Biohydrogen Production: A Potential Energy Resource

Shayra Fatima[1], Ankit Kumar[2], Ravi Kant Singh[1]*

[1]Amity Institute of Biotechnology, Amity University Chhattisgarh, Raipur, Chhattisgarh, India

[2]Department of Biotechnology, Noida Institute of Engineering & Technology, Greater Noida, U.P., India

: rksingh@rpr.amity.edu

Abstract

Biohydrogen production from biomass is accepted as an eco-friendly and sustainable approach. The future of biohydrogen production depends on research advances, economic considerations and the development of hydrogen energy systems. However, the process for biohydrogen production is slow due to the recalcitrant biomass structure hindering the liberation of readily fermentable sugars for fermentation. To overcome these bottlenecks intensive research work has already been carried out on the advancement of these processes such as the development of genetically modified microorganisms, the improvement of the bioreactor design, molecular engineering of the key enzyme hydrogenases, and the development of two stage processes**.** This paper deals with critical review of potential biomass resources, biomass pretreatment options and fermentation processes used for biohydrogen gas production. The different approaches towards improvement of the hydrogen production are also outlined. Currently, biohydrogen has been successfully produced by the anaerobic fermentation process using kitchen waste as substrate.

Keywords: Biohydrogen, Biophotolysis, Genetically engineered microorganisms, Energy conversion efficiency, and two stage process.

1.0 INTRODUCTION

Non-conventional energy resources have received great attention by the international community in the last decades which includes solar energy, wind energy, hydroelectricity, geothermal energy, biomass energy etc. Currently hydrogen is considered as a dream fuel by virtue of the fact that it is renewable, does not evolve the green house gases, has high energy content per unit mass of any known fuel and is easily produced electricity by fuel cells and on combustion it gives water as byproduct (1). Presently, 40% hydrogen is produced from natural gases, 30% produced from heavy oil & naphtha, 18% from coal, 4% from electrolysis and about 1% is produced from biomass (2,3). The utilization of biomass as feedstock for hydrogen production is not only cost effective but also eco-friendly option, because the processes have net zero CO_2 emission due to the fact that CO_2 is fixed in the atmosphere by plants during photosynthesis (4). Besides, agricultural crops & their byproducts, lignocellulosic wastes, aquatic plants like algae & water weeds, industrial or municipal solid wastes and animal wastes are accepted as biomass resources (3,5,6). Over the last few decades, the investigations on biohydrogen production as a by-product of microbial metabolism have been accelerated for generating energy to meet global energy demand (7).

Interest in biohydrogen started getting prominence in early 90s, when it becomes apparent that atmospheric pollution by fossil fuels is not only dangerous for health but might also cause significant climate changes globally.

This review article briefly describes the processes for the optimum production of biohydrogen and also been made to highlight the significance and bottlenecks of each processes towards improvement of hydrogen production and process efficiency (8). The advantages and disadvantages of various processes for biohydrogen production are outlined in table-1.

Table-1: Advantages & Disadvantages of different biohydrogen production processes

S. No.	Process	Advantages	Disadvantages
1	Pyrolysis	Produces carbonaceous materials along with bio-oil, chemicals & minerals	Chances of catalyst deactivation
2	Solar gasification	Good biohydrogen yield	Required effective collector plates
3	Thermochemical gasification	Maximum conversion can be achieved	Significance gas conditioning is required
4	Super critical conversion	Can process sewage sludge, which is difficult to gasify	Selection of supercritical media.

2.0 Process available for biohydrogen production

Hydrogen is a natural, though transient, byproduct of several microbial driven biochemical reactions, mainly in anaerobic fermentation processes. In addition, certain microorganisms can produce enzymes that can produce H_2 from water if an outside energy source, like sunlight, is provided to them. The specific ways by which microorganisms can be produced H_2 are as follows:

2.1 Biophotolysis - Photoautotrophic organism such as microalgae and cyanobacteria are capable to generate hydrogen and carbon dioxide in biophotolysis process. They utilize the light as energy source and carbon dioxide as carbon source to split water into hydrogen [6].

$$4H_2O + \text{“light energy”} \rightarrow 2O_2 + 4H_2$$

1. Biophotolysis of water using green algae & blue green algae (cynobacteria)
   i. Direct Biophotolysis
   ii. Indirect Biophotolysis
2. Photo-fermentation
3. Dark fermentation
4. Hybrid systems
   i. Using dark fermentation & photo fermentation bioreactors
   ii. Using bio-electrochemically assisted microbial bioreactors

The biological hydrogen production processes are summarized with overall reactions involved therein, microorganism used & their relative advantages in table-2.

Table-2: Different processes for biological hydrogen production

S. No.	Process	General reaction & Microorganisms	Advantages
1	Direct Biophotolysis	$2H_2O$ + Light = $2H_2 + O_2$ (Microalgae)	Can produce H_2 directly from water & sunlight Solar conversion energy increased by 10-folds as compare to trees, crops
2	Indirect Biophotolysis	$6H_2O + 6CO_2$ + Light = $C_6H_{12}O_6$ $C_6H_{12}O_6 + 2H_2O = 4H_2 + CH_3COOH + 2CO_2$ $2CH_3COOH + 4H_2O$ +Light = $8H_2 + 4CO_2$ Overall reaction $4H_2O$ + Light = $12H_2 + 6O_2$ (Microalgae, Cynobacteria)	Can produce H_2 directly from water Has affinity to fix N_2 from atmosphere
3	Dark Fermentation	$C_6H_{12}O_6 + 6H_2O = 12H_2 + 6CO_2$ (Fermentative bacteria)	It can produce H_2 all day long without light A variety of carbon sources can be used as substrate It produces valuable metabolites such as butyric, lactic, acetic acids as byproducts It is anaerobic process, so there is no O_2 limitations problems
4	Photo-fermentation	$CH_3COOH + 2H_2O$ + Light = $4H_2 + 2CO_2$ (Purple bacteria, Microalgae)	A wide spectral light energy can be used by these bacteria Can used different waste materials like distillery effluents, wastes etc.
5	Hybrid Reactor System	$C_6H_{12}O_6 + 2H_2O = 4H_2 + 2CH_3COOH + 2CO_2$ $CH_3COOH + 2H_2O$ + Light = $4H_2 + 2CO_2$ (Fermentative bacteria followed by anoxygenic phototrophic bacteria)	Two stage fermentation can improve the overall yield of hydrogen

2.1.1 Direct Biophotolysis

A direct biophotolysis is a biological process for hydrogen production which utilizes solar energy and photo-synthetic systems of algae to convert water into chemical energy.

$$2H_2O + \text{Solar energy} = 2H_2 + O_2 \ldots\ldots\ldots\ldots(1)$$

Two photosynthetic systems (PSI, PSII) are responsible for direct photosynthesis process. First photo system (PSI) produces reductant for CO_2 & second photo system (PSII) splits water to evolve O_2. The two photons obtained from the splitting of water can either reduce CO_2 by PSI or form H_2 in the presence of hydrogenase. The green algae and cynobacteria (blue green algae) contain hydrogenase and thus have the ability to produce H_2 (6). In these organisms, electrons are generated when PSII absorbs light, which is then transferred to ferredoxin (fd). A reversible hydrogenase accepts electrons directly from the reduced ferredoxin to generate H_2 in the presence of hydrogenase. Since hydrogenase is sensitive to oxygen, it is necessary to maintain the oxygen content at a low level (under 0.1%), so that the hydrogen production can be sustained (9). This condition can be obtained by the use of green algae, *Chlamydomonas reinhardtii* which can deplete oxygen during the oxidative respiration (10). However, the reaction is very short-lived & the rate of the hydrogen production is very low, less than one-tenth than that of other photosynthetic reactions (11). The processes of direct biophotolysis involved in the production of biohydrogen from renewable source are given below in figure 1.

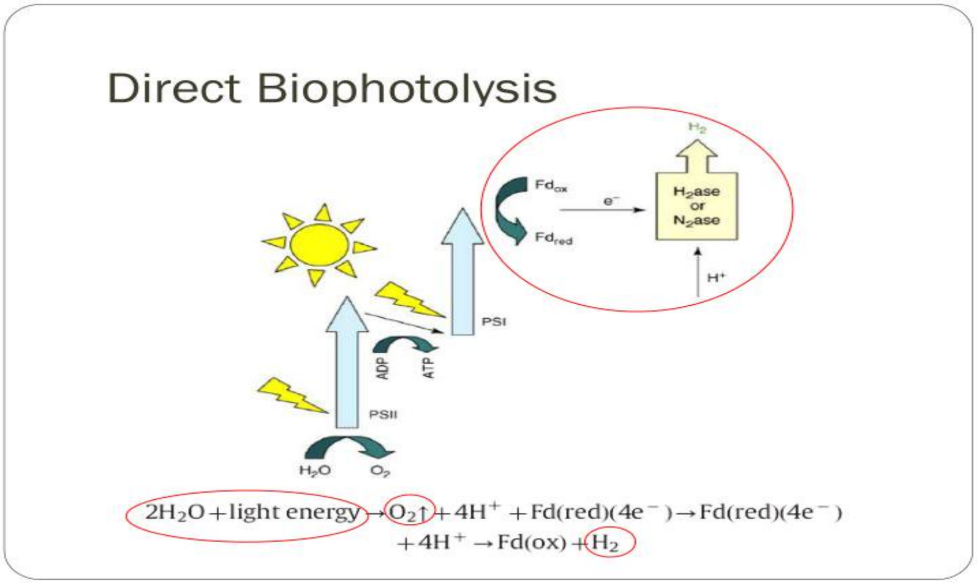

Figure-1: Direct biophotolysis

2.1.2 Indirect Biophotolysis

In the indirect Biophotolysis, the problem of sensitivity of the H_2 evolving process to O_2 is usually circumvented by separating O_2 & H_2 (12). In this process, CO_2 is fixed and released as the electro carrier between the O_2 producing reaction and O_2 sensitive hydrogenase reactions.

Indirect biophotolysis is the production of hydrogen from water via microalgae and cyanobacteria photosynthetic system to convert solar energy into chemical energy which is hydrogen by two steps in series. The first step is the biomass production through photosynthetic system (4) and follow by second step which utilise the biomass rich-carbohydrate for hydrogen-producing fermentation.

$$6H_2O + 6CO_2 + light \rightarrow C_6H_{12}O_6 + 6O_2$$

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

In this process, the evolutions of hydrogen and oxygen are temporally and/or spatially separation at different steps. In this way, the inhibition of hydrogenase enzyme activity to oxygen produced during photosynthesis process can be avoided. The examples of cyanobacteria organism that perform indirect biophotolysis such as *Gloebacter sp., Synechocystis sp.,* and *Synechococcus sp.* [3].

The advantage of biophotolysis is the simplicity of the process to produce hydrogen without requirement of additional substrates as nutrient and can produce hydrogen directly from abundant source of water as electron donor; sunlight and carbon dioxide as basic needed for growth of microalgae and cyanobacteria. However, the process is requires high light intensity and the green algae and cyanobacteria have lower light conversion efficiency. The lower performance of the photobioreactors for light penetration in dense culture is contributed to lower hydrogen production.

In such concepts the algae undergo a cycle of CO_2 fixation into storage carbohydrates (starch, glycogen) followed by its conversion to H_2 by dark anaerobic fermentation processes. Many types of green algae and cynobacteria, besides having the ability to fix CO_2 via photosynthesis, also have the ability to fix nitrogen from the atmosphere and produce enzymes that can catalyze the second H_2 generating step. Since these nitrogen fixing enzymes, nitrogenases, are localized within the heterocyst, they provide an O_2 free environment to carry out the H_2 evolution reactions. The processes involved in the production of biohydrogen by indirect biophotolysis are given below in figure 2.

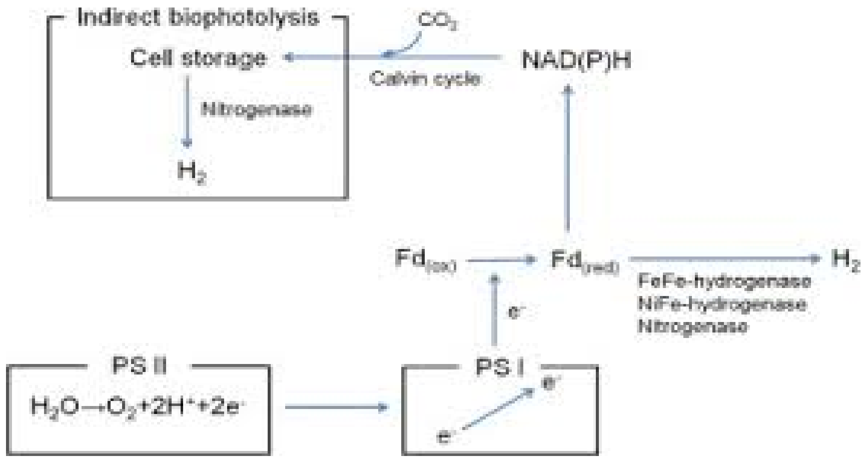

Figure-2: Indirect biophotolysis

2.2 Photo-fermentation

Photo-fermentation is carried out by non-oxygenic photosynthetic bacteria that use sunlight and biomass to produce hydrogen. Purple non-sulfur (PNS) and green sulfur (GS) bacteria such as *Rhodobacter spheroids* and *Chlorobium vibrioforme* respectively, are capable of producing hydrogen gas by using solar energy and reduced compounds. Their photosynthetic systems differ from oxygenic photosynthesis due to their requirement to reduce substrates and their inability to oxidize water (13). Photosynthetic bacteria have long been studied for their capacity to produce hydrogen through the action of their nitrogenases system (14). The efficiency of light energy used for hydrogen production by photosynthetic bacteria is theoretically much higher compared with cynobacteria. A generalized expression for photochemical efficiency has been put forward by Akkerman et al (15).

% efficiency = H_2 production rate x Hydrogen energy content/ Absorbed light energy

For the photoautotrophic hydrogen production, photochemical efficiencies are only 3-10% when the oxygen is totally & immediately removed. Such low efficiencies of photoautotrophic process pose a major limitation towards its commercial acceptance. On the other hand, photochemical efficiency of photo heterotrophic bacteria is comparatively higher than that of photoautotrophs (15). The energy conversion efficiency of light energy into hydrogen in the presence of photosynthetic bacteria varies under different light sources. It is believed that the biohydrogen production by photosynthetic bacteria may depend on the spectral distribution, since the bacteria utilize the specific light wavelengths for photosynthesis. An approach for the improvement of hydrogen production by photosynthetic bacteria is the control of photosynthetic protein expression to allow efficient absorption of light energy. A method for the enhancement of the bacterial light-dependent hydrogen production is proposed by Miyake et al (16) by rearrangement of light harvesting systems. Genetic manipulation of photosynthetic pigment content of bacteria by 'promoter competition method' can be controlled for making the light penetrating easy (16). The hydrogen produce in photo fermentation is by photosynthetic organisms, in which using photosynthetic bacteria with additional of light. These photosynthetic bacteria are lack of Photosystem II (PSII) and perform the photosynthesis with Photosystem I (PSI) for hydrogen production. The process still requires light as source energy likely to biophotolysis, but the photosynthetic bacteria in this process not powerful enough to split water such in biophotolysis, so the process utilise organic acids, like acetic acid as electron donor to generate hydrogen.

$$CH_3COOH + 2H_2O + \text{light energy} \rightarrow 4H_2 + 2CO_2$$

The main bottleneck for bacterial application of photo biological hydrogen production is the required scaling up of the system. A large surface area is needed to collect light. Construction of a photo bioreactor with a large surface / volume ratio for the direct absorption of sunlight is expensive. A possible alternative is the utilization of solar collectors.

2.3 Dark Fermentation

Dark fermentation is a ubiquitous phenomenon under anoxic or anaerobic conditions. The oxidation of the substrate by microorganism generates electrons which need to be disposed off in order to maintain the electrical neutrality. Under the anaerobic conditions the other compounds such as protons, act as the electron acceptor and are reduced to molecular H_2 (17,18). Carbohydrates, mainly glucose, are the preferred carbon sources for this process, which predominantly give rise to acetic and butyric acids together with H_2 evolution (19). In this process, glucose is converted to pyruvate by the glycolysis. Pyruvate is further oxidized to acetyl CoA, which can be converted to acetyl phosphate. The oxidation to acetyl-CoA requires a ferredoxin (Fd) reduction. Reduced Fd is oxidized by hydrogenase which regenerates Fd (ox) and releases electrons as molecular H_2 (19,20). The overall reaction of the process can be described as follows:

Pyruvate + CoA + 2Fd (ox) = Acetyl CoA + 2Fd (red) + CO_2 …………………(2)

$2H^+$ + Fd (red) = H_2 + Fd (ox)…………………………………………………...(3)

The anaerobic bacteria use in dark fermentation is classified into strict anaerobes or facultative anaerobes. The examples of strict anaerobes are from Clostridial species which produce hydrogen by PFOR (pyruvate ferredoxin oxidoreductase) pathway, thus produce 4 moles of hydrogen per mole glucose. Whereas facultative anaerobes such as Escherichia coli have PFL (pyruvate formate lyase) pathway, thus is limited to give 2 moles of hydrogen per mole glucose.

All the superiority of dark fermentation makes it great potential to be developed for practical biohydrogen production application. Besides that, long-term research and development are required for biophotolysis and photofermentation to encounter intractable problems such as oxygen sensitivity of hydrogenase and photobioreactor design if they are establish for practical application [16]. However, the dark fermentation processes produce a mixed biogas which containing mainly hydrogen and carbon dioxide, and possible also contain lesser amounts of carbon monoxide, methane, and/or hydrogen sulfide. Small amount CO_2 present in fuel of the fuel cell is able to reduce it performances. In order to utilize hydrogen from biohydrogen production process as fuel in fuel cell, so the separation and purification system is very important. Thus, in the next section, the various separation and purification of biohydrogen process will be presented and discussed along with potential candidate to integrate with biohydrogen production technology.

2.4 Hybrid Reactor System

2.4.1 Dark & Photo-Fermentative Bioreactor

The hybrid reactor system is the combination of dark & photo fermentation system for improving the efficiency of biohydrogen production. The photosynthetic bacteria could provide an integrated system for maximization of hydrogen yield (21). In such system, anaerobic fermentation of carbohydrate (or organic wastes) produces intermediates such as low molecular weight organic acids, which are then converted to hydrogen by photosynthetic bacteria in the second step in a photo bioreactor. Dark hydrogen fermentation is an incomplete oxidation, yielding not only hydrogen & CO_2 but also organic acids like acetic acids like acetic acid. Therefore, for an economically second process, the remaining carbonaceous compounds are to be converted, either in photo-bioreactor to H_2 and CO_2 or in a methane reactor to CH_4 & CO_2. If the dark hydrogen fermentation is not followed by further conversion the H_2 yield will not warrant economic feasibility.

2.4.2 Biochemically assisted microbial bioreactors

Microbial fuel cells (MFCs) have gained much attention because of its ability to generate power from organic or inorganic compounds via microorganisms. MFCs are devices that use bacteria catalysts to oxidize organic & inorganic matter and generate current (22, 23, 24, 25, 26). Electrons produced by the bacteria from these substrates are transferred to the anode (negative terminal) and follow to the cathode (positive terminal) linked by a conductive material containing a resistor, or operated under a load (i.e. producing electricity than run a device). By convention, positive current flows from the positive to the negative terminal, a direction opposite to that of electron flow. These electrons can be transferred to the anode by electron mediators or shuttles (27, 28), by direct membrane associated electron transfer (29), or by so-called nanowires (29, 30, 31) produced by the bacteria. The chemical mediators, such as neutral red or anthraquinone-2, 6 disulfonate (AQDS), can be added to the system to allow electricity production by bacteria (32, 33). If no exogenous mediators are added to the system the MFC is classified as a “mediator-less” MFC even though the mechanism of electron transfer may not be known (34, 35).

The performance of MFCs can be influenced by several factors. Gil et al (36) reported that the factors include the rates of substrate oxidation, electron transfer to the electrode by the microbes, the resistance of the circuit, proton transport to the cathode through the membrane, oxygen supply and reduction in the cathode. This fundamental scientific information must now be applied to the development of biohydrogen production processes that could deliver biohydrogen at acceptable cost.

3.0 Major Challenges and Perspectives in Biohydrogen production

Biological hydrogen production (BHP) is the most challenging area of biotechnology and it is perceived worldwide as a developmental process that requires extensive biotechnological interventions through research and innovations. The production of biohydrogen faces various technical challenges includes selection of microbes, conversion efficiencies, feed stock types and their availability, identifying the right conditions, molecular understanding, engineering applications and the need to safely integrate hydrogen production systems with hydrogen purification, storage and technologies for efficient utilization. A major challenge in biohydrogen production by both dark and light fermentation is the improvement in the rate of hydrogen production. The microbiological part has to be addressed for the

- Sensitivity of hydrogenase enzyme to hydrogen partial pressure and oxygen that severely decreases the efficiency of the process.
- Scaling up competition with other microorganisms under non-sterile conditions.
- Amenability for metabolically engineered process etc.

The hydrogen can be used in either internal combustion engines or fuel cells. Since fuel cell applications are not commercially standardized yet and a distribution infrastructure for hydrogen cannot be realized in the short term.

Biohydrogen has several properties that project it as a potential alternative to our largely used fossil fuels. However, its production technology must overcome a number of limitations before it could successfully compete in the fuel market and be deployed on a large scale. Although the potential of microbes to produce biohydrogen has raised a lot of excitement, yet a lot needs to be done on technical, economical, policy making, and legislative fronts to make it replace the existing fossil fuels. Review of published literature suggests that the biohydrogen production system holds great promise for industrial application. In this chapter, we have attempted to elucidate the major challenges being faced for using biohydrogen on a commercial scale by making an assessment of its economics taking into account the different processes like production, storage, transportation, and delivery to the user and also assess its future commercialization perspectives.

4.0 Conclusion

The cost effective biohydrogen production is still in its nascent stage and a number of issues still have to be addressed. Among these, utilization of organic load, use of starch rich solid wastes, industrial wastewaters with pre-treatment, forms an attractive approach for biohydrogen production in the future. The development of a sequential or combined hybrid bioprocess for hydrogen and methane production would prove to be viable in the long run. However, some major aspects need indispensable optimization including identification of a suitable substrate, suitable pre-treatment methods, development of ideal microbial culture/ consortia that can convert the substrate effectively to hydrogen & methane, suitable hybrid bioreactors to achieve the desired results etc. Future biological hydrogen production also depends on economic considerations, social acceptance, and developments in hydrogen & hydrogen associated energy used system. Also the dark fermentation would appear the potential candidate to generate hydrogen compare to other biohydrogen production technologies to power the fuel cells at sufficient size and would be applicable for portable fuel cell and stationary power generator. However, more research and development are requiring such as increasing the rates and yields of hydrogen production and optimizing the bioreactor sizing so that hydrogen from biohydrogen production process is able replace non-renewable energy source, fossil fuels and also able to sustain the world power energy supply. Besides that, it is essential to study and extensive research on membrane gas technology in hydrogen separation together with hydrogen fermentation process to determine the possibility of integrated applications.

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