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ROLES OF MICROALGAE AND BACTERIA IN HYDROGEN PRODUCTION AS ONE OF THE RENEWABLE ENERGY RESOURCES

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REVIEW ARTICLE:

ABSTRACT:

Hydrogen gas is considered to be one of the most desired alternate sources of the limited fossil energy resources of today. It shows great promise as a non-polluting fuel, but to reduce carbon dioxide releases hydrogen gas will need to be produced from renewable sources. The limited fossil fuel prompts the prospecting of various unconventional energy sources to take over the traditional fossil fuel energy source. Photosynthetic microbes can produce hydrogen using the nature plentiful resources, sunlight, the included greens, and blue-green algae (Cyanobacteria), either via direct or indirect biophotolysis. In addition, Cyanobacteria produced hydrogen through decomposing the organic compounds (Photodecomposition). The hydrogen production by green algae could be considered as an economical and sustainable method, water utilization as a renewable resource and recycling CO_2, a greenhouse gas. Rates of hydrogen production by photoheterotrophic bacteria are higher in the case of immobilized cells than that of the suspended cells. Cyanobacteria are highly promising microorganism for hydrogen production. Cyanobacterial hydrogen production is commercially viable, in comparison to the traditional ways of hydrogen production (chemical, photoelectrical). The present review shows the basic biology of microalgae and bacterial hydrogen production and its future prospects. While integrating the existing knowledge and technology, much future improvement and progress is to be done before hydrogen is accepted as a commercial primary energy source.

INTRODUCTION:

Hydrogen gas shows great promise as a non-polluting fuel, but to reduce carbon dioxide releases hydrogen gas will need to be produced from renewable sources. Due to the consumption of fossil fuel and production of green house gases (i.e., methane and carbon dioxide), developing clean and new energy will be one of the important researches in the further. Hydrogen, high energy yield (about 2.75 times greater than that of hydrocarbon fuels), is considered a promising candidate as an

ideal and clean source of energy. Biohydrogen production process not only can solve environmental pollution, but also achieve resource recycling (Chang, 2001).

Hydrogen (H_2) offers tremendous potential as a clean, renewable energy currency. Hydrogen has the highest gravimetric energy density of any known fuel and is compatible with electrochemical and combustion processes for energy conversion without producing carbon-based emissions that contribute to environmental pollution and climate change. Hydrogen fuel cells and related hydrogen technologies provide the essential link between renewable energy sources and sustainable energy services. The transition from a fossil fuel-based economy to a hydrogen energy-based economy, however, is fraught (Dunn, 2002).

It can fill an important role in the "greening" of the global energy and industrial base. H_2 is not a greenhouse gas, it has 2.4 times the energy content of methane (mass basis) and its reaction with oxygen in fuel cells produces only harmless water. Not only can pollutants from fuels used in high-temperature combustion engines be avoided using hydrogen-based fuel cells, but the elimination of combustion also avoids the generation of NO_2. As a result of these advantages of hydrogen-based fuel cells, there is a global transition occurring to hydrogen-based technologies. However, hydrogen is currently produced mostly from fossil fuels, an inherently non-sustainable technology.

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% (Momirlan and Veziroglu, 2002). Presently, hydrogen is produced 40% from natural gas, 30% from heavy oils and Naphtha, 18% from coal, and 4% from electrolysis (Nath and Das, 2003).

Hydrogen intensive research work has already been carried out on the advancement of these processes, such as the development of genetically modified microorganism, metabolic engineering, improvement of the reactor designs, use of different solid matrices for the immobilization of whole cells, biochemical assisted bioreactor, development of two-stage processes, etc. for higher H_2-production rates (Debabrata and Veziroglu, 2008). Hydrogen may be produced by a number of processes, including electrolysis of water, thermocatalytic reformation of hydrogen-rich organic compounds, and biological processes. Currently, hydrogen is produced, almost exclusively, by

electrolysis of water or by steam reformation of methane. Biological production of hydrogen (biohydrogen), using (micro) organisms, is an exciting new area of technology development that offers the potential production of usable hydrogen from a variety of renewable resources.

However, major bottlenecks for the commercialization of these processes are lower H_2 yield and rate of H_2 production. Suitable microbial cultures are required to handle waste materials efficiently, which are usually complex in nature. This will serve dual purposes: clean energy generation and bioremediation. Scale-up studies on fermentative H_2-production processes have been done successfully. Pilot plant trials of the photo-fermentation processes require more attention. Use of cheaper raw materials and efficient biological hydrogen production processes will surely make them more competitive with the conventional H_2 generation processes in near future (Debabrata and Veziroglu, 2008).

Biohydrogen has gained attention due to its potential as a sustainable alternative to the conventional methods for H_2 production. It gives unassailable flexibility for a sustainable energy system, considering the present energy crisis and environmental tribulations. Biological processes, unlike their chemical or electrochemical counterparts, are catalyzed by microorganisms in an aqueous environment at ambient temperature and atmospheric pressure. 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. In addition, these are becoming important mainly due to utilize renewable energy resources. These processes are usually carried out by different anaerobic bacteria and/or algae. The characteristics of these microorganisms widely differ from each other with respect to substrates and process conditions. The merits and demerits of the processes have already been discussed (Das and Veziroglu, 2001, Nandi and Sengupta, 1998)

Biological hydrogen production processes offer a technique through which renewable energy sources like biomass can be utilized for the generation of the cleanest energy carrier for the use of mankind (Debabrata and Veziroglu, 2008). Hydrogen has been produced through thermal cracking or water electrolysis, which requires much energy and emits global warming gases such as carbon dioxide. One of the most promising methods is that the gas is produced from annually renewed biomass using microorganisms.

Microorganisms producing hydrogen:

The types of microorganism-producing hydrogen are divided into four groups: cyanobacteria, anaerobic bacteria and photosynthetic bacteria. Cyanobacteria split water into hydrogen and oxygen gas by photosynthesis. Anaerobic bacteria use organic substrates as the sole source of electrons and energy and convert them into hydrogen. The reaction is rapid and the process does not require solar radiation, which makes it useful

for treating large quantities of wastewater. Finally, the photosynthetic bacteria lie somewhere between cyanobacteria and anaerobic bacteria. Although photosynthetic bacteria also convert organic substances to hydrogen at fairly high rates, they also require light energy to assist or promote the reactions involved in the hydrogen production. Some nonphotosynthetic bacteria can produce hydrogen from different organic substrates, such as *Enterobacter Aerogenes* from glucose or a *Clostridium beijerinckii* from glucose and starch. Non-photosynthetic bacteria, like *Clostridium butyricum*, produce hydrogen from carbohydrates at a high rate, but the yield is low because they also produce organic acids. Photosynthetic bacteria such as Rhodobacter sphaeroides RV are powerful hydrogen producers, showing 7% energy conversion efficiency in the presence of lactate and glutamate. The high rate of hydrogen production makes them suitable for photobioreactor applications (Jeong *et al.,* 2008).

Hydrogen Bioproduction pathways:

A major route for hydrogen production is biological nitrogen fixation (Prince and Kheshgi, 2005). This is catalyzed by the enzyme nitrogenase, and hydrogen is an obligatory, but not advantageous, product of a reaction that evolved to enable cells to synthesize ammonia from nitrogen gas (Simpson and Burris, 1984). So, Photosynthetic microbes can produce the clean-burning fuel hydrogen gas (hydrogen) using one of nature, most plentiful resources, sunlight (Das and Veziroglu, 2001).

Hydrogen production rates of various biohydrogen systems are compared by first standardizing the units of hydrogen production and then by calculating the size of biohydrogen systems that would be required to power proton exchange membrane (PEM) fuel cells of various sizes. (Levin *et al.,* 2004).

Generally; Biological hydrogen production processes can be done by:

- **Biophotolysis; green algae and blue-green algae (Cyanobacteria) split water molecules into hydrogen ion and oxygen.**
- **Photodecomposition photosynthetic bacteria decompose the organic compounds by; Dark fermentation; and Hybrid systems.**

Direct biophotolysis:

Green algae, under anaerobic conditions, can either use H_2 as an electron donor in the CO_2-fixation process or evolve H_2. Hydrogen production by green microalgae requires several minutes to a few hours of anaerobic incubation in the dark to induce the synthesis and/or activation of enzymes involved in H_2 metabolism, including a reversible hydrogenase enzyme.

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

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

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 (Winkler, *et al*; 2002). 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). A reversible hydrogenase accepts electrons directly from the reduced ferredoxin to generate H_2 in presence of hydrogenase. This enzyme is very sensitive to O_2. Hydrogenase activity has also been observed in other green algae like *Scenedesmus obliquus*, *Chlorella fusca* (Winkler *et al.,* 2002), *Chlorococcum littorale* and *Platymonas subcordiformis* (Nandi and Sengupta, 1998). On the other hand, there are several green algae types that do not have hydrogenase activity such as *Dunaliella salina* and *Chlorella vulgaris* (Nandi and Sengupta, 1998).

The hydrogen production by green algae could be considered as an economical and sustainable method, in terms of water utilization as a renewable resource and recycling CO_2, a greenhouse gas. However, strong inhibition effect of generated oxygen on hydrogenase is the major bottleneck for the process. It has been reported that inhibition of the hydrogenase by oxygen can be partially overcome by cultivation of algae under sulfur deprivation for 2–3 days to provide anaerobic conditions under the light (Pinto *et al.,* 2000). Major drawbacks of this process are low hydrogen production potential and inability to use organic wastes. The hydrogenase activity of *C. reinhardtii* [200 nmol/(g chl a h)] is higher than *Scenedesmus* sp. [150 nmol/(g chl a h) (Winkler *et al.,* 2002). Rates of hydrogen production by photoheterotrophic bacteria are higher in the case of immobilized cells than that of the suspended cells.

Indirect biophotolysis:

General reaction for hydrogen formation from water by cyanobacteria (blue-green algae) can be represented by following reactions:

$$12\ H_2O + 6CO_2 + \text{light energy} \rightarrow C_6H_{12}O_6 + 6\ O_2 \quad \text{and}$$

$$C_6H_{12}O_6 + 12\ H_2O + \text{light energy} \rightarrow 12H_2 + 6\ CO_2$$

Cyanobacteria are large and diverse group of photoautotrophic microorganism, contain photosynthetic pigments such as Chl *a*, carotenoids and phycobiliproteins, and can perform oxygenic photosynthesis. Photosynthetic bacteria have long been studied for their capacity to produce significant amounts of hydrogen (Bolton, 1996). 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.

Morphologically these organisms include unicellular, filamentous and colonial species. Hydrogen is produced both by hydrogenase and

nitrogenase enzymes. Within the filamentous Cyanobacteria, vegetative cells may develop into structurally modified and functionally specialized cells. 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 (Pinto *et al.,* 2002). Heterocystous filamentous *Anabaena cylindrica* is a well-known hydrogen producing cyanobacterium. But, *Anabaena variabilis* has received more attention in recent years, because of higher hydrogen yield (Liu *et al.,* 2006). Hydrogen production by vegetative cells can take two routes:

A- Heterocystous nitrogen fixing bacteria.

B-Nonheterocystous nitrogen fixing bacteria:

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 (Liu *et al.,* 2006). 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 (Levin *et al.;* 2004). With dinitrogen:

$$N_2 + 8\,H^+ + 8\,e\_ + 16\,ATP \rightarrow 2\,NH_3 + H_2 + 16\,ADP + 16\,Pi$$

Or, without dinitrogen

$$8\,H^+ + 8\,e\_ + 16\,ATP \rightarrow 4\,H_2 + 16\,ADP + 16\,Pi$$

A very wide variety of Cyanobacterial species and strains has been studied for Hydrogen production. It occurs within at least 14 Cyanobacteria genera, under a vast range of culture conditions (Lopes *et al.,* 2002). Quantitatively, studies were investigated between aerobic and anaerobic hydrogen-producing bacteria. That were concluded that the aerobic bacteria will be used in the actual system for hydrogen production from carbohydrate, while the anaerobic bacteria may be a good choice for the production of hydrogen from wastewater containing innumerable compound (Jeong *et al.,* 2008). Some strains

produced up to five times more hydrogen than did wild-type cells growing under nitrogen-fixing conditions (Rey *et al.,* 2007). They revealed that in addition to the nitrogenase genes, 18 other genes are potentially required to produce hydrogen.

Different microorganisms participate in the biological hydrogen generation system, green algae, Cyanobacteria (or blue-green algae), photosynthetic bacteria and fermentative bacteria were collected and illustrated in Table (1).

Photodecomposition of organic compounds by photosynthetic bacteria:

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 (Liu *et al.,* 2006), in batch processes (Zurrer and Bachofen, 1979), continuous cultures (Fascetti and Todini, 1995), or immobilized whole cell system using different solid matrices like carrageenan (Francou and Vignais, 1984), agar gel (Vincenzini *et al.,*1986), porous glass (Tsygankov *et al;* 1994), and polyurethane foam (Fedorov *et al.,* 1998). 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 (Winkler *et al.,*2002). The oxidation of CO to CO_2 with the release of H_2 occurs via a water gas shift reaction as shown below:

$$CO + H_2O \rightarrow CO_2 + H_2$$

Table 1: Reported hydrogen yield by different microorganisms (cited from; Debabrata and Veziroglu 2008)

Microorganisms	Raw material used	Maximum rate of H_2 production (ml/l.h)	Maximum H_2 yield (mol H_2/mol substrate)
Green algae			
Scenedesmus obliquus	TAP-S medium	3.6a	–
Chlamydomonas reinhardii	TAP-S medium	4.5a	–
Chlamydomonas moewusii	TAP-S medium	10.0a	–
Cyanobacteria			
Heterocystous			
Anabaena variabilis	BG-11 medium	20	-
Anabaena cylindrical	Nutrient medium	8	-
Nonheterocystous			
Oscillotoria Miami	BG7 Medium-A except NH_4Cl	90	0.3
Photosynthetic bacteria			
Rhodobacter sphaeroides	Minimal medium	5	-
Rhodobacter capsulatus	Minimal medium	25	-
Rhodobacter palustris	Rhodospirillaceae medium	24.9	-
Rhodospirillum rubnum	CO & H_2O	358b	-
Fermentative bacteria			
Enterobacter aerogenes	Glucose 390	390	-
Enterobacter cloacae IIT-BT 08	Sucrose	660	6.0
Clostridium butyricum	Glucose	205	-
Citrobacter spY19	Glucose	-	2.49
Bacillus coagulans	Glucose	-	2.28

Clostridium acetobutylicum ATCC 824	Glucose	220	-

a ml/mg Chl a.l. b ml/g cell.h

Combined photosynthetic and anaerobic bacterial hydrogen production:

Anaerobic bacteria metabolize sugars to produce hydrogen gas and organic acids, but are incapable of further breaking down the organic acids formed. The combined use of photosynthetic and anaerobic bacteria for the conversion of organic acids to hydrogen. Theoretically, one mole of glucose can be converted to 12 moles of hydrogen (Fig. 1) through the use of photosynthetic bacteria capable of capturing light energy in such a combined system. From a practical point of view, organic wastes frequently contain sugar or sugar polymers. It is not however easy to obtain organic wastes containing organic acids as the main components. The combined use of photosynthetic and anaerobic bacteria should potentially increase the likelihood of their application in photobiological hydrogen production.

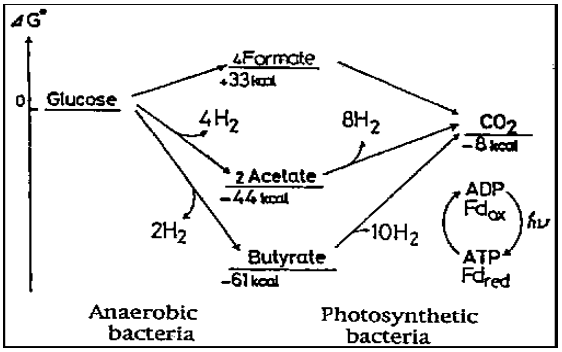

Figure (1): Free energy changes in hydrogen-producing reactions by anaerobic an photosynthetic bacteria

Ecology of Hydrogen-producing Bacteria:

The morphology of thermophilic anaerobic hydrogen producing bacteria was rod and enodspore-formation. From DGGE fingerprint, the GC content of thermophilic anaerobic hydrogen producing bacteria was higher than mesophilic anaerobic hydrogen producing bacteria (Chang, 2001). In addition to several known species of the genera Bacteroides, *Clostridium*, *Enterococcus*, *Escherichia*, *Eubacterium* and *Klebsiella*, two strains which classified based on phenotypic and phylogenetic considerations in a new genus Dorea as *Dorea longicatena* sp. nov.. Experiments with a specific 16S rRNA directed oligonucleotide indicate that *D. longicatena* sp. nov. is present in all human volunteers studied so far at average cell counts of 1,55 x 10^9/g of dry weight faces. They form with 0,58% of the total cell count a considerable proportion of the total flora. (Taras, 2001).

Thermophilic bacteria that are able to utilize CO under strictly anaerobic conditions, producing H_2 and CO_2 are of potential interest in astrobiology. Many volcanic exhalations contain high levels of CO_2, and anaerobic, CO-utilizing, hydrogen-producing thermophiles can be isolated on the Kuril and Kermadec Islands, south of the Kamchatka Peninsula, in terrestrial hydrothermal springs (Bonch-Osmolovskaya *et al.*, 1999). *Carboxydothermus. hydrogenoformans,* the prototype for these strains, was characterized as a Gram positive thermophile with the potential for H_2 production (Svetlichny *et al.*, 1991, 1994). Since

then such isolates have been obtained from deep sea hydrothermal vents (Sokolova *et al.*, 2001) and hot springs in Yellowstone National Park, USA. *C. hydrogenoformans* was the first strictly carboxydotrophic strain to be described, with an optimal growth temperature of 72°C (Svetlichny *et al.*, 1991). Later, another species, *Carboxydothermus restrictus* was isolated from hydrothermal outlets on Raoul Island on the Kermadec Archipelago (Svetlichny *et al.*, 1994). These strains grow optimally at 75°C, using CO as an energy and carbon source, with H_2 and CO_2 as the only end detectable products. Chemolithotrophic growth by means of CO oxidation is coupled to hydrogen and carbon dioxide formation according to the following equation:

$$CO + H_2O \longrightarrow CO_2 + H_2 \quad \Delta G = -20 \text{ kJ/mole}$$

The sequence of *Carboxydothermus hydrogenoformans* confirmed. The earlier observation that the strain was capable of spore formation, for it has a set of genes encoding for each of the major stages of endospore formation (DiRuggiero *et al.,* 2002).

Hydrogen synthesis via the water–gas shift reaction of photoheterotrophic bacteria:

Certain photoheterotrophic bacteria within the superfamily Rhodospirillaceae can grow in the dark using CO as the sole carbon source to generate ATP with the concomitant release of H_2 and CO_2 (Champine and Uffen, 1987). The oxidation of CO to CO_2 with the release of H_2 occurs via water–gas shift reaction. In these organisms, however, the reaction is mediated by proteins coordinated in an enzymatic pathway. The reaction takes place at low (ambient) temperature and pressure. Thermodynamics of the reaction are very favorable to CO-oxidation and H_2 synthesis since the equilibrium is strongly to the right of this reaction. Stoichiometric amounts of CO_2 and H_2 are produced during CO-oxidation. The enzyme that binds and oxidizes CO, carbon monoxide: acceptor oxidoreductase (carbon monoxide dehydrogenase = CODH) is part of a membrane bound enzyme complex (Wakim and Uffen, 1982). *Rubrivivax gelatinosus* CBS is a purple non-sulfur bacterium that not only performs CO–water–gas shift reaction in darkness, converting 100% CO in the atmosphere into near stoichiometric amounts of H_2, it also assimilates CO into new cell mass I, the light (via CO_2 6xation) when CO is the sole source of carbon (Maness and Weaver, 1997). Even when an organic substrate is available with CO, *R. gelatinosus* CBS will utilize both substrates simultaneously, indicating that the CO-oxidation pathway is fully functional even when a more favorable substrate is included. *R. gelatinosus* CBS exhibits a doubling time of 7 h in light when CO serves as the only carbon source (Maness and Weaver, 2002). The mass transfer of CO may be enhanced by a high ratio of gas phase to liquid bacterial suspension, and by stirring the culture vigorously. The hydrogenase from this organism is tolerant to O_2, exhibiting a half-life of 21 h when whole cells were stirred in full air (Maness *et al.*, 2002).

A specific rate of CO oxidation to H_2 production of 0:8 mmol/min/g of cells, dry weight (cdw) was measured using a low-density culture (final OD660 ‹0:2), stirred at a high rate (250 rpm), and supplemented with 20% CO in the gas phase. Because the conversion of CO to H_2 is stoichiometric, this corresponds to a rate of CO uptake and conversion of approximately 1:34 g CO/h/g cdw, or 48 mmol CO/h/g dcw. An OD660 of 2.0 yields 2:0 g *R. gelatinosus* CBS cdw/l. This corresponds to a H_2 production rate of 96 mmol H_2/2 g cdw/h or 96 mmol H_2/l/h). Advantages and disadvantages of different hydrogen production processes are shown in Table (2).

Biological hydrogen production resources:

Cost of the raw materials play a very important role for the overall economy of the hydrogen generation process. There are various applications where the process of biological hydrogen production by cyanobacteria can be well utilized. The examples can be included from food and chemical industries, which employ the process of hydrogenation to produce derivatives that are used as food additives, commodities, and fine chemicals (Dutta *et al.,* 2005). Biological hydrogen production from the fermentation of renewable substrates is one promising alternative although the use of commercially produced food products, such as corn and sugar, is not yet economical (Benemann, 1996). Also hydrogen produced from sweet sorghum by thermophilic bacteria (Claassen *et al.,* 2004). The food processing industry produces highly concentrated, carbohydrate-rich wastewaters. It can use for hydrogen production. Biohydrogen was produced by a domestic wastewater that had apple, potato processing and confectioners with both. Biogas produced consistently contained 60% hydrogen, with the balance as carbon dioxide. (Van Ginkel *et al.,* 2005).

Starch based wastewater has great potentiality for the H_2 production (Yu *et al.,* 2002). The major problem of using industrial wastewater is the presence of components in the reaction mixture. *Ruminococcus albus* has been found suitable for the production of H_2 from energy crop such as sweet sorghum by utilizing its free sugar, cellulose and hemicelluloses (Ntaikou *et al.,* 2008). H_2 yield is varied from 0.47 to 2.52 mol/mol glucose in continuous and batch experiments, respectively.

Microcrystalline cellulose (Wang *et al.,* 2008) and corn stover biomass pretreated with a steam-explosion process are found suitable substrates for the hydrogen production (Datar *et al.,* 2007). The H_2 yields of 2.84 and 3.0 are obtained using the mixed sugar present in the hydrolysates derived from neutral and acidic steam explosion, respectively. Delignified wood fiber is found to produce an average yield of 1.6 mol H^2/mol glucose by Clostridium thermocellum 27405 in a batch system (Levin *et al.,* 2007). Comparison between the activity of some organisms applied with different wastes are shown in Table (3).

Table 2: Advantages and disadvantages of different hydrogen production processes from biomass

Process	Advantages	Disadvantages
Microbial conversion	Can be operated at ambient temperature and atmospheric pressure.	▪ Lower rate of hydrogen production and yield.
Direct biophotolysis	▪ Can produce H_2 directly from water and sunlight Solar conversion energy increased by ten folds as compared. ▪ o 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 3: Use of different waste materials for the biohydrogen production processes (cited from; Debabrata and Veziroglu 2008)

Name of the wastes	Organism used	Process	Maximum rate of H_2 production (ml H_2/l h)	Maximum H_2 yield (mol H_2/mol substrate)
Dairy wastewater	Anaerobic mixed consortia	UASB	-	0.122a
Starch manufacturing wastes	*Closdridium butyricum* and *Enterobacter aerogenes* HO-39 and *Rhodobacter* sp. M-19	Repeated batch culture	-	7.2
Sewage biosolids	Mixed culture	Batch	-	0.7b
Rice winery wastewater	Sludge from wastewater treatment plant	Up-flow reactor	-	2.14
Potato processing wastewater	Sludge from wastewater treatment plant	Batch		6a
MSW	Mixed culture	PBR		99c
Food wastes	Sewage sludge	Batch	112.2d	5.48a
Food wastes	Mesophilic and thermophilic cultures	Batch		1.8
Jackfruit peel	Cow dung	Anaerobic contact filter		400e
Olive mill wastewater	Activated sludge and *Rhodobacter sphaeroides* O.U. 001	Two-stage process	11	-

a mmol H_2/g COD. b mol H_2/g COD. c mlH_2/g VS removed.d mlH_2/g VSS h. e ml/g VS destroyed

Wastewaters show great potential for economical production of hydrogen because producing a product from a waste could reduce waste treatment and disposal costs (WERF, 1999). Different waste materials have been successfully used in different processes for the hydrogen generation (Table 3). Some author observed that sewage sludge in combination with molasses improves the hydrogen yield of the process to a great extend (Yokoi *et al.,* 2002).

Enhancement of hydrogen-producing capabilities through genetic engineering:

Although genetic studies on photosynthetic microorganisms have markedly increased in recent times, relatively few genetic engineering studies have focused on altering the characteristics of these microorganisms, particularly with respect to enhancing the hydrogen-producing capabilities of photosynthetic bacteria and cyanobacteria. Some nitrogen-fixing cyanobacteria are potential candidates for practical hydrogen production. Hydrogen production by nitrogenase is, however, an energy-consuming process due to hydrolysis of many ATP molecules. On the other hand, hydrogenase-dependent hydrogen production by cyanobacteria and green algae is "economic" in that there are no ATP requirements. This mechanism of hydrogen production is not however sustainable under light conditions. Water-splitting by hydrogenase is potentially an ideal hydrogen-producing system. Asada *et al.,* (1986a; 1986b) attempted to overexpress hydrogenase from *Clostridium pasteurianum* in a cyanobacterium, *Synechococcus* PCC7942, by developing a genetic engineering system for cyanobacteria. These workers also demonstrated that clostridial hydrogenase protein, when electro-induced into cyanobacterial cells is active in producing hydrogen by receiving electrons produced by photosystems.

Another strategy being examined is the enhancement of hydrogen-producing capabilities of photosynthetic bacteria. In nitrogenase-mediated hydrogen-producing reactions, a considerable amount of light energy which is converted to biochemical energy by the photosystem, is lost through various biochemical processes. Control of the photosystem at an appropriate level for nitrogenase activity, would result in reduced energy losses, and thus improved light energy conversion. To this end, with the objective of utilizing genetic engineering techniques in controlling the photosystem level in the potent hydrogen-producing photosynthetic bacteria *Rhodobacter sphaeroides* RV, the *puf* operon encoding photoreaction center and light-harvesting proteins was isolated and characterized (Nagamine *et al.,* 1996).

As most organic substrates undergo combustion with the evolution of energy, the biocatalyzed oxidation of organic substances by oxygen or other oxidizers at two-electrode interfaces provides a means for the conversion of chemical to electrical energy. Abundant organic raw materials such as methanol, organic acids or glucose can be used as

substrates for the oxidation process, and molecular oxygen or H_2O_2 can act as the substrate being reduced. Intermediate formation of hydrogen as a potential fuel is possible as well.

***Escherichia coli*, *Enterobacter aerogenes, Clostridium butyricum, Clostridium acetobutylicum, and Clostridium perfringens* are examples of bacteria used in microbial biofuel cells. On the other hand, *Alcaligenes eutrophus Escherichia coli, Anacystis nidulans, Proteus vulgaris, Bacillus subtilis, Pseudomonas putida, Pseudomonas aeruginosa and Streptococcus lactis* are examples of bacteria used in biofuel cells upon application of membrane-penetrating electron transfer mediators.**

Fuel cells are electrochemical devices that create an electron flow using charged ions. A variety of different fuel cells systems have been developed. They differ in the type of electrolyte used, in the operating conditions, in their power density range, in their application, and each has its advantages and disadvantages (reviewed by Larminie and Dicks, 2000). Alkaline fuel cells (AFC) utilize hydroxyl ions (OH^-) as the mobile ion (derived from potassium hydroxide, KOH), operate in the 50 to 200^oC range, and are extremely sensitive to the presence of CO_2. Phosphoric acid fuel cells (PAFC) utilize protons (H^+) as the mobile ion and operate at approximately 200^oC. PAFC systems were the first fuel cells produced commercially and are used as stationary power sources, generating up to 200 kW of electricity. Many are in use in the USA and in Europe. The high operating temperature and corrosive nature of the electrolyte makes them unsuitable for use in mobile and transportation applications. Molten carbonate fuel cells (MCFC) utilize carbonate ions (CO_2^{-3}) as the mobile ion, operate at approximately 650^oC, and can take H_2, CO_2, CO, and/or CH_4 as fuel, which means they can use natural gas, coal gas, or biogas as fuel sources. Like PAFC, MCFC are used as stationary power sources, generating electricity in the MW range. Solid oxide fuel cells (SOFC) utilize oxygen radicals (O_2^-) as the mobile ion and operate between 500^oC and 1000^oC. Like MCFC, SOFC can utilize H_2, CO, and/or CH_4 as fuel, which means they can use methane, coal gas, or biogas as fuel sources. Carbon dioxide is not utilized as a fuel and is discharged as a waste gas. Like other high-temperature fuel cell systems (PAFC and MCFC) SOFC systems are used as stationary power sources, generating electricity from the low kW to the MW range. PEMFC utilize hydrogen protons (H^+) as the mobile ion, operate in the 50–100^oC range, require pure H_2 and are extremely sensitive to the presence of CO. Of all the fuel cell systems that are available, PEMFC systems are especially suitable for mobile and transportation applications, and PEMFC engines have been demonstrated successfully in both cars and buses. Small PEMFCs, in the 1–10 kW range, are also under commercial development as small stationary power units to provide electricity to homes and small businesses. Because of their imminent commercial applications, PEMFC technologies

are under intense research and development. The rate of H_2 consumption by PEMFCs is usually expressed in kg of H_2/s or in mol of H_2/s.

Biohydrogen: prospects for practical application:

The analyses of Levin *et al.,* (2004) indicate that photosynthesis-based systems do not produce H_2 at rates that are sufficient to meet the goal of providing enough H_2 to power even a 1 kW PEMFC on a continuous basis. This does not mean that these systems should be abandoned. There may be applications other than our hypothetical objective to which they may b more suited. Moreover, continued research will no doubt result in significant improvements in their respective technologies, and thus in the rates of H_2 production. Thermophilic and extreme thermophilic biohydrogen systems would require bioreactors in the range of approximately 2900–14; 600 l to provide sufficient H_2 to power PEMFCs of 1.5–5:0 kW, and a bioreactor of approximately 5700 l would be required to power the 5:0 kW fuel cell using the in particular appears most promising. A bioreactor of approximately 500 l (495 l) would provide enough H_2 to power a 2:5 kW PEMFC, while a bioreactor of approximately 1000 l (989 l) would provide sufficient H_2 to power a 5:0 kW PEMFC. The CO–water shift reaction of R. gelatinosus CBS is intriguing as it offers the potential to capture and reform CO, and produce H_2. A bioreactor of approximately 624 l would be required provide enough H_2 to power the 2:5 kW EMFC, while a bioreactor of approximately 1250 l (1247 l,) would provide sufficient H_2 to power a 5:0 kW PEMFC. By way of comparison, the current state-of-the-art for distributed, on-site production of hydrogen is via stationary electrolyzers which can generate H_2 from H_2O at a rate of 1000 l/h, or 40:3 mol/h. Equivalent rates of H_2 synthesis could be achieved by a bioreactor of approximately 334 l using a dark-fermentation system, or by a bioreactor of approximately 420 l containing *R. gelatinosus* CBS. The CO-water shift reaction of *R. gelatinosus* CBS offersan additional advantage. The CO–water shift reaction also yields equi-molar amounts of CO_2, which *R. gelatinosus* CBS can assimilate into new cell biomass using organic compounds, such as butyrate or propionate, as a carbon source and ATP as an energy source (Madigan *et al*., 1996). Thus, in addition to reformation of CO and H_2 synthesis, this process could be incorporated into an integrated energy system to sequester CO_2 in light, after H_2 is recovered from the system. Bioreactors of 1000–1500 l in the basement of a home are not unthinkable. Before electrical and natural gas heating, it was normal for North American homes located in northern latitudes to have, in their basements, tanks of heating oil that were approximately this size, while dark-fermentation systems and the CO-water were shift.

Reaction may have practical applications, there are a number of technical challenges that must be considered and overcome before these systems can be used to produce H_2 to power a

PEMFC. The most significant of these problems is whether the systems can be scaled up to volumes large enough to generate the required flow rate (22:1 mol H_2/h for the 5:0 kW fuel cell). The working volume of the bioreactor described that produced 121 mmol H_2/l/h) was 3l, and used sucrose (at 20 g/l of culture) as the carbon source for bacterial growth (Chang *et al*., 2002). The biogas produced was 25–35% H_2 and 65–75% CO_2. Further research is required to determine if the rate of H_2 production will remain at high levels if these systems are scaled up to much larger volumes (183 l or more), and if carbon sources other than pure sucrose can be used. The major challenge for the CO–water shift reaction is the problem of mass transfer: the gas must be available to the bacteria, in solution, at a sufficient concentration that the bacteria can absorb and metabolize efficiently. This may require radically new technologies and bioreactor designs.

Effect of physico-chemical parameters:

The effect of several physicochemical parameters on the photo-biological processes has already been reported (Das and Veziroglu, 2001). Physically; Temperature, pH and medium composition play very important roles for the H_2 production. The lower temperature and lower pH value would make the activity of hydrogen producing bacteria decrease (Chang, 2001). The fermentative H_2 reduction carried out at different temperatures: 20, 30, 35 and 55°C indicated that higher temperatures tend to improve the H_2 yield. (Yu *et al.,* 2002). Fermentative bacteria usually produces hydrogen under acidic conditions (pH 4.5–6.5), but photosynthetic bacteria works well at pH above 7 (Younesi *et al.,* 2008). Partial pressure of H_2 effects the efficiency of the process to a great extends (Mandal *et al.,* 2006). The energy conversion efficiency by photosynthetic bacteria is inversely proportional to the intensity of light (Nath and Das, 2005).

Chemically; addition of oxygen to an H_2-evolving culture, as well as the addition of nitrate to cells (which had formed the dissimilatory nitrate reductase system during the preceding growth), caused immediate cessation of hydrogen evolution (Kuhn *et al.,* 1984). Fertilizer helped gas production because of nitrates and phosphates, iron filings, and heat in the form of sunlight all, while lye (pH approx. 9) and lemon juice (pH approx. 5) prevented gas production (Dreszer, 2005). Comparing the addition of sodium and potassium ion for thermophilic hydrogen producing bacteria, the hydrogen producing bacteria were more sensitivity with potassium ion.

Substrate concentration affects the fermentative H_2-production processes to a great extent. Malate and glutamate play important roles in this fermentation process. The initial acetic acid concentration present in the spent medium of dark fermentation process has a profound effect on hydrogen production. Acetate concentration up to 55 mM is found non-toxic to the photofermentation of hydrogen (Nath and Das, 2005). H_2 production is found to be increased through redirection of metabolic

pathways by blocking formation of alcohol and some organic acids in E. cloacae (Kumar *et al.,* 2001).

Hydrogen production from cellulose by microflora is performed by a consortium of several species of microorganisms. (Ueno *et al.,* 2001).

Concluding remarks:

Biohydrogen technologies are, still in their infancy. Thus, further research and development aimed at increasing rates of synthesis and final yields of H_2 are essential. There are many technical challenges, from the production of sufficient quantities of hydrogen to its storage, transmission, and distribution. One of the major limitations to the practical application of biohydrogen systems is that scientists who study biohydrogen systems do not talk to engineers who develop hydrogen fuel cell technologies (and vice versa). Thus, the rates of hydrogen reduced by biological systems are unknown to fuel cell engineers and the amounts of H_2 required for practical applications, such as fuel cells, are unknown to biohydrogen researchers. The rates of hydrogen produced by the various biohydrogen systems are expressed in different units, making it difficult to assess and compare the rates and mounts of hydrogen synthesized by different biohydrogen technologies. 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.

Future prospects:

-The future of biological hydrogen production depends on research advances, and improvement in efficiency through genetically engineering microorganisms and/or the development of bioreactors.

-Progress on biological hydrogen production processes economically is still not attractive as compared to the conventional H_2-production processes.

-Hydrogen production depends also on economic considerations (the cost of fossil fuels), social acceptance, and the development of hydrogen energy systems. So that the following points require immediate attention:

- **Improvement of H_2 yield of the processes using cheaper raw materials,**
- **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.**
- **Optimization of bioreactor designs, rapid removal and purification of gases, and genetic modification of enzyme pathways that compete with hydrogen producing enzyme systems offer exciting prospects for biohydrogen systems.**
- **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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دور الطحالب الدقيقة و البكتريا في إنتاج الهيدروجين كإحدى مصادر الطاقة المتجددة

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غاز الهيدروجين يعد حالياً واحداً من أهم مصادر الطاقة البديلة للبترول. ولقد أتضح أنه ليس فقط من الوقود الغير ملوث، بل أنه يحد من انبعاثات ثانى أكسيد الكربون ومنتجا لغاز الهيدروجين كواحد من مصادر الطاقة المتجددة. إن التناقص المستمر في الوقود الحفري يلزمه إيجاد مصادر جديدة وغير تقليدية للطاقة عوضاً عن تلك التي تتمثل في الوقود الحفري. ولقد ثبت أن الكائنات النباتية الدقيقة لها القدرة على إنتاج غاز الهيدروجين من خلال عملية البناء الضوئي معتمدة على ضوء الشمس كمصدر طبيعي ودائم للطاقة. وتشمل تلك الكائنات الطحالب الخضراء والخضراء المزرقة (السيانوبكتريا)، من خلال عملية الاختزال الضوئي المباشر والغير مباشر. بالإضافة إلى أن تلك الطحالب الخضراء المزرقة تنتج الهيدروجين خلال تحلل المركبات العضوية (التحلل الضوئي).

ويعتبر إنتاج الهيدروجين من الطحالب الخضراء من الوسائل الاقتصادية والدائمة، وكذلك استخدام المياه وإعادة استخدام ثاى أكسيد الكربون من المصادر المستدامة للطاقات النظيفة. كما أن معدلات إنتاج الهيدروجين باستخدام بكتريا التحلل الضوئي من الخلايا الثابتة أكثر منه في العالقة. وتعد الطحالب الخضراء المزرقة من أفضل الكائنات الدقيقة لإنتاج الهيدروجين، كما انه بالا مكان تطبيقاتها على المستوى التجاري. وهي أفضل اقتصادياً مقارنة بالطرق التقليدية مثل الكيميائية والكهروضوئية. هذا البحث المرجعي يوضح الأسس البيولوجية لإنتاج الهيدروجين من الطحالب الدقيقة والبكتريا وإلقاء الضوء على بعض التوقعات المرئية لتطبيق هذه السبل. ويتوقف استخدامها على الجهد المعرفي والتكنولوجيات ومدى التقدم والتطور المستقبلي الواجب قبيل اعتمادها كمصدر أساسي للطاقة.