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Review

Biological hydrogen synthesis as an alternative energy of the future without environmental pollution?

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Abstract
Direct photobiological hydrogen production would be an ideal solution for the environment, especially since it releases oxygen as a by-product with low environmental impact. The efficiencies achieved so far are not yet sufficient to compete economically - yield/expenditure - with the established processes of H_2 production or even fossil fuels. Aspects of the safety, storage and transport of hydrogen must also be taken into account. In the field of photobiological hydrogen production, further scientific efforts are needed to understand molecular biology and increase the effectiveness of biophotolysis. In addition to genetic modifications, biophysical-biochemical methods such as the optimization of the environment (pH, temperature, gas and ion activities, etc.) or electric and magnetic fields are also discussed in order to optimize the yield of photosynthetic hydrogen. Ultimately, only innovative ideas – which will also contribute to the understanding of photosynthesis – in the field of the photosynthetic hydrogenase system can help this environmentally friendly and desirable approach to hydrogen production to achieve lasting economic success.

Keywords: *bio-hydrogen, microorganisms, photosynthesis, environment*

INTRODUCTION

The National Hydrogen Strategy describes hydrogen (H_2) as the energy source of the future, which is intended to replace fossil fuels [1]. First and foremost, it refers to green hydrogen, CO_2-free hydrogen produced by electrolysis from renewable energies. Due to the expected high demand, Germany will temporarily have to resort to traditional production methods of H_2, especially since the economic viability is called into question [2÷4].
The main traditional production methods of hydrogen are listed below.
A: Production from fossil raw materials (natural gas, oil, HFs) by oxidation or steam reforming: disadvantage CO_2 release (grey H_2)
B: CO_2 released is stored (e.g. as solid, gas or for synthetic fuel [electro-fuel]): blue H_2, less CO_2 release
C: thermal splitting of methane (CH_4). The result is solid carbon that must be used or stored (carbon capture and storage): turquoise H_2
D: The German Federal Environment Agency also describes the white H_2 (H_2 is released as a by-product) and the pink H_2 (obtained from nuclear energy).
E: Quaschning also discusses the Kavaerner process, in which CH_4 bound to activated carbon is split at 1600 °C and split "CO_2-free" ($CH_4 \rightarrow C + 2\ H_2$); the thermal processes are generally characterized by high energy consumption [2].
The production of biohydrogen is currently being researched worldwide and has not yet reached the status of an industrially feasible method. In principle, enzymatic processes that reduce the activation energies are suitable in plants or microorganisms; initial attempts have also been made with artificial

photosynthetic cells [5÷9]. The thesis will investigate the question of whether environmentally friendly photobiological production can be optimized and whether these processes can be implemented economically and technically?

Does green hydrogen solve environmental problems?
Technically, hydrogen produced with alternative energy would be an innovative contribution to reducing the environmental impact in the energy sector or striving for this goal. However, it is not enough to focus only on CO_2. On the one hand, intrinsic problems of hydrogen production, transport and storage must be considered, and on the other hand, all ecological footprints of alternative energy production must be considered and included in the overall balance. PV systems and wind power plants also represent a relevant environmental impact in the overall balance [10, 11]. However, the mining and production methods, the life cycle of the plants and the recycling balance can still be substantially optimized technically. Nevertheless, green energy is a central prerequisite for the production of green hydrogen and is therefore also associated with its environmental balance.

Water demand and energy comparison
Water demand is particularly critical if hydrogen is to be produced in water-scarce regions with high solar activity and transported to Europe [12]. To produce one kg of H_2, we need 9 kg of water [13]. Fairly emphasizes that the demand for water would increase by another 15 kg due to purification processes and that another 124 L and 11 L would be added by the production of the PV systems or wind turbines. The energy hunger of desalination plants (about 4 kWh/L of fresh water) and the problems associated with desalination should not be underestimated ecologically [14]. In principle, water availability is a factor that is "hidden" in the H_2 strategies [15].
Under normal conditions (lower calorific value LHV), 1 kg H_2 corresponds to approximately 11.2 m3 of hydrogen or 33.3 kWh or 120 MJ; in comparison, 1 kg of diesel contains 11.9 kWh or 43 MJ of energy [16]. For a distance of 100 km, the fuel cell needs about 1 kg of (compressed) hydrogen, which is equivalent to about 7 L of diesel or 5.9 kg of diesel. In the combustion engine, the H_2 consumption would add up to about 2.2 kg. However, H_2 has to be stored in (expensive) pressure tanks, diffuses easily and there are some technical problems to be solved in the combustion process. It should not be forgotten that sickly oxides are produced during the combustion process with air [17]. In the long term, biological hydrogen could represent a technical alternative that combines the advantages of hydrogen with good environmental compatibility. The energetic framework is provided by photosynthesis (see appendix), whereby the potential difference is not used for the synthesis of carbohydrates, but rather the maximum possible energy flows into the biochemical byway of hydrogen synthesis.

Enzymes of hydrogen synthesis
Numerous microorganisms possess hydrogenases (cyanobacteria, algae) or nitrogenases (bacteria, archaea, cyanobacteria), which can produce hydrogen. Biochemically, a distinction is made between [NiFe] hydrogenases and [FeFe] hydrogenases. Experimentally, green algae ([FeFe] type) as well as purple bacteria and cyanobacteria ([NiFe] type) are suitable. Bidirectional hydrogenases – production or uptake of H_2 – are distinguished from uptake hydrogenases, which are only found in bacteria and take up H_2 to provide electrons to N-fixing bacteria; this hydrogenase should be suppressed as much as possible. Normally, oxygen is formed via PS II and PS I and carbohydrates via the Calvin cycle. If PS II is blocked, hydrogenase can generate H_2 by the bidirectional shunt.
Nitrogenases fix N_2, normally form ammonia and nitrate; well-known are the nodule bacteria (rhizobia). Biochemically, it is a multienzyme complex (dinitrogenase – [dinitrogenase reductase]) that converts molecular nitrogen into ammonia [9, 18, 19]. In addition to "molybdenum nitrogenase" or "iron nitrogenase", vanadium nitrogenase in particular has proven to be beneficial for hydrogen production. Nitrogenase also produces H_2 under certain conditions (absence of O_2 and N_2, electrons, ATP energy).

Example of a model organism

The green algae Chlamydomonas reinhardtii often serves as a model organism, especially since the cells can use carbon sources such as acetate – already photosynthesized compounds – in the dark. The green algae are easy to cultivate and double about every 5÷8 hours under optimal conditions [20, 21]. Under standard conditions, they bind CO_2 in the Calvin cycle to synthesize (long-chain) carbohydrates, with photosynthesis using the ATP (energy) and NADPH (reduction equivalents) from the light reaction.

Under anaerobic conditions, the bacterium can "switch" to photosynthesis-associated H_2 production by a hydrogenase, whereby the O_2 formed in photosynthesis inhibits this reaction [6, 21÷22]. It is important to suppress PS II (photosynthesis system II) – which is partly responsible for the release of O_2 – which is achieved by sulfate or phosphate limitation [21]. However, hydrogenase activity can be influenced by numerous factors that are also interesting with regard to the control of H_2 production: intensity; pH; temperature; medium and cell density, sulfate concentration, phosphate (adjust); starch incorporation (when starch is the electron source); presence of chemicals and/or nutrients; oxygen deprivation, removal of H_2 out of balance.

Biological processes of hydrogen synthesis

In principle, green algae can take several paths to produce H_2 [24, 25]:

1: the **direct splitting (biophotolysis)** of water, where PS II (water splitting) and PS I interact (fig. 1)

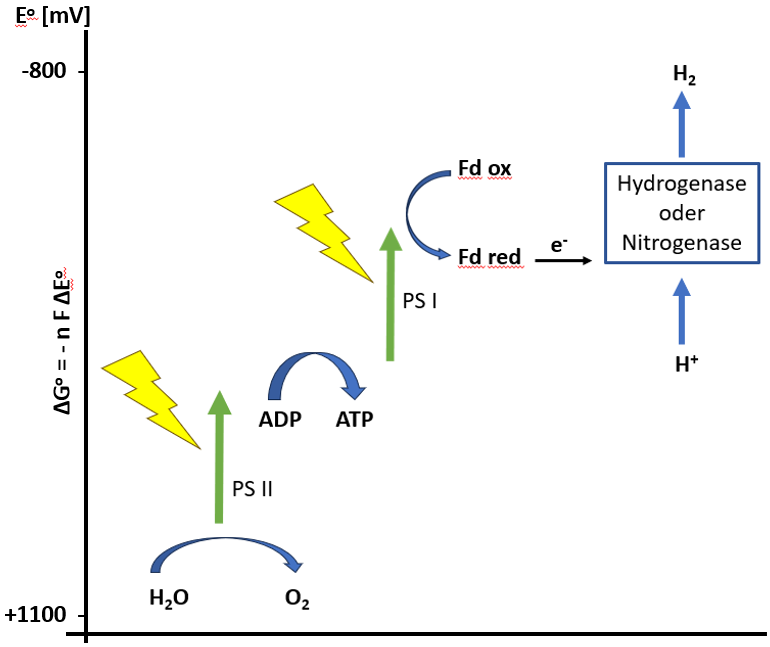

Fig. 1. Schematic representation of H_2 production in the alga Chlamydomonas reinhardtii

The light is absorbed in the light-harvesting complexes (LHC I and II of the PSI). There is a PSII-dependent pathway and a PSII-independent pathway. Electrons are made available to PSII for PSI way. Ferridoxine is reduced via PSI with electrons, which passes the electrons to the hydrogenase. Under anaerobic conditions, the electrons are provided by the pyruvate ferridoxin oxidoreductase. Both the PSII-independent pathway and the dark reaction of the hydrogenase rely on the electrons from the degradation of organic compounds – not drawn, according to Batyrova and Hallebeck [26]. Oxygen-forming cyanobacteria ("blue-green algae") have hydrogenases and nitrogenases.

$2\ H_20 \leftarrow\rightarrow 2\ H_2 + O_2$ (2 e- are transferred to the hydrogen [6])

$H_2O \rightarrow 2\ H+ + 1/2\ O_2 + 2\ e^-$

$2\ H+ + 2\ e^- \rightarrow H_2$

2: **PS II independent fission** of H_20 (electrons originate from the citrate cycle or glycolysis via starch). In this case, the photosynthetic systems I and II are separated and H_2 is indirectly formed [27].

3: **Dark fermentation** of decarboxylated pyruvate (glycolysis) by ferridoxine oxyreductase. In this case, H_2 is produced in the dark under anaerobic conditions by anaerobes, e.g. from lignocellulose ("wood waste") or carbohydrates, wastewater, sugary waste according to the sum equation:

$$C_6H_{12} + 2\ H_20 \rightarrow 2\ CH_3C00H + 2\ C0_2 + 4\ H_2$$

but this produces CO_2 and acetic acid [28].
Sulfur-free purple bacteria have only one photosystem; due to cyclic electron transport, no electron gap has to be filled by donors (fig. 2). Organic compounds act as electron donors in many bacteria, H_2S in sulfur bacteria; reverse electron transport is necessary because a redox potential difference has to be bridged [29÷31].

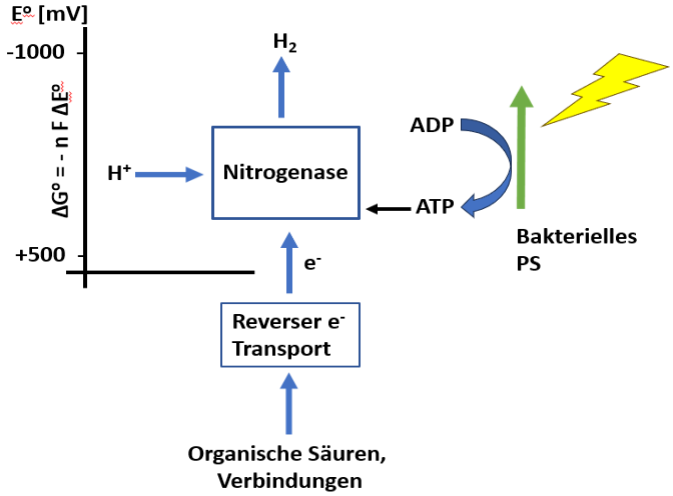

Fig. 2. Schematic principle of photobiosynthesis of H_2 in bacteria (except cyanobacteria, whose biochemistry is more similar to algae and plants)

Possible variations of hydrogen biosynthesis to increase yield
The great challenge of the future is to increase the productivity of hydrogen production. This can be done by optimizing process control (see above) and effective bioreactors. From a procedural point of view, continuous processes would be advantageous, e.g. a helically arranged flow reactor (translucent flow tube) with H_2 sampling, measuring and stirring points under control of the process conditions (light energy and temperature, etc.). Of course, the biological process can be optimized by bioprocess-technological measures [32]. Below are some options and theoretical approaches.
1: Approach in **the electric field** ("electrobiosynthesis") and/or introduction of cathodes and anodes into the reactor [33]. In this process, the free energy of the potential ΔE is additionally used ($\Delta G = n*F*\Delta E$). Magnetic fields can also increase the growth of algae - as well as photosynthesis - and change electrical potentials [34]. One idea that has so far been little discussed in this context is the electron or complex mobility in the membrane [35]. Basically, the free energy or potential depends on the mobility (mobility coefficients) of the landing carriers, i.e. also on the vertical and lateral fluidity and the electrical properties of the thylakoid membrane.
2: Approach with and without **UV irradiation** to increase mutation rate - possibilities under good laboratory conditions and deduction: irradiation with alpha emitters, chemicals, etc. Mutants, e.g. of the **D1 protein** (necessary for the release of O_2 in PS II), could increase production by targeted switching off [21]. New methods (e.g. CRISPR-Cas; **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats - gene scissors) offer numerous starting points for genetically optimizing hydrogenase. In the broadest sense, antisense strategies should also be mentioned here, e.g. to inhibit sulfate permease (sulfate uptake) [31].
3: Influence of growth factors such as auxins, gibberellines, ethylene in plants that affect algae growth and H_2 production [9]. Phytohormones promote the growth of plants and, last but not least, influence gene expression by blocking repressors. It is interesting to note that there are mutants that react in a special way to phytohormones.

4: Influence of **salt stress** on algae growth and hydrogen production [36]. This is also in view of the desirable production of H_2 in seawater. Salt-loving plants (halophytes) are to be discussed in this context.
5: Elimination of reactive oxygen species (ROS) e.g. glutathione ascorbate cycle or superoxide dismutase [31]. Reducing the amount of photopigments (purple bacteria) to avoid harmful light effects has a similar effect.
6: Suppression or modification of hydrogenase-inhibiting metabolic pathways, e.g. suppression of uptake hydrogenase. Overexpression of nitrogenase increases energy consumption and promotes hydrogen synthesis [31]. A reduced activity of ribulose-1,5-bisphosphate-carboxylase-oxygenase (binds CO_2 for carbohydrate synthesis) also has a beneficial effect.
7: Oxygen - and for nitrogenases also nitrogen - inhibits hydrogenases or nitrogenases. If it is possible to find O_2- or N_2-tolerant enzyme variants, this would not only facilitate hydrogen synthesis, but also simplify process engineering.

DISCUSSION AND PROSPECTS

According to initial research, the method has not yet been able to establish itself in process engineering/industry. The production rates of microbiological systems measured to date in comparison to the effort (area/productivity, energy demand, environmental impact) and the current potential do not yet justify economic viability. In principle, higher plants should also be able to produce hydrogen, possibly, after genetic modification, too; this would significantly reduce the experimental and later also procedural effort [36, 37]. An alternative would also be the creation of algae lawns, i.e. the immobilization of organisms, especially since a higher yield of H_2 would be expected [38].
Nevertheless, most experts assume that the photosynthetic production of H2 is still in its infancy today [39], table 1.

Table 1. Advantages and disadvantages of biological hydrogen production processes (supplemented by Hüsing et al. [39], Ahmed et al. [40])

Production	Microorganisms	Initial product	Disadvantages
Direct biophotolysis	Green algae (bacteria)	Light, $C0_2$ (Bacteria: CO)	O_2 inhibits process Economy?
Photofermentation	Photochromic bacteria	Organic material, waste	Light requirement $C0_2$ is created
Indirect biophotolysis	Cyanobacteria (algae)	1. Photosynthesis of sugars 2. Fermentation to H_2	O_2 inhibits process Economy? Wastewater pollution
Dark Fermentation	Bacteria	Organic Substrates Light-independent Co-products Acids such as butyric acid	Wastewater pollution H_2 with CO_2 content
Nitrogenase	Bacteria, Archaea	N_2 fixation, H_2 as a by-product	Yield?, O_2 sensitivity

It should be borne in mind that direct biophotolysis of water is not very productive compared to photofermentation (table 2).
Other authors see biological hydrogen production as a real economic alternative, also with regard to waste recycling [41]. According to the research center in Jülich, the annual production rate of a conventional hydrogen manufacturer is about 2900 t. If one optimistically assumes that we obtain 200 ml of H_2 per liter of algae suspension in one day, we would come to about 72 liters per year. Under special conditions (factor 8÷10), we achieve about 720 L H_2 when extrapolating batch tests [21]. We would have to use an unrealizable amount of algae suspension (4,107 L) to produce the

technical order of magnitude of H_2. In order to increase the biological H_2 production rate or to be able to operate it economically, the efficiency would have to be increased considerably.

Table 2. Comparison of hydrogen production rates and economics of some biological processes [3, 40]

Method	**H_2 production rate (mmol/l h)**	**Cost-effectiveness (USD/kg H_2)**
Direct biophotolysis	0.07	2.13 to 7.24 depending on the source
Indirect biophotolysis	0.355	1.42
Dark fermentation	3 to 120 depending on the source	7.54÷7.61
Integration of dark and photofermentation	47.92 to 51.2	

Benemann [42, 43] is somewhat more optimistic about the forecast and estimates a higher efficiency of 10 % (1.5 % in real terms) of biophotolysis and possibly also of open pond plants, although these theoretical assumptions are unlikely to be put into practice [21]. Current publications remain skeptical about the economic viability of biohydrogen production – with an optimistic undertone – and still see a considerable need for optimization [23, 38]. Touloupakis et al. cite calculations comparing commercially viable H_2 production ($0.3/kg) with actual microbiological production ($2.99/kg to $8.44 in gallon gasoline equivalents) [38]. The energy balance of photolysis includes not only the splitting of water (286 kJ/mol H_2), but also the transport of 8 electrons (173 kJ/2 mol), so that the maximum efficiency is 40%. If you include all losses, you get a light conversion efficiency of up to 13%.

Possibly, artificial photosynthesis modules or combined processes (fermentation, biophotolysis) offer an alternative, especially to avoid fuel consumption [44, 45]? Moreover, fermentation processes may help to metabolize waste and get hydrogen does not abolutely need light [44]. Chocois et al. characterize mutants of green algae that degrade starch compounds and produce hydrogen [27]. They do not depend on PS II and may facilitate „circular economy“ [46]. Hupp et al. put forward a continuous procedure to produce hydrogen using starch [47]. This kind of „microalgae reactor“ may be more efficient than batch techniques. There are efforts to make biophotolysis more effective by constructing "artificial leaves", i.e. to significantly increase the efficiency in relation to the area required [8]; here there is a certain analogy to the algae lawns. Touloupakis et al. propose further optimizations such as co-cultures of anaerobes with aerobes – O_2 consumption improves H_2 yield – or alternatives to SO_4/PO_4 stress such as ethanol or dithionite [38].

If natural – unmanipulated – hydrogen production were very high, the effect of H_2 – as an indirect greenhouse gas via methane – as a climate-damaging gas should have already been felt [48], so the low efficiency of biological hydrogen production is not surprising. However, this results in a risk factor for H_2 losses in the production (explosive gas) and transport (diffusion tendency) of hydrogen [15]. The transport and storage problem could perhaps be solved by light metal hydrides [49].

The vision would be to perfect the effectiveness of biophotolysis, perhaps combining it with photocatalysis [41]. In this case, catalysts – copper oxide (CuO) and gold were used in the work of Chen et al. – could act as electron donors and significantly increase efficiency. Another theoretical option would be to use the electron potential of the photosynthesis of algae or plants to directly produce electricity - "biological solar cell" [37]. For both theoretical approaches, however, the efficiencies achieved so far are not sufficient to produce electricity economically.

CONCLUSIONS

Biosynthetic hydrogen production remains interesting in the context of research into cellular energetics and metabolic processes, however all pathways have to be improved with regard to economic and substrate output [50]. With regard to climate change, insights into the regulation of hydrogenases or nitrogenases are definitely important and interesting and a challenge for the future.

APPENDIX

Notes on the Biophysics of Photosynthesis

The energy of one mole of photons (h Planck quantum of action, c speed of light, λ wavelength) can be combined with the Avogadro constant N_A with the relationship:

$$E = h\frac{c}{\lambda} * N_A \,, usable\ energy:\ E * \Phi F$$

Where: the quantum yield ΦF (= irradiated photons/absorbed photons) of photosynthesis is about 0.3 [9, 30]. For hydrogenase and nitrogenase (see below), the yield is currently likely to be significantly lower.

In relation to the potential difference, ΔE with ΔG is given by the Faraday constant F (n number of electrons transferred) by $\Delta E = \frac{\Delta G}{n\,F}$ or as function of λ: $\Delta E = h * c * \frac{N_A}{n\,F*\lambda}$ linked.

The range of energy per mole of photons in visible light is then between 170 and 300 kg/mol or a potential difference of about 3.4 V; in reality, about 1.2 V of this can ultimately be used for ATP synthesis [30].

In addition to total enthalpy (free Gibbs enthalpy) and proton motor force (PMK) for the production of ATP, pH and temperature must be taken into account in the process, derived from the chemical potential.

$$\Delta G = -2{,}3 * RT * \Delta pH + F * \Delta E \quad \text{or} \quad PMK = \Delta G/F = -2.3\ RT/F\ *\ \Delta pH + \Delta E$$

Under physiological conditions, PMK yields about 14 kJ/mol, so that at least 4 protons per ATP are needed [30]. In order to optimize hydrogen production by bacteria or algae, the goal remains to increase the quantum yield by appropriate measures, which at the same time means suppressing photosynthesis itself (ATP synthesis, oxygen production).

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