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Some considerations on fermentative biohydrogen production

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Abstract: Biohydrogen production has been established as a prospective alternative and integral component of green sustainable energy. A challenging problem in making biohydrogen production a more economically feasible is the generation of large quantities of hydrogen gas. Compared with photosynthetic hydrogen production, fermentative hydrogen production has higher hydrogen production efficiency, higher hydrogen production stability, and higher feasibility for industrialization, simpler control requirement and lower operating costs. This method of hydrogen production becomes more economically viable when the process is combined with dark fermentation developed in the fermentation tanks used in high organic load waste water treatment processes.

Keywords: biohydrogen, dark fermentation, photo-fermentation, biophotolysis, anaerobic metabolism

1. INTRODUCTION

Hydrogen is the simplest element and also the most plentiful gas in the universe. Because hydrogen gas (H_2) is lighter than air and, as a result, it rises in the atmosphere, it is not found by itself on earth but only in compound form with other elements. Hydrogen combined with carbon, forms different compounds such as methane (CH_4), coal, and petroleum [1]. Hydrogen is also found in all growing things-biomass [2, 3]. It has the highest energy content per unit weight of any known fuel (142 kJ/g or 61,000 Btu/lb) and can be transported for domestic/industrial consumption through conventional means [4, 5, 6]. H_2 gas is safer to handle than domestic natural gas and is universally accepted as an environmentally safe, renewable energy resource and an ideal alternative to fossil fuels that does not contribute to the greenhouse effect [7, 8, 9]. Hydrogen satisfies the above requirements because it produces only water, when it is combusted as a fuel or converted to electricity. Its use in fuel cells is inherently more efficient than the combustion currently required for the conversion of other potential fuels to mechanical energy. Hydrogen breakdown generates no pollutants; unlike ethanol for example, whose large scale use is predicted to release large amounts of carcinogenic acetaldehyde with the generation of large amounts of smog. All presently studied biofuels are largely carbon neutral since the carbon released by their combustion is derived, directly or indirectly, from recently fixed atmospheric CO_2. However, the emitted carbon associated with hydrogen produced by microbial fermentation is released during the production of this biofuel rather than during its utilization, thus potentially permitting easy capture of CO2 and sequestration. Thus, in this scenario, biological hydrogen production could even be carbon negative [10].

2. HYDROGEN PRODUCTION TECHNOLOGIES

Presently, 40% H_2 is produced from natural gas, 30% from heavy oils and naphtha, 18% from coal, and 4% from electrolysis and about 1% is produced from biomass [11]. Thus, currently H_2 is produced, almost exclusively, by steam reformation of methane or by water electrolysis. Biological production of H_2 (biohydrogen), using microorganisms, is an area of technology development that offers the potential production of renewable H_2 from biomass [12].

Conventional steam methane reforming (SMR) processes use steam at high temperatures (800ºC) to react with the methane (CH_4), which forms H_2 and carbon monoxide (CO); see reaction (1), below. The CO further reacts with vaporized water to form carbon dioxide (CO_2), a greenhouse gas, and additional H_2 (reaction (2)).

$$CH_4 + H_2O \rightarrow CO + 3H_2 \qquad (1)$$

(Reforming reaction)

$$CO + H_2O \rightarrow CO_2 + H_2 \qquad (2)$$

(Water–Gas Shift reaction)

Hydrogen may also be produced by steam or aqueous reforming of various oxygenated hydrocarbons, such as methanol, ethanol, glycerol, or glucose using a wide variety of temperatures and catalysts. The catalytic pathway for the production of H_2 and CO_2 by aqueous-phase reforming of oxygenated hydrocarbons involves cleavage of C–C bonds, as well as C–H and/or O–H bonds to form adsorbed species on the catalyst surface. Oxygenated hydrocarbons can be reformed in the aqueous phase in the presence of a platinum-based catalyst to produce H_2 gas [13, 14]. The conversion takes place at

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moderate temperatures, around 225–265ºC, and at pressures of 27–54 bar, conditions that prevent steam formation and ensure that the reaction sequence takes place in the aqueous phase.

Production of H_2 by water electrolysis relies on passing an electric current through a conductive electrolyte in water (alkaline or polymeric), which results in splitting water molecules into H_2 and oxygen (O_2). Hydrogen produced by electrolysis of water is of relatively high quality, as no carbon, sulphur, or nitrogenous compounds are generated in the process. Purification costs for fuel cell grade H_2 are thus much less than for SMR. Electrolysis by itself is emission free, but lifecycle emissions are a direct function of the source of the electricity used in the process.

In case of hydrogen production by biomass gasification, traditional gasification reactors generate a complex mixture of gases, called syn-gas, including CO, CO_2, CH_4, and small amounts of longer chain hydrocarbons. A key problem with gasification is how to separate and purify the H_2 from the other gases in the syn-gas. Ishida et al. [15] propose an innovative method for the synthesis of H_2 without CO or CO_2 for fuel cells through the reactions of biomasses, alkali metal hydroxides and water vapor at relatively low temperatures (200-350ºC) under atmospheric pressure. The main reaction for this new method can be expressed as:

$$\underset{\text{Cellulose}}{C_6H_{12}O_6} + \underset{\text{Alkali}}{12NaOH} + \underset{\text{Vapor}}{H_2O} \rightarrow \underset{\text{Sodium carbonate}}{6Na_2CO_3} + 12H_2 \qquad (3)$$

The reactions of cellulose with sodium hydroxide (NaOH) and water vapor were carried out with a conventional mass controlled gas flow system under atmospheric pressure. The use of nickel (Ni), cobalt (Co), rodium (Rh), or ruthenium (Ru) catalysts supported on aluminum oxide (Al_2O_3) specifically enhanced the formation of H_2. The total yields of H_2 produced through the reactions over these catalysts at 100-500ºC were increased from 62% to almost 100%.

Supercritical water partial oxidation is a process that occurs in water at temperatures and pressures above a mixture's critical point [16]. Under these conditions water becomes a fluid with unique properties that can be used to advantage in the destruction of hazardous wastes such as PCBs, or for the production of H_2 [17], although little data for hydrogen via this process are available. The fluid has a density between that of water vapor and liquid at standard conditions, and exhibits high gas-like diffusion rates along with high liquid-like collision rates. Efficient oxidation reactions occur at low temperature (400-650ºC) with reduced NOx production.

3. BIOLOGICAL HYDROGEN PRODUCTION

Among various hydrogen production processes, biological method is known to be less energy intensive, for it can be carried out at ambient temperature and pressure [18, 19]. Furthermore, these techniques are well suited for decentralized energy production in small-scale installations in locations where biomass or wastes are available, thus avoiding energy expenditure and costs for transport [20].

Biohydrogen technologies provide a wide range of approaches to generate hydrogen, including direct and indirect photolysis, photo-fermentation, and dark-fermentation [21, 6].

3.1. BIOPHOTOLYSIS OF WATER USING GREEN ALGAE AND BLUE-GREEN ALGAE (CYANOBACTERIA)

Green algae and blue-green algae split water molecules into hydrogen ion and oxygen via direct and indirect biophotolysis.

3.1.1. DIRECT BIOPHOTOLYSIS

The conversion of water to hydrogen by green algae may be represented by the following general reaction:

$$2H_2O + \text{lightenergy} \rightarrow 2H_2 + O_2 \qquad (4)$$

The well-known H_2-producing green algae, *Chlamydomonas reinhardtii*, under anaerobic conditions, can either generate H_2 or use H_2 as an electron donor [22]. The generated hydrogen ions are converted into hydrogen gas in the medium with electrons (donated by reduced ferredoxin) by hydrogenase enzyme present in the cells. This enzyme is very sensitive to O_2. Hydrogenase activity has also been observed in other green algae like *Scenedesmus obliquus* [22], *Chlorococcum littorale* [23] *Platymonas subcordiformis* [23] and *Chlorella fusca* [22]. On the other hand, there are several green algae types that do not have hydrogenase activity such as *Dunaliella salina* and *Chlorella vulgaris* [23].

3.1.2. INDIRECT BIOPHOTOLYSIS

The general reaction for hydrogen formation from water by cyanobacteria can be represented by following reactions:

$$12H_2O + 6CO_2 + \text{lightenergy} \rightarrow C_6H_{12}O_6 + 6O_2 \qquad (5)$$

And

$$C_6H_{12}O_6 + 12H_2O + \text{lightenergy} \rightarrow 12H_2 + 6CO_2 \qquad (6)$$

Cyanobacteria are also known as blue-green algae, cyanophyceae or cyanophytes. It is a large and diverse group of photoautotrophic microorganism. Cyanobacteria contain photosynthetic pigments, such as chl a, carotenoids and phycobiliproteins, and can perform oxygenic photosynthesis. Hydrogen is produced both by hydrogenase and nitrogenase enzymes. The nutritional requirements of cyanobacteria are simple: air (N_2 and O_2), water, mineral salts and light. Hydrogen producing cyanobacteria may be either nitrogen fixing or non-nitrogen fixing. The examples of nitrogen fixing organisms are non-marine *Anabaena sp*., marine cyanobacteria *Calothrix sp., Oscillatoria sp*. Non-nitrogen fixing organisms are *Synechococcus sp., Gloebacter sp.* and *Anabaena sp.* They are found suitable for higher hydrogen evolution as compared to other cyanobacteria species [21, 22, 23, 24].

In recent years, some species of *Anabena* genus had received more attention regarding hydrogen production [23, 25]. The growth conditions for *Anabaena* are simple which include nitrogen free media, illumination, CO_2 and N_2. Nitrogenase plays important role for the hydrogen generation. Activity of the nitrogenase is inhibited by oxygen. Hydrogen production takes place under anaerobic conditions. Some cultures require CO_2 during hydrogen evolution phase, although CO_2 is reported to give some inhibition effects on photo-production of H_2. Lower CO_2 concentrations (4–18% w/v) have been reported to increase cell density during growth phase, resulting in higher hydrogen evolution in the later stage. Simple sugars have been found suitable for hydrogen production. Recently more emphasis has been given to increase hydrogenase activity and bidirectional hydrogenase deficient mutants of *Anabaena sp*. to increase the rate of hydrogen production. However, at the present time the rate of hydrogen production by Anabaena sp. is considerably lower than that obtained by dark or photo-fermentations [21, 22, 23, 24, 25].

Even though photosynthetic hydrogen production is a theoretically perfect process with transforming solar energy into hydrogen by photosynthetic bacteria, applying it to practice is difficult due to the low utilization efficiency of light and difficulties in designing the reactors for hydrogen production [8, 9, 18, 19, 26, 27, 28].

3.2. PHOTODECOMPOSITION OF ORGANIC COMPOUNDS BY PHOTOSYNTHETIC BACTERIA

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:

$$CH_3COOH + 2H_2O + \text{lightenergy} \rightarrow 4H_2 + 2CO_2 \quad (7)$$

Photosynthetic bacteria have long been studied for their capacity to produce significant amounts of hydrogen [29]. The advantage of their use is in the versatile metabolic capabilities of these organisms and the lack of Photosystem II (PSII), which automatically eliminates the difficulties associated with O_2 inhibition of H_2 production. Phototrophic bacteria require organic or inorganic electron source to drive their photosynthesis. They can utilize a wide range of cheap compounds. These photoheterotrophic bacteria have been found suitable to convert light energy into H_2 using organic wastes as substrate [29, 30, 31] in batch processes [32], continuous cultures [33], or immobilized whole cell system using different solid matrices like carrageenan [34], agar gel [35], porous glass [31], and polyurethane foam [30].

3.3. FERMENTATIVE HYDROGEN PRODUCTION FROM ORGANIC COMPOUNDS

Dark hydrogen fermentation is a ubiquitous phenomenon under anoxic conditions (i.e., no oxygen present as an electron acceptor) [36]. When bacteria grow on organic substrates (heterotrophic growth), these substrates are degraded by oxidation to provide building blocks and metabolic energy for growth. This oxidation generates electrons which need to be disposed of to maintain electrical neutrality. In oxic environments, oxygen is reduced and water is the product. In anoxic environments, other compounds, e.g., protons, which are reduced to molecular hydrogen (H_2), need to act as electron acceptor [27, 21]. In the hydrogen fermentation process, glucose is initially converted to pyruvate by the glycolytic pathways. This is oxidized to acetyl-CoA, which can be converted to acetyl phosphate and results in the generation of ATP and the excretion of acetate (Fig. 1).

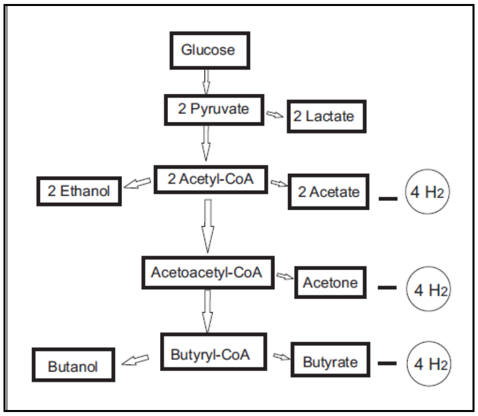

Fig. 1. The metabolic pathways representing hydrogen production fermentation in strictly anaerobic *Clostridium acetobutylicum*; adapted from [46]

Pyruvate oxidation to acetyl-CoA requires ferredoxin (Fd) reduction. Reduced Fd is oxidized by hydrogenase which generates Fd(ox) and releases electrons to produce molecular hydrogen [37, 38]. The overall reaction of the process can be described as follows:

$$\text{Pyruvate} + \text{CoA} + 2\text{Fd(ox)} \rightarrow \text{acetyl - CoA} + 2\text{Fd(red)} + CO_2 \quad (8)$$

$$2H + \text{Fd(red)} \rightarrow H2 + \text{Fd(ox)} \quad (9)$$

Anaerobic fermentation enables the mass production of hydrogen via relatively simple processes from a wide spectrum of potentially utilizable substrates, including refuse and waste products [39]. Moreover, fermentative hydrogen production generally proceeds at a higher rate and does not rely on the availability of light sources. Carbohydrates, mainly glucose, are the preferred carbon sources for fermentation processes, which predominantly give rise to acetic and butyric acids together with hydrogen gas [38], as follows:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (10)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2COOH + 2CO_2 + 2H_2 \quad (11)$$

The end-products of glucose fermentation by anaerobic and facultative anaerobic chemoheterotrophs, e.g., clostridia and enteric bacteria, are produced through pyruvate. Facultative anaerobic bacteria give 2 mol of hydrogen per mol of glucose, whereas strictly anaerobic bacteria give four. Facultative anaerobes are less sensitive to oxygen, and are sometimes able to recover hydrogen production activity after accidental oxygen damage to them by rapidly depleting oxygen present in the broth. As a consequence, a facultative anaerobe is considered a better microorganism than a strict anaerobe to carry out fermentative hydrogen production process [40]. A fermentative hydrogen production process can be conducted by using either pure cultures or mixed cultures. However, in a fermentative hydrogen production process using mixed cultures, the hydrogen produced by hydrogen-producing bacteria can be consumed by hydrogen-consuming bacteria. Thus, in order to harness hydrogen from a fermentative hydrogen production process, the seed sludge often needs a pretreatment to suppress as much hydrogen consuming bacterial activity as possible while still preserving the activity of the hydrogen-producing bacteria [41]. One of the main constraints of fermentative biohydrogenation process is the lower yield of hydrogen, maximally 4 mol/mol glucose, compared with other processes (Table 1).

Table 1. Comparison of different hydrogen production technologies; adapted from [47]

Process	Feedstocks	Conversion efficiencies	Co-products
Electrolysis	H_2O + electricity	50-60%	only H_2
Steam methane reforming	Methane, glycerol, alcohols, polyols, sugars, organic acids	70-85%	CO, CO_2, C_{10}–C_{22} carbon chains
Aqueous reforming	Glycerol, alcohols, polyols, sugars, organic acids	35-100%	CO, CO_2, alkanes, alcohols, polyols, organic acids
Biomass gasification	Lignocellulosic biomass	35-50%	CO, CO_2, CH_4
Partial oxidation	Glycerol, alcohols, polyols, sugars, organic acids	60-75%	NO and NO_2
Biophotolysis	H_2O + sunlight	0,5%	only H_2
photo-fermentation	Organic acids + sunlight	0,1%	CO_2
dark fermentation	Biomass	60-80%	CO_2

This yield is too low to be economically viable as an alternative to existing chemical or electrochemical processes of hydrogen generation [42]. Therefore, the ultimate goal, and challenge, for fermentative hydrogen research and development focuses essentially on attaining higher yields of hydrogen.

Comparison of the rates of H_2 production by various biohydrogen systems, however, suggests that dark-fermentation systems offer an excellent potential for practical application and integration with emerging hydrogen and fuel cell technologies [21, 4].

3.4. HYBRID SYSTEM USING FERMENTATIVE AND PHOTOSYNTHETIC BACTERIA

The light independent bacteria could provide an integrated system for maximizing the hydrogen yield [43]. In such a system, the anaerobic fermentation of carbohydrate (or organic wastes) produces intermediates, such as low molecular weight organic acids, which are then converted into hydrogen by photosynthetic bacteria in the second step in a photo-bioreactor. The overall reactions of the process can be represented as:

(1) Stage I. Dark fermentation (facultative anaerobes):

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

(2) Stage II. Photo-fermentation (photosynthetic bacteria):

$$2CH_3COOH + 4H_2O \rightarrow 8H_2 + 4CO_2 \qquad (13)$$

So, theoretically it is evident that using glucose as the sole substrate in dark anaerobic fermentation, where acetic acid is the predominant metabolite product, a total of 12 mol hydrogen could be expected in a combined process from one mol of glucose. In recent years, a number of studies concerning the combination of purple non-sulfur (PNS) photosynthetic bacteria and anaerobic bacteria for the efficient conversion of wastewater, sewage sludge and glucose into hydrogen had been made [44, 45, 38].

4. CARACTERISTICS OF THE BIOHIDROGEN PRODUCTION METHODS

The discussed biohydrogen production methods are characterized by some fundamental quantitative and qualitative characteristics. The advantages and disadvantages of different biohydrogen production methods are summarized in table 2.

Compared with photosynthetic hydrogen production, fermentative hydrogen production has higher hydrogen production efficiency, higher hydrogen production stability, higher feasibility for industrialization, simpler control requirement and lower operating costs. Thus fermentative hydrogen production is more feasible and widely used. In addition, it is of great significance to produce hydrogen from organic wastes by fermentative hydrogen production, because it plays the dual role of

Table 2. Advantages and disadvantages of different biohydrogen production processes; adapted from [26]

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, etc.	Requires high intensity of light; O_2 can be dangerous for the system; Lower photochemical efficiency;
Indirect biophotolysis	low nutrient input requirements; 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.

waste reduction and energy production. However, the reported H_2 production rates, stabilities and efficiency of these processes are far below commercialization.

4. CONCLUSION

Biohydrogen production has been established as a prospective alternative and integral component of green sustainable energy. A challenging problem in establishing biohidrogen as a source of energy from the renewable substrate and environmentally friendly immobilized solid matrices is the generation of large quantities of hydrogen gas.

The major bottlenecks of the biohydrogen production processes for the commercialization are:

- development of mixed microbial consortia or metagenomic approaches may be used to develop efficient microbial strains for the better utilization of industrial wastewater, which has different carbon content;
- improvement of H_2 yield of the processes using cheaper raw materials;
- in two-stage processes, the major bottleneck lies on the photo-fermentation process. Improvement of these processes surely will improve overall hydrogen yield as well as economy of the process.

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