

Review

Biohydrogen Production from Lignocellulosic Biomass: Technology and Sustainability

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Abstract: Among the various renewable energy sources, biohydrogen is gaining a lot of traction as it has very high efficiency of conversion to usable power with less pollutant generation. The various technologies available for the production of biohydrogen from lignocellulosic biomass such as direct biophotolysis, indirect biophotolysis, photo, and dark fermentations have some drawbacks (e.g., low yield and slower production rate, *etc.*), which limits their practical application. Among these, metabolic engineering is presently the most promising for the production of biohydrogen as it overcomes most of the limitations in other technologies. Microbial electrolysis is another recent technology that is progressing very rapidly. However, it is the dark fermentation approach, followed by photo fermentation, which seem closer to commercialization. Biohydrogen production from lignocellulosic biomass is particularly suitable for relatively small and decentralized systems and it can be considered as an important sustainable and renewable energy source. The comprehensive life cycle assessment (LCA) of biohydrogen production from lignocellulosic biomass and its comparison with other biofuels can be a tool for policy decisions. In this paper, we discuss the various possible approaches for producing biohydrogen from lignocellulosic biomass which is an globally available abundant resource. The main technological challenges are discussed in detail, followed by potential solutions.

Keywords: biohydrogen; biofuels; lignocellulosic biomass; technology; sustainability; life cycle assessment

1. Introduction

Recent years have seen a rapid surge in research activities focusing intensely on alternative fuels in order to reduce the dependency on fossil fuels, mainly by providing local energetic resources. This is mainly due to two reasons, the first being that new fuels are needed to supplement and ultimately replace depleting oil reserves and secondly, fuels capable of low or nil CO₂ emissions are urgently required to reduce the impact of global warming [1–7]. Hydrogen (H₂), which can be used in fuel cells mainly to operate machines, is a fascinating alternative, particularly because its combustion provides high amounts of energy and water is the only reaction product. Among all

biofuels, H₂ has the highest gravimetric energy density at 141 MJ/kg. Despite this, its volumetric energy density, at only 12 MJ/m³ (at normal temperature and pressure) is low. This is an important aspect particularly in reference to transportation fuel. It is considered to be one of the cleanest energy carriers if produced using energy generated from renewable sources. In summary, H₂ is interesting due to its potentially high efficiency of conversion to usable power, low generation of pollutants and high energy density [8]. Global H₂ production today amounts to around 700 billion Nm³ and is based almost exclusively on fossil fuels [9]. However, for H₂ to be accepted as a sustainable substitute for fossil fuels, it has to be produced from renewable feedstock other than fossil fuels [10].

Hydrogen has been suggested as the ideal fuel of the future. It is considered as one of the cleanest energy carriers to be generated from renewable sources [11]. It has a high energy yield (122 kJ/g) which is 2.75 times greater than hydrocarbon fuels. It can be easily used in fuel cells for generation of electricity. Though not a primary energy source, it serves as a medium through which primary energy sources (such as H₂ produced from nuclear power and/or solar energy) can be stored, transported and utilized to fulfill our energy needs. The major problem facing H₂ as a fuel is its unavailability in Nature. H₂ can be produced safely, is environmentally friendly when combusted, and versatile *i.e.*, has many potential energy uses, including powering non-polluting vehicles, heating homes and offices, and fueling aircraft. Current H₂ production technologies such as steam reforming of natural gas, thermal cracking or coal gasification are not environmentally friendly. Biological H₂ production is a promising alternative. There are two methods to produce H₂ from microorganisms. The first method uses photosynthetic microorganisms such as bacteria or algae (photofermentative processes) and the second method uses fermentative organisms (dark fermentation processes). Fermentative H₂ production has the advantage of producing H₂ under mild conditions with the additional benefit of allowing residual biomass valorization. The dark fermentation process is more attractive as it has the potential to use wastewater and organic wastes and has higher production rates compared to photofermentative processes. So far, few studies have used real wastewater for the production of H₂ due to inhibition by both substrate and/or product in the fermentation process [12]. Studies on bioH₂ production have been focused on photodecomposition of organic compounds by photosynthetic bacteria, dark fermentation from organic compounds with anaerobes and biophotolysis of water using algae and cyanobacteria [13–17].

Lignocellulosic biomass is the most abundant in Nature and it is present in hardwood, softwood, grasses, and agricultural residues. The global annual yields of lignocellulosic biomass residues were estimated to exceed 220 billion tons, equivalent to about 60–80 billion tons of crude oil [12]. Lignocellulosic feedstocks consist mainly of glucose and xylose and thus microbial strains that can effectively degrade glucose and xylose are important for development of renewable H₂ production processes [18]. Direct conversion of lignocellulosic biomass to H₂ needs pretreatment to hydrolyze the incorporated heterogeneous and crystalline structure [19,20]. The lignocellulosic biomass hence presents an attractive, low-cost feed stock for H₂ production.

Aim of the Paper

In recent past, several reviews have appeared which have discussed the prospects and challenges of biomass-based H₂ [21,22]. Earlier, Kraemer and Bagley gave a thorough description of the yield improvement approaches in fermentative H₂ production [23]. Wang and Wan summarized the main factors influencing fermentative H₂ production [24]. A special issue of the journal *International Journal of Hydrogen Energy*, recently dealt with “Biohydrogen: From Basic Concepts to Technology” [25]. Biohydrogen can be generated by adopting different technologies and different technologies can perform differently. Thus, the aim of this paper is to discuss specifically the technological aspect of biohydrogen production from lignocellulosic biomass and its sustainability on the basis of a life cycle assessment (LCA).

2. Feed Stock for Biohydrogen Production

Glucose is the ideal substrate, but it is too costly at present. Many agricultural residues and food wastes are rich in carbohydrates that could serve as feedstock. Lignocellulosic biomass is another sustainable feedstock for H₂ production [26]. The criteria for an ideal feedstock for sustainable H₂ production, which include high carbohydrate content, minimum pre-treatment requirement, sustainable resources, low cost and sufficient concentration of carbohydrate for fermentative conversion, have been suggested by Bartacek [27]. The substrates usable for fermentative H₂ production were further divided into four main groups, namely, pure substrates such as glucose; energy crops such as *Miscanthus*, solid wastes like food waste and industrial wastewaters such as wastewater from the pulp and paper industry.

A variety of substrates has been used as feedstock for H₂ production. For example, the fermentation of household wastes under different temperature conditions has been well studied [28–30]. An increase in H₂ yields as temperature increased to thermophilic regimes was reported.

Wastewaters and residual biomass with high carbohydrate content have also been demonstrated to be a suitable candidate for dark fermentation. This includes molasses [31,32] and cheese whey [33,34], which have been evaluated under continuous stirred tank reactor (CSTR) and immobilized system configurations. Besides, H₂ production from soluble and particulate starch and cellulose [35,36], xylose [37], sugar beet [38], wastewater from a sugar beet refinery [39] and the bottom layer from a beer manufacturing plant [40] has also been demonstrated.

3. Technology

3.1. Biohydrogen Production Systems

The conventional methods for producing H₂ gas include steam reforming of methane and hydrocarbons, non-catalytic partial oxidation of fossil fuels and autothermal reforming. However, most of these methods are energy intensive processes requiring high temperatures (>850 °C). A general scheme of H₂ production from renewable sources is shown in Figure 1. Biological methods of H₂ production are preferable to chemical methods because of the possibility to use sunlight, CO₂ and organic wastes as substrates for environmentally benign conversions, under moderate conditions.

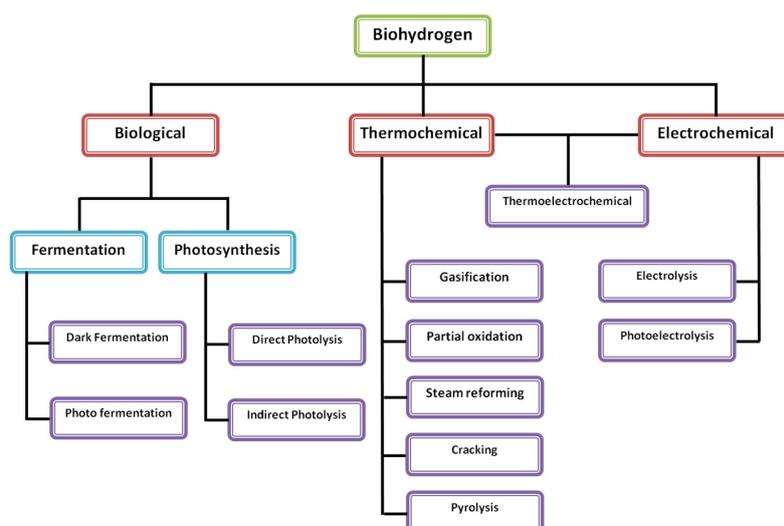


Figure 1. The main alternative methods of H₂ production from energy sources.

The biological production of H₂ involves light-dependent methods: direct and indirect biophotolysis, and photo fermentation. The other routes are light-independent methods, including

the dark fermentation process and water-gas shift reaction of photoheterotrophic bacteria. This biologically produced H₂, generally referred to as “biohydrogen”, is characterized by low H₂ yields which present a challenge for commercial applications.

3.1.1. Dark/Anaerobic Fermentation

Dark fermentation is one of the most common processes for bio-H₂ production. Although only 15%–20% of the theoretical H₂ potential of carbohydrates can be harvested, dark fermentation is considered as a promising process in a two phase anaerobic treatment system [23]. Also, since the CO₂ produced in dark fermentation has already been fixed by the waste treated originally from the atmosphere, the emissions associated with global climate change are virtually zero [41]. Microbial species analysis of hydrogen-producing cultures (using anaerobic sludge as inoculum) shows the presence of *Clostridium cellulosi*, *Clostridium acetobutylicum*, *Clostridium tyrobutyricum*, *Enterobacteriaceae* and *Streptococcus bovis* [42]. Fermentative H₂ production usually proceeds from the anaerobic glycolytic breakdown of sugars. The theoretical complete oxidation of 1 mole of hexose to CO₂ can produce 12 moles of H₂. Nevertheless, the theoretical yield of H₂ via acetic acid fermentation cannot be higher than 4 moles. Actual H₂ yields are quite lower, typically ranging from 1.0 to 2.5 moles per mole of hexose consumed. Recently, Varanasi *et al.* reported production of 2.95 mol H₂/mol hexose equivalent by thermophilic dark fermentation using cellubiose as substrate [43]. If butyric acid is produced as the major fermentation product instead of acetic acid, only 2 moles of H₂ can be produced [44]. H₂ yield is even lower when more reduced organic compounds such as lactic acid, propionic acid and ethanol are produced, because these metabolites represent end products of metabolic pathways that bypass the major H₂-producing reaction [45]. Recently, it was concluded that to maximize net energy gain via dark fermentation, appropriate cultures capable of high-H₂ yield have to be employed and the process has to be operated at near-ambient temperatures with the lowest feedstock concentration as possible [10]. In an experiment with *Thermotoga neapolitana* sparged with N₂ and supplemented with 40 mM sodium bicarbonate a 2.8 and 2.7 mol/mol glucose yield of hydrogen with a lactic acid/acetic acid ratio of 0.26 was obtained, challenging the currently accepted dark fermentation model that predicts reduction of this gas when glucose is converted into organic products different from acetate [46]. Pradhan *et al.* reviewed the hydrogen production efficiency of a similar bacterium (*Thermotoga neapolitana*) with different feedstocks and found 1.9–3.5 mol H₂/mol hexose yields achievable with a range of feedstocks and variable substrate loads [47]. Byproducts of the reactions are acetic acid, lactic acid and ethanol.

3.1.2. Photo Fermentation

Photo fermentation is carried out by purple non-sulfur (PNS) photosynthetic bacteria which can grow as photoheterotrophs, photoautotrophs or chemoheterotrophs [48]. These bacteria produce H₂ under photoheterotrophic conditions (light, anaerobiosis, organic electron donor) [49]. The advantages of this process over photolysis of water using green algae and cyanobacteria, are that oxygen does not inhibit the process and that these bacteria can be used in a wide variety of conditions (*i.e.*, batch processes, continuous cultures, and immobilized systems) [50]. The hydrogenase and nitrogenase enzymes produced in photosynthesis by green algae and photosynthetic bacteria, respectively, play a crucial role in biohydrogen production. The main PNS bacteria that participate in H₂ production are *Rhodospirillum rubrum*, *Rhodospseudomonas palustris*, *Rhodobacter sphaeroides* O.U 001, *Rhodobacter sphaeroides* RV, *Rhodobacter sulfidophilus* and *Rhodobacter capsulatus*. Kapdan *et al.* used three different pure strains of *Rhodobacter sphaeroides* (RV, NRLL and DSZM) in batch experiments to select the most suitable strain [51]. *R. sphaeroides* RV resulted in the highest cumulative hydrogen gas formation (178 mL), hydrogen yield (1.23 mol·H₂·mol⁻¹ glucose) and specific hydrogen production rate (46 mL·H₂·g⁻¹ biomass·h⁻¹) at 5 g·L⁻¹ initial total sugar concentration among the other pure cultures. Using *Rhodobacter capsulatus* JP91, Keskin and Hallenbeck compare the photofermentative biohydrogen yield of different feedstocks in a batch culture experiment [52].

Overall yield of biohydrogen was 10.5, 8 and 14.9 mol H₂/mol sucrose using beet molasses, black strap molasses and sucrose respectively. Optimization of process parameters such as availability of solar light, bioreactor configuration and proper C/N ratio in substrate (synthetic and derived from waste products) still needs to be studied at higher scale.

3.1.3. Combined Biotechnologies

Combination of two or more of the abovementioned techniques have also been studied for improved H₂ yields. Theoretically 12 moles of H₂ per mole of glucose can be generated by combining dark fermentation with photo fermentation (using PNS bacteria) [48]. For instance, Nath *et al.* studied combined dark and photo fermentation using glucose as substrate [53]. The effluent from the dark process (containing unconverted metabolites, mainly acetic acid) underwent photo fermentation by *Rhodobacter sphaeroides* in a column photo-bioreactor demonstrating the feasibility of this combination to achieve higher yields of H₂ by complete utilization of the chemical energy stored in the substrate. A sequential process using glucose as substrate and an immobilized system for the photo fermentation step evaluating key factors such as diluted ratio of dark fermentation effluent, ratio of dark and photo fermentation bacteria, light intensity, and light/dark cycle has also been studied [54]. During the combined process, maximum total H₂ yield was 5.374 moles of H₂/moles of glucose. However, the sterilization step applied to the dark fermentation effluent may pose a constraint to a scale-up the process. The combined system can also be run in continuous mode and achieve more combined H₂ yield [49]. These further combinations will reduce the overall cost of H₂ production but more field studies are required to obtain an economical H₂ production process.

3.1.4. Bioelectrochemical Production

Bioelectrochemical production of H₂ is the latest technology using systems called microbial electrolysis cells (MEC). This is an emerging field where the oxidation of organic material is carried out by the bacteria present at the anode and results in formation of protons, CO₂ and electrons (Figure 2). Protons migrate through a proton exchange membrane (PEM) to the cathode and the electrons are transported through the external circuit to the cathode [55]. By applying an external voltage of approximately 0.5–0.9 V, these electrons combine at the cathode with protons producing H₂ gas (Table 1). The advantage here is the low energy consumption (0.3–0.9 V) necessary for microbial electrohydrogenesis to produce H₂ in comparison to the theoretical minimum voltage of 1.23 V required for water electrolysis [56]. An overall scheme of H₂ production from lignocellulosic biomass is shown in Figure 3. Figure 3 summarized the pathways for biohydrogen production by using lignocellulosic biomass, depending on the nature of feed (solid or liquid), pre-treatment methods were used and then followed by the dark fermentation.

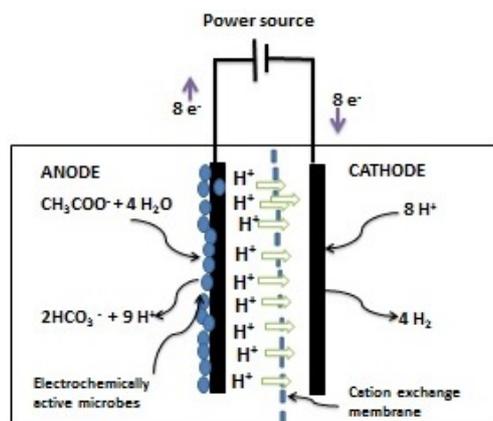


Figure 2. Schematic of hydrogen production in MEC (Adapted from [56]).

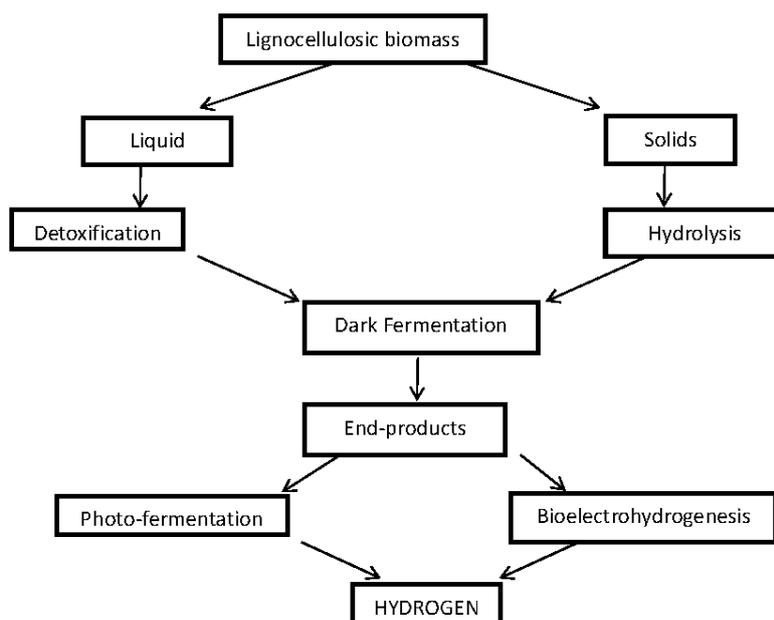


Figure 3. General scheme for biohydrogen production from lignocellulosic biomass (adapted from [12]).

Table 1. A comparison between the three major routes for biological hydrogen production (adapted from [57]).

Production Routes	Main Reaction	H ₂ Production Rates (mmol/h·L)	Remark
Direct photolysis	$2\text{H}_2\text{O} + \text{"light energy"} \rightarrow 2\text{H}_2 + \text{O}_2$	0.07	Similar to the processes found in plants and algal photosynthesis.
Photo fermentation	$\text{C}_6\text{H}_{12}\text{O}_6 + 6\text{H}_2\text{O} + \text{"light energy"} \rightarrow 12\text{H}_2 + 6\text{CO}_2, \Delta G_0 = +3.2 \text{ kJ}$	145–160	Bacteria evolve molecular H ₂ catalyzed by nitrogenase under N-deficient conditions using light energy and reduced compounds (organic acids).
Dark fermentation	Pyruvate + CoA → acetyl-CoA + formate OR Pyruvate + CoA + 2Fd(ox) → Acetyl-CoA + CO ₂ + 2Fd (red)	77	H ₂ is produced by anaerobic bacteria, grown in the dark on carbohydrate rich substrate.

Table 2 shows the various substrates used for H₂ production in different MEC volumes. The rate of H₂ production differs with substrate due to their degradation pathways. To date MECs have shown H₂ production from initial volumes ranging from 5 mL to 1000 L (pilot plant) reactors, which shows that MECs can be a better solution for producing H₂ in the cathodic chamber while treating wastewater in the anodic chamber [58]. Still, there are many issues to be addressed for the long term real time application such as electrode and membrane stability for longer duration and reactor configuration design for higher volumes. Before scale up, mathematical models are required, which need to be validated first for the present lab scale studies and then on the basis of data obtained, higher volume MECs may be designed and validated.

Table 2. Various substrates used in MEC for hydrogen production (adapted from [59]).

Substrate	Concentration (g/L)	Applied Voltage (V)	MEC Volume (mL)	Hydrogen Production Rate (m ³ H ₂ /m ³ /day)	Reference
A de-oiled refinery wastewater	04–1	0.7	5	79% (Hydrogen production based on COD removal)	[60]
Sodium Acetate	1	0.6	18	2.0	[61]
Glucose	2	0.6	26	0.25 ± 0.03	[62]
Glucose	2	0.8	26	0.37 ± 0.04	[62]
Fermentation effluent	1	0.6	26	1.41	[63]
Sodium Acetate	1	0.6	28	1.99 ± 0.02	[64]
Sodium Acetate	1	0.8	28	3.12 ± 0.002	[64]
Sodium Acetate	1	0.5	28	1.7	[65]
Glucose	1	0.5	28	0.83 ± 0.18	[66]
Glucose	1	0.9	28	1.87 ± 0.30	[66]
Potato wastewater	1.9–2.5 (COD)	0.9	28	0.74	[67]
Swine wastewater	2 (COD)	0.5	28	0.9–1.0	[68]
Sodium Acetate	1	0.6	48	0.76	[65]
Sodium Acetate	1	0.7	76	-	[69]
Sodium Acetate	1	0.8	240	0.0231 ± 0.003	[70]
Sodium Acetate	1	1	400	1.58	[71]
Sodium Acetate	2	0.6	500	0.53	[72]
Winery wastewater	8	0.9	1000 Lt	0.19 ± 0.04	[58]
Sodium Acetate	1	0.5	6600	0.02	[56]

3.2. Microbiology of Biohydrogen Production

Perera *et al.* evaluated three main routes for biological H₂ production [10]. These are (1) direct photolysis, in which cyanobacteria decomposes water to generate hydrogen and oxygen in presence of light; (2) photo fermentation, where anoxygenic photoheterotrophic bacteria utilizes organic feedstock to produce H₂ in presence of light and (3) dark fermentation, in which anaerobic heterotrophic bacteria utilizes organic feedstock without any light to produce H₂. A comparison of these three main routes is shown in Table 1.

The microbiology and biochemistry of dark fermentative H₂ production was discussed in detail by Hawkes *et al.* [42]. H₂ production in *Clostridia* is due to the presence of hydrogenase enzymes. These transfer electrons from reduced ferredoxin or NADH to protons to regenerate the oxidized forms (Fd_{ox} and NAD⁺) required so that glycolysis and oxidative decarboxylation of pyruvate can proceed to generate ATP.

Pure microbial cultures have mainly been used in lab-scale reactors for studying the effect of environmental and operational parameters on fermentation profiles and carbon metabolism. One of the successful tests using pure culture in a pilot-scale bioreactor using a non-sterilized feedstock employed *Caldicellulosiruptor saccharolyticus* [73]. However, most studies on H₂ production on biowaste have been performed using mixed cultures under mesophilic conditions [74,75]. Only a few studies have focused on mixed thermophilic consortia [76,77]. It has been demonstrated that the extreme thermophile *C. saccharolyticus* can produce H₂ from mono- and disaccharides [78]. Hexose is the predominant component in the cellulose hydrolysates. A highest H₂ yield of approximately 83% of the theoretical value (4 mol·mol⁻¹ hexose) has been reported using thermophilic anaerobic bacteria [78].

3.3. Limiting Factors in Biohydrogen Production Systems

The most challenging barrier of fermentative H₂ production is its low H₂ molar yield [26]. Thauer *et al.* predicted that 4 moles of H₂ per mole of glucose is the biological maximum in *Clostridial* microbes if acetate is the only waste by-product [79]. In practice, even that figure is rarely achieved. A number of factors adversely affect and inhibit H₂ fermentation [44]. H₂ itself, when it reaches high concentrations not only makes its production thermodynamically unfavourable but also acts as an

inhibitory agent as do other metabolic products, such as acetic acid and propionic acid [17,80]. Partial pressure of H₂ is one of the most critical parameters in fermentative production of H₂ as high H₂ partial pressures make H₂ production thermodynamically unfavourable. Removal of produced H₂ from the liquid phase lowers the H₂ partial pressure which in turn increases H₂ yield [81]. Moreover, the H₂ remaining in the system might be consumed by some bacteria [82]. Removal of dissolved H₂ and reduction of H₂ partial pressure can be achieved by nitrogen flushing, adsorption of H₂ by metals and H₂ stripping by boiling or by introduction of steam [83–85]. Low H₂ partial pressure also needs to be maintained because hydrogenases (such as NiFe-hydrogenase) may re-oxidize the produced hydrogen into protons and electrons [86]. Gas sparging has proved to be an efficient method to maintain maximum hydrogen production even though it leads to biogas dilution and higher cost for hydrogen recovery [87]. Depending on the nature of the flushing gas, the flow rate and the reactor configuration, volumetric production of biogas up to 120% has been achieved [85,88]. Non-sparging techniques such as headspace modification under vacuum, high pressure or gas adsorption (reviewed in [87]), hydrogen-separating membranes [83] and using mechanical stirring [89,90], have also showed significant improvements in hydrogen yield. Argon has been often used to flush both oxygen and nitrogen and to keep a low H₂ partial pressure in the reactors, but it increases production costs and hinders H₂ purification [91]. Some researchers have reported reduced pressure and CO₂ for flushing the headspace and maintaining low H₂ partial pressure in dark fermentation [92,93], but the information on photofermentation is deficient. Montiel-Corona *et al.* suggested that flushing with Ar could be replaced with reduced pressure, which can be less expensive and practical for hydrogen recuperation [91]. Coupling the dark and photo fermentation showed an increased total hydrogen yield. One of the major drawbacks in coupling the dark and photo fermentation processes is the need of keeping apart the H₂-producing microflora and the presence of NH₄⁺, which may be naturally present in wastewater and may also be generated in the dark fermentation process when hydraulic retention time (HRT) is high enough to achieve protein degradation, especially when particulate substrates (as in the case of food wastes) are being considered, since HRT may be as high as 5 days [94].

In case of bioelectrochemical production of H₂ in MEC, the main challenge is avoiding methane formation via methanogenesis [24], though researchers are now shifting more towards methane formation rather than H₂ in these systems [95,96]. Another issue limiting the large-scale application of this technology is the use of precious metal catalyst such as platinum which is usually used on the cathode [97]. Though there have been efforts to use low cost materials such as stainless steel [98] and Ni-based electrodes [61], the results are much lesser from the targets.

3.4. Role of Metabolic Engineering

The application of genomic and molecular tools has made it possible to steer the metabolic pathways towards maximal H₂ production and avoid waste and by-product accumulation. This is especially true when genetic engineering is conducted on cellulolytic microbes [26]. The main principles of genetic engineering include: (1) overexpression of cellulases, hemicellulases and lignases to maximize substrate availability, (2) elimination of H₂-consuming hydrogenases and (3) overexpression of H₂-producing hydrogenases [53]. Metabolic engineering modifications have been used to increase H₂ production in fermentative systems [99]. These include over-expression of H₂-evolving enzymes [100], the knockout of metabolic pathways that compete for reducing equivalents [81] and the introduction/over-expression of genes (cellulases, hemicellulases and lignases) to enhance carbohydrate availability to the cell [101]. Inactivation of the gene lactate dehydrogenase (*ldhA*) in *E. coli* by introducing mutations could lead to a modest increase (20%–45%) in net hydrogen production (reviewed in [102]).

Ryu *et al.* combined several known approaches to construct a superior hydrogen-producing strain of the purple nonsulfur photosynthetic bacterium *Rhodobacter sphaeroides* HPCA* (mutant expressing *NifA* L62Q) [103]. In this strain maximum hydrogen levels are reached almost twice as

fast as in wild type cells and final hydrogen levels are ~39% higher than in the wild type as well. As increased number of genomes for H₂ producing microorganisms are sequenced and compared and as more specific enzymes are functionally characterized, the distinctive metabolic strategies used and enzymological contexts through which H₂ evolution is controlled in different organisms will become clearer. This will allow researchers to construct more effective strategies to modulate competing pathways, and help in the designing molecular engineering strategies leading to enhanced H₂ evolution.

4. Kinetic Models for Hydrogen Production by Fermentation

Different factors such as substrate and inhibitor concentrations, temperature, pH and reactor type affect H₂ production by fermentation. Modeling of the H₂ production is very important to improve, analyze and predict H₂ production during fermentation. Mathematical models include the kinetic of cell growth and product(s) formation, substrate utilization and inhibition. In addition some models are developed to describe the effect of pH, temperature and dilution rate on H₂ production. The obtained model kinetic constants can be used in the design, operation and optimization of the fermentative H₂ production process. Different kinetic models have been proposed to describe growth of H₂ producing bacteria, substrate degradation and H₂ production. H₂ production is reported as growth associated product.

Monod (or Michaelis–Menten equation) (Equation (1)) which is an unstructured, non-segregated model of microbial growth, fits a wide range of data. The kinetic constants of this equation, K_s and μ_{max} , can be obtained by linear regression. Wang and Wan reported on previous studies using a Monod model to describe H₂ production with time in bio-H₂ fermentation [104]:

$$\mu = \frac{1}{X} \frac{dX}{dt} = \mu_m \frac{S}{K_s + S} \quad (1)$$

where μ is the specific growth rate, X is the biomass concentration, S is the substrate concentration, K_s is the saturation constant, μ_m is the maximum specific growth rate.

Recently, the logistic model (Equation (2)) became the most popular in describing cell growth. This equation has a sigmoidal shape that includes the lag phase, exponential and stationary phase of the batch growth:

$$\mu = \frac{1}{X} \frac{dX}{dt} = \mu_m \left(1 - \frac{X}{X_m}\right) \quad (2)$$

where X_m is the maximum biomass concentration.

At high substrate concentration, the cell growth is inhibited and production of H₂ is reduced. Different substrate inhibition models have been proposed. The Haldane-Andrew model (Equation (3)) is widely used to describe the substrate dependence of the specific growth rate of H₂ fermentations. Wang and Wan have reported that previous studies used an Andrews model to describe H₂ production with time [104]. Other substrate inhibition models are used in the literature such as modified Han-Levenspiel model (Equation (4)):

$$\mu = \frac{1}{X} \frac{dX}{dt} = \mu_m \frac{S}{K_s + S + \frac{S^2}{K_i}} \quad (3)$$

where K_i is the inhibition constant.

The presence of other inhibitors such as salts and the product cause reduction of H₂ production. Some models have been proposed to describe the effect of inhibitors such as the modified Han-Levenspiel model (Equation (4)):

$$\mu = \frac{1}{X} \frac{dX}{dt} = \mu_m \left(1 - \frac{C}{C_m}\right) \quad (4)$$

where C is the inhibitor concentration, C_m is the maximum inhibitor concentration or the concentration of inhibitor above which there is no biomass growth

The modified Gompertz model (Equation (5)) is widely used to describe the progress of cumulative H_2 production in batch fermentations [104]:

$$H_t = H_{\max} \exp \left\{ -\exp \left[\frac{R_{\max} \times e}{H_{\max}} (\lambda - t) + 1 \right] \right\} \quad (5)$$

where H_t is the cumulative volume of H_2 produced at any time (mL), H_{\max} is the gas production potential (mL), R_{\max} is the maximum gas production rate (mL/h), λ is the lag time (h). t is the incubation time (h)

The Luedeking-Piret model (Equation (6)) has been widely used to describe the relation between cell growth rate and H_2 production:

$$\frac{dP}{dt} = Y_{P/X} \frac{dX}{dt} + \beta X \quad (6)$$

where P is the product, $Y_{P/X}$ is the growth associated yield coefficient; β is the non-growth associated product yield coefficient.

Wang and Wan reported that previous studies used the Luedeking–Piret model to relate cell growth rate and H_2 production rate [104]. The effect of temperature on the fermentative H_2 production has been widely described using the Arrhenius model, while the effect of pH on the substrate consumption rate is described by an Andrew model using the concentration of H^+ as the limiting substrate concentration. According to this model, the rate of substrate consumption passes through maximum with increasing H^+ concentration.

5. Sustainability and Life Cycle Assessment

The concept of sustainable development is an attempt to combine growing concerns about a range of environmental issues with socio-economic issues and implies smooth transition to more effective technologies from a point view of an environmental impact and energy efficiency [105,106]. H_2 can be considered one of the pillars of a future sustainable energy system [107]. H_2 production could be a possible avenue for the large-scale sustainable generation of H_2 needed to fuel a future H_2 economy [106]. Despite its many obvious advantages, there remains a problem with storage and transportation. Pressurized H_2 gas occupies a great deal of volume compared with other fuels. For example, gasoline that with equal energy content, needs about 30 times less volume at 100 bar gas pressure. Due to its high explosivity there are also obvious safety concerns with the use of pressurized or liquefied H_2 in vehicles as well as additional energy use for pressurizing or liquefaction. Furthermore, the overall energy balance of using H_2 as vehicle fuel does indeed seem to be less beneficial than gasoline, but being the only non-carbon fuel it may still make sense to produce H_2 from waste streams if some of the obstacles can be solved and it can be used effectively for energy production to feed into grid or to use in stationary requirements, e.g., industries, etc.

Though this paper is focused on bio- H_2 production from lignocellulosic biomass, it is important to compare it to other production methods using various substrates. Such a comparison has been made in Table 3 by presenting the various H_2 production systems, which show different H_2 yields from different feedstocks by adopting different production systems. Therefore, life cycle assessment (LCA) could be a tool to scrutinize the best H_2 production system for a particular feedstock in terms of environmental impact and indirect natural resource costs towards different services and commodities [108]. LCA allows the possibility of comparing different H_2 production approaches and identifying the environmental “hot spot” of the whole process, which helps in development of a sustainable H_2 production process [106,109]. Investigations of the environmental benefits and impacts from a life cycle perspective are scarce. Only a few LCA-studies have been performed

specifically on H₂ production. The feedstocks investigated so far are steamed potato peel, wheat straw and sweet sorghum stalks [110–112].

Table 3. Comparison of different biohydrogen production systems.

Reactor	Feed Stock	Maximum H ₂ Yield	Reference
Fermentation			
Dark fermentation			
CSTR	Starch	0.52 L/h/L and 13.2 mmol H ₂ /g total sugar	[113]
Batch	Glycerol	0.41 mol H ₂ /mol glycerol	[114]
FBR	Sucrose	4.26 mol H ₂ /mol sucrose	[115]
Batch	Food waste	593 mL H ₂ /g carbohydrate	[116]
Fed-batch	Swine manure	18.7 × 10 ⁻³ g H ₂ per g TVS	[117]
Batch	Sucrose	4.3 mol H ₂ /mol sucrose	[118]
Batch	Fructose, sorbitol, glucose	1.27, 1.46 and 1.51 mol H ₂ /substrate	[119]
Fed-batch	Starch, glucose	465 mL H ₂ /g starch, 3.1 mol H ₂ /mol glucose	[120]
Batch	Food waste	39.14 mL H ₂ /g food waste (219.91 mL H ₂ /VS _{added})	[121]
Batch	Crude Glycerol	64.24 mmol H ₂ /L and 5.74 mmol H ₂ /g COD consumed	[122]
Batch	Distillery wastewaters	1 L H ₂ /L medium	[123]
Batch	Cheese whey	94.2 L H ₂ /kgvs	[124]
Batch	Water hyacinth (leaves and stems)	76.7 mL H ₂ /g TVS was obtained at 20 g/L of water hyacinth	[125]
Batch	waste ground wheat solution	SHPR = 25.7 mL H ₂ /g cells/h	[126]
Photo fermentation			
CSTR	Sucrose	5.81 mol H ₂ /mol hexose	[127]
Fed-batch operation	Wheat starch	201 mL H ₂ g/L starch	[128]
Batch	Molasses	0.50 mmol H ₂ /L _c h	[129]
Batch	Beet molasses	10.5 mol H ₂ /mol sucrose	[52]
Batch	Black strap	8 mol H ₂ /mol sucrose	[52]
Batch	Sucrose	14 mol H ₂ /mol sucrose	[52]
Batch	Ground wheat starch	46 mL H ₂ /g biomass/h, 1.23 mol H ₂ /mol glucose	[51]
Batch	lignocellulose-derived organic acids	7 mL H ₂ /mL of the fermentation effluent	[130]
Photosynthesis			
Direct Photolysis			
Batch	Lactate	0.07 mmol H ₂ (l × h) or 54 mL/h·g dry weight	[131]
Indirect Photolysis			
Batch	arabinose and xylose	14.55 mmol/g (arabinose); 13.73 mmol/g (xylose)	[132]
Thermochemical			
Gasification			
Continuous supercritical water gasification	glucose	10.5–11.2 mol/mol glucose	[133]
Partial Oxidation			
Batch	municipal sludge	Not reported the amount	[134]
Steam reforming			
molten carbonate fuel cell (MCFC) system	ethanol	5 mol H ₂ /mol fed ethanol	[135]
Cracking			
fixed-bed quartz micro reactor	Methane	500 μmoles/min	[136]
Pyrolysis			
stainless steel tank reactor	Biomass (redwood sawdust; cole stalk and rice husk) feed	65.39 g/Kg biomass for redwood sawdust; 40.0 g/Kg biomass for cole stalk and rice husk	[137]
Thermoelectrochemical			
membrane electrode assembly	sulfur dioxide	0.4 A/cm ² at 0.835 V (H ₂ production rate did not reported)	[138]
membrane electrode assembly	anhydrous hydrogen bromide	2.0 A/cm ² at 1.91 V (H ₂ production rate did not reported)	[138]

Table 3. Cont.

Reactor	Feed Stock	Maximum H ₂ Yield	Reference
Electrochemical Electrolysis			
The BiOx–TiO ₂ electrode and stainless steel (SS, Hastelloy C-22) were used as an anode and a cathode in the electrochemical system, respectively	arsenite (As(III))	9.4 μmoles/min	[139]
Photoelectrolysis			
The TiO ₂ (ns) was prepared in the form of a sol-gel	photoelectrode system TiO ₂ (ns)–VO ₂	6 L·h ⁻¹ ·m ⁻² for the TiO ₂ (ns); 13.0 L·h ⁻¹ ·m ⁻² for the TiO ₂ (ns)–VO ₂ photoelectrode	[140]

In connection with a European research study, HYVOLUTION, the life cycle environmental impacts of pilot production of H₂ through thermophilic fermentation, and photo fermentation of potato peel was compared to production of H₂ from natural gas through steam methane reforming (SMR) [112]. It was demonstrated that the bio-H₂ production had approximately 5.7 times higher environmental impacts (negative impacts on the environment) than a centralized SMR. The processes involved in steam (pretreatment), phosphate buffer (used in photo fermentation) and potassium hydroxide (used in thermophilic fermentation), were the main causes of the environmental impact (98.3%). Recirculation of the sewage reduces the environmental impacts considerably to having only approximately two times more environmental impact than SMR. If instead biomethane were produced for use in the SMR the environmental impact would be reduced to less than 1/3 of the traditional SMR [112]. On the other hand alternative use of the peel would be as animal feed and Djomo *et al.* showed that the production of bio-H₂ is more beneficial than the use as animal feed by a factor of 2–3 [110]. In a more recent study Djomo and Blumberga investigated potential differences in environmental performance between the three different feedstocks [111]. They performed a “well-to-tank” study *i.e.*, the system boundary is at supplying H₂ to road vehicles meaning that the combustion and transportation of H₂ in the vehicles, was not included. Further, the production of feedstock was excluded as they are considered wastes. Their conclusion is in contrast to the earlier study they find that H₂ produced from any of the feedstock reduced GHG-emissions by approximately 55% compared to SMR and a few percent less for gasoline. When the subsequent use of the remains from the H₂ production were considered as animal feed, an environmental benefit could be observed. The energy ratio calculated was 1.08–1.17, *i.e.*, the energy gain is between 8 and 17%. Though steamed potato peel was slightly better, no significant environmental differences were observed between the feedstocks [111]. The results compare well with those of Manish and Banerjee who investigated the energy balance of H₂ and found an energy ratio of 3.1 (excluding the gas treatment and the compressing) [57].

The conclusion from these studies from an environmental view point is that the production of H₂ for renewable energy production from potato peel could be preferred to using SMR or as direct animal feed due to the lesser environmental impacts. The LCA studies can further be used for identification of the main environmental improvements in the technology development (e.g., recirculation of the sewage and reuse of the remains for animal feed). The LCA of H₂ is very important before taking them into consideration for commercial scale production and policy decisions on H₂ promotion.

6. Future Directions and Perspectives

One option proposed to lower feedstock costs is to identify microbes that can directly utilize hemicellulose and cellulose [26]. This would eliminate the need for cellulase enzymes and simplify biomass pretreatment. As cellulose is the most abundant biopolymer in the world [141], its

bioconversion provides a viable approach to produce renewable H₂ from organic matter. The combined dark fermentation coupling with photo fermentation, or dark fermentation coupling with bioelectrohydrogenesis is a promising H₂ production process from lignocellulosic biomass if the technological barriers can be overcome [12]. Overall, to develop a mature H₂ production technology, bioconversion performance from lignocellulosic biomass need to be further improved in terms of production rates, cost-effectiveness, and system scale-up. Based on the limited number of LCA studies done on H₂ production, it can be assumed that the bioconversion of lignocelluloses-to-H₂ on industrial scale is a feasible option to produce H₂ via biotechnology. However, more in-depth studies need to be carried out to confirm this.

7. Conclusions

Although considerable progress has been made on H₂ production from lignocellulosic biomass, several challenges remain for its commercial application. Among the various techniques available for H₂ production from lignocellulosic biomass, dark fermentation seems to have an edge over the others and is the closest to commercialization. Photo fermentation is the next best option, though it has to overcome the problems associated with reactor design and operation. Bioelectrochemical H₂ production is still in its infancy and needs much more research and development. The kinetic models for H₂ production provide insights on substrate utilization and factors limiting higher yields. The models will help in scale up studies for validating the proposed data and later on with the experimental data. The few environmental assessment studies performed from a LCA perspective show that H₂ production from lignocellulosic biomass also may be preferable to other renewable energy production pathways. Such studies can furthermore help identifying technological improvement options. The results of LCA studies could also help policy makers in taking decision on policies related to promotion of renewable energy.

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