



Review

Biological Hydrogen Production from Biowaste Using Dark Fermentation, Storage and Transportation

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Abstract: Hydrogen is widely considered as the fuel of the future. Due to the challenges present during hydrogen production using conventional processes and technologies, additional methods must be considered, like the use of microorganisms. One of the most promising technologies is dark fermentation, a process where microorganisms are utilized to produce hydrogen from biomass. The paper provides a comprehensive overview of the biological processes of hydrogen production, specifically emphasizing the dark fermentation process. This kind of fermentation involves bacteria, such as *Clostridium* and *Enterobacterium*, to produce hydrogen from organic waste. Synthetic microbial consortia are also discussed for hydrogen production from different types of biomasses, including lignocellulosic biomass, which includes all biomass composed of lignin and (hemi)cellulose, sugar-rich waste waters, and others. The use of genetic engineering to improve the fermentation properties of selected microorganisms is also considered. Finally, the paper covers the important aspect of hydrogen management, including storage, transport, and economics.

Keywords: biological hydrogen; biowaste; dark fermentation; storage hydrogen



Citation: Talapko, D.; Talapko, J.; Erić, I.; Škrlec, I. Biological Hydrogen Production from Biowaste Using Dark Fermentation, Storage and Transportation. *Energies* **2023**, *16*, 3321. <https://doi.org/10.3390/en16083321>

Academic Editors: Shie Jelueng and Min-Hao Yuan

Received: 23 February 2023

Revised: 29 March 2023

Accepted: 5 April 2023

Published: 7 April 2023



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1. Introduction

There is an increasing energy demand in the modern world. It is expected to be even more pronounced in the future as it is imposed by modern lifestyle and economic development [1]. However, natural resources for energy production are limited [2]. The major problem is posed by the use of non-renewable fossil fuel energy sources and the technologies used for production, which are direct polluters of the environment, leading to the generation of greenhouse gases and global warming [3–5].

The development of alternative, clean energy sources represents the right path toward solving the energy problems of humankind [6]. A practical alternative to fossil fuels is the utilization of hydrogen, which has been under discussion for a long time. It is included in the plans of countries in the European Union and beyond [7]. In line with the European Green Deal of 2020, the European Commission presented the Hydrogen Strategy for a Climate-Neutral Europe by 2050. Hydrogen is highlighted as one of the key levers for a successful energy transition and the European strategy for integrating energy systems [8]. The global hydrogen production in 2021 was about 90 megatons per year (Mt/y). However, most hydrogen is produced from fossil fuels, and less than 1% comes from renewable resources and microorganisms [9].

Hydrogen (H₂) is the lightest element in the periodic table of elements and the most abundant chemical element in the universe [10]. It is a colorless, odorless, non-toxic, and highly flammable gas at standard pressure and density. It is difficult to find in its pure molecular form as it is lighter than air and rises from the atmosphere [11]. In the energy industry, hydrogen is nominated with a color designating the primary sources of energy used for hydrogen generation (Table 1).

Table 1. An overview of the classification of hydrogen by color as one of the main methods for describing the types of hydrogen based on currently available production technologies [12].

Hydrogen Type (Designated Color)	Hydrogen Derived from
White	Natural source
Black	Coal
Gray	Methane
Brown	Lignite
Blue	Methane with carbon capture
Turquoise	Methane pyrolysis
Green	Renewable energy
Pink	Nuclear

Based on its properties, hydrogen is increasingly considered a promising alternative to fossil fuels in scientific, economic, and political circles [13]. It is considered an ideal fuel for the future and one of the cleanest energy carriers derived from renewable sources [14]. In addition, it has a high energy density (122 kJ/g) [15].

It is important to emphasize the environmental acceptability of hydrogen as it does not produce CO₂ during combustion. The energy released during hydrogen combustion in air, or reaction with oxygen, enables hydrogen to be used as a fuel without harmful emissions [16]. Instead, hydrogen combustion only produces water when it can also be used directly to produce electricity by means of fuel cells [17].

Fuel cells use hydrogen to produce electricity and heat, generating water as waste. Since fuel cells do not burn fuel in the same way as gas turbines, their electrical efficiency is high [18]. In addition, hydrogen is used as a fuel due to its high reactivity, which reduces the need for expensive catalysts [19]. The installed capacity of power plants with fuel cells that produce electrical and thermal energy depends on their design and can range from 1 kW to 10 MW [20]. Hydrogen derived from renewable energy sources, or green hydrogen, is a significant factor in driving the energy transition and the global decarbonization process [21].

As of the end of 2021, the majority (47%) of global hydrogen production was derived from natural gas, while 27% of production came from coal. As a byproduct, 22% of hydrogen was obtained from oil, and only 4% was obtained from electrolysis (Figure 1).

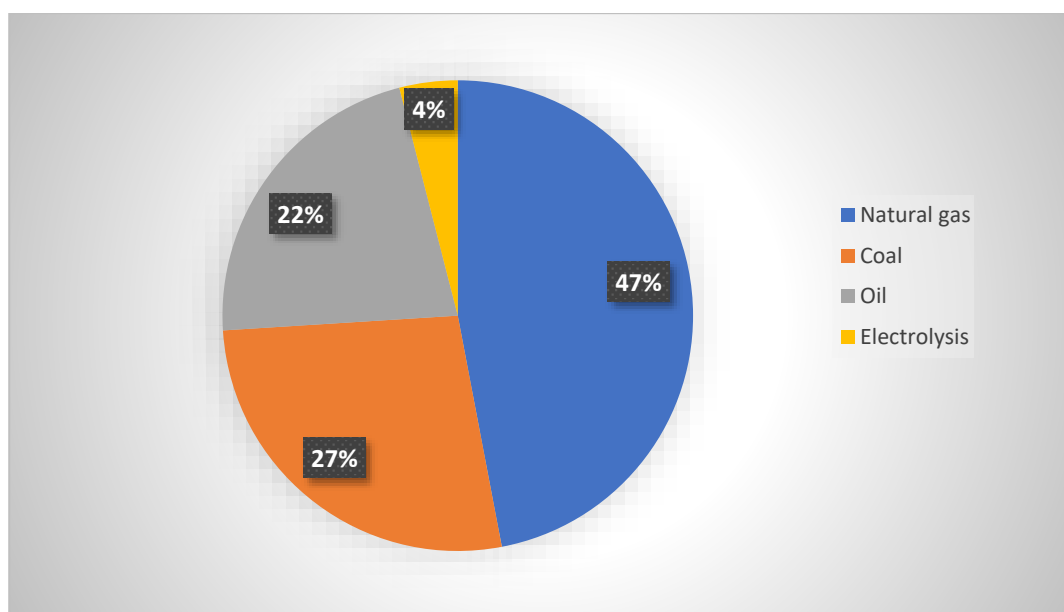


Figure 1. Sources of global H₂ production in 2021.

In 2021, renewable energy sources accounted for approximately 33% of electricity production, meaning that only 1% of hydrogen was produced from renewable sources [9]. Several technologies for producing hydrogen from renewable resources do not involve microorganisms. These include electrolysis, thermolysis, photoelectrochemical water splitting, pyrolysis, biomass gasification, and hydrogen production from geothermal sources [22,23]. Each technology has its advantages and limitations, and the choice of technology depends on factors such as the availability of renewable resources, the cost of the technology, and the desired scale of production. However, microorganisms can have several advantages over other green technologies for producing energy or fuels, such as low energy input, high efficiency, versatility, renewability, and carbon neutrality (e.g., carbon dioxide produced during combustion is captured and stored) [24]. However, green hydrogen production technologies are becoming increasingly accessible, and renewable energy production costs are decreasing, making them increasingly economically competitive [25]. In order to continue the trend of reducing reliance on fossil fuels, a shift towards resource transformation through the microbial transformation of renewable resources is needed [26]. Such hydrogen production presents a promising alternative to fossil fuels [27]. Thus, sustainable processes through biomass fermentation are crucial for developing environmentally friendly, more sustainable, and more energy-efficient hydrogen production [28].

Recently, new technologies have been introduced for the production of hydrogen from wastewater. Aqueous phase reforming (APR), which is one of the most researched today, is a catalytic reaction that allows hydrogen to be produced from oxygenated compounds. The catalytic system is very stable, which is an advantage in industrial applications [29,30].

Since the problem of energy production is present in all areas of modern society, science is striving to find optimal solutions. Accordingly, several scientific papers have been published recently on the treatment of wastewater by the process of dark fermentation, in which the production of hydrogen by the said process is presented in a very systematic way [31–33]. This paper was written with the same aim: to explore new possibilities of hydrogen production by dark fermentation, describing also transport and storage issues.

2. Biological Hydrogen Production

Based on the process of biological H₂ production, we can classify them as photosynthetic processes that involve direct or indirect bio-photolysis of water using cyanobacteria and algae or as fermentation, whether it is a photo-fermentation using photosynthetic bacteria or dark fermentation that uses various groups of heterotrophic bacteria [34,35].

Compared to bio-photolysis and photo-fermentation, dark fermentation represents a more attractive option, although solar light (natural source) could be used in hydrogen production in these light-dependent processes. However, these methods have proven to be poorly efficient and require very expensive equipment for hydrogen production [27] (Table 2).

Table 2. Advantages of dark fermentation compared to photo-fermentation and bio-photolysis.

	Advantages of Dark Fermentation Compared to Photo-Fermentation and Bio-Photolysis	Ref.
1.	The rate of dark fermentation is significantly higher compared to photo-fermentation and bio-photolysis processes	[36]
2.	Dark fermentation has the potential to utilize a wide range of potential substrates, including renewable biomass and organic waste materials, resulting in relatively lower costs.	[37]
3.	Dark fermentation does not require direct input of light energy. Therefore, it is capable of continuously producing hydrogen day and night.	[38]
4.	Its simple reactor design gives it efficiency and economic feasibility	[39]
5.	The estimated cost of hydrogen production by dark fermentation is 340 times lower than that of photosynthetic processes.	[40]

Dark fermentation is carried out without oxygen and light, with facultative anaerobes and strict anaerobes acting on the substrate to produce hydrogen [41]. Various substrates can be used for dark fermentation [42]. This can include industrial wastewater containing carbohydrates, lignocellulosic biomass, municipal solid waste, and lignocellulosic biomass containing sugars [43]. The efficiency of dark fermentation is significantly influenced by biomass pretreatment [44], the sugar concentration in the substrate, the microorganisms participating in the reaction [45,46], and the pH, which affects the activity of hydrogenase enzymes, which play a vital role in hydrogen metabolism in dark fermentation [47]. Based on the “Thauer limit”, the maximum yield of 4 and 2 moles of H₂ can be produced from dark fermentation, depending on acetate and butyrate as byproducts [48,49] (Table 3).

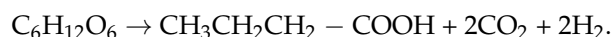
Table 3. Hydrogen yield based on biomass used and dark fermentation conditions [32].

Substrate	Organism	pH	Temp	Hydrogen Yield
Cornstalk 20 g/dm ³	<i>Thermoanaerobacterium thermosaccharolyticum</i>	7.5	55 °C	6.38 mmol/g substrate
Rice straw 1% w/v	<i>Thermotoga neapolitana</i>	7.5	75 °C	2.27 mmol/g straw
Wheat straw 5 g/dm ³	<i>Thermoanaerobacterium thermosaccharolyticum</i>	7.0	60 °C	3.53 mmol/g substrate
Cornstalk 15 g/dm ³	<i>Clostridium sartagoforme</i>	6.5	35 °C	87.2 cm ³ /g substrate
Sugarcane bagasse 1%	<i>Caldicellulosiruptor saccharolyticus</i>	-	70 °C	2.3 mol/mol glucose
Corn leaves 0.9%	<i>Caldicellulosiruptor saccharolyticus</i>	-	70 °C	1.80 mol/mol glucose
Soybean straw	Mixed cultures	7.0	35 °C	5.46 cm ³ /g substrate
Pine tree wood	Mixed cultures from sewage sludge digester	7.0	35 °C	0.99 mol/mol substrate
Cattle wastewater	Sewage sludge	5.5	45 °C	12.41 mmol/g substrate
Wastewater from the brewery plant	Mixed cultures (from activated sludge)	5.5	35 °C	2 mol/mol hexose

The equation represents the dark fermentation process with acetic acid as a byproduct [50]:



The dark fermentation process with butyric acid as a byproduct can be represented by the equation [50]:



In conclusion, dark fermentation is an anaerobic conversion of organic matter into VFA, H₂, and CO₂ through the action of microorganisms, which can be written as [38,51]:



anoic acid + κH_2 + λCO_2 + μ Microbial biomass + π Others (e.g., ethanol).

It is important to note that the hydrogen production rates from dark fermentation are significantly higher than those obtained from photo-fermentation [48]. The hydrogen yield in photo-fermentation is between 0.5 and 2 moles of hydrogen per mole of substrate used [52]. Hence, it has been reported that hydrogen production in dark fermentation by pure cultures such as *Bacillus*, *Caldicellulosiruptor*, *Clostridium*, *Thermotoga*, and *Enterobacter* can reach up to 3.80 mol/mol hexose [48].

2.1. Microorganisms Participating in Dark Fermentation

Fermentation can be performed with pure or mixed bacterial cultures. The issue that arises with the use of mixed cultures relates to the reduction in hydrogen yield due

to the potential exploitation of hydrogen by hydrogenotrophic bacteria [53]. To prevent this problem, an inoculum pretreatment could be necessary; thermal treatment is often used [54]. In addition, hydrogen-producing enzymes (hydrogenases) could be utilized in dark fermentations. Since neither oxygen production nor consumption takes place in these reactions, there is a reduced likelihood of hydrogenase deactivation by oxygen [27].

In the case of pure cultures, the microorganisms used for biological hydrogen production through dark fermentation can be strict and facultative anaerobic bacteria [55]. Among strict anaerobic bacteria, *Clostridia*, thermophilic archaea, *Thrtmotoga*, and *Ruminococcus* are capable of producing H_2 (Table 3). However, *Clostridia* are the main H_2 -producing bacteria [56]. *Clostridium* belongs to gram-positive, spore-forming bacteria and plays a highly significant role in hydrogen production [57]. Specifically, *Clostridia* are naturally capable of producing hydrogen through mixed acid fermentation at a high rate [58]. Analysis of anaerobic microbial species of the genus *Clostridium* that produce hydrogen (using anaerobic sludge as an inoculum) shows the presence of *Clostridium cellulosi*, *C. acetobutylicum*, *C. tyrobutyricum* [55]. In addition, *C. butyricum* is known for its high hydrogen yield regardless of whether the substrate is a simple carbohydrate (glucose, xylose, sucrose) or complex biomass such as waste food [59]. *Clostridia* species significant for the production of biohydrogen from organic waste under mesophilic conditions include *C. beijerinckii*, *C. pasteurianum*, *C. acetobutylicum*, and *C. saccharoperbutylacetonicum*, while under thermophilic conditions, *C. thermocellum* is considered the ideal strain [59].

The hydrogen production reaction through fermentation involving the H_2 -evolving hydrogenase enzyme is characteristic of *Clostridia* [60]. Specifically, [FeFe]-hydrogenases produce hydrogen [61]. The internal production of H_2 using [FeFe]-hydrogenase via proton reduction increases the intracellular pH and provides an oxidized ferredoxin for central metabolism [62]. The oxidized ferredoxin is required for the decarboxylation of pyruvate through pyruvate ferredoxin oxidoreductase (PFOR) [63] (Figure 2). During H_2 production through charge-based fermentations, hydrogen by [FeFe]-hydrogenase isoform 1 (HydA1) is significant, and the enzymatic reaction is reversible and important for the H_2 -dependent reduction of nitroaromatic compounds [64]. An important factor in the production of hydrogen from waste materials is that most strains of *Clostridium* produce spores, allowing them to survive after thermal processing because they can tolerate very high temperatures [65].

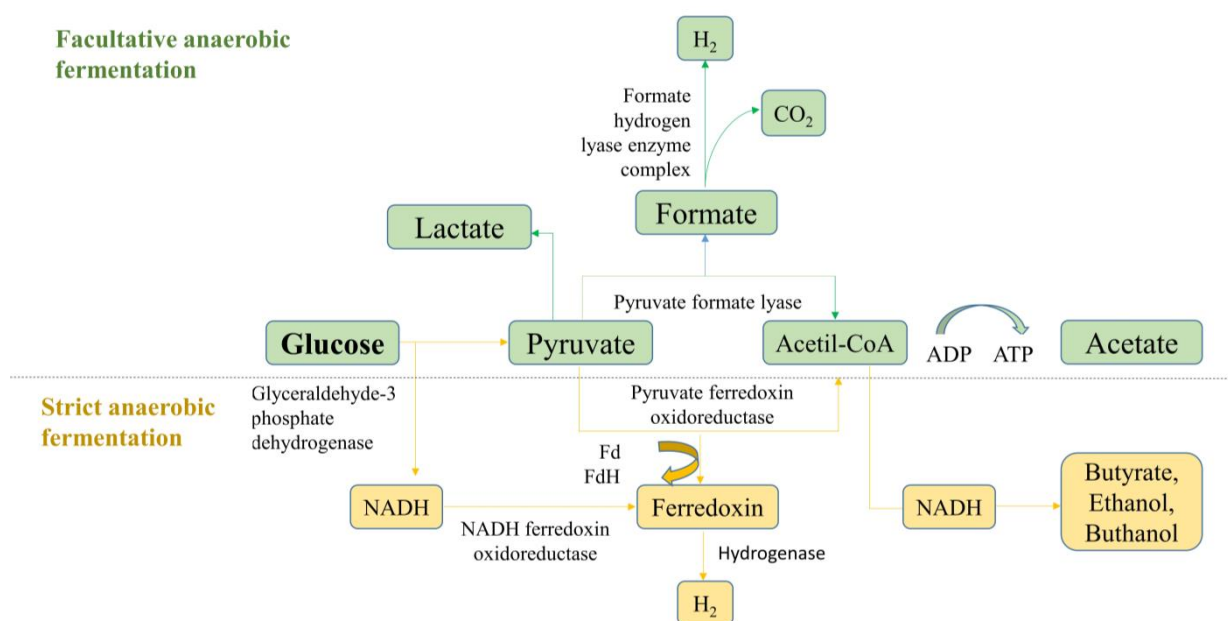


Figure 2. Presentation of the metabolic pathways of facultatively anaerobic and strictly anaerobic fermentation.

Waste material sterilization is carried out according to EU regulations on sterilizing municipal waste (European Commission, 2005) at 85 °C for 1 h [51]. The *Clostridia* present in the substrate resists sterilization by creating spores. Thus, these clostridia add to those of the inoculum, becoming the dominant bacteria because most of the various bacteria are killed at these temperatures [27], and spores generated at high temperatures can be activated for hydrogen production [27]. *Clostridium* can use various substrates such as glucose, xylose, lactose, and sucrose for hydrogen production, with relatively high yields and more than 2 moles per mole of substrate [27]. Strict anaerobes do not carry out oxidative phosphorylation and primarily produce ATP by substrate-level phosphorylation and a flavin-based electron bifurcation process during fermentation [66].

Bacterial genera such as *Ruminococcus albus* can hydrolyze cellulose, i.e., use widely available plant biomass directly as substrate [67], and are potential candidates for hydrogen production [68]. However, their hydrogen yield after cellulose fermentation is low (0.59 mol/mol cellulose) [27] (Table 4).

Table 4. Strict anaerobic hydrogen-producing bacteria and their maximum hydrogen yields.

Genus	Species	Hydrogen Yields	Ref.
<i>Clostridium</i>	<i>C. acetobutylicum</i>	2.0 mol/mol glucose	
	<i>C. beijerinckii</i>	2.31 mol/mol xylose	
	<i>C. butyricum</i>	2.78 mol/mol saccharose	
	<i>C. thermolacticum</i>	3.0 mol/mol lactose	
<i>Ruminococcus</i>	<i>R. albus</i>	2.01 mol/mol glucose	
	<i>R. albus</i>	0.59 mol/mol cellulose	[27]
<i>Thermotoga</i>	<i>T. maritima</i>	2.2 mol/mol glucose	
<i>Thermoanaerobacterium</i>	<i>T. thermosaccharolyticum</i>	2.4 mol/mol glucose	

The facultatively anaerobic bacteria that exhibit the best characteristics significant for hydrogen production are enteric bacteria such as *Citrobacter* sp., *Escherichia coli*, *Enterobacter* sp., and *Klebsiella* sp. [27]. It should be noted that the theoretical hydrogen yields from facultatively anaerobic bacteria are lower than those from strictly anaerobic bacteria due to the different metabolic pathways followed to produce hydrogen [56]. For example, *Enterobacter cloacae* can increase the hydrogen yield to 3.9 moles/mole of glucose [27] (Table 5).

Table 5. Facultatively anaerobic hydrogen-producing bacteria and their maximum hydrogen yields.

Genus	Species	Hydrogen Yields	Ref.
<i>Citrobacter</i>	<i>C. amalonaticus</i>	1.24 mol/mol glucose	[27]
	<i>C. freundii</i>	0.83 mol/mol glucose	
	<i>C. intermedius</i>	1.1 mol/mol glucose	
<i>Klebsiellae</i>	<i>K. pneumoniae</i>	2.07 mol/mol glucose	[27]
	<i>Enterobacter aerogenes</i>	2.16 mol/mol glucose	[69]
<i>Enterobacter</i>	<i>Enterobacter cloacae</i>	3.9 mol/mol glucose	[27]
<i>Escherichia</i>	<i>Escherichia coli</i>	2 mol/mol glucose	[27]

The typical reaction for the hydrogen production from facultative anaerobes through dark fermentation involves the carbon source from waste materials or plant biomass entering the glycolytic pathway, forming ATP, NADH, and pyruvate [41]. Pyruvate is the final product of glycolysis and is split into acetyl-CoA and formate: formate lyase (PFL) [70]. Subsequently, formate is divided into H₂ and CO₂ by the formate: hydrogen lyase (FHL) complex, yielding 2H₂ per glucose [55,70].

2.2. Artificial Microbial Consortium

Research has determined that the energy yields of artificial microbial consortia containing well-defined microorganisms, precisely engineered with specific ecological and

metabolic functions, are significantly higher than the wild-type and undefined microbiomes [71,72] (Table 6).

Table 6. Hydrogen yield from artificial microbial consortia [73,74].

Microorganisms Consortium	Hydrogen Yield
<i>E. aerogenes</i> – <i>C. acetobutylicum</i>	5.58 mol/mol glucose
<i>Ruminococcus albus</i> – <i>Wolinella succinogenes</i>	3.91 mol/mol glucose
<i>Caldicellulosiruptor saccharolyticus</i> – <i>C. kristjanssonii</i>	3.7 mol/mol glucose
<i>Citrobacter freundii</i> – <i>C. butyricum</i>	2.12 mol/mol glucose
<i>C. saccharolyticus</i> – <i>C. owensensi</i>	4.42 mol/mol glucose
<i>Thermatoga neapolitana</i> – <i>C. saccharolyticus</i>	2.8 mol/mol glucose
<i>Enterobacter cloacae</i> – <i>Bacillus cereus</i>	3.0 mol/mol glucose
<i>Escherichia coli</i> – <i>C. butyricum</i>	1.65 mol/mol glucose

Ergal and colleagues designed a microbial consortium consisting of *C. acetobutylicum* and *E. aerogenes*, which gave a 40% higher hydrogen yield than the Thauer limit, with a value of 5.6 mol H₂/mol glucose [73].

3. Hydrogen Production from Biomass

First-generation biomass mainly consists of Lignocellulosic biomass with high starch and sugar content, such as sweet sorghum, sugar beet, potato, wheat, pumpkin, and oilseed rape, as well as the residues and byproducts of their processing, which are traditionally utilized for food and feed [75]. Second-generation biomass is mainly represented by lignocellulosic biomass with low commercial value but high abundance. Third-generation biohydrogen production is derived from algal biomass rich in polysaccharides [76]. Algae have the ability to grow rapidly compared to other biomasses and sequester large amounts of CO₂. In addition, they can contain a low amount of lignin and lignin oligomers [77,78], which makes them easy to hydrolyze [79]. The fourth-generation biomass feedstock comprises genetically modified organisms to improve biohydrogen production processes [80]. Second and third-generation biomasses, after genetic modification, become part of the fourth-generation feedstock. Genetic/metabolic modifications in microorganisms capable of contributing to the development of new technologies are achieved through genetic engineering or nanotechnology [35,81].

3.1. Lignocellulosic Biomass and Crop Residues Containing Sugars

Lignocellulosic biomass is considered “the most abundant organic component of the biosphere”, with an annual production of 1–5·10¹³ kg, which is considered an attractive and cheap substrate for the production of biofuels [82,83]. It is the most prevalent in nature and is present as hardwood, softwood, grasses, and agricultural residues. The estimated global annual yields of lignocellulosic biomass residues are more than 220 Bt [84], which is equivalent to 60–80 Bt of crude oil [15].

Based on dry matter composition, lignocellulosic biomass primarily consists of cellulose (40–60%), hemicellulose (20–40%), and lignin (10–25%) [85]. Cellulose and hemicellulose can be enzymatically hydrolyzed into smaller sugar molecules [86]. They mainly contain glucose and xylose, readily available for microorganisms that can effectively ferment glucose, and xylose are for bio-hydrogen production [87] (Figure 3).

In order to improve substrate hydrolysis and facilitate biological hydrogen production through dark fermentation in reactors, pretreatment of the lignocellulosic biomass matrix is used to release cellulose molecules into solution, breaking the crystal structure of cellulose and helping depolymerization [87]. Pretreatment can also be applied to the inoculum to enrich hydrogen-producing bacteria and inhibit hydrogen-consuming bacteria [88]. Mixed cultures have an advantage over pure cultures because they allow easier control of the process and higher substrate efficiency, but pretreatment should be performed in this case. Acid or alkaline treatment, thermal shock, aeration, chemical inhibition, or inhibition with

long-chain fatty acids can be used to increase hydrogen production by dark fermentation. Pretreatment aims to suppress the growth of hydrogen consumers that do not form spores. Spore-forming hydrogen-producing bacteria survive this pretreatment [89].

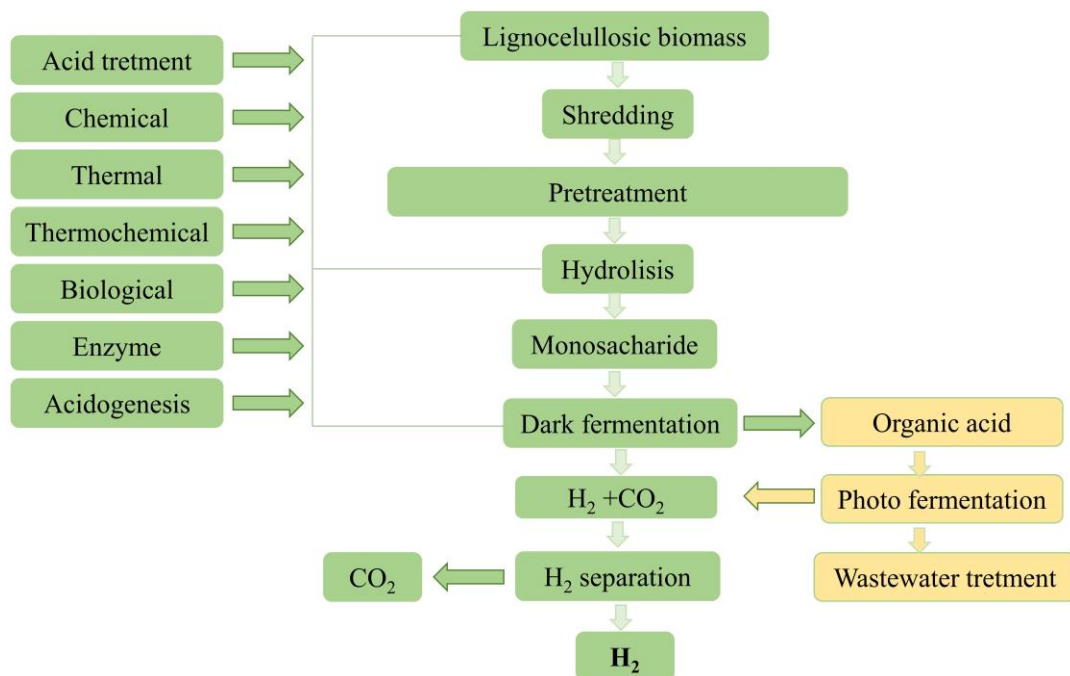


Figure 3. Process for obtaining hydrogen from lignocellulosic biomass.

Hydrogen can be produced from lignocellulosic biomass through dark fermentation with a yield close to the maximum theoretical yield of 4 moles of hydrogen per mole of hexose [15]. It is worth mentioning that a wide range of monosaccharides, disaccharides, and polysaccharides (including cellulose) can be used for hydrogen production [90].

Caldicellulosiruptor saccharolyticus is an example of an interesting bacterium for bio-hydrogen production. *C. saccharolyticus* is a thermophile, strictly anaerobic, Gram-positive, cellulolytic bacterium bio-hydrogen producer [90]. Genome analysis revealed that *C. saccharolyticus* has a high ability to hydrolyze polysaccharides such as cellulose, hemicellulose, pectin, and starch, along with a large number of ABC transporters for the uptake of monomeric and oligomeric sugars [91]. Catabolic pathways have been identified for a range of sugars, including rhamnose, fucose, arabinose, glucuronic acid, fructose, and galactose, which lead to the production of NADH and reduced ferredoxin. NADH and reduced ferredoxin later use two different hydrogenases to form hydrogen [92]. Whole-genome transcriptome analysis revealed significant regulation of the glycolytic pathway and ABC-type sugar transporters during growth on glucose and xylose, indicating that *C. saccharolyticus* coferments these sugars without repression based on glucose catabolite [82]. In addition, *C. saccharolyticus* is an extreme thermophile. It is known that H_2 yields are higher in thermophilic than in mesophilic conditions, although the volumetric productivity is reversed [56]. Thus, transferring the metabolic pathway of *C. saccharolyticus* to a mesophilic bacterium would be advantageous. The role of genetic engineering in cellulolytic microorganisms, in fact, is significant and enables the redirection of metabolic pathways toward maximum hydrogen production [93]. For example, introducing mutations in mesophilic *E. coli* by inactivating the lactate dehydrogenase gene (*ldhA*) increases hydrogen production by 20–45% [94].

To increase H_2 production in fermentation systems, modifications to metabolic engineering as the introduction/overexpression of genes (cellulases, hemicellulases, and lignases), can be used to increase the availability of carbohydrates for the cell, overexpres-

sion of enzymes that produce H₂, and disruption of metabolic pathways that compete for reductive equivalents [15].

3.2. Wastewaters

Wastewater, particularly those from industrial facilities, presents a promising avenue for water treatment companies to apply methods of wastewater recycling to produce green hydrogen directly using microorganisms [95]. Wastewater consists of 70% organic compounds and 30% inorganic compounds, with organic compounds primarily being carbohydrates, fats, and proteins, which can be utilized through dark fermentation for hydrogen production [96]. In dark fermentation methods, bacteria such as *Clostridium thermocellum* break down organic materials and produce hydrogen [32].

The wastewater microbial consortium contains a large number of bacteria, some of which inhibit hydrogen production (i.e., hydrogenophilic methanogenesis) through consumption (homo-acetogens and methanogens) [95]. Therefore, for optimal hydrogen production, the activity of inhibitory microorganisms is suppressed or killed, most commonly through pre-heating of the inoculum [97] or, in a more cheap way, optimizing the parameters processes as the initial pH [98].

Potentially, wastewater constitutes readily accessible and inexpensive biomass from which hydrogen can be produced [99]. This would reduce the negative impact of wastewater treatment on the environment while increasing hydrogen production, thus making wastewater a fuel of the future [100]. Essentially, green hydrogen from wastewater has tremendous potential for further development [101].

Organic wastewater contains a potentially high energy content, with each kg of chemical oxygen demand producing about 1.4×10^7 kg of metabolic heat, which has immense practical significance [41,102,103]. It has been noted that the energy contained in wastewater is 9.3 times greater than the energy consumed for its treatment [104]. If 10% of the energy can be utilized, it could power the wastewater treatment facility. As a result, energy extraction from organic waste is undoubtedly critical for developing low-carbon “energy saving and emissions reduction” models and for developing renewable energy [41].

4. Hydrogen Management

It would be ideal to use bio-hydrogen on the production site [105]. However, these are mainly energy generation systems by combustion, and the produced energy is transferred to other systems through a heat exchanger: biological, technical, and physical processes [31]. In this way, energy losses are minimized, and the costs of preparation for transport, transport, and storage, as well as the costs of re-releasing pure hydrogen as a fuel at the destination, are avoided [106].

However, quantities of produced gas from 100,000 m³ to several million cubic meters must be stored in natural or technically advanced geological formations, ensuring the conditions of impermeability, preservation of the purity of stored hydrogen from bacterial, organic, and inorganic pollution, and the possibility of increasing storage space [107].

4.1. Hydrogen Storage

The final choice of the method and form of hydrogen handling—storage depends on its final use, the form of energy, and the energy conversion method [108].

4.1.1. Natural Caves and Salt Mine Caves

The walls of such caves ensure the impermeability and preservation of the storage gas's purity [109]. Such storage facilities are already used in the USA and the UK [110]. Such a form of storage is considered the most economical option and the safest gas storage facility that is well protected from external influences (terrorist attacks, fires, and military actions) [110]. The capacities of such caves are around one million cubic meters [111]. In general, pure hydrogen is in the form of fuel cells ready for technological application (vehicles, etc.) [112].

4.1.2. Underground Hydrogen Storage in a Gas Mixture

Besides its nascent form, hydrogen can be stored in depleted natural gas deposits (natural gas: a mixture of methane and higher homologs) or a mixture with methane, CO₂, and CO (synthetic gas) [113], as well as in a mixture with city gas (methane, CO) [114].

All of these forms of stored gas can be utilized in gas turbines or fuel cells to produce electrical energy [115]. Such mixtures can be stored in salt caverns and aquifers (so-called depleted geological environments saturated with free subterranean waters) [116]. They form in water-impermeable rocks as well as in natural gas storage facilities (hydrogen content of 5–15%) [108].

Hydrogen must be separated from the mixture with natural gas in pure form for further use [117]. This can be achieved through membrane filtration, adsorption, and electrochemical separation [118].

4.1.3. Special Cases of Hydrogen Storage in Subsurface Reactors for the Methanation Process

In underground storage sites of natural gas and aquifers, hydrogen and carbon dioxide are subjected to hydrogenotrophic methanogenic bacteria that, through the process of methanization, create methane [119]. This process takes place at low temperatures, which is more acceptable than some high-temperature processes with catalysts [120].

4.2. Hydrogen Transport

Hydrogen transport can be considered a special form of hydrogen storage [121]. Long-distance transportation is carried out based on the technical principles of natural gas transport [122]. Hydrogen can be transported as a liquefied gas or as hydrogen converted into ammonia, methanol, or another transportable fluid. However, additional costs such as “energy loss” are incurred [123].

The most favorable cost-to-output ratio for hydrogen transport is transporting in liquefied form or the form of ammonia, with the possibility of hydrogen production via cracking at the consumption site [124]. However, the most significant drawbacks of these technologies are the costs of liquefaction and cracking, and the more promising technology is liquefaction [125]. Currently, efforts are underway to develop technologies that reduce costs when hydrogen is used as an energy source rather than a raw material in the form of ammonia [126].

Hydrogen can also be transported and stored using other organic carriers such as methanol and methylcyclohexane [127]. In hydrogen transport systems, such as pipelines, the phenomenon of diffusion occurs in the material the pipeline is made of [128]. This phenomenon is known as “hydrogen brittleness” and manifests as a reduction in the mechanical properties of pipelines. The degree of hydrogen brittleness depends on the operation regime of the pipeline (fluctuation of pressures, the type of material the pipeline is made of) [129]. Therefore, processes for handling hydrogen after production in terms of transport and storage represent an enormous challenge in finding the most efficient, safe, and cost-effective methods [130]. However, given the growing commitment to hydrogen as an energy source of the future, we are confident that optimal solutions will also be found for handling hydrogen [105].

4.3. Hydrogen Economics

According to Kayfeci et al., hydrogen production can cost between 1.25 USD/kg and 23.27 USD/kg, respectively [126,131]. The choice of the production method, in this case, is impacting the H₂ production costs, where fossil fuel-based technologies tend to have relatively low costs, 1.34–2.27 USD/kg, while solar-based technologies (PV, thermal, solar thermolysis, and photo-thermolysis) tend to be with the highest cost varying between 5.78–23.27 USD/kg) [132]. Among the most competitive options are indirect bio-photolysis, with a cost of 1.42 USD/kg, and direct bio-photolysis, with a cost of 2.13 USD/kg [24]. The dark fermentation method is estimated to cost 2.57 USD/kg, which is in the same range as

nuclear thermolysis option 2.17–2.63 USD/kg, while nuclear electrolysis is estimated to have costs 4.15–7.00 USD/kg, respectively [42] (Table 7).

Table 7. Cost of hydrogen production by a different process [24,131].

Process/Method	Cost (USD/kg)
Dark fermentation	2.57
Photo-fermentation	2.83
Biomass pyrolysis	1.25–2.20
Biomass gasification	1.77–2.05
Direct biophotolysis	2.13
Indirect biophotolysis	1.42
Dark fermentation	2.57
Photo-fermentation	2.83

5. Conclusions

Hydrogen is the only chemical element that is oxidizable (flammable) in cosmic, biological, and technical systems. This universal property directs the attention of science and technology to use it as a ubiquitous source of energy and no longer as an indirect source of global pollution (primarily the atmosphere–greenhouse gases) in the form of hydrocarbons (gas, oil). Biological systems are the most powerful systems for hydrogen production and manipulation. They produce hydrogen in the fermentation process, of which dark fermentation is the most productive and technically available. In this way, dark fermentation also solves the problem of disposal and saturation of the environment with organic waste rich in carbohydrates. The waste becomes valuable biomass (starch, sugars, cellulose, lignocellulose) from which bacteria, mainly *Clostridium* and *Enterobacterium*, can reach efficiencies up to Thauer’s limit (4 molH₂/mol hexose) in anaerobic conditions. A pretreatment of inoculum allows to have some selected dark fermentative bacteria without other microorganisms that obstruct dark fermentation). Genetic engineering, on the other hand, can further improve the fermentative properties of selected microorganisms. The hydrogen produced must be handled (transported and stored) in the most convenient forms: molecular hydrogen, hydrogen in the form of ammonia, mixed with other gases, etc., in natural or mining deposits. It can be stored in a liquefied form in special containers. The complexity of transport and storage depends on the form of handling, and pipeline materials require special protection due to diffusion into the walls. Among different technologies for hydrogen production, dark fermentation is one of the technologies with the lowest cost of production. In further work, these costs should also be benchmarked against the CO₂ footprint, where the dark fermentation process should provide additional benefits. For future research on the biological production of hydrogen, it is important to choose techniques based on reasonable estimates of the production cycle. This includes an assessment of the impact on the environment, i.e., low greenhouse gas emissions and high energy efficiency in processing waste biomaterials into hydrogen.

Author Contributions: Conceptualization, J.T. and D.T.; writing of the manuscript, J.T., D.T., I.E. and I.Š.; updating of the text, J.T.; literature searches, J.T., D.T., I.E. and I.Š.; figure drawings, J.T.; critical reviewing of the manuscript, J.T. and D.T.; organization and editing of the manuscript, J.T. and I.Š. All authors have read and agreed to the published version of the manuscript.

Funding: This research study was funded by a grant from the Croatian Ministry of Science and Education and dedicated to multi-year institutional financing of scientific activity at Josip Juraj Strossmayer University of Osijek, Faculty of Dental Medicine and Health, Osijek, Croatia, grant number IP2-FDMZ-2022.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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