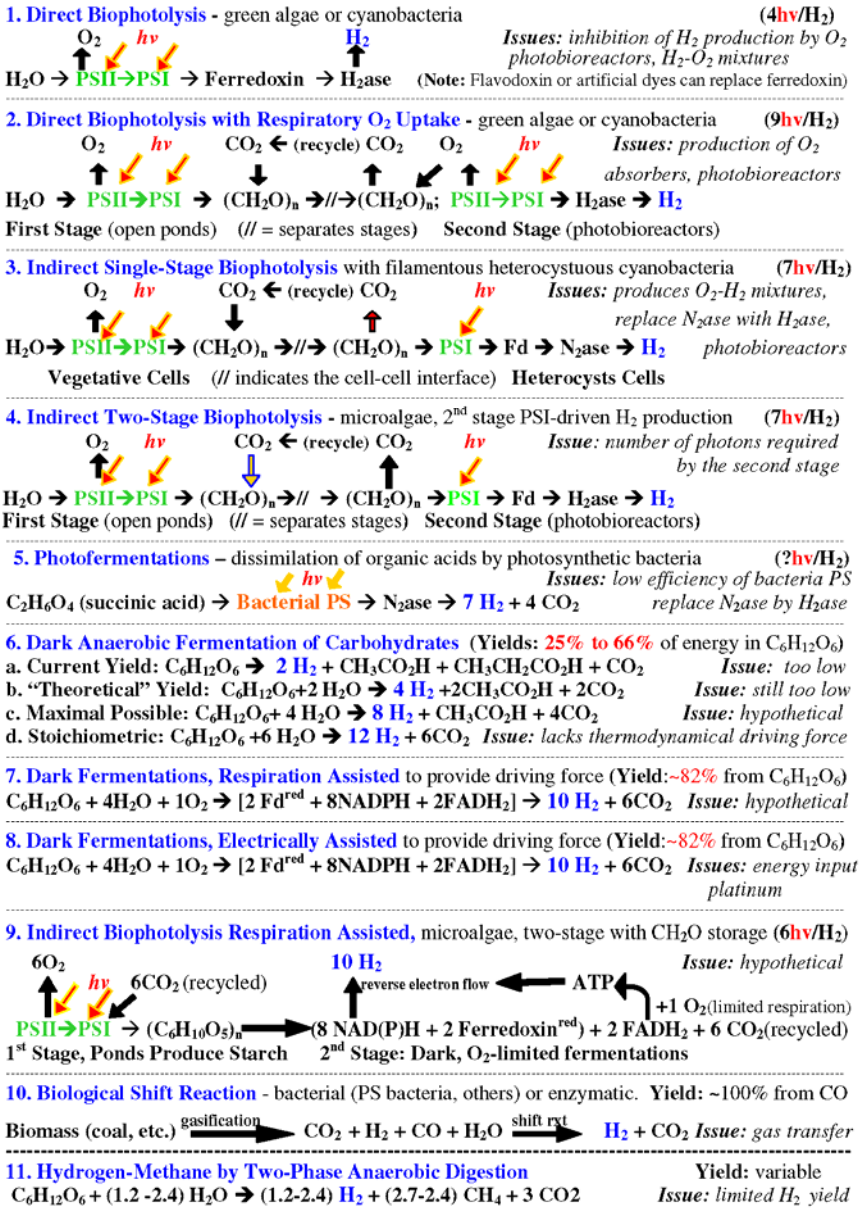

Figure 1. Proposed Mechanisms of Biological H_2 Production: Efficiencies, Yields, Issues

2. Biological Catalysts for Hydrogen Production

Biohydrogen production is based on H_2 producing enzymes, the hydrogenases and nitrogenases. Hydrogenases are present in all three domains of life, archaea (methanogens and some extremophiles), the bacteria (the rest of the procaryotes) and the eucaryotes (in particular the green algae). Hydrogenases are catalysts that can reduce protons to H_2 by oxidizing a suitably strong reductant including the natural electron carrier proteins ferredoxin and/or flavodoxin, which have redox potentials near that of the H_2 electrode, -420 mV. NADPH (reduced nitcotinamide phosphate) is too positive (–320 mV) to serve as a direct reductant of hydrogenase, except in hyperthermophiles, bacteria growing >65^{o}C, where the H_2 redox potential is near this potential.

Hydrogenases can be classified by their metal content as Ni-Fe or Fe-only hydrogenases, the latter now referred to as [Fe-Fe] hydrogenases. The two types of enzymes differ in molecular weight, cellular location, subunit composition, electron carrier specificity, sensitivity to O_2 inactivation (the [Fe-Fe] being generally more sensitive), and perhaps most important, their physiological role. Ni-Fe hydrogenases are typically involved in H_2 uptake reactions, but can also function in H_2 evolution, while [Fe-Fe] hydrogenases are more often involved in H_2 evolution, and their specific H_2 evolution rates are over a hundred times faster than that of the [Ni-Fe] enzymes. They are thus the logical choice for biohydrogen production. The [Fe-Fe]-hydrogenases, are typically found in strictly anaerobic bacteria, but are also present in some aerobic cyanobacteria and green algae. They contain iron-sulfur centers binding cyanide and carbon monoxide, in a unique structure for enzyme active sites.

Nitrogenases are found only in prokaryotes and are irreversible catalysts that can only evolve, but not take up, H_2. Their natural function is to fix N_2, and some H_2 evolution takes place during N_2 fixation, but in much larger quantities in the absence of N_2. The specific activities (H_2 evolved per weight of enzyme per unit time) of nitrogenases are three to four orders of magnitude lower than those of [Fe-Fe] hydrogenases. Even worse, nitrogenases consume large amounts of metabolic energy (ATP) during H_2 production, doubling the energy required to evolve H_2, compared to H_2 production by hydrogenases. Thus, nitrogenases are impractical for biohydrogen production. However, nitrogen fixing bacteria are excellent models for H_2 production processes [see also– *Cell Thermodynamics and Energy Metabolism*], as they have evolved the reductant generation pathways, described next, required to generate H_2 at high rates. Thus, nitrogen-fixing bacteria are candidates for biological H_2 production, provided that the inefficient nitrogenase is replaced with preferably a[Fe-Fe] hydrogenase.

Hydrogenases and, somewhat surprisingly, nitrogenases, provide a site for H_2 absorption/ desorption that has a free energy change similar or even lower than platinum, the most efficient metal catalyst known for electrochemical H_2 production. In recent years major advances have been made in the genetics, regulation and molecular characterization of hydrogenases and nitrogenases, including their 3-dimensional structures at high resolution showing the positions of the iron-sulfur clusters, the site of docking of the reductant (e.g. ferredoxin or flavodoxin), and, for the hydrogenases, the putative gas channels through which H_2 molecules are believed to flow out of the active

site, among other details. There are increasing expectations that it will be possible to engineer completely new hydrogenases, including enzymes not inhibited by O_2 (discussed under "Direct Biophotolysis", Reaction #1 in Figure1).

Even more important, for practical applications, than the structures, genetics or specific activities of the hydrogenases are the metabolic reactions that generate the low redox potential reductant (e.g. reduced ferredoxin or flavodoxin) required for H_2 production. Although the study of these electron transport pathways channeling reductant from sugars in case of heterotrophs and water through photosynthesis for microalgae, has lagged somewhat behind that of the hydrogenases, we now have a reasonable understanding of these metabolic processes in most H_2 producing microbes. One area of uncertainty, even confusion, is the role of so-called "reverse electron transport" reactions in generating the reductant needed by the hydrogenases or nitrogenases. This topic, and electron transport pathways generally, is discussed in more detail in later sections.

3. Bioreactors for H_2 Production

Before discussing in detail the processes listed in Figure 1, it is useful to consider some practical aspects of the bioreactors (design, costs, and operations) that must both contain the microbial culture, and capture the H_2 generated [see also – *Instrumentation and Control of Bioprocesses; Bioreactors*] . Indeed, the entire system, including front- and back-end subsystems, such as production of the catalysts (e.g. the microbial biomass used in the processes) and H_2 purification and storage, must be considered. Although the microbial metabolic mechanisms that actually produce the H_2 are at the heart of the processes listed in Figure 1, it is the engineering and economics of bioreactor designs and operations that determine their eventual practicality. Most fundamental, is the form of the energy supplied for H_2 production: light (Reactions #1 to 4 in Figure 1), organic substrates (Reactions #6 and 7), a combination of both (Reactions #5 and 8), or even carbon monoxide (Reaction #9) and electricity (Reaction #11).

For light-driven H_2 production processes (Reactions #1-#5, #8), the necessity for capturing both light and H_2 simultaneously requires transparent, closed photobioreactors, capable of efficiently collecting solar energy while also collecting the H_2 output and providing a suitable environment for the biological catalyst. One major issue is that the maximum unit scale of such reactors is limited by gas exchange (O_2, H_2, and CO_2, in or out, as needed) to at most a few hundred square meters, compared to tens of thousands of square meters for open pond systems. This greatly affects the capital and operating costs of closed photobioreactors, the key issue for practical applications. The actual costs of photobioreactors would depend on their design and overall system scale, which could range from a few hundred m^2 for residential-commercial systems to millions of m^2 (hundred of hectares) for large facilities.

A plausible minimum capital cost of \$100/m^2 for closed photobioreactor systems for biohydrogen production can be anticipated based on experience with commercial units of 1 hectare in scale. This would result in annualized capital costs (depreciation, return on investment, maintenance and other fixed costs) of close to \$20/m^2-year. Even assuming a theoretical 10% solar-to-H_2 conversion at a favorable location with 5

kWhr/m^2/day annual average solar insolation, this capital charge translates into a H_2 price of $33/GJ, or about $200/barrel oil equivalent. Adding operating costs, including inoculum production, temperature control (overheating is a major issue), cleaning, mixing, gas exchange, process control, system management, H_2 processing, storage and distribution, could double overall H_2 production costs. It must be noted that this projection is highly optimistic, both in capital and operating costs, and, most important, in the high H_2 outputs assumed. Thus processes that require that the entire system be enclosed in a photobioreactor (Reactions #1-3) are inherently limited by high costs, even without considering the costs of catalyst production and H_2 handling, among others.

As discussed below, the, relatively, more attractive systems are two-stage photobiological processes, in which large open mass culture ponds serve as a first stage producing carbohydrates followed by smaller closed photobioreactors in a second stage that produce the H_2 (e.g. Reaction #4 in Figure 1). Large open pond systems have capital costs projected at US$ 10/m^2, including infrastructure and supporting systems. Thus, such two-stage processes would be of lower cost than single stage processes.

Bioreactors for dark fermentation are also much less expensve than closed photobioreactors. Here the determining costs and productivities are per unit volume, rather than per unit area. Assuming achievement of a rate of H_2 production of between 10 to 100 volumes per volume bioreactor per day, in analogy with ethanol fermentations, this would thus produce approximately between 30 and 300 GJ/m^3 bioreactor per year. Fermentation bioreactors cost can range from under $100/$m^3$ for anaerobic digesters used for animal waste treatment, to about $1000/$m^3$ for fermenters used in ethanol production, to over $10,000 m^3 for sterilizable reactors used for high value (e.g. pharmaceutical products), where contamination is the key problem [see also – *Process Optimization Strategies for Biotechnology Products*]. In brief, bioreactors similar to ethanol fermenters, the most directly comparable process, could plausibly be economical for even the projected low H_2 productivities, but sterilizable reactors would not be practical at any plausible production rate. Thus any process which requires operation of bioreactors, either fermenteors or photobioreactors that require sterilization of the system to exclude contaminants is not feasible in a practical process.

Fundamental to the design and operation of bioreactors, both in fermentation and photobiological processes, is gas exchange to manage the concentrations in solution of the H_2 but also O_2 and CO_2 due to gas transfer limitations in any practical process, these gases, when produced by microbial metabolism, will accumulate in the liquid phase and can exceed the calculated concentrations in equilibrium with the gas phase by one to two orders of magnitude. This is critical for biohydrogen production, as H_2 and O_2 are strong inhibitors of fermentative and photobiological H_2 evolution, respectively, and thus will reduce the rates and yields of H_2 production. This problem cannot be overcome, outside the laboratory, through such artifices as sparging with inert gases, increased mixing, or applying vacuum, as these are inherently expensive. In practice, a dissolved H_2 concentration equivalent to 10 atmospheres of H_2 can be anticipated in fermentation processes, equivalent to a redox potential of -450 mV, making this the key issue in such processes (where already a 1 atmosphere partial pressure is inhibitory). For photobiological processes, high H_2 liquid side partial pressures are tolerable, but O_2

accumulation and/or CO_2 gas supply are major problems, even with open ponds.

In conclusion, the design and operation of affordable bioreactors for biohydrogen production will constrain the type of process that can be scaled-up and, in turn, the attributes of the microbes catalyzing these reactions. Thus not all the processes demonstrated in the laboratory, and some reviewed below, can't be scaled-up for practical biohydrogen production.

4. Photobiological Hydrogen Production Processes

4.1. Introduction: Photosynthesis and Solar Conversion Efficiency

Over the past several decades many scores of photosynthetic microorganisms have been studied for their ability to evolve H_2. Photobiological H_2 production can be classified into several distinct mechanisms, based, on the immediate source of electrons for the hydrogenase (water or organic substrates, the latter involving intermediate CO_2 fixation), on the enzymes used (nitrogenases or hydrogenases), and on the microbes used (green algae, cyanobacteria, photosynthetic bacteria, etc.). Figure 1 classifies photobiological H_2 production mechanisms into five distinct processes (reactions #1 to #5), based mainly on the pathway for reductant generation. (Reaction #8 involves a mixed photobiological-dark fermentation process and is discussed after the review of the latter processes).

In photobiological H_2 production the efficiency by which the photosynthetic process can convert solar energy into chemical energy is more critical than the H_2 production process itself. In green algae and cyanobacteria, photosynthesis involves two light capture and conversion processes working in tandem, first splitting water into O_2 and then generating reduced ferredoxin, a strong reductant that can then generate the NADPH used for fixation of CO_2 into sugars or, alternatively, can reduce hydrogenase to produce H_2. A major assumption in this field has been that it will be possible to achieve a 10% solar-to-H_2 conversion efficiency, near the theoretical maximum for photosynthesis. However, the highest achieved solar conversion efficiencies by algal mass cultures fixing CO_2 into biomass are only 2 to 3%, while current solar efficiencies for H_2 production are only about $1/10^{th}$ this level. Thus, increasing photosynthetic solar-to-H_2 conversion efficiencies to even approaching 10% would be a major challenge, and, indeed, does not appear feasible in the foreseeable future. Achieving even half this goal appears plausible but will require a major R&D effort to overcome currently limiting factors in photosynthetic energy conversion efficiencies.

Of these, the so-called light-saturation effect is perhaps the major single factor that limits photosynthetic efficiencies: at light intensities above about one tenth of full sunlight the rate of photosynthesis by individual algal cells saturates - it does not further increase with increasing light intensity. This means that much, indeed most, of the full sunlight received by an algal culture is actually not used in photosynthesis, resulting in an overall low efficiency of photosynthesis. The reason for this is that the photosynthetic apparatus uses large arrays of chlorophyll and/or other pigment molecules to capture as many photons as possible. In brief, the photon energy (the "exciton") captured by these arrays (the "antenna" or "light harvesting" pigments) is

transferred to so-called “reaction centers” chlorophylls where the exciton energy is converted into chemical energy. The key problem is that the light harvesting pigment arrays are relatively large, optimized to capture photons at low light intensities, when an algal cells finds itself in a deep layer in a pond or photobioreactor, shaded by other cells above it. However, this adaptation results in cells that find themselves at or near the surface absorbing many more photons than can be processed by the reaction centers, and this excess is wasted as heat and fluorescence. This results in cells near the surface absorbing most of the light, and wasting most of it, those in the deeper layers compensate by increasing their antenna size to as large as possible to capture what photons remain. As the culture is mixed the position of individual algal cells changes, from light limitation in the deeper layers to light saturation when they are near the surface. Adaptation to such changing conditions is possible, but rather slow, requiring a major structural change in the photosynthetic apparatus. The overall optimal strategy for individual algal cells is to maintain at all times as large an array of light capture pigments (antenna size) as possible. This however greatly reduces the overall productivity (e.g. light conversion efficiency) of the entire culture, as the excess photons captured by the algae near the surface are wasted.

This problem was recognized many years ago, as has the apparent solution: it should be possible to select or genetically alter algal cells with a reduced content of light harvesting pigments, such that the rate of photon absorption under full sunlight better matches the rate of photon utilization. Although neglected for many years, research has been ongoing for the past decade to develop algal strains with reduced pigment content. The main approach has been to mutagenize cells and screen on plates for low pigment (pale) colonies, from which a few have been identified as having reduced antenna pigments and increased photosynthetic efficiency at higher light intensity. However, mutagenesis typically results in multiple mutations and these mutants have exhibited slower growth and other detrimental characteristics that make them unsuitable for algal mass culture. Work is now onogoing around the world on using genetic engineering techniques to develop strains that demonstrate actual increased solar conversion efficiencies under sunlight intensities in algal mass cultures. Eventually these strains could also be used for biohydrogen production. However, first a plausible mechanism for such a process must be demonstrated

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Biographical Sketches

Patrick C. Hallenbeck, *Academic Career*: BSc in Physics and PhD in Biophysics. Professor at the Université de Montréal since 1987. Active in both fundamental and applied research, and teaching at both the undergraduate and graduate level. Trained numerous undergraduate students, technicians, Masters and Doctoral students and Post-Doctoral Fellows. *Expertise and Interests*: Microbial physiology, applied microbiology and biotechnology, biological energy production, anaerobic metabolism, nitrogen fixation, molecular genetics, and protein chemistry

John R. Benemann, B.S. in Chemistry and a Ph.D. in Biochemistry, both from the University of California Berkeley; three years as postdoctoral fellow, Dept. Chemistry, U.C. San Diego; rejoined U.C. Berkeley, in Civil Engineering, and as an independent researcher supervised half dozen Ph.D. thesis students in Civil Engineering, Biophysics and Plant Physiology. In 1980 started a small biotechnology company, 1983-1988 Associate Professor at Georgia Inst. of Technology and for past 20 years full time consultant in the fields of biofuels, microalgae, waste treatment, landfill bioreactors, nutraceutical products, bioremediation, photosynthesis, and greenhouse gas abatement.