## Chapter 6

# Lignocellulose Materials as a Potential Feedstock for Hydrogen Production

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There are numerous natural lignocellulosic materials that are readily available and have been proven to be effective feedstock for bio-hydrogen production. Currently, the focus, particularly in developing nations, is on utilizing lignocellulosic waste as feedstock and developing appropriate techniques for processing it into bioenergy products. The economic viability of lignocellulosic bioenergy can be a challenge owing to the high cost of enzyme production; however, this obstacle may be addressed with the development of an effective bio-system for simultaneous saccharification and fermentation as well as consolidated biomass processing. Lignocellulosic materials, which primarily consist of cellulose, hemicellulose, and lignin, are a promising resource for renewable bioenergy and can be sourced from agricultural, forestry, or wood waste. The present chapter will cover the details on bio-hydrogen from lignocellulose materials.

## 1. Introduction

The global energy crisis stems from the increasing demand for limited natural resources (*1*). As the demand for these resources increases, they edge closer to depletion, presenting a pressing concern. Another problem that dominates the public discussion on energy is climate change. It is the production of energy that is responsible for 87% of global greenhouse gas emissions. Unfortunately, non-sustainable fossil fuels like coal, oil, and natural gas are the main energy source for a large chunk of our country. In addition to depleting fossil resources, their excessive usage results in increased costs, reduced productivity and lower living standards. Given these conditions, current research is

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directed toward clean and sustainable energy sources, with biofuels emerging as a feasible alternative (*1*, *2*). At the G20 summit, the global biofuel alliance was formed with the objective of advocating for the biofuel economy to achieve a sustainable future. This coalition seeks to drive significant progress in harnessing biomass as a cost-effective alternative feedstock. Various renewable and cost-efficient energy sources hold potential, including bio-methane, bio-hydrogen, bio-ethanol, bio-butanol, and bio-methanol (*2*).

Bio-hydrogen stands out among these alternatives due to its high energy content and cleaner nature. The climate change imperative has been the main driver of the focus on hydrogen. It is envisaged that clean hydrogen could meet up to 12% of final energy consumption by 2050. While hydrogen can be derived from fossil fuels as well as biomass, the production cost of hydrogen from fossil fuels is considerably higher compared to biomass. The production of hydrogen from biomass is envisioned to be more sustainable and economically viable. Extensive research is underway on lignocellulosic materials as feedstock in hydrogen production because they are abundant sources of complex polysaccharides such as cellulose, hemicellulose, pectin, and lignin (*3*, *4*, *5*). Lignocellulosic feedstock includes agricultural waste, forest wood, forest waste, and other organic sources like agriculture-based industries. Since lignocellulosic material is challenging to degrade, it can be converted to biofuel (Table 1). This approach supports sustainable farming methods while also addressing the energy crisis and pollution issues (*6*).

On a global scale, India possesses significant potential to accumulate extensive quantities of agricultural lignocellulosic residual biomass, being the world's second-largest producer of rice, sugarcane, and other food grains (*4*, *5*). The Indian government has recently announced a substantial initiative aiming to replace fossil fuels like crude oil, diesel, and gasoline with lignocellulosic biofuel by 2040. This endeavor holds the potential of reducing air pollution levels by up to 90%, achieved through the cessation of stubble burning by farmers and the curbing of emissions from transportation vehicles, including carbon monoxide, volatile hydrocarbons, nitrogen oxides, formaldehydes, and particulate (*4*).

Lignocellulose presents as a complex polymeric substance necessitating preprocessing to transform it into simpler monomers, referred to as "platform molecules," which can subsequently undergo further processing into biofuels. The feedstock must undergo depolymerization to match the platform atoms by disassembling it into its constituent parts, namely cellulose (30–50%), hemicelluloses (20–30%), and lignin (10–20%) (*7*). Phenolic chemicals serve as the fundamental components of lignin; however, controlled delignification and lignin depolymerization may present technical challenges that require resolution, particularly concerning biofuel blends (*8*, *9*). The controlled depolymerization of cellulose and hemicellulose, achieved through a combination of hexose (glucose) and pentose (xylose) sugars, yields essential molecules such as furfural, 5-hydroxymethylfurfural (5-HMF), and levulinic acid. These intermediate monosaccharides serve as precursors for catalytic conversion into biofuels. However, a challenge arises in utilizing lignocellulosic materials for biofuel production, as the highest fermentable sugars are predominantly present in cellulose and hemicellulose. The efficiency of sugar extraction relies on the breakdown of lignin and complex polysaccharides into simpler monomers. In this context, bio-hydrogen production from organic wastes emerges as a promising and environmentally friendly alternative garnering global attention. Biohydrogen, derived from biological sources, represents a viable substitute for conventional fossil fuels, characterized by clean combustion that generates only water. The utilization of biomass as feedstocks for $H_2$ production offers numerous environmental and economic benefits and holds substantial potential in meeting current fuel demands (*3*).

In the present chapter, an attempt has been made to describe the feasibility of using lignocellulosic feedstock as a replacement for petroleum-based products in biohydrogen production. The details of sources of lignocellulosic biomass, composition, natural pre-treatment techniques, and its conversion to bio-hydrogen are provided, paving the way to new avenues in the energy sector.

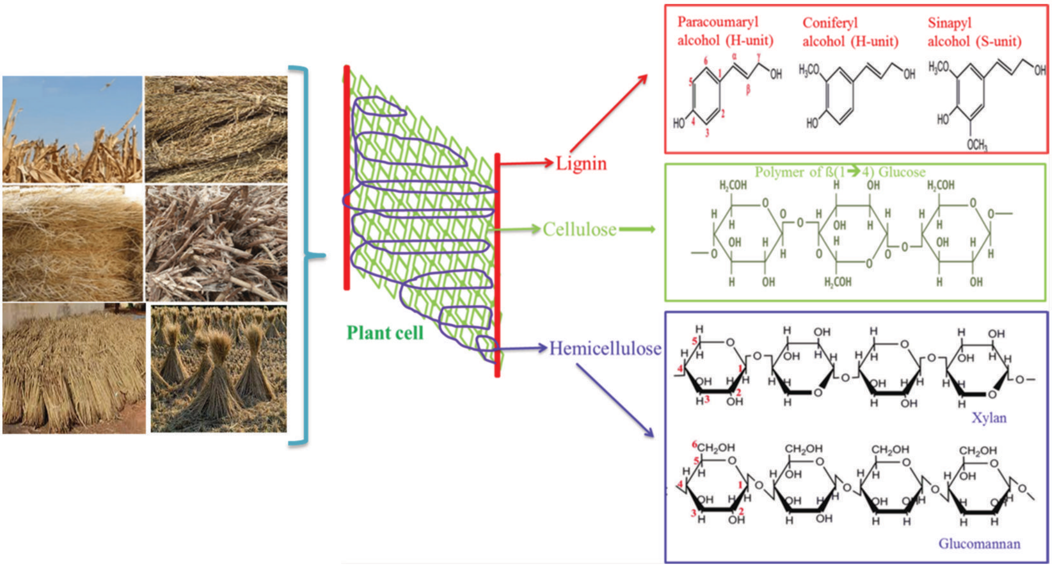

*Figure 1. Lignocellulosic biomass sources and structural arrangement.*

## 2. Lignocellulosic Biomass

Lignocellulosic biomass consists of plant dry material comprising carbohydrate polymers such as cellulose, lignin, and hemicellulose. Each component exhibits distinct chemical behavior, with cellulose serving as the key element in plant cells. The mechanical strength of structures is attributed to a linear homopolymer composed of recycled glucose residues linked by β-1,4 linkages, forming solid stringy bundles (Figure 1). Cellulose particles commonly exhibit two shapes: crystalline and amorphous. While the majority of cellulose molecules are held together by van der Waals forces and intermolecular hydrogen bonds in their local environment, a small percentage tends to form intramolecular hydrogen bonds, enhancing cellulose's rigidity and stiffness. This characteristic renders cellulose largely insoluble in water and resistant to most natural solvents (*3*, *10*, *11*). It is widely acknowledged that standard cellulase enzymes are less effective in degrading the crystalline portion of cellulose, resulting in reduced bio-ethanol yields. Nevertheless, cellulose constitutes nearly half of the organic carbon in soil, emphasizing the importance of transforming this feedstock into biofuels. In contrast to cellulose, hemicellulose can be a heteropolymer with short lateral branches composed of various saccharides, including uronic acids (such as 4-o-methylglucuronic, D-glucuronic, and D-galacturonic acids), pentoses (xylose, rhamnose, and arabinose), and hexoses (glucose, mannose, and galactose). These saccharides are linked by (1,4)-glycosidic bonds and occasionally -(1,3)-glycosidic bonds (Figure 1).

About 90% of its backbone chain comprises xylan linkages, with L-arabinose and β-xylose constituting the remaining 10%. The type and source of crop residues influence the degree of branching and backbone structure. For instance, xylan is the primary hemicellulose in hardwoods, whereas glucomannan predominates in softwoods. Unlike cellulose, hemicellulose contains polymers that are readily depolymerized. Through non-covalent interactions, hemicelluloses strongly adhere to the cellulose fiber surface. These heteropolysaccharides are believed to play an intermediary role in cellulose production. Lignin, on the other hand, is a rigid, aromatic, heterogeneous polymer primarily composed of phenolic monomers such as p-coumaryl, coniferyl, and synapyl alcohols, typically linked by ether and ester linkages. Lignin provides rigidity to the cell wall and forms a protective barrier around cellulose and hemicellulose (Figure 1). This polymer, integral to the cell wall, contributes to its impermeability, resistance against pathogen invasion, and structural support (*6*, *11*, *12*). However, the phenolic monomers resulting from lignin polymerization inhibit cellulolytic enzymes, hindering fermentation, enzymatic hydrolysis, and ethanol yield significantly. To produce biofuel through microbial fermentation of sugars, lignin molecules obstructing enzymatic hydrolysis and fermentation-associated microorganisms must be removed. Given its presence in various agricultural residues, this non-carbohydrate polymer is considered a byproduct or residue in bioethanol production (*6*, *11*, *13*, *14*).

**Table 1. Different Types of Lignocellulose Biomass Used for Biohydrogen Production**

| *S. No.* | *Lignocellulosic Feedstock* | *Biohydrogen Productivity* | *Refs* |
|---|---|---|---|
| 1 | Sugarcane bagasse | 0.733 mmol $H_2$/g of sugarcane bagasse | (*14–20*) |
| 2 | Waste date seeds | 146.19 mmol/L | |
| 3 | Wheat straw | 128 mL/L | |
| 4 | Agave biomass | 150 L $H_2$/Kg of Biomass | |
| 5 | Corncob | 132 L $H_2$/Kg of Biomass | |
| 6 | Corn stover hydrolysate | 8.78 – 9.17 mmol $H_2$/g utilized sugar | |
| 7 | Rice straw | 0.66–6.42 mmol $H_2$/g substrate | |
| 8 | Corncob pine | 0.61–5.55 mmol $H_2$/g substrate | |
| 9 | Wood waste | 0.58–5.32 mmol $H_2$/g substrate | |

## 3. Bio-hydrogen Generation Using Biomass

The emergence of biohydrogen resulting from microbial metabolism has sparked increased interest in sustainable energy production from renewable sources in recent years (*21*). In comparison to thermochemical processes, microbial processes are considered to be more energy-efficient and environmentally advantageous. The availability, affordability, carbohydrate content, and fermentability of biomass all influence its suitability for biohydrogen production (*22*, *23*). This method primarily focuses on hydrogen production and is recognized for its energy efficiency and environmental friendliness. The two most commonly used biobased hydrogen production techniques are dark fermentation and photofermentation. Dark fermentation has gained popularity due to its high yield, rapid reaction rate, and ability to utilize various organic waste materials as feedstock (*24–30*).

## Table 2. General Principles, Advantages, and Disadvantages of Various Pretreatment Techniques

| | *Pretreatment Factors* | *Effect* | *Advantages* | *Disadvantages* | *Refs* |
|---|---|---|---|---|---|
| **Biological** | Microbe (fungi, at neutral pH and in both aerobic and anaerobic condition) or enzyme | Remove lignin | Low energy consumption. No equipment corrosion problems.<br>Do not produce inhibitors | Long time; Low efficiency | (*13*, *18–20*) |
| **Physical** | Irradiation (electron beam, gamma-ray, microwave)<br>Electric (pulsed electrical field)<br>Hydrothermolysis (liquid hot water)<br>Steam explosion (high pressure steam) | Soften lignin and lignocellulose structure | Do not produce inhibitors.<br>Simple process. | Only soften lignin and lignocellulose structure, still not break structure to remove lignin | (*13*, *18*, *20*, *21*) |
| **Chemical** | Alkaline extraction (calcium, sodium and ammonia hydroxide) | Decrease cellulose crystallinity; Partial or complete hydrolysis of hemicelluloses; Delignification | Requires short reaction time; High conversion of hemicelluloses | Corrosive | (*13*, *18*, *20–22*) |
| | Acid hydrolysis (carbonic, hydrochloric, hydrofluoric, nitric, phosphoric, sulfuric) | | Requires long reaction time at low temperatures and low pressures. | Part of irrecoverable salt formed.<br>Corrosive. | (*13*, *18–22*) |
| | Ammonia fiber explosion (AFEX) | | Requires short reaction time<br>Not corrosive for equipment<br>Do not produce inhibitors<br>Allows recovery of lignin and ammonia | Not effective with high lignin biomass;<br>Requires high pressure | (*13*, *18–20*) |
| | Oxidant (ozone, wet oxidation) | | Highly effective<br>Requires short reaction time<br>Do not produce inhibitors | Expensive;<br>Good only for low lignin content. | (*13*, *19*) |
| | Organic solvent (ethanol–water, benzene–water, ethylene glycol, butanol–water) | | High delignification efficiency<br>Allows solvent reuse<br>Require short reaction time<br>Do not produce inhibitors | Some solvents are explosive and flammable | (*13*, *18–20*) |

**Table 2. (Continued). General Principles, Advantages, and Disadvantages of Various Pretreatment Techniques**

| *Pretreatment Factors* | *Effect* | *Advantages* | *Disadvantages* | *Refs* |
|---|---|---|---|---|
| Protic ionic liquid (PIL) | Simple extraction of lignin from lignocellulosic biomass | High delignification efficiency;<br>Recovers lignin and recycled PIL;<br>Requires short reaction time<br>Do not produce inhibitors | Complicated process | (*13*, *18*, *19*, *21*, *22*) |

**Table 3. Summary of Pretreatment Methods Employed for Lignocellulosic Materials**

| *Pretreatment* | *Lignocellulose* | *Experimental Conditions* | *Lignin Removal (%)* | *Sugar Retaining (%)* | *Refs* |
|---|---|---|---|---|---|
| **Biological** | Corn stover | Myrothecium verrucaria; 4 days; 29 °C | 42.3% | - | (*31*) |
| | Radiata pine | Trametes versicolor; 5 weeks; 25 °C | 22% | 77% | (*32*) |
| | Bamboo culms | Punctularia sp. TUFC20056; 12 weeks; 21 °C | >50% | - | (*33*) |
| **Chemical** | Corn stover | $NH_3$ 15%;12 h; 60 °C; 1:6 w/v | 62% | 100% glucan and 85% of xylan | (*25*) |
| | Rice straw | KOH 0.2 M; 4 h; 30 °C; 1:10 w/v | 80% | 98% | (*26*) |
| | Corn stalk | NaOH 5%; 24 h; 60 °C; 1:20 w/v | 71.8% | 79.6% | (*34*) |
| | Corn stalk | $H_2SO_4$ 5%; 24 h; 60 °C; 1:20 w/v | 64.3% | 71.6% | (*34*) |
| | Sugarcane bagasse | NaOH 1%; 0.5 h; 121 °C; 1:10 w/v | 62.3% | - | (*35*) |
| | Polar | NaOH 0.4 M; 170 °C; 7 min (combined microwaves) | 61.9% | - | (*36*) |
| | Elephant grass | NaOH 3%; 1 h; 121 °C; 1:10 w/v | 81.0% | 72.3% glucan<br>Xylan: no data | (*17*) |

Lignocellulosic biomass, an abundant and cost-effective raw material, can be efficiently utilized for the environmentally friendly production of high-energy-density biohydrogen through the dark fermentation process (*31*, *32*).

However, the complex structure and presence of lignin in biomass pose a significant challenge due to their recalcitrant nature (33), which restricts the access of enzymes to hydrolyzable sugars. Prior to fermentation, biomass necessitates pretreatment to overcome this obstacle. Various pretreatment methods, have been documented to reduce biomass recalcitrance. The dark fermentation of lignocellulosic biomass yields biohydrogen along with other by-products such as acetate, butyrate, lactate, ethanol, and CH4, which are influenced by the microbes and operating conditions employed (*34–42*).

### 3.1. Pre-treatment

Pretreatment serves as the initial step in preparing lignocellulosic biomass for biohydrogen production (*14*). Its primary objective is to disintegrate the lignin matrix, thereby producing monomers that enzymes can readily target during the subsequent hydrolysis process (*13*, *15*, *16*). The majority of the lignin content dissolves in the aqueous medium and becomes separated from the cellulose and hemicellulose. To maximize the value of lignocellulose, it is essential to consider the recovery and utilization of lignin as a value-added product alongside sugars (*16*, *17*). Table 2 presents a compilation of various pretreatment methods, which can be categorized into three primary classes based on the nature of the reaction: biological (*13*, *18–20*), physical (*13*, *18–21*), and chemical (*13*, *18–22*) processes.

Microorganisms, particularly fungi such as brown-, white-, and soft-rot fungi, are employed in biological pretreatment techniques to degrade the lignin matrix using enzymes like peroxidases and laccase. While this method is cost-effective and environmentally friendly, it typically exhibits lower delignification efficiency and requires longer treatment times compared to other methods. However, a study demonstrated that Myrothecium verrucaria can secrete three lignin-degrading enzymes simultaneously, resulting in the removal of 42.3% of the lignin in maize stover after 96 hours of treatment. Hydrothermal physical pretreatment methods like steam explosion and irradiation do not extensively break down lignin, but they do induce material swelling, which facilitates subsequent treatment steps.

Chemical pretreatment techniques utilize chemical reagents such as acids, alkalis, and solvents, which are highly efficient and require shorter treatment times. Alkaline pretreatment is the most extensively researched method due to its cost-effectiveness, although it necessitates large quantities of acid to adjust the pH to approximately 5 for enzymatic hydrolysis. Acid hydrolysis is more suitable for acid pretreatment. Pentoses dissolve in the pretreatment liquid phase, and the hemicellulose structure is readily broken down by chemical pretreatment. However, a significant portion of pentoses may be lost if the material is washed to remove lignin following chemical delignification. The efficient synthesis of hydrogen from glucose and xylose derived from lignocellulose is hindered by the loss of pentoses, primarily xylose. Chemical treatments and size reduction procedures such as milling and chopping are employed to enhance the pretreatment of lignocellulosic materials (*15*, *23*, *24*). Kim and Lee conducted a study on alkaline pretreatment to evaluate delignification, employing aqueous ammonia soaking (AAS) as a chemical pretreatment method. AAS effectively breaks down the lignin structure surrounding cellulose and hemicellulose by using aqueous ammonia as a reagent. Unlike other chemical pretreatment methods, AAS operates at lower temperatures, and ammonia is not a strong chemical. Consequently, AAS retains a significant fraction of hemicellulose in the solid material. Kim and Lee's test on corn stover demonstrated that this method removed 62% of lignin while preserving 100% of glucan and 85% of xylan after 12 hours (*25*). In a study by Yadav et al. (2011), 0.2 M potassium hydroxide (KOH) was utilized to remove lignin from rice straw at room temperature (around 30 °C) for 4 hours. The result was an 80% removal of lignin with only a 2% loss of sugars (*26*). In addition to these studies, numerous other research pieces have explored pretreatment methods for lignocellulosic materials. Table 3 provides a summary of some of these studies.

Hydrolysis techniques play a pivotal role in the production of biohydrogen from lignocellulosic-derived glucose and xylose (*27*, *28*). This process involves the liberation of hexoses (mainly glucose) and pentoses (mainly xylose) from cellulose and hemicelluloses through various methods such as chemical, biological, and physical methods. Enzymes or chemical reagents serve as catalysts in the combination of physical procedures with biological or chemical methods. Chemical reagents include alkalis like sodium hydroxide and acids like sulfuric and hydrochloric acids (*24*, *29*, *30*, *43*, *44*). The advantages and disadvantages of these methods are delineated; chemical methods are preferred for hydrolysis. While enzymatic hydrolysis is environmentally friendly, it remains costly due to the high expense of enzymes. Conversely, chemical methods yield higher sugar quantities in shorter reaction times, albeit necessitating corrosion-resistant reaction vessels. Nuwamanya et al. (2012) employed hydrolysis methods involving enzymes, dilute, or concentrated chemical reagents (such as NaOH and HCl) to extract sugar from non-food parts of cassava (untreated) (*30*). They reported a 47% sugar recovery through the enzymatic method and a 56% recovery through either NaOH or HCl hydrolysis.

Acid hydrolysis surpasses alkali hydrolysis in reducing the amount of xylose produced from hemicellulose. Lignocellulosic materials contain significant amounts of xylose, and releasing these fermentable sugars can enhance biohydrogen production, rendering it more economically feasible to derive biohydrogen from lignocellulose. Several studies have employed the two-stage sulfuric acid hydrolysis method to achieve a high yield of sugars from lignocellulosic materials. For instance, in a study on cassava stem hydrolysis, complete hydrolysis was attained at a dosage of 20 g CS $L^{-1}$, with glucan and xylan hydrolysis slightly reduced at higher dosages of 100 g $CS{\cdot}L^{-1}$ and 200 g $CS{\cdot}L^{-1}$. However, as the photocatalytic process performs optimally at a slightly higher pH, a mildly basic environment is more advantageous for biohydrogen production via photocatalytic reforming. Alkali hydrolysis may prove beneficial in this scenario to adjust pH. Despite significant efforts to comprehend the mechanistic and kinetic aspects of lignocellulosic-derived glucose and xylose hydrolysis, further research is necessary to implement this technology on a large scale. Nonetheless, numerous techniques, including steam reforming, water electrolysis, materials and catalyst development, integration with renewable energy, and hydrogen storage, can be utilized to efficiently produce hydrogen from waste. Various obstacles hinder the mass production of hydrogen in rural areas, including sustainability and cost concerns. However, hydrogen production can be accomplished using various treatment methods, including microbial electrolysis cells, biomass gasification, water electrolysis, and bio-digestion. In rural settings, these treatments can establish a more sustainable and energy-efficient hydrogen manufacturing system by leveraging locally available resources and renewable energy sources.

### 3.2. Photofermentation

Optimization and maintenance of strict environmental conditions are necessary for photofermentation, including the type of light source, light intensity, and lighting regime. The composition of the fermentation media also plays a crucial role, with the addition of elements such as Fe and Mo improving hydrogen yield (*45*–*49*). Nitrogenase activity is responsible for mediating hydrogen evolution by photosynthetic bacteria. Purple nonsulfur bacteria, such as *Rhodobactersphaeroides, Rhodobactercapsulatus, and Rhodospirillum rubrum* (*49*, *50*), are the primary microorganisms involved in hydrogen production through photofermentation. Organic acids like acetate, butyrate, and lactate serve as the main substrates for this processas shown in the eqs. i-vi (*50*, *51*, *52*, *53*–*63*), and a wide range of effluents rich in organic acids can be utilized. However, it is important to note that despite the potential for high yields, low light conversion efficiency (3-10%) and production volumes can pose challenges in photofermentation. Previous studies have reported hydrogen generation rates ranging from 145-160 mmol/h/L (*53*). Additionally, certain chemicals such as iron, molybdenum, EDTA, vitamins, and buffer solutions have been found to have significant positive effects on biohydrogen production rates (*51*–*54*). Table 4 provides an overview of hydrogen yields achieved through photofermentation in the literature.

$$\text{Butyric acid } CH_3CH_2CH_2COOH + 6H_2O \longrightarrow 10H_2 + 4CO_2 \quad \text{(i)}$$

$$\text{Propionic acid } CH_3CH_2COOH + 4H_2O \longrightarrow 7H_2 + 3CO_2 \quad \text{(ii)}$$

$$\text{Malic acid } COOHCH_2CHOHCOOH + 3H_2O \longrightarrow 6H_2 + 4CO_2 \quad \text{(iii)}$$

$$\text{Succinic acid } COOHCH_2CH_2COOH + 4H_2O \longrightarrow 7H_2 + 4CO_2 \quad \text{(iv)}$$

$$\text{Lactic acid } CH_3CHOHCOOH + 3H_2O \longrightarrow 6H_2 + 3CO_2 \quad \text{(v)}$$

$$\text{Acetic acid } CH_3COOH + 2H_2O \xrightarrow{\hspace{3cm}} 4H_2 + 2CO_2 \quad \text{(vi)}$$

**Table 4. Hydrogen Yields Obtained by Photofermentation**

| *Feedstock* | *Culture* | *$H_2$ Yield (mol $H_2$/mol glucose equiv)* | *Refs* |
|---|---|---|---|
| **Corncob** | *Rhodospirillum rubrum*, | 229 mmol $H_2$/L | (*56*) |
| **Sorghum stover** | *Rhodobacter capsulatus*, | 149.67 | |
| **Corn stover** | *Rhodopseudomonas palustris* | 145.67 | |
| **Rice straw** | | 140.45 | |
| **Soybean stalk** | | 131.12 | |
| **Cotton stalk** | | 118.46 | |
| **Wheat straw** | *R. capsulatus*—PK | 372 mL $H_2$/L | (*57*) |
| **Cassava wastewater** | Mixed bacterial consortium | 36.1 mmol $H_2$/L culture | (*58*) |
| **Combined palm oil plus pulp and paper mill effluent** | *Rhodobacter sphaeroides* NCIMB 8253 | 8.72 mL $H_2$/mL medium | (*59*) |
| **Sugar beet molasses** | *R. capsulatus* JP91 | 10.5 mol $H_2$/mol sucrose | (*60*) |
| **Corn stalk pith** | Mixed bacterial consortium | 2.61 mol $H_2$/mol glucose | (*61*) |

### 3.3. Dark Fermentation

Fermentation stands out as one of the most effective techniques for sustainable biohydrogen production, with dark fermentation being extensively researched. According to Nandi and Sengupta (*63*), studies on anaerobic bacterial hydrogen generation initially emerged in the 1980s. Dark fermentation holds precedence over photosynthetic processes due to its simplicity and higher production rates, as emphasized by Chong et al. (2009) (*64*). Glucose, the primary compound in lignocellulosic biomass, can undergo conversion into hydrogen. Theoretically, the conversion of 1 mole of glucose ($C_6H_{12}O_6$) yields 12 moles of hydrogen. As noted by Ntaikou et al. (2010) (*65*) and Sveinsdottir et al. (2011) (*66*), the fermentation pathway and the resultant products determine the actual yield. Acetate and butyrate constitute over 80% of the end-products, as reported by Balat and Kırtay (2010) (*47*). Other fermentation by-products include ethanol and lactic acid, which do not contribute to hydrogen production, and propionic acid, which consumes hydrogen (*49*). For instance, if acetic acid and butyric acid are the final products, the theoretical yield of hydrogen can be calculated using Equations (vii) and (viii), respectively.

$$C_6H_{12}O6 + 4H_2O \xrightarrow{\hspace{3cm}} 2CH_3COO^- + 2HCO_3 + 4H_2 + 4H^+ \quad \text{(vii)}$$

$$C_6H_{12}O_6 + 2H_2O \xrightarrow{\hspace{3cm}} CH_3CH_2CH_2COO^- + 2HCO_3 + 2H_2 + 3H^+ \quad \text{(viii)}$$

However, the yield of hydrogen from 1 mol of glucose is achieved at approximately 2.0-2.5 mol, which is lower than the expected theoretical yield (*67*). This lower yield can be attributed to various factors such as the production of a mixture of acetate and butyrate, which contributes to the hydrogen yield, the production of non-hydrogen forming or hydrogen-consuming end-products, or the utilization of the feedstock substrate for microbial growth instead of organic acid formation (*49*).

**Table 5. Hydrogen Yields Obtained by Dark Fermentation**

| *Feedstock* | *Culture* | *$H_2$ Yield (mol $H_2$/mol glucose equiv)* | *Refs* |
|---|---|---|---|
| **Corn stover** | *Thermoanaerobacterium thermosaccharolyticum*<br>Mixed | 2.24<br>1.53 | (*69*, *70*) |
| **Napier grass** | Mixed | 1.2 | (*71*) |
| **Wheat straw** | *Caldicellulosiruptor saccharolyticus*<br>Mixed<br>*Clostridium* sp. IODB03<br>*Bacillus coagulans* NCIM 2323 and *Enterobacter aerogens* NCIM5139 | 3.8<br>1.0–2.54<br>2.52 mol/mol sugar<br>0.23–1.40 mol/mol glucose | (*72*)<br>(*73*)<br>(*74*)<br>(*75*) |
| **Barley hulls** | *Clostridium thermocellum* | 1.24 | (*76*) |
| **Grass** | *Clostridium AK14* | 0.8–0.9 | (*77*) |
| **Food waste** | Mixed | 0.6–2.4 | (*78*) |
| **Vegetable waste** | Mixed | 1.7 | (*79*) |
| ***Miscanthus*** | *Thermotoga elfii*<br>*T. neapolitana*<br>*Caldicellulosiruptor saccharolyticus* | 1.1<br>3.2<br>3.4 | (*80*)<br>(*81*) |
| **Sweet sorghum stalk** | *Rumicococcus albus* | 3.15 | (*65*) |
| **Bagasse** | Mixed | 13.39 | (*82*) |
| **Maize leaves** | *C. saccharolyticus* | 3.6 | (*72*) |
| **Rice straw** | Heat—treated sludge | 0.44 mol/mol sugar | (*83*) |
| **Soybean straw** | *Clostridium buytricum* | 47.65 mL/g substrate | (*84*) |

Various microbes, including species like Enterobacter, Thermoanaerobacterium, and Thermotoga, are capable of conducting the fermentation process. The substrate, pH, and temperature requirements of these microbes vary, leading to alterations in the metabolic pathways they undertake and the resulting hydrogen yields (*65*). Studies on fermentation processes have revealed that extremophilic bacteria, thriving in high-temperature conditions, may produce larger quantities of hydrogen. In pure cultures, these bacteria can generate up to 4 mol of hydrogen and 2 mol of acetate. Their ability to tolerate increased hydrogen partial pressure, exhibit enhanced reaction kinetics at high temperatures, and display reduced susceptibility to pollutants contribute to this phenomenon. Conversely, mesophilic and moderate thermophilic bacteria produce fewer ethanol, lactic acid, or butyric acid byproducts when exposed to high hydrogen concentrations (*66*).

Dark fermentation, involving anaerobic bacteria converting organic matter into hydrogen, also yields carbon dioxide, organic acids, and solvents as by-products. These by-products accumulate in the medium since anaerobes cannot utilize them, causing a decline in medium pH and impeding the achievement of maximum theoretically possible hydrogen yields (*68*). Furthermore, by-products generated during initial biomass pretreatment, such as furfural and HMF, have been found to inhibit microbial growth (*66*). Numerous investigations have explored the potential of hydrogen production via fermentation. As outlined in Table 5, various pretreatment methods have been explored to obtain hydrogen yields from diverse lignocellulosic biomass sources. However, the organic load in the substrate persists during dark fermentation, reducing hydrogen yields as a substantial amount of hydrogen is trapped in these molecules. Techniques such as implementing a second stage for hydrogen extraction or producing high-value products from effluents, like polyhydroxyalkaonates, can harness residual organic matter in the medium, increasing energy output and economic viability.

These techniques facilitate hydrogen generation from various types of cellulosic biomass; however, achieving a high yield remains a challenging aspect due to the inherently low efficiency of biomass-based cellulose fermentation. Therefore, in addition to pretreatment techniques, the effective fermentation of waste biomass-based cellulose necessitates the use of selective bacteria with genetic modifications.

## 4. Summary and Future Prospects

In summary, biohydrogen stands out as a promising alternative to fossil fuel-based hydrogen, presenting a sustainable energy option with zero carbon emissions. Lignocellulose-derived monosugars have garnered recognition as a valuable feedstock in biohydrogen production. A comprehensive review has provided an overview of current material treatment approaches for biohydrogen production from lignocellulose, successfully assessing various pretreatment procedures, including hydrolysis and detoxification methods, while microbial fermentation emerges as the most environmentally friendly post-treatment method. A thorough understanding of suitable biomass lignocellulose, pretreatment procedures, and post-methods, along with the optimization of operating parameters, is crucial to enhance conversion efficiencies and reduce costs. Therefore, continuous extensive research is critically important to overcome limitations and enhance the productivity of biohydrogen.

## References

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