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Biohydrogen from Algae: Fuel of the Future

Sharma Shaishav, Singh R.N. and Tripathi Satyendra
School of Energy and Environment Studies, Devi Ahilya Vishwavidyalaya, Takshashila Campus, Indore, INDIA

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Abstract

Hydrogen holds much promise as a future fuel because of the increase in pollution caused by fossil fuels and continuous decrease in the availability of fossil fuels. The biggest drawback of hydrogen is that its production involves fossil fuels thereby causing pollution of environment. Biohydrogen produced from algae is not only a clean source of energy but can also be a major substitute for the continuous depleting gasoline. Third generation biohydrogen from algae have provided solutions to drawbacks of first and second generation biofuels up to an extent. This review critically updates different processes available for biohydrogen production from algae with special emphasis on their merits and demerits. Some approaches to overcome existing problems have also been outlined.

Keywords: Biohydrogen, algae, biophotolysis.

Introduction

Energy is the most important factor for sustaining a civilization as well as for its development but our dependence on fossil fuels as our primary energy source contributes to global climate change, environmental degradation and health problems. The limited amount of fossil fuels also is a cause of concern[1]. Hydrogen (H_2) offers tremendous potential as a clean, renewable energy source. The higher heating value of hydrogen is 3042 cals/m^3 (considering water as a product). In combustion, water is the main product, thus, hydrogen is regarded as a clean non-polluting fuel[2]. Apart from being used as a clean fuel, Hydrogen has following uses[3,4]. Used for the manufacture of ammonia. Used in oil refineries for removal of impurities. Used in methanol production, used as a fuel in rocket engines.

99% of the total hydrogen is produced from fossil fuels. Catalytic steam reforming of naphtha or natural gas, gasification of coal and electrolysis of water are some of the classical methods of hydrogen production but these methods are energy extensive and affect environment. On the contrary, biological hydrogen production is particularly useful because they are catalyzed by microorganisms in aqueous environment at ambient pressure and temperature.

The interest in large scale production of biohydrogen developed when the limited availability of fossil fuels and the pollution caused by them started putting a negative impact on the sustainability of the world population. The discovery that algae can produce hydrogen upon illumination was done 70 years ago by Gaffron and coworkers[5]. Hydrogen evolution in algae was induced when prior incubation in dark was given to the cells[6,7,8]. A high specific activity enzyme, Hydrogenase was expressed due to incubation and catalyzed light mediated hydrogen evolution. The monomeric form of the enzyme belongs to the class of Fe hydrogenase[9,10] is encoded in the nucleus of the unicellular green algae. However, the mature protein is localized and functions in the chloroplast stroma[10]. The absorption of light by the photosynthetic apparatus is an essential step for the production of hydrogen because the absorbed light energy oxidizes the water molecules and the released electrons are transported to ferredoxin. The ferredoxin transfers the electrons to the Fe-hydrogenase there by linking it to the electron transport chain of the chloroplast[11]. The activity of the hydrogenase last only for a short span of time (few seconds to minutes) because the light dependant oxidation of water leads to formation of molecular oxygen which unfortunately is a strong inhibitor of the Fe-hydrogenase[12]. This review critically deals with different approaches for biohydrogen production from algae, their advantages and drawbacks. Attempts have been made to highlight some techniques for improving the current drawbacks.

General Characters of Algae: Green algae and cyanobacteria (also called blue-green algae) are made up of a large group of photosynthetic organisms. Algae are some of the most robust organisms on earth, able to grow in a wide range of conditions. Algae usually grow in damp places or water bodies and hence found both in terrestrial as well as aquatic environments. Algae lack the various structures that characterize land plants, such as phyllids (leaves) and rhizoids in nonvascular plants, or leaves, roots, and other organs that are found in tracheophytes (vascular plants). Its uniqueness that separates them from other microorganisms is due to presence of chlorophyll and having photosynthetic ability in a single algal cell, therefore allowing easy operation for biomass generation and effective genetic and metabolic research in a much shorter time period than conventional plants. Well defined nucleus, a cell wall, chloroplast containing chlorophyll and other pigments, pyrenoid, a dense region containing starch granules on its surface, stigma, and flagella are the major components of green algae[13].

Biohydrogen production from Algae: Production of hydrogen biologically is mainly the domain of microalgae and bacteria. Many microbial species of these groups consisting of different taxonomic and physiological types are involved in hydrogen metabolism[3]. Table 1 summarizes different processes of hydrogen production from Algae, general reactions and their advantages:

Direct Biophotolysis: This involves the dissociation of water molecules under sunlight in the presence of microalgae. Microalgae possesses the genetic, enzymatic, metabolic and electron transport machinery to photo produce hydrogen gas. Biophotolysis is an inherently attractive process since solar energy is used to convert a readily available substrate, water to oxygen and hydrogen as shown in figure-1.

The overall general reaction of biophotolysis can be described as:
$2H_2O + light = 2H_2 + O_2$

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[10]. 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. Light energy absorbed by photosystem II (PSII) generates electrons which are transferred to ferredoxin using light energy absorbed by photosystem I (PSI). This enzyme is very sensitive to O_2. Hydrogenase activity has also been observed in other green algae like *Scenedesmus obliquus, Chlorococcum littorale, Platymonas subcordiformis and Chlorella fusca.* On the other hand, there are several green algae types that do not have hydrogenase activity such as *Dunaliella salina* and *Chlorella vulgaris*[15].

Indirect Biophotolysis: Cyanobacteria (also known as blue green algae) are a large and diverse group of photoautotrophic microorganisms, which can evolve hydrogen by indirect biophotolysis. The general reaction for hydrogen formation from water by cyanobacteria can be represented by following reactions:

$12H_2O + 6CO2 + light\ energy = C_6H_{12}O_6 + 6O_2$
$C_6H_{12}O_6 + 12H_2O + light\ energy = 12H_2 + 6CO$

Photosystem II utilizes the energy of sunlight in photosynthesis to extract electrons from water molecules. Electrons released upon the oxidation of water are transported to the Fe-S protein in ferredoxin on the reducing side of photosystem I. The hydrogenase in the stroma of the algal chloroplast accepts electrons from reduced ferredoxin and donates them to two protons to generate one H_2 molecule. For photobiological hydrogen production, cyanobacteria have been adjudged as one of the ideal candidates since they can grow in air (N_2 and CO_2), water and mineral salts with light as the energy source[16]. The cultivation of cyanobacteria in nitrate free media under air and CO_2, followed by incubation in light under argon and CO_2 atmosphere has rapidly become standard, since it results in immediate hydrogen production. Localization of nitrogenase in heterocysts provides an oxygen free environment and the ability of heterocystous cyanobacteria to fix nitrogen in air[17].

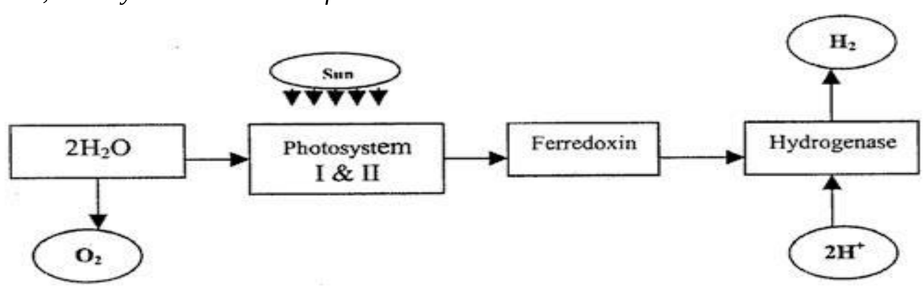

Figure-1
Mechanism of Biophotolysis

Table-1
Different biohydrogen production processes from Algae and their advantages

S.No.	Process	Reactions	Advantages
1.	Direct Biophotolysis	$2H_2O + light = 2H_2 + O_2$	Can produce H_2 directly from water and sunlight. Solar conversion energy increased by 10 folds as compared to trees.
2.	Indirect Biophotolysis	$6H_2O+6CO_2+light = C_6H_{12}O_6+6O_2$ $C_6H_{12}O_6+2H_2O=4H_2+2CH_3COOH+2CO_2$ $2CH_3COOH+4H_2O+light = 8H_2+ 4CO_2$ Overall Reaction: $12H_2O+light = 12H_2+6O_2$	Produced by both microalgae and cyanobacteria. Can produce H_2 from water. Cyanobacteria have the ability to fix N_2 from atmosphere.
3.	Photo-fermentation	$CH_3COOH+2H_2O+light = 4H_2+ 2CO_2$	Produced by both Purple bacteria and Microalgae. A wide spectral light energy can be used by these bacteria.

Table-2
Major drawbacks associated with these processes and the ways to overcome it

Process	Major limitations	Approaches to overcome
Direct Biophotolysis	Sensitivity of hydrogenase for O_2. Light conversion efficiency is low.	Use of O_2 absorbers. b) Use of O_2 tolerant hydrogenase. Genetic manipulation of light gathering antenna. Optimization of light input into photobioreactor.
Indirect Biophotolysis	Enzyme inhibition by O_2. Overall low production rate.	To achieve O_2 tolerant hydrogenase activity by classical mutagenesis. Genetic modification to increase levels of bidirectional hydrogenase activity.
Photo fermentation	O_2 is a strong inhibitor of hydrogenase.	Use of O_2 absorbers. b) Use of O_2 tolerant hydrogenase.

Photo fermentation: The general reaction for photo fermentation can be written as:
$CH_3COOH+2H_2O+light = 4H_2+ 2CO_2$

A number of microalgae have been examined for photofermentative hydrogen production. The most promising candidates are *Rhodopseudomonas capsulate, Rhodobacter spheroides* and *Rhodospirillum rubrum*[18]. The most promising of all of the current body of research is the use of *Rhodobacter spheroides* immobilized on porous glass.

Major Bottlenecks of these processes: There are certain shortcomings associated with these processes which are an obstacle in the path of large scale production of biohydrogen from algae. Table 2 summarizes the shortcomings of these processes as well as the ways to overcome it. The hydrogenase enzyme responsible for hydrogen production is extremely sensitive to O_2. Therefore, the concurrent production of O_2 poses a serious limitation. This is a major drawback associated with photolysis. Removing the O_2 as it is produced can solve the problem to a large extent. But this won't be possible in a large scale operation unit. Some other methods to overcome this include use of O_2 absorbers and the use of O_2 tolerant hydrogenase enzymes. Another promising solution is to develop O_2 tolerant hydrogenase[19].

Photobiological technology holds great promise but because of the production of oxygen along with hydrogen causes a major setback for the technology. The technology must overcome the limitation of oxygen sensitivity of the hydrogen evolving enzyme systems. Researchers are addressing the issue by screening the algae that is more tolerant of oxygen, and by creating new genetic forms of algae that can sustain hydrogen production in the presence of oxygen[3].

Conclusion

Hydrogen has tremendous potential to become major contributor of clean and renewable source of energy. Biohydrogen production from algae on commercial scale can be useful as it fulfills most of the criteria of a clean and renewable source of energy. The processes through which hydrogen is produced by algae has its pros and cons both in terms of technology and productivity. These processes are yet to be evaluated and modified for productivity and cost of commercialization.

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