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Review

Biohydrogen from Microalgae: Production and Applications

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Abstract: The need to safeguard our planet by reducing carbon dioxide emissions has led to a significant development of research in the field of alternative energy sources. Hydrogen has proved to be the most promising molecule, as a fuel, due to its low environmental impact. Even if various methods already exist for producing hydrogen, most of them are not sustainable. Thus, research focuses on the biological sector, studying microalgae, and other microorganisms' ability to produce this precious molecule in a natural way. In this review, we provide a description of the biochemical and molecular processes for the production of biohydrogen and give a general overview of one of the most interesting technologies in which hydrogen finds application for electricity production: fuel cells.

Keywords: green fuel; biohydrogen; microalgae; *Chlamydomonas reinhardtii;* hydrogenase; fuel cell

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1. Introduction

Nowadays, searching for renewable energy represents one of the major challenges for the global scientific community. Population growth and industrial activities, with consequential high-energy demand, require solutions and proposals in the short-term. It is assumed that the world population could reach 8–10 billion in 2040, while the increase in global energy demand, within the same year, is estimated between 100 and 170 qBtu. However, models cannot accurately predict sudden changes in the global energy situation, as different socio-economic scenarios and political choices modulate each country's response [1,2]. Considering the slow oil, coal, natural gas formation processes, the excessive exploitation of fossil fuels has triggered a drastic and irreversible reserve reduction. Another side aspect of fossil fuel use concerns greenhouse gas (GHG) emissions, particularly carbon dioxide (CO_2), during the combustion reaction [3]. In particular, in the years between 1998 and 2018, CO_2 emissions increased by 48%, making it necessary to implement carbon capture and storage (CCS) technology to limit serious and detrimental effects on climate change [2]. In this worrying scenario, fossil fuels still provide more than 80% of primary energy needs, although the interest in renewable sources, together with their consumption, are steadily increasing [4].

Among renewable options, hydrogen shows the important advantage of CO_2-free combustion, with water as a by-product. Thermodynamic properties, compared to traditional fuels, confirm the interest in its investments in research, although several aspects related to production and storage are still to be mastered. Today, several industrial applications depend on hydrogen, but the most is obtained by techniques, such as steam reforming or electrolysis, not entirely free from the involvement of fossil fuels [5]. Some living organisms, such as microalgae and bacteria, are the basis of processes

capable of producing hydrogen in a completely eco-sustainable way. Microalgal hydrogen production is made possible by biological processes directly or indirectly, depending on sunlight, or by fermentation processes and thermochemical technologies for biomass conversion (Figure 1).

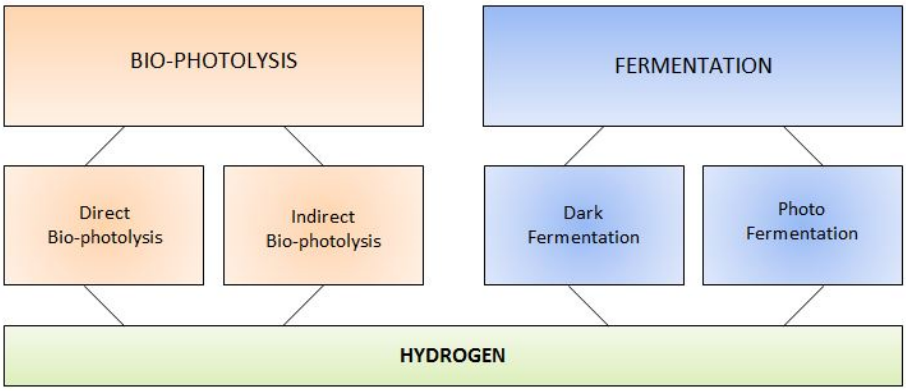

Figure 1. Hydrogen production processes in microalgae.

In recent years, many green systems have focused on algal biomass to obtain energy from living matter. Microalgae store and exploit light energy thanks to the photosynthetic process. To support cell growth and metabolic activities, they use resources widely available in nature, such as water and CO_2. These organisms can concentrate considerable CO_2 amounts and obtain nutrients necessary for growth, even from substrates or waters deriving from industrial waste. Algal cultivation systems, which are simple and relatively inexpensive, ensure advantageous growth rates. Furthermore, they can be set up on infertile territories without competing with agricultural areas that can be exploited for food resources. In particular, the general interest in microalgae has increased significantly in recent decades due to the variety and versatility of the metabolites present in various and numerous species [6].

The present review article is a collection of the most recent evidence on hydrogen production in green microalgae and the efforts to understand and improve the above-mentioned processes in the most widely used species.

2. Hydrogen Metabolism in Green Microalgae: Biophotolysis

Hydrogen metabolism was firstly observed in eukaryotic microalgae in 1939 by German physiologist Hans Gaffron [7]. Due to the oxygen incompatibility, it occurs only transiently in photosynthetic organisms.

2.1. Photosynthetic Electrons and Hydrogenase

The pivotal process of microalgal metabolism consists of oxygenic photosynthesis and complex redox reactions that take place at the level of the thylakoid membranes in chloroplasts through two successive phases. During the first light-depending reactions, ATP and reduced NADH, and NADPH, are generated to be involved in the next dark reactions where the atmospheric CO_2 is fixed by a RuBiSco (ribulose-1,5- bisphosphate carboxylase/oxygenase) enzyme to ultimately generate energy rich-carbohydrate stores. Specifically, during the light phase, an electron transport chain is generated along with photosystems II (PSII) via the plastoquinone (PQ) pool, cytochrome b6f complex (Cyt b6f), and photosystems I (PSI) due to the light energy received as photosystems are associated with light-harvesting complexes I and II (LHC I and LHCII), consisting of numerous photoreceptive pigments. These electrons through PSI leave the electron transport chain and reach the final acceptor ferredoxin (Fd) [8,9].

In anoxic conditions, Fd is able to address electrons to the hydrogenase enzyme. This kind of enzyme catalyzes a reversible reaction in which hydrogen can also be split into electrons and protons:

$$2H^+ + e^- \leftrightarrow H_2 \quad (1)$$

Hydrogenases from the green algae *Chlamydomonas reinhardtii* are the most well-characterized among microalgae. This model organism expresses two different Fe-hydrogenases genes: *HydA1*, the isoform mainly involved under anoxic conditions, and *HydA2* [10,11]. Hydrogenase activity is part of the various responses that microalgal organisms carry out in response to anoxic or stress conditions, and is highly regulated on several levels. After the ribosomal translation in the cytoplasm, the protein is driven to chloroplasts through a transit peptide at the N-terminal end [12]. Assembling of the protein and the catalytic center, H cluster, are required to obtain the functional 47–48 kDa enzyme. *HydEF* and *HydG* genes encode for maturation proteins, crucial for its activation [13]. H cluster is composed of [4Fe4S] unit cysteine-linked to a di-iron unit able to receive electrons via Fd, but easily reversibly inactivated by oxygen [14,15]. Evidence of hydrogen metabolism is reported also in less investigated species, such as *Scenedesmus obliquus* [16,17], *Chlorella fusca* [18], *Chlamydomonas moewusii*, *Lobochlamys culleus* [19], *Chlorococcum littorale* [20], *Tetraselmis subcordiformis* [21].

2.2. Direct and Indirect Biophotolysis

Hydrogenase can receive electrons from different metabolic sources deriving upstream from the biophotolysis of water. At the PSII level, the splitting of water simultaneously produces O_2 and electrons, which, in lighting conditions, reach the Fd and are processed for carbon fixation through the ferredoxin-$NADP^+$ reductase. Under anoxic conditions, oxygen depletion generates a favorable environment for the expression of hydrogenases that start to receive photosynthetic electrons. This process is the direct biophotolysis.

Biophotolysis can also indirectly feed the hydrogen evolving process through the electrons deriving from the breakdown of complex stored carbohydrates, such as starch, which reach Fd and, finally, the hydrogenase. Moreover, the electron load is transferred to the PQ pool via a specific type-II calcium-dependent NADH dehydrogenase (Nda2), and then is transferred to Fd, bypassing PSII without oxygen cogeneration. This process is called indirect biophotolysis [22,23]. Hydrogen evolution is triggered and promoted by modulating different growth strategies, largely experimented on *Chlamydomonas reinhardtii* strains. The most commonly adopted strategy is sulfur deprivation that forces cells to reduce protein synthesis and recover sulfur (S) from protein degradation, such as PSII linked protein D1, impairing the photosystem functions. Exposure to light after dark incubation causes the formation of electrons, but also reactive oxygen species near the defective photosystems with potentially harmful consequences on cell structures. In this context, hydrogenase activity works as a safety valve to preserve structures from oxidative stress, combining electrons with protons to produce hydrogen [24].

3. The Role of Growth Parameters in Hydrogen Production

Acting on cultivation parameters is the simplest way to produce immediate effects on the growth or the specific metabolite production.

Regarding media components, the aforementioned S deprivation gives the expected effects only if preceded by a photosynthetic growth phase, providing all of the necessary elements for growth. More recently, alongside this strategy, which envisages separate growth and a deprivation step with several operational issues, a different modality that involves minimal quantities of S to simulate a condition of starvation has also been tested [25,26]. Moreover, particular attention should be paid to the carbon source used: it has been observed that an increase in starch reserves during the phase preceding S

deprivation can be crucial in supporting production. Mixotrophic conditions involving both inorganic and organic carbon sources appear to be preferred. [27,28].

The less studied condition of nitrogen (N) deprivation induces hydrogen production in *Chlamydomonas reinhardtii,* albeit with a delayed effect and lower yields, suggesting a different mechanism in establishing process [29]. In *Scenedesmus obliquus,* N deprivation modulates metabolism towards lipids accumulation to be used as an energy reserve, suggesting that this strategy is not advantageous for the purposes of hydrogen production [30]. In marine *Chlorella* spp., where S deprivation is inapplicable due to the sulfates-rich presence in seawater, the effect of phosphorus (P) deficiency was evaluated. Similarly, P deprivation was able to establish anaerobic metabolism with a sustained hydrogen photoproduction, even if low-density culture were required to reduce the effect linked to cellular P reservoirs [31]. Less common element deprivations, such as magnesium (Mg), were also tested. Mg-deprived *Chlamydomonas reinhardtii* cells exhibited a longer hydrogen production over time than the same cells in the case of S deprivation. This response may depend on the lesser Mg importance for cellular activities compared to S [32].

Various chemical compounds added in the cell media can affect protons and electrons mobilization inside the cell and, consequently, hydrogen metabolism. The protonophores carbonyl cyanide m-chlorophenylhydrazone (CCCP) and 2,4-dinitrophenol (DNF) increase hydrogen production, owing to proton mobilization [33]. The action of other similar decoupling agents, such as 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DMCU) and carbonyl cyanide p-trifluoromethoxy phenylhydrazone (FCCP) allow to stimulate hydrogen production through the PS II independent pathway, although the underlying mechanisms are not deeply understood [34].

Parameter changes to produce hydrogen require several operational steps, especially during cell transfer upon different media. To handle culture more easily, some attempts have demonstrated the feasibility of cell cultivation through different immobilization systems [35]. Most of the attempts have been applied in *Chlamydomonas reinhardtii* through the use of fiberglass matrix, thin alginate films, or, more recently, sodium alginate beads. In particular, the latter study selected beads of few millimiters diameter to further investigate the process in the classical photobioreactors, bringing out the need to adapt bioreactors to new immobilization systems [36–38].

Indeed, photobioreactor design must take into account downstream applications and process needs. Closed systems are used in lab-scale hydrogen production experiments and represent a better alternative to open systems. Growth parameters, especially temperature and pH, are strictly monitored and, at the same time, the collection of the gas produced is less subject to dispersions. Particular importance is attached to the agitation mode and the intensity and quality of the light radiation. Vertical column, tubular, and flat panel, are the most investigated photobioreactor modalities for hydrogen production. The latter seems to be preferred as it provides the highest surface/volume ratio, although it is not a solution to all technical issues, and this area of research still faces numerous challenges [9,39].

4. Genetic Engineering Approaches

4.1. Random Mutagenesis

The approaches described in the previous paragraph act in a transitory way, since it is not possible to manage microalgal culture by applying stress conditions permanently. Indeed, depriving a cell culture of the optimal growth conditions for a long time leads to cellular suffering that can culminate in cell death. In this way, only a discontinuous hydrogen production is possible, alternating stress and recovery phases to avoid permanent damage to the cells. One strategy to overcome this limitation concerns the selection of organisms with a higher aptitude to withstand these stress conditions; another

one could be the selection of organisms which are less susceptible to the conditions that usually hinder hydrogen evolution in photosynthetic green microalgae.

Physical and chemical treatments induce transmissible genomic mutations that favor the appearance of new traits in an organism. These treatments are not oriented towards a specific target and it is necessary to take into account an expensive screening phase among the mutants produced to select the ones with characteristics of interest. However, a recent study demonstrated that *Chlamydomonas reinhardtii* mutants obtained via atmospheric and room temperature plasma (ARTP) showed a lighter green coloration, compared to wild types, indicative of lower chlorophyll content. The lower chlorophyll content is associated with better photosynthetic performance (probably due to the improved light transmittance and the consequent increased solar energy conversion efficiency), as confirmed by transcriptomic analyses, with consequent benefits in hydrogen production [40].

4.2. Targeted Mutagenesis

On the other hand, targeted approaches turn out to be more advantageous and several proposals have been provided by genetic engineering tools to increase yields and overcome current limitations.

As already mentioned, Fd receives electrons from the electron transport chain to address them to hydrogenase. Even under optimal conditions for enzyme expression, other assimilatory pathways compete for the reductants impairing the overall yield. Ferredoxin-NAD(P)$^+$ reductase (FNR) represents the main competitor shuttling electrons from Fd towards CO_2 fixation. To bypass this limitation, fusion protein has been designed. Among the most recent attempts, a fusion complex between Fd and hydrogenase in an in vivo culture of *Chlamydomonas reinhardtii* was evaluated. The complex demonstrated higher production rates and greater oxygen tolerance than the sole enzyme [41]. Similar behavior was also observed in the *Chlamydomonas reinhardtii* protein D1 mutant. A double amino acid substitution (L159I – N230Y) gave the mutant several new characteristics, including greater oxygen tolerance than the wild strain [42]. Similarly, *Chlamydomonas reinhardtii* mutants knock out for flavodiiron protein (FDP) showed higher photoproduction of hydrogen than wild types. In this way, it is again demonstrated how by eliminating a competing pathway (for example the FDP-mediated O_2 photoreduction pathway), the electrons are preferentially conveyed towards the production of hydrogen. Furthermore, it has been seen that, even exposure to prolonged light pulses in these mutants do not direct the metabolism towards CO_2 fixation. It is, therefore, demonstrated how genetic engineering approaches together with actions on growth parameters are jointly useful to increase production [43].

LHC complexes exhibit a poor light energy conversion with more than 80% absorbed light energy wasted as fluorescence or heat and not addressed towards hydrogen production. Conversely, a truncated light-harvesting antenna has demonstrated improvements in terms of photoinhibition and light saturation phenomena. Afterward, the same approach has also shown encouraging results for hydrogen production: tla1 CC-4169 *Chlamydomonas reinhardtii* mutant has exhibited to produce until six-time more hydrogen compared to the wild type strain with a light intensity of 350 $\mu E\ m^{-2}\ s^{-1}$ [44,45].

Non-coding RNA molecules, such as microRNA (miRNA) or long non-coding RNA (lncRNA), with regulatory function, are part of the most recent discoveries in microalgae and several studies have already exploited them for innovative approaches. In particular, miRNAs exhibit a regulatory function on the translation process by binding or degrading the messenger RNA and avoiding the corresponding protein synthesis. Transcriptomic studies showed that stressful situations in microalgae lead to an increase in these molecules which reflects the need to obtain immediate responses by the cell [46]. In *Chlamydomonas reinhardtii*, some endogenous miRNAs have overexpressed in S deprivation conditions. These observations led to the design of several artificial miRNAs (amiRNAs) to increase hydrogen yields by stimulating a faster oxygen consumption or

repressing *psbA* gene expression that encodes for PSII linked D1 protein [47,48]. Similarly, optogenetic systems have also developed using properly blue light-inducible expression amiRNAs. This gene control system has enhanced hydrogen production, confirming as a most promising tool [49]. Approaches related to genetic engineering require a fine upstream design and considerable resources. Certainly, specific approaches compared to random ones allow for better management of resources. Genome-scale metabolic reconstructions have already been used to direct choices in this sense for many species of prokaryotes and eukaryotes. For algal organisms, a similar tool has been developed using information from the literature. In particular, the AlgaGEM software is configured as a tool capable of defining the primary metabolism of Chlamydomonas reinhardtii and allowing the in silico prediction of any changes in the growth parameters or the engineering of specific metabolic pathways [50].

Engineering strategies generally allow the establishment and introduction of new traits that are advantageous for different research fields. Although they are not easy to implement techniques and require huge resources, new bioinformatics tools to support this area seem to push further to consider this strategy as one of the most promising for the energy sector.

5. Fermentation Processes and Biomass-Applied Technologies

Direct and indirect biophotolysis processes are intrinsically linked to the photosynthetic process and the connected electron transport chain. Together with these two photosynthetic pathways that contribute to the production of hydrogen, another one linked to fermentative metabolism has also been identified. In dark conditions, the enzymatic activity of pyruvate:ferredoxin oxidoreductase (PFR) in *Chlamydomonas reinhardtii* is responsible for the reduction of Fd and the passage of electrons towards hydrogenase. Overall, this pyruvate-dependent hydrogen production acts in ways similar to those observed in bacteria, and though the yield is low, its contribution is not negligible [51]. The accumulation of complex carbohydrates, such as starch, or endogenous substrates, is positively associated with the production of hydrogen, while, it was also observed that exogenous carbon-rich media further stimulate its production in the early anaerobic stages. Several fermentative bacteria use anaerobic processes to transform carbon sources into various by-products, including hydrogen. Processes, such as photo- and dark- fermentation, are commonly exploited and widely investigated in bacteria species, such as *Escherichia coli*, *Clostridium* spp., *Thermococcales* spp., *Rhodobacter* spp., and *Rhodopseudomonas* spp. [52,53]. The consumption of organic substrates, also deriving from waste, by photofermentation, include the transformation into organic acids, alcohols, CO_2, and H_2 in presence of light, but with a low overall yield of solar energy conversion. In a similar way, but without light, dark fermentation uses various substrates and waste too, leading to the release of different components and gaseous mixtures in which hydrogen is present [46,54]. It has recently been observed that hydrogen production can be increased by up to 60% compared to *Chlamydomonas reinhardtii* monoculture systems, by using co-culture systems with *Escherichia coli*. Growth media glucose-rich are exploited by bacteria that produce acetic acid, which can be used in algal metabolism [55]. Synergistically, different photobiological and fermentative microbial metabolisms may interact and cooperate increasing the hydrogen yields [56].

Microalgae show an enormous biodiversity being present in different habitats, even extreme and hostile to most living organisms, suitably adapting their metabolism. It is therefore possible to modulate the growth conditions to obtain biomass of the desired composition, based on the requirements of the downstream process also using industrial and agricultural processing water and waste. In this perspective, various strategies have been applied by combining bioenergy production and bioremediation approaches [57,58]. Unicellular green alga *Scenedesmus obliquus* managed to biodegrade the phenolic content present in the olive oil mill wastewater. This strategy makes it possible to remedy a problem particularly encountered in the Mediterranean area and, since the

biotransformation carried out consumes oxygen, favorable conditions to trigger a concomitant production of hydrogen are also generated [59]. A consortium of microalgae, mainly composed of *Scenedesmus* and *Chlorella* species, grown in pig manure showed good growth, without the addition of external nutrients, and significant fermentative hydrogen production [60,61].

One of the main problems associated to these approaches is the elevated costs in terms of management and purification of the components obtained, which include the presence of several by-products, also toxic. Moreover, although the biomass of the microalgae contains a reduced lignin content compared to other lignocellulosic feedstock previously used for energy purposes, preliminary treatments are often necessary to facilitate the extraction and conversion of the microalgae content. Mechanical, thermal, chemical, or biological treatments are often applied to biomass separately or in combination as a preliminary step [57,58].

6. Fuel Cell

Hydrogen is an energy carrier with a high calorific value of 122 kJ/g, which is about 2.75 folds than fossil fuels, and it is also an environmentally friendly molecule since it only gives water as a by-product of its combustion [62]. For this reason, hydrogen environmental damage ratio has been estimated as 1, compared to that of several hydrocarbon fuels about 20 times higher [8,63]. Hence, hydrogen is considered as the best alternative to fossil fuels, which usage is supposed to be drastically reduced by 2050, according to the 2015 Paris Climate Agreement. Several technologies are currently available for hydrogen production (Figure 2), which can be classified according to the starting material: on the one hand, from fossil fuels, hydrogen can be obtained by thermochemical conversions, such as partial oxidation, autothermal reforming, or steam reforming. On the other hand, exploiting renewable sources, hydrogen is made by water electrolysis or biomass thermochemical conversion [64].

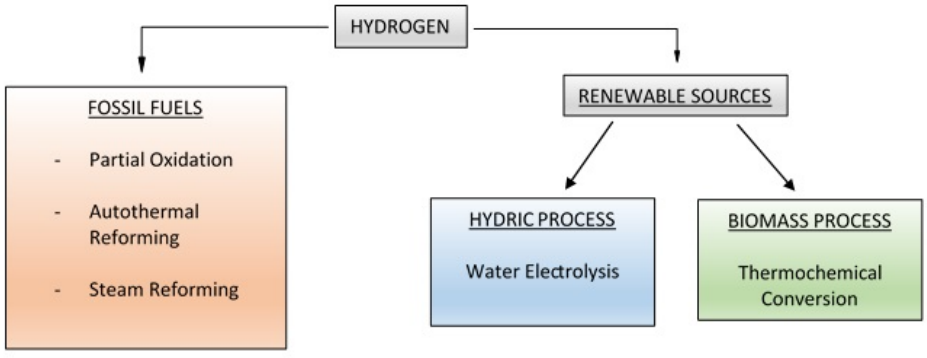

Figure 2. Major hydrogen production technologies**.**

However, none of these processes is sustainable: they all are energy-intensive process, usually requiring high temperatures. For example, water electrolysis requires temperatures ranging from 20 °C to 100 °C [65], while thermochemical processes can reach 2000 °C [66]. Moreover, fossil thermochemical conversions release high amounts of CO_2 [62]. Theoretically, it could be possible to convert these technologies into green ones by pairing them to mechanism that prevent CO_2 releasing into the atmosphere. This is the CSS approach, according to whom CO_2 can be stored into adequate geological sites, or reutilized for the chemical synthesis of useful products such as CO, urea, methanol, polymer, and carbonates [67]. Nevertheless, CSS approach has high design and operational costs that make his application unfeasible on the long term [68]. To implement a completely green sustainable economy, it is necessary to switch to biohydrogen, the

biological hydrogen produced by microorganisms, including microalgae, according to the methods described in the previous paragraphs.

An interesting technology that has raised attention in recent years is that of fuel cells, in which hydrogen is often used as fuel. A fuel cell is an electrochemical technology capable of energy conversion. It is indeed capable to transform the chemical energy of a fuel into electricity [69]. A fuel cell may vary for it architecture, for the kind of fuel or for its catalyst; but it always consists of a few simple main parts (Figure 3): electrodes (an anode and a cathode), electrolytes, and an external circuit [70].

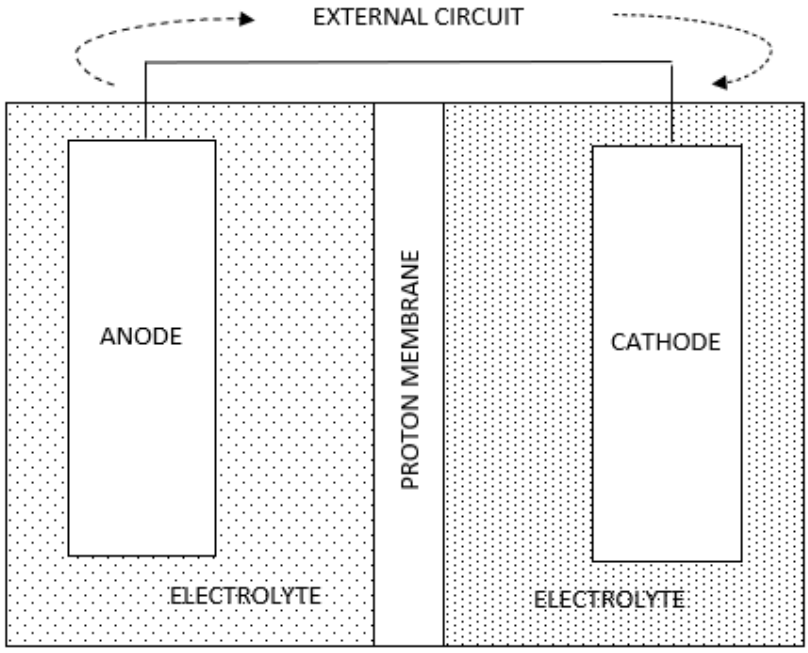

Figure 3. A basic fuel cell.

The reactions that happen in a fuel cell are simple: the fuel (usually hydrogen) at the anode is oxidized; then the electrons, through the circuit, reach the cathode, where (usually) oxygen is reduced to water [71]. Despite the simplicity of the processes, fuel cells currently present numerous criticalities that limit their use on a large scale. Their main problems are durability of the materials and high costs, often both related to the catalyst used. The most commonly used catalyst is platinum, both pure and alloyed, due to its maximum activity and chemical stability. In some cases, platinum group metals (PGMs) have even been used, including palladium, ruthenium, rhodium, iridium, and osmium. However, all of these materials have high costs due to their global scarcity [72]. The second issue of this technology concerns how to get hydrogen to the fuel cell itself. Storage is, at the moment, the main limitation to the development of an effective hydrogen economy. Many technologies are being studied in order to reach the highest volumetric density possible: physical methods, such as high-pressure cylinders for gaseous hydrogen or cryogenic tanks for liquid hydrogen, chemical reaction with metal and alloys, new materials, such as carbonaceous nanostructures for hydrogen absorption [73–75].

For this reason, technological research continues unabated. Based on the various innovative solutions that are proposed, it is possible to give a classification, to the different types of fuel cells currently under development. There are six of them [72]:

1. Proton Exchange Membrane Fuel Cell (PEMFC);
2. Alkaline Fuel Cell (AFC);

3. Phosphoric Acid Fuel Cell (PAFC);
4. Molten Carbonate Fuel Cell (MCFC);
5. Solid Oxide Fuel Cell (SOFC);
6. Microbial Fuel Cell (MFC).

6.1. Proton Exchange Membrane Fuel Cell

In a PEMFC, platinum is substituted by an ion exchange membrane that facilitates ion migration. This membrane is a polymer that usually has negatively charged group in order to let protons to flow toward cathode; but there also exists anion exchange membranes that hold positively charged groups so that the anion can be transported [76]. The most commonly used membrane is that made of perfluorosulfonic acid polymers—commonly known as Nafion®. It was first commercialized by the DuPont company in the 1960s and it soon received wide acceptance because of its qualities. Nafion® is a robust polymer with high chemical and mechanical resistance, good conductivity, and little water or fuel crossover [70,77]. However, it is a costly material, covering about 20% of the cost of a fuel cell [77], and it is also thermolabile since it works only at a low temperature (50–80 °C) [78]. Alternative polymers are under development, mainly to allow the fuel cell to operate at high temperatures in order to optimize its yield. The most promising materials are currently aromatic-based membranes consisting of aryl rings and polybenzimidazole linkages [77].

6.2. Alkaline Fuel Cell

AFCs work at high pH using anion exchange membranes generally based on poly(olefine), poly(arylene ether), poly(phenylene oxide), poly(phenylene), polysulfone, and poly(ether imide). These fuel cells have lower costs than PEMFCs, and they are more resistant to high temperature. However, their main weak point is intrinsic due to the lower conductivity of OH- compared with protons [79].

6.3. Phosphoric Acid Fuel Cell

Phosphoric acid as an electrolyte in fuel cell consent to elevate the working temperature to high temperature around 220 °C. Therefore, it is possible to connect the PAFC directly to a steam reformer, to easily take up hydrogen from the source. The main flaws of this type of fuel cell are the necessity to use a metal catalyst on the electrode, and the hydrogen source that is not sustainable [80]. Recent studies have evaluated how the yield of PEMFCs improve by doping the membrane with phosphoric acid [81].

6.4. Molten Carbonate Fuel Cell

The electrolyte of this fuel cell is a molten carbonate salt solubilized in a lithium aluminate matrix. It can reach very high working temperatures (650 °C); thus, it is not necessary to connect it to an external hydrogen source because it self-reforms gases, also functioning with different hydrocarbon fuels [82,83].

6.5. Solid Oxide Fuel Cell

It is a high-temperature fuel cell and exists in two different types, the oxygen ion conducting fuel cell, and the proton-conducting one. Both have high-energy conversion efficiency and fuel flexibility, but the high fabrication cost makes them commercially not competitive [84].

6.6. Microbial Fuel Cell

These are the greenest and most sustainable types of fuel cells, and undoubtedly represent the future of energy production. MFCs can be double-chambered, with separated anodes and cathodes, or single-chambered, having the electrodes in the same container [85]. In both cases, they exploit microorganisms and their metabolism to

produce the fuel necessary for the fuel cell to function. Most MFCs are mixed, using anode bacterial cultures for hydrogen production and cathode microalgae strains for oxygen supply [86–88]. However, prototypes of fuel cells that work only with algal strains are in development [89,90]. MFCs have significant environmental benefits. Thanks to the biological processes underlying their functioning, they can combine energy production with other functions, such as bioremediation activities [91–93]. It also bypasses the hydrogen storage limitation, since hydrogen is produced and utilized almost at the same time in the anodic chamber. However, this technology is not yet widely applied due to high costs and ineffective yields, which require further study for improvement [86,94–97].

7. Conclusions

In this review, we offered a general summary of the current status of biohydrogen applications. Hydrogen is undoubtedly the future fuel for its green and environmentally friendly properties. However, its production technology is still based on fossil fuel; thus, carbon releasing. Scientific research is working hard to improve new strategies to reach green and sustainable hydrogen production and exploitation technologies.

Microalgae seem to be an attractive solution to this problem. As previously described, they can produce biological hydrogen without carbon emissions; rather, by fixing it during the process. The limiting factor for large-scale applications of this ability is that of low production yield, and, therefore, scientific research must focus in this direction. Solutions described in this review represent the most promising developments for implementing hydrogen yield.

The extreme versatility of microalgae also consents to combine several applications; thus, multiplying the benefits. The use of microalgae in dedicated fuel cells allows the development of an ecological energy production system, which can be associated with bioremediation advantages. In fact, microalgae can grow even in wastewater, purifying them from heavy metals and other dissolved substances.

Since current wastewater treatment plants present some critical issues concerning GHG emissions [98–100], developing an integrated purification and energy production facility based on microalgae could represent a promising ecological technology for the future.

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