Biological Hydrogen Production Methods

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Abstract

As a sustainable energy source, hydrogen is a promising alternative to fosil fuels. It is a clean and environmentally friendly fuel. Currently, most hydrogen is produced by electrolysis of water and by steam reformation of natural gas. But, biological production of hydrogen has significant advantages over thermochemical and electrochemical. Hydrogen can be produced biologically by biophotolysis (direct and indirect), photo fermentation, dark fermentation, combination of dark and photo fermentation and biocatalyzed electrolysis. In this study, biological hydrogen production methods have been investigated.

Keywords: Biological hydrogen, biophotolysis, photofermentation, dark fermentation.

1. Introduction

Today global energy requirements are mostly dependent on fossil fuels (about 80% of the present world energy demand). This will eventually lead to the foreseeable depletion of limited fossil energy resources. Presently, the utilization of fossil fuels are causing global climate change mainly due to the emission of pollutants like CO_x, NO_x, SO_x, C_xH_x, soot, ash, droplets of tars and other organic compounds, which are released into the atmosphere as a result of their combustion [1]. Hydrogen has the highest energy content per unit weight of any known fuel and can be transported for domestic/industrial consumption through conventional means. H_2 gas is safer to handle than domestic natural gas. H_2 is now universally accepted as an environmentally safe, renewable energy resource and an ideal alternative to fossil fuels that doesn't contribute to the greenhouse effect. The only carbon-free fuel, H_2 upon oxidation produces water alone. H_2 can be used either as the fuel for direct combustion in an internal combustion engine or as the fuel for a fuel cell. The largest users of H_2, however, are the fertilizer and petroleum industries with, respectively, 50% and 37%. Sales of H_2 have increased by 6% annually in the last five years, which is closely related to the increased use of H_2 in refineries as a result of stricter standards for fuel quality [2].

At present hydrogen is produced mainly from fossil fuels, biomass and water. The methods of hydrogen production from fossil fuels are

(a) Steam reforming of natural gas.
(b) Thermal cracking of natural gas.
(c) Partial oxidation of heavier than naphtha hydrocarbons.
(d) Coal gassification.

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Methods of hydrogen production from biomass are

(e) Pyrolysis or gassification (which produces a mixture of gases, i.e., H_2; CH_4; CO_2; CO; N_2).

Methods of hydrogen production from water are

(f) Electrolysis.
(g) Photolysis.
(h) Thermochemical process.
(i) Direct thermal decomposition or thermolysis.
(j) Biological production.

Conventionally hydrogen is produced from natural gas by steam reforming. Other industrial methods are coal gasification and water electrolysis. However, these methods use non-renewable energy sources to produce hydrogen and are not sustainable. Therefore, it is necessary to explore hydrogen production from renewable energy sources. Processes for biological hydrogen production mostly operate at ambient temperatures and pressures, and are expected to be less energy intensive than thermochemical methods of hydrogen production. These processes can use a variety of feedstocks as carbon sources. Waste materials can also be used as a carbon source which facilitates waste recycling [3]. However, the rate of H_2 production is low and the technology for this process needs further development. Production of clean energy source and utilization of waste materials make biological hydrogen production a novel and promising approach to meet the increasing energy needs as a substitute for fossil fuels [4]. In this study, biological production methods are reviewed.

2. Biological Hydrogen Production Methods

Biological hydrogen production methods can be classificated as below:
2.1. Direct biophotolysis
2.2. Indirect biophotolysis
2.3. Photo fermentation
2.4. Dark fermentation
2.5. Two stage process (integration of dark and photo fermentation)
2.6. Biocatalyzed electrolysis

2.1. Direct biophotolysis

This method is similar to the processes found in plants and algal photosynthesis. In this process solar energy is directly converted to hydrogen via photosynthetic reactions (Eq. (1)).

$$2H_2O + \text{‘light energy’} \rightarrow 2H_2 + O_2. \quad (1)$$

Algae split water molecules to hydrogen ion and oxygen via photosynthesis. The generated hydrogen ions are converted into hydrogen gas by hydrogenase enzyme. *Chlamydomonas reinhardtii* is one of the well-known hydrogen producing algae [4]. Hydrogenase activity has also

been observed in other green algae like *Scenedesmus obliquus*, *Chlorococcum littorale*, *Platymonas subcordiformis* and *Chlorella fusca* [2].

The advantage of this method is that the primary feed is water, which is inexpensive and available almost everywhere (Table 1).

A direct biophotolysis method must perforce operate at a partial pressure of near one atmosphere of O_2, which is a thousand-fold greater than the maximum likely to be tolerated. Thus, the O_2 sensitivity of the hydrogenase enzyme reaction and supporting reductant generating pathway remains the key problem, as it has been for the past 30 years [5].

Hydrogen production by direct photolysis using green algae is currently limited by three parameters: (i) solar conversion effciency of the photosynthetic apparatus; (ii) H_2 synthesis processes (i.e. the need to separate the processes of H_2O oxidation from H_2 synthesis); and (iii) bioreactor design and cost. A number of approaches to improve H_2 production by green algae are currently under investigation. These include genetic engineering of light gathering antennae, optimization of light input into photobioreactors, and improvements to the two-phase H_2 production systems used with green algae [6]. In direct biophotolysis, hydrogen production rates of the order of 0.07 mmol/h per liter has been reported in the literature (Table 2) [7,8].

2.2. Indirect Biophotolysis

In indirect biophotolysis, problems of sensitivity of the hydrogen evolving process are potentially circumvented by separating temporally and/or spatially oxygen evolution and hydrogen evolution. Thus indirect biophotolysis processes involve separation of the H_2 and O_2 evolution reactions into separate stages, coupled through CO_2 fixation/evolution. Cyanobacteria have the unique characteristics of using CO_2 in the air as a carbon source and solar energy as an energy source (Eq. (2)). The cells take up CO_2 first to produce cellular substances, which are subsequently used for hydrogen production (Eq. (3)). The overall mechanism of hydrogen production in cyanobacteria can be represented by the following reactions:

$$12H_2O + 6CO_2 + \text{‘light energy’} \rightarrow C_6H_{12}O_6 + 6O_2 \quad (2)$$

$$C_6H_{12}O_6 + 12H_2O + \text{‘light energy’} \rightarrow 12H_2 + 6CO_2 \quad (3)$$

Cyanobacteria possess key enzymes (nitrogenase and hydrogenase) that carry out metabolic functions in order to achieve hydrogen generation [9]. Because of the higher rates of H_2 production by *Anabaena* species and strains, these have been subject to intense study [6]. In indirect biophotolysis mutant strains of *A. Variabilis* have demonstrated hydrogen production rate of the order of 0.355 mmol/h per liter [10].

2.3. Photo fermentation

H_2 production by purple non-sulfur bacteria is mainly due to the presence of nitrogenase under nitrogen-deficient conditions using light energy and reduced compounds (organic acids). The reaction is as follows (Eq. (4)) [2]:

$$CH_3COOH + 2H_2O + \text{'light energy'} \rightarrow 4H_2 + 2CO_2 \quad (4)$$

Photosynthetic bacteria have long been studied for their capacity to produce significant amounts of hydrogen. The advantage of this method are that oxygen does not inhibit the process [2]. These photoheterotrophic bacteria have been found suitable to convert light energy into H_2 using organic wastes as substrate [11,12,13] in batch processes [14], continuous cultures [15], or immobilized whole cell system using different solid matrices like carrageenan [16], agar gel [17], porous glass [11], and polyurethane foam [12]. The disadvantages are the limited availability of organic acids, the nitrogenase enzyme is slow, the process requires a relatively high amount of energy, and hydrogen re-oxidation [18,19]. To increase the nitrogenase activity and decrease the energy requirements, the proper ratio of carbon to nitrogen nutrients must be maintained [2]. Another major factor affecting the photo-fermentation process is light intensity. Although an increase in light intensity has shown some stimulatory effect on the overall hydrogen production rate of photosynthetic micro-organisms, an adverse effect was also reported on their light conversion efficiency at high light intensities.[20,21] However, the light conversion efficiency can be improved by genetic manipulation of the light-harvesting antennae, thereby reducing the saturation effect of light.[22,23]. Hydrogen production rates of the order of 145–160 mmol/h per liter by this methods have been reported [6,11].

Certain photoheterotrophic bacteria within the superfamily Rhodospirillaceae can grow in the dark using CO as the sole carbon source to generate ATP with the simultaneous release of H_2 and CO_2 [24]. The oxidation of CO to CO_2 with the release of H_2 occurs via a water gas shift reaction as shown below (Eq. (5)):

$$CO + H_2O \rightarrow CO_2 + H_2 \quad (5)$$

2.4. Dark fermentation

Hydrogen can be produced by anaerobic bacteria, grown in the dark on carbohydrate-rich substrates. Bacteria known to produce hydrogen include species of *Enterobacter*, *Bacillus*, and *Clostridium* [6]. Carbohydrates, mainly glucose, are the preferred carbon sources for fermentation processes, which predominantly give rise to acetic and butyric acids together with hydrogen gas [25]. Theoretically bioconversion of 1 mol of glucose yields 12 mol of hydrogen gas (H_2). According to reaction stoichiometry, bioconversion of 1 mol of glucose into acetate yields 4 mol H_2/mol glucose (Eq. (6)), but only 2 molH_2/mol glucose is formed when butyrate is the end product (Eq. (7)) [4]. Currently fermentative processes produce 2.4 to 3.2 moles of hydrogen per mole glucose [26].

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (6)$$
$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3CH_2COOH + 2CO_2 + 2H_2 \quad (7)$$

While direct and indirect photolysis systems produce pure H_2, dark fermentation processes produce a mixed biogas containing primarily H_2 and carbon dioxide (CO_2), but which may also contain lesser amounts of methane (CH_4), CO, and/or hydrogen sulfide (H_2S). Dark-fermentation proves to be superior over photo-fermentation as this requires no light and the energy produced is

relatively higher, due to the fermentation of sugar and carbohydrates. The process is initiated by the hydrolysis of organic polymers to monomers, thereafter acetogenic conversion of monomers to organic acids, alcohols, and release of hydrogen. Although biohydrogen production by dark-fermentation is promising and advantageous over photo-fermentation [27]. However, the requirement of organic biomass as a feedstock makes this process quite expensive [28]. Hydrogen production by these bacteria is highly dependent on the process conditionss such as pH, hydraulic retention time (HRT), and gas partial pressure, which affect metabolic balance. The partial pressure of H_2 (pH_2) is an extremely important factor for continuous H_2 synthesis. Hydrogen synthesis pathways are sensitive to H_2 concentrations and are subject to end-product inhibition. As H_2 concentrations increase, H_2 synthesis decreases [6]. Sugars and carbohydrate rich biomass are reported to be the most suitable feedstock for the formation of biohydrogen from darkfermentation [29]. In laboratory experiments, hidrogen production rates of the order of 21 mmol/l-h [30], 64.5 mmol/l-h [31], 121 mmol/l-h [32], 8.2 mmol/l-h [33] and 2.7-8.4 mmol/l-h [34,35] have been achieved.

2.5. Two stage process with integration of dark and photo fermentation

In fermentation, complete oxidation of 1 mole of glucose yields 12 moles of hydrogen. However, complete oxidation of glucose into hydrogen and carbon dioxide is not possible as the corresponding reaction is not feasible thermodynamically (Eq. (8)).

$$C_6H_{12}O_6 + 6H_2O \rightarrow 12H_2 + 6CO_2, \quad (8)$$

With external energy supply (photon-energy in photofermentation) theoretically 12 moles of hydrogen per mole of glucose can be produced. However this process cannot be operated in the absence of light. On the other hand, in the absence of external energy (in the case of dark-fermentation), oxidation of glucose by fermentative bacteria results in other by-products also and maximum 4 moles of hydrogen are produced per mole of glucose consumption (Eq. (9)) with acetate as the sole by-product.

$$C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CO_2 + 2CH_3COOH \quad (9)$$

Acetate produced in the dark-fermentation stage can be oxidized by photosynthetic bacteria to produce hydrogen (Eq. (10)).

$$CH_3COOH + 2H_2O + \text{‘light energy’} \rightarrow 4H_2 + 2CO_2 \quad (10)$$

Hence continuous production of hydrogen at maximum yield can be achieved by integrating dark- and photo-fermentation methods. Hydrogen production rates obtained in this method were 47.92 mmol/l-h [36] and 51.20 mmol/l-h [25].

2.6. Biocatalyzed electrolysis

Another way of oxidizing the acetate (or the effluent of dark fermentation process) to produce hydrogen is to provide external energy (in Eq. (10)) in the form of electrical energy instead of solar energy.

In this approach, the bioreactor containing acetate forms the anodic compartment of an electrolyzer cell and protons and electrons produced by bacteria (Eq. (11)) are collected at cathode (a platinum electrode catalyzing hydrogen evolution reaction). Anodic and cathodic reactions are as follows:

$$\text{Anot: } 2CH_3COOH+2H_2O \rightarrow 2CO_2 +8H^+ +8e^- \quad (11)$$

$$\text{Katot: } 8H^+ + 8e^- \rightarrow 4H_2 \quad (12)$$

From Eqs. (11) and (12), it can be concluded that an external supply of around 100 mV is required to produce hydrogen at cathode. However, because of over-potentials at the electrodes a voltage higher than 100 mV is required to produce hydrogen. In this method, it was obtained the yield %73 H_2 per mole of acetate at an external supply of 250 mV [37] and the yield of 53 ± 3.5% with acetate at an external supply of 500 mV [38].

Table 1. Advantages and disadvantages of different hydrogen production processes [2].

Process	Advantages	Disadvantages
Direct biophotolysis	Can produce H_2 directly from water and sunlight Solar conversion energy increased by ten folds as compared to trees, crops	Requires high intensity of light O_2 can be dangerous for the system Lower photochemical efficiency
Indirect biophotolysis	Cyanobacteria can produce H_2 from water Has the ability to fix N_2 from atmosphere	Uptake hydrogenase enzymes are to be removed to stop degradation of H_2 About 30% O_2 present in gas mixture
Photo-fermentation	A wide spectral light energy can be used by these bacteria Can use different organic wastes	O_2 has an inhibitory effect on nitrogenase Light conversion efficiency is very low, only 1–5%
Dark fermentation	It can produce H_2 all day long without light A variety of carbon sources can be used as substrates It produces valuable metabolites such as butyric, lactic and acetic acids as by products It is anaerobic process, so there is no O_2 limitation problem	O_2 is a strong inhibitor of hydrogenase Relatively lower achievable yields of H_2 As yields increase H_2 fermentation becomes thermodynamically unfavorable Product gas mixture contains CO_2 which has to be separated

Table 2. Hydrogen production rates in biohydrogen production processes

Method	Hidrojen production rate (mmol/l-h)	Reference
Direct biophotolysis	0.07	[7,8]
Indirect biophotolysis	0.355	[10]
Photo-fermentation	145-160	[6,11]
Dark fermentation	21 64.5 121.0 8.2 2.7-8.4	[30] [31] [32] [33] [34,35]
Integration of dark and photo-fermentation	51.20 47.92	[25] [36]

3. Conclusions

Heavy dependence on fossil fuels has caused growing environmental concerns worldwide due to the release of carbon dioxide in the atmosphere resulting in global warming. Hydrogen production through biological processes exemplifies a promising area for bioenergy generation due to its clean, recyclable and high efficient nature. Existing technologies offer potential for practical application, but if biohydrogen systems are to become commercially competitive they must be able to synthesize H_2 at rates that are sufficient to power fuel cells of sufficient size to do practical work. Further research and development aimed at increasing rates of synthesis and final yields of H_2 are essential. If the technological potential of hydrogen is realized, it will contribute to the sustainable growth of the world economy by facilitating a stable supply of energy and by helping to reduce future emissions of greenhouse gases.

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