Production of Clean Energy from Cyanobacterial Biochemical Products

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ABSTRACT

In this article, an improved technology to produce hydrogen biologically will be discussed as a source of clean energy. The photochemical reaction among photons, ultraviolet (UV) light, and cyanobacterial biomaterials in photobioreactors offer a unique methodology for producing hydrogen energy. A photobioreactor is a bioreactor that utilizes a light source to cultivate phototrophic microorganisms. Using this technology, hydrogen production is significantly higher than any other technology that has ever been used. This hydrogen evolution is a product of the ultimate reaction of agitated photon electrons into the cyanobacterial biomolecules, where hydrogenase enzymes function as an active catalyst. The evolved hydrogen is then clarified using an electronic semiconductor-based sensor gas chromatograph with the efficiency recorded using a computerized data acquisition (DAQ) system. The results confirmed that this larger amount of hydrogen formation could be an interesting source of clean energy production. It is suggested that producing hydrogen using cyanobacteria could be a method of meeting future global energy demand. The purpose of this article is to describe this process and discuss its benefits.

Keywords: Photon Particle, Cyanobacteria, Biochemical Mechanism, Photo Bioreactor, and Clean Energy.

INTRODUCTION

During the last 150 years, the combustion of fossil fuels has resulted in more than a 25% increase in the levels of atmospheric carbon

dioxide. Climate scientists predict that if atmospheric carbon dioxide levels continue to increase, Earth's atmosphere will become much warmer during the next century. This will likely result in severe human health issues, melting glaciers, sea-level rise, floods, air pollution and ecosystem destruction [27].

Unlike fossil fuels, renewable energy comes from natural resources (i.e., wind, sunlight, geothermal power and biomass) that are environmentally benign. Using these resources to supply our energy needs will not only meet global energy demand, it will further support sustainability by lowering greenhouse gas emissions. The development and use of renewable energy provides benefits to the world's nations including incremental energy production, environmental protection, and reduction in pollution. Renewable energy sources such as solar (thermal or photovoltaic), wind, hydroelectric, biomass, and geothermal energy constitute the most common sustainable sources of energy. The characteristics of specific energy resources can be evaluated in terms of sustainability indicators [2]. In 2006, sustainable energies represented about 18% of the global total energy consumption [33]. They substitute for traditional fuels (e.g., coal, natural gas, petroleum, etc.) providing power generation, heating and transportation fuels. Due to its common use in developing countries for local energy supplies, biomass represents a major source of renewable energy constituting as much as 75% of the renewable energy used [18,19].

Cyanobacteria could produce third generation eco-energy since its photo biological hydrogen (H_2) production is considered to be a candidate for renewable energy production [15,18]. Cyanobacteria possess certain properties which have entitled them to be one of the most promising feedstocks for energy generation. Cyanobacteria, being photosynthetic organisms, use the sun's energy, water (H_2O) and carbon dioxide (CO_2) to synthesize their energy storage components. These energy storage components form a potential feedstock which can be converted into bioenergy [35]. Cyanobacteria possess unique properties which make them a promising model to transform carbohydrate sources into valuable fuels.

Hydrogen is produced by many strains of cyanobacteria by the reversible activity of hydrogenase. When cyanobacteria are grown under nitrogen (N_2)-limiting conditions, H_2 is formed as a byproduct of N_2 fixation by nitrogenase. Several reports have considered cyanobacterial species capable of producing H_2 [1,14,15]. Cyanobacteria should not

only be used as a biocatalyst of sunlight. They possess other properties which make them ideal candidates for the development of bio-friendly systems for the generation of clean fuels for 21st century.

METHODS AND MATERIALS

Central Metabolism of Photosynthetic Cyanobacterial for Hydrogen Production

The main constraint for H_2 production in cyanobacteria is that hydrogenases are highly intolerant to the oxygen (O_2) produced during photosynthesis. I have used the reducing agents ferredoxin and nicotinamide adenine dinucleotide phosphate (NADPH) as these are involved in respiration to reduce the O_2 by cyanobacteria. In order to enhance H_2 production, it is important to redirect part of the electron flow towards the H_2-producing enzymes and to oxygen-tolerant hydrogenases [5,43]. An attempt to eliminate pathways that consume reducing agents has

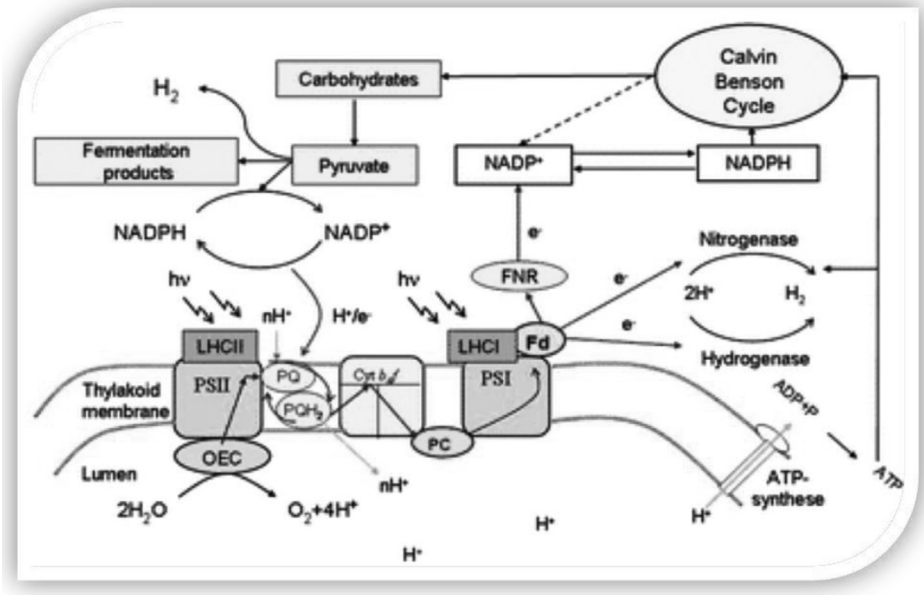


Figure 1. The scheme of H_2 production during oxygenic photosynthesis and subsequent formation of carbohydrates in microalgae [from 5,6,9,13,26].

also been performed.

Figure 1 shows the oxygenic "light reactions" of photosynthesis driven by the solar energy captured by the light-harvesting complexes of PSI and PSII. Electrons extracted from H_2O by the oxygen-evolving complex of PSII are passed along to the photosynthetic electron transport chain via plastoquinone (PQ), the cytochrome b6f complex (Cyt b6f), plastocyanin (PC), photosystem I (PSI), and ferredoxin (Fd), then by ferredoxin-NADP+ oxidoreductase to NADP+ ultimately producing NADPH. H+ are released into the thylakoid lumen by the PSII and PQ/PQH_2 cycles and used for adenosine triphosphate (ATP) production via ATP synthase.

The ATP and NADPH generated during primary photosynthetic processes are consumed for CO_2 fixation in the Calvin-Benson cycle which produces sugars and starch. Under anaerobic conditions, hydrogenase accepts electrons from reduced Fd molecules and uses them to reduce protons to H_2. Certain algae under anaerobic conditions can use starch as a source of H+ and e– for H_2 production (via NADPH, PQ, cytb6f, and PSI) using the hydrogenase. In cyanobacteria the H+ and e– derived from H_2O can be converted to H_2 via a nitrogenase. Nitrogenase is responsible for nitrogen-fixation and hydrogenase is responsible for hydrogen production. Molecular nitrogen is reduced to ammonium with consumption of reducing power (e' mediated by ferredoxin) and ATP. The reaction is substantially irreversible as follows:

$$N_2 + 6H1^+ + 6e^- = 2HN_3$$
$$12ATP \rightleftharpoons 12(ADP+Pi)$$

However, nitrogenase catalyzes proton reduction in the absence of nitrogen gas (i.e., in an argon atmosphere).

$$2H^+ + 2e^- = H_2$$
$$4ATP \rightleftharpoons 4(ADP+Pi)$$

Hydrogen production catalyzed by nitrogenase occurs as a side reaction at a rate of one-third to one-fourth that of nitrogen-fixation, even in a 100% nitrogen gas atmosphere. It is important to analyze the nitrogenase function on cyanobacterial metabolism for the proper clarification of hydrogen production. As Figure 2 shows, the nitrogenase itself is extremely oxygen-labile which ultimately consumes O_2 and accelerates

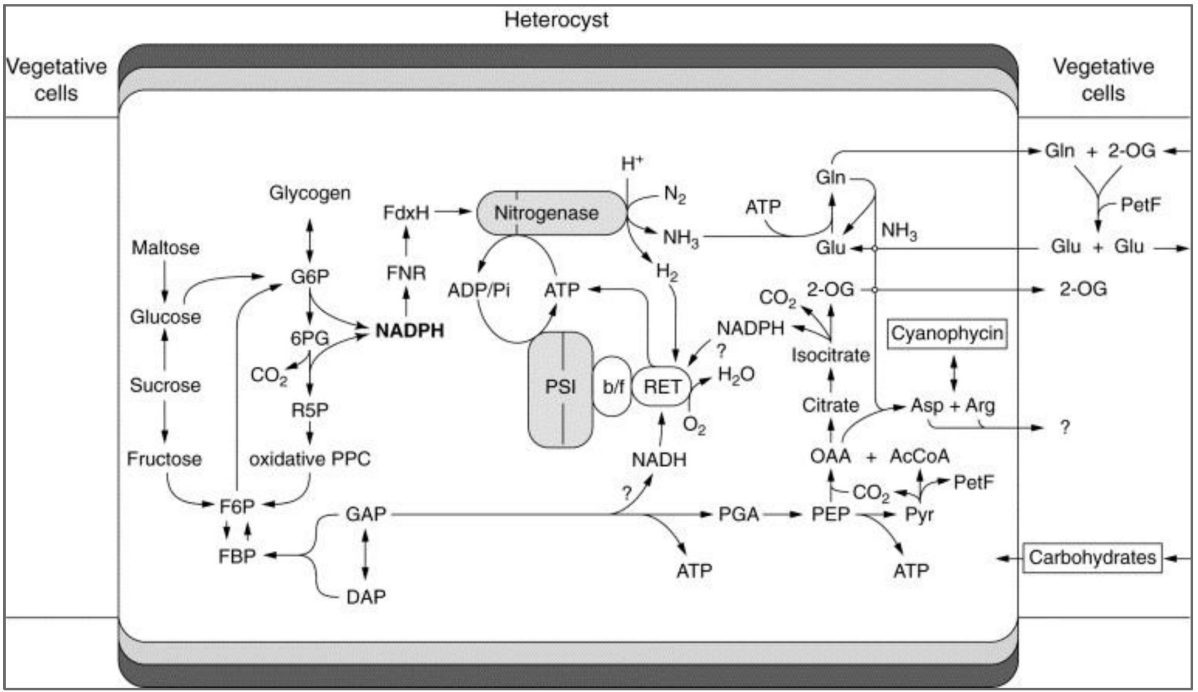


Figure 2. Cyanobacterial metabolism for nitrogen fixation. Abbreviations: AcCoA, acetyl coenzyme A; Arg, arginine; Asp, aspartate; b/f, cytochrome b6f complex; F6P, fructose 6-phosphate; PetF, vegetative cell type ferredoxin; Glu, glutamate; Gln, glutamine; OAA, oxaloacetate; 2-OG, 2-oxoglutarate; 6PG, 6-phosphogluconate; PGA, 3-phosphoglycerate; Pi, inorganic phosphate; R5P, ribose 5-phosphate [5,8,11].

H_2 production. Unlike hydrogenase, cyanobacteria have developed mechanisms for protecting nitrogenase from oxygen gas and supplying it with energy (ATP) and reducing power.

Vegetative cells (ordinary cells) in filamentous cyanobacteria perform oxygenic photosynthesis. Organic compounds are produced by carbon monoxide (CO); reductions are transferred into cyanobacterial heterocyst and are decomposed to provide nitrogenase with reducing power. ATP can be provided by PSI-dependent and anoxygenic photosynthesis within heterocyst. Investigations into prolongation and optimization of hydrogen production revealed that the hydrogen-producing activity of cyanobacteria was stimulated by nitrogen starvation. Therefore, we continuously sparge argon-based gas while the hydrogen content of the effluent gas was measured. The average conversion efficiency over a period of one month (combustion energy of hydrogen gas produced by cyanobacteria/incident solar energy into the photobioreactor area) significantly produced the highest amount of H_2 (68 μmol mg^{-1} chl a h^{-1}).

Cyanobacteria are photoautotrophic microorganisms [23] that use two sets of enzymes to generate hydrogen gas. The first is ***Nitrogenase***. It is found in the cyanobacterial heterocyst of filamentous cyanobacteria when grown under nitrogen limiting conditions. Hydrogen is produced as a byproduct of fixation of nitrogen into ammonia. The reaction consumes ATP and has the general form:

$$16ATP + 16H_2O + N_2 + 10H_2 + 8e^- \xrightarrow{\text{Nitrogenase}} 16ADP + 16Pi + 2NH_4^+ + H_2 \text{ [24]}$$

A Nitrogenase enzyme consists of two parts: one is dinitrogenase (MoFe Protein, encoded by the genes *nifD* and *nifK*, □ and □ respectively) and the other is dinitrogenase reductase (Fe Protein, encoded by nifH). Dinitrogenase is a $□_2□_2$ heterotetramer, having molecular weight of 220 to 240 kDa respectively, breaking apart the atoms of nitrogen. Dinitrogenase reductase is a homodimer of about 60 to 70 kDa and mediates the transfer of electrons from the external electron donor (a ferredoxin or a flavodoxin) to the dinitrogenase [17,25,30]. There are three types of dinitrogenase found in Nitrogenase, which vary depending on the metal content. Type I contains molybdenum [39], Type II contains vanadium (V) instead of molybdenum [22,39], and Type III has neither molybdenum nor V but contains iron [10,22].

The other hydrogen-metabolizing/producing enzymes in cyanobacteria are *Hydrogenases*; they occur as two distinct types in different cyanobacterial species. One type uptakes hydrogenase encoded by *hupSL* [38] and has the ability to oxidize hydrogen. The other type of hydrogenase is reversible or bidirectional hydrogenase (encoded by *hoxFUYH*) and it either absorbs or produces hydrogen. *Uptake hydrogenase* enzymes are found in the thylakoid membrane of cyanobacterial heterocyst, where they transfer electrons from hydrogen for the reduction of oxygen via the respiratory chain in a reaction known as oxyhydrogenation or Knallgas reaction. The enzyme consists of two subunits. The *hupL*-coded protein uptakes hydrogen and the smaller *hupS*-coded subunit is responsible for reduction. Under ambient conditions, the hydrogen formed is reoxidized by an uptake hydrogenase via a Knallgas reaction. This is counterproductive when the goal is to produce hydrogen on a commercial scale. The reaction catalyzed by the uptake hydrogenase takes the following form:

$$H_2 \xrightarrow[\text{Nitrogenase}]{} 2H + 2e^-$$

The biological role of *bidirectional* or *reversible hydrogenase* is thought to control ion levels in the organism. Reversible hydrogenase is associated with the cytoplasmic membrane and likely functions as an electron acceptor from both NADH and H_2 [12]. The reversible hydrogenase is a multimeric enzyme consisting of either four or five different subunits depending on the species [12,34]. On a molecular scale, it is a NiFe-hydrogenase of the $NAD(P)^+$ reducing type which consists of a hydrogenase dimmer coded by *hoxYH* gene. Maturation of reversible hydrogenases requires the action of several auxiliary proteins collectively termed as hyp (products of genes: *hypF, hypC, hypD, hypE, hypA*, and *hypB*) [44]. Unlike uptake hydrogenase, reversible hydrogenases are helpful in hydrogen production. Most cyanobacterial species preferentially absorb red light near 680 nm [31], the light required for hydrogen production.

Photobioreactor Design

The effects of light on nitrogenase mediated hydrogen production by most types of cyanobacteria are well studied [36]. Since light is essential for cyanobacterial growth, a unique photo bioreactor was designed

for large-scale hydrogen production [3,7]. Our photobioreactors require sunlight along with some controlled illumination (i.e., florescent light).

Inside photobioreactors there are photic zones, close to the illuminated surface and dark zones, those further away from this surface. The hydrogen productivity of a photobioreactor is light limited and tends to decrease at higher light intensities. Photosynthesis diverts the hydrogen production pathway. The light regime is determined by the light gradient which must be diluted and distributed as much as possible since the highest production levels occur in the darkest conditions. Rates of aeration (hydrogen producing enzymes are oxygen susceptible; anaerobic conditions or inert gas environments are preferred) impact hydrogen productivity. The red light needed by cyanobacteria is generated by panels constructed in specialized bioreactors [37].

The photobioreactor (PBR) was mainly divided into two parts: a vertical column reactor (VCR), and a tubular type and flat panel photobioreactor. The reactor for photo biological hydrogen production that was used met two conditions:

1. The photobioreactor is enclosed so that the produced hydrogen may be collected without any loss;

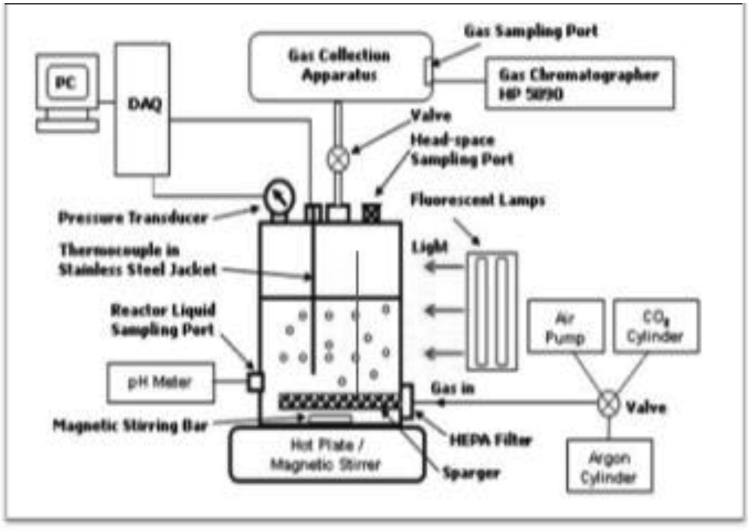


Figure 3. Schematic diagram of the Photobioreactor and Production of Hydrogen.

2. To maximize the area of incident light (thus allowing high growth and hydrogen production) the photobioreactor design provides a high surface to volume ratio.

Vertical column reactor (air-lift loop reactor and bubble column) part: This PBR's part of the VCR consists of a transparent column usually composed of high quality glass and surrounded by a water jacket. This configuration allows the temperature to be maintained with circulating water and provides adequate light entry. The reactor has medium inlets and outlets for the gases such as argon and for the hydrogen. Fresh medium is added from a reservoir from above the VCR [6, 28]. Cyanobacteria are inoculated through a septum that helps maintain sterility and prevents contamination. The bottom section of the VCR column retains outlets for the culture and an inlet/outlet for argon gas. In bubble columns using sunlight as light source, the presence of gas bubbles enhances internal irradiance at sunset and sunrise. As the position of sun changes from the horizon in the morning to overhead at noon, the bubbles diminish the internal column irradiance relative to the un-gassed state.

The biomass productivity varies substantially during the year. The peak productivity in the summer may be several times greater than the lower productivity in the winter. An example of this type of VCR is hydrogen production using cyanobacteria [6,7,28]. This reactor column consists of a glass cylinder with an inner volume of 400 ml surrounded by a water jacket. The optimal dimensions of the vertical column are about 0.2 m in diameter and 4 m in column height. The optimal column height depends on factors including the wind speed and the strength of optically transparent materials (e.g., glass or thermoplastics).

A typical flat-panel photobioreactor consists of a stainless-steel frame with polycarbonate panels. These sections of reactor are placed side by side. Water is circulated via a temperature controlled water bath through the bioreactor compartment to maintain the desired temperature of the culture. This design for a PBR often uses direct photon particles from sunlight. The average light intensity provided at the reactor surface is 175 W/m^2. A red light emitting diode (LED) that peaks at 665 nm is used as the light source on one side. In addition, the extreme ultra-violet (wave length 10-121 nm) and photon energy (see calculation described in Figure 4) is introduced.

A membrane gas pump circulates the gas through the spargers

(hypodermic needles) at the bottom of the reactor. The gas produced is collected in a gasbag. In this reactor system, pressure vessels prevent pressure fluctuations in the gas recirculation system and a pressure valve maintains a constant input pressure to the mass flow controller. A condenser prevents water vapor from entering the gas recirculation system. The reactor is autoclaved prior to cyanobacterial cultivation and hydrogen production. The culture medium is separately autoclaved and fed to the reactor. Sampling is performed via the sample port which is attached to the outflow tube. Bacterial growth is monitored by a computer.

Results and Discussion

Substantial progress has been made over the last decade in understanding the fundamental reaction of photosynthesis that evolved in cyanobacteria 3.7 billion years ago. This process uses water molecules as a source of electrons to transport energy derived from sunlight. Light energy (E_{photon}) introduced to cyanobacteria give bacteria their blue ("cyano") color, enabling plants to evolve by "kidnapping" bacteria for their photosynthetic engines to produce H_2. The emission of photon energy into the hydrogen electron state is higher than any other technology previously used for renewable energy production.

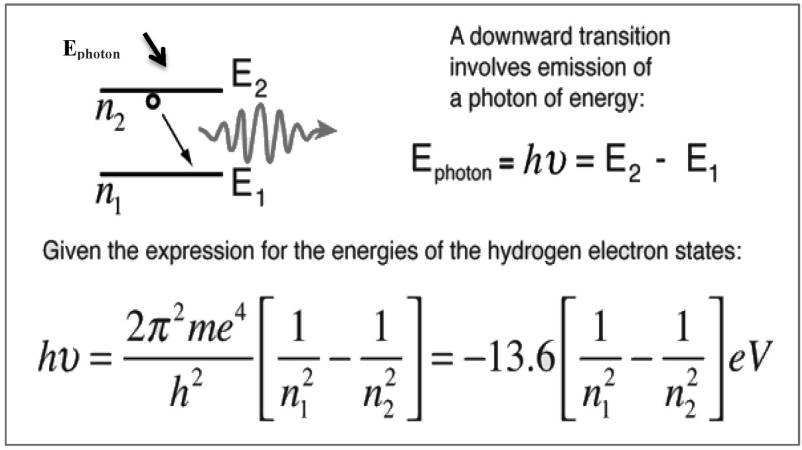


Figure 4. Photo reaction over the hydrogen electron states confirms that photobacteria hydrogen is a way to produce energy. The equation shows that the high rate of electron deliberation occurs once the photon energy passes through the hydrogen electron state.

It is known that cyanobacteria possess thylakoid, granum and other pigments, capturing the energy from sunlight using photosynthetic systems (PSII and PSI) to perform photosynthesis [20,21,39,41]. The pigments in PSII (P680) absorb the photons, generating a strong oxidant capable of splitting water into protons (H^+), electrons (e^-) and O_2 as shown in Figure 5. The electrons or reducing equivalents are transferred through a series of electron carriers and cytochrome complex to PSI. The pigments in PSI (P700) absorb the photons, which further raises the energy level of the electrons to reduce the oxidized ferredoxin (Fd) and/or nicotinamide adenine dinucleotide phosphate ($NADP^+$) into their reduced forms. The proton gradient formed across the cellular (or thylakoid) membrane drives adenosine triphosphate (ATP) production via ATP synthase.

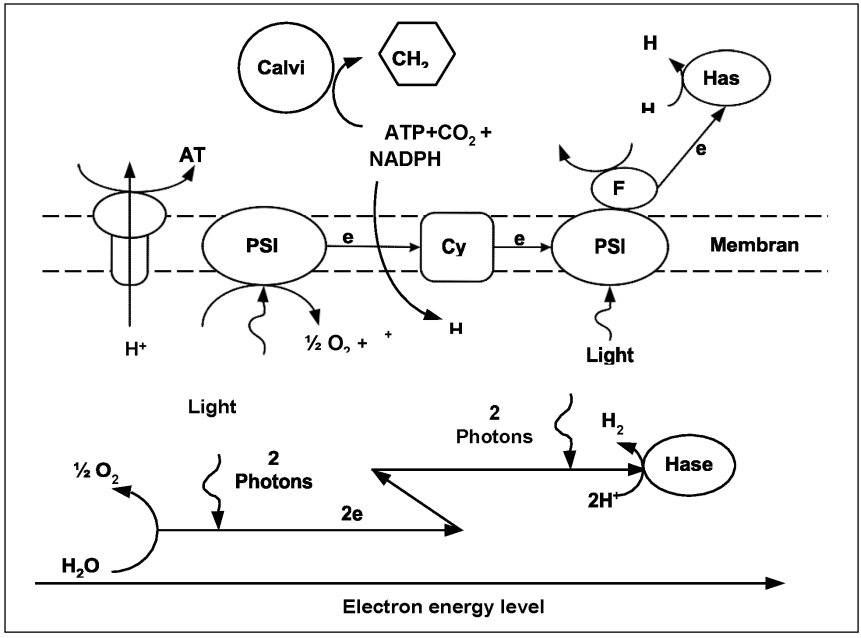


Figure 5. Schematic mechanisms of photosynthesis and biophotolysis of photoautotrophic cyanobacteria. The energy level of electrons or reducing equivalents from water oxidation is raised by the adsorbed photons at PSII and PSI. The reducing equivalent (NADPH) is used for CO_2 reduction in photosynthesis and carbohydrates (CH_2O) are accumulated inside the cells. The reducing power (Fd) could also be directed to hydrogenase (Hase) for hydrogen evolution.

Consequently, biophotolysis produced hydrogen within 24 hours of light irradiation. This occurred when light energy was absorbed by the pigments at PSII, or PSI or both, raising the energy level of electrons from water oxidation when transferred from PSII via PSI to ferredoxin. This biochemical process in *Cyanobacteria* provides an oxygen-free environment to the oxygen-sensitive nitrogenase reducing molecular nitrogen into NH_2 as well as protons into H_2 [23,39,40] and results in much higher hydrogen formation in the absence of molecular nitrogen [Figure 6].

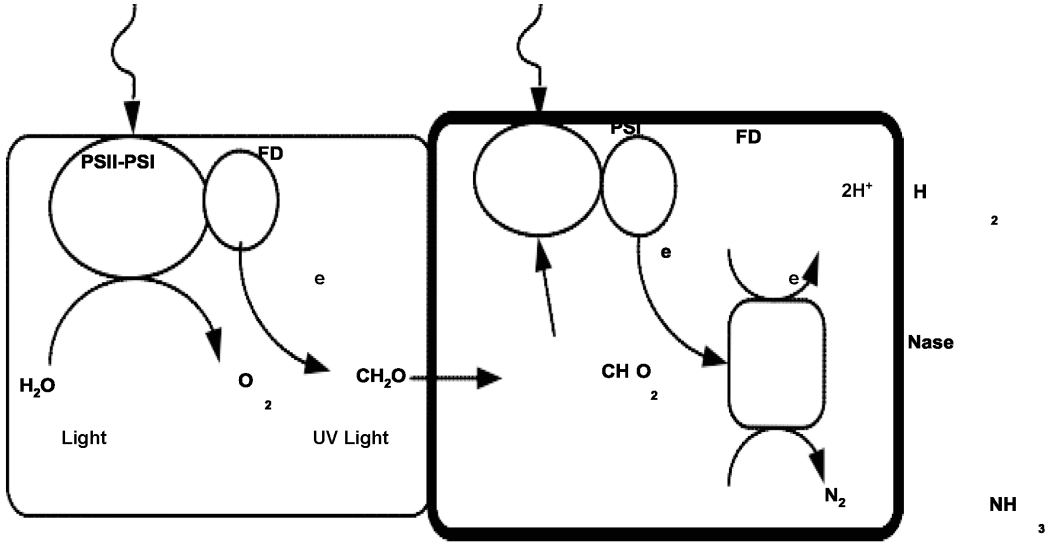
The hydrogen productivity was then calculated based on the reactor surface area from the volumetric productivity and a critical optical length. The latter has a range from 2 to 6 cm [41], depending on factors that include cell density, cell size, light intensity and light saturation [16,29,34,44]. The hydrogen collected is indicated by gas chromatography. The H_2 energy productivity is calculated by multiplying the volumetric productivity (mmol H_2/L/hr) by the heat of combustion of hydrogen at 25 degrees C.

Conversion of H_2 into Electricity

I have used a fuel cell to produce hydrogen as a fuel along with electrons, protons, heat and water. Fuel cell technology is based upon the following combustion reaction:

$$2H_2 + O_2 \leftrightarrow 2H_2O$$

The proton exchange membrane (PEM) fuel cell produces electricity by chemical reaction. Hydrogen and oxygen pass over the electrodes producing electricity, heat and water. Hydrogen fuel is supplied to the anode (negative terminal) of the fuel cell while oxygen is supplied to the cathode (positive terminal) of the fuel cell. Through a chemical reaction, the hydrogen is split into an electron and a proton. Each takes a different path to the cathode. The electrons take a path bypassing the electrolyte to produce electricity. The proton passes through the electrolyte and both are reunited at the cathode. The electron, proton, and oxygen combine to form water as a byproduct. Significantly cleaner emissions result from using a PEM fuel cell rather than from a fossil fuel combustion process. PEM fuel cells are considered one of the best technologies to produce electricity from H_2 due to their high efficiency (80%) of electricity production.



$$N_2 + 8H^+ + 8e + 16ATP = 2NH_3 + H_2 + 16ADP + 16Pi$$

$$8H^+ + 8e + 16ATP = 4H_2 + 16ADP + 16Pi$$

$N_2 + 8H^+ + 8e + 16ATP = 2NH_3 + H_2 + 16ADP + 16Pi$
$8H^+ + 8e + 16ATP = 4H_2 + 16ADP + 16Pi$

Figure. 6. Nitrogenase (Nase)-mediated hydrogen evolution in a heterocyst of nitrogen-fixing cyanobacteria [10, 30, 32]. The oxygen and hydrogen evolution occur separately and the energy-rich carbohydrate (CH2O) is used as the electron source in the oxygen-free heterocyst.

Table 1. Design and construction cost of the photo bioreactor for hydrogen production.

List of Component	Materials Cost	Labor Cost	Equipment Cost	GC & OH Cost	Total Cost
Site Preparation	$5,000	$3,000	$2,000	$2,000	$12,000
Photo Bioreactor Equipment	$20,000	$5,000	$2,500	$5,400	$32,900
Instrumentation	$2,000	$1,000	$2,000	$1,000	$6,000
Electrical, Mechanical, Plumbing and Control	$2,500	$1,000	$1,000	$ 900	$5,400
Supply for 30 Years cost at $0.05/kWh for monthly 4000 kWh for 100 people					$72,000
				Total Cost	$128,300

This estimate was prepared using January 2016 material costs from leading manufacturers and labor installation costs using union labor wages. The equipment rental was calculated using market rental costs in conjunction with production rate standard construction practice.

Energy Cost Savings

The total cost for 30 years of electrical energy consumption from a conventional source for a standard industry (100 people capacity) at 0.12/kWh using 4,000 kWh per month is equal to $172,800 (30 x 12 x 4,000 x 0.12). This comparison between conventional energy use and cyanobacterial energy production clearly indicates a cost savings of $44,500 when cyanobacterial energy is substituted as the energy source.

CONCLUSIONS

Cyanobacteria provide unique opportunities for researching biological production of hydrogen. Cyanobacterial hydrogen production is poised to be a very useful and effective method of producing hydrogen. Hydrogen produced by cyanobacteria as described in this article is economically preferable when compared to traditional hydrogen production technologies (fermentative, photocatalytic water splitting). Hydrogen gas can be a future energy provider since it does not emit greenhouse gases when combusted. It frees large amounts of energy per unit weight in combustion, is easily converted to electricity, and offers

an inexhaustible energy resource.

Cyanobacterial hydrogen production has several advantages over traditional hydrogen generation processes. The photobioreactor is a closed transparent box with low energy requirements. The process is very cost effective. The most appealing aspect of biological hydrogen production is the simplicity of this technology, using nothing but photons, water and cyanobacterial biochemical reactions. The current knowledge of cyanobacterial biochemistry and photosystems are tested in this article to create light-driven electrochemical devices to produce high efficiency hydrogen. As sunlight is abundant, our insight into light conversion and hydrogen production by cyanobacteria is essential for creating renewable alternatives to fossil fuels. Biomechanisms and photochemical reactions achieve high rates of hydrogen production, thus providing environmentally benign energy [24].

The best methodologies for photobacterial hydrogen production yield zero emissions of greenhouse gases and compare favorably to other renewable technologies such as hydroelectric plants and solar photovoltaics over their production life-cycle. Hydroelectricity plants and solar photovoltaics emit as much as 0.7% greenhouse gases during their reaction process while phtotobioreators emit 0.0% greenhouse gases. Considering the costs of biological hydrogen energy production, this is another reason to deploy this technology globally. The U.S. Energy Information Administration (EIA) depicts photobiogically produced hydrogen energy power as the only generation technology that has a levelized avoided cost of electricity (LACE) greater than levelized cost of electricity (LCOE) in its 2014 forecast. It is the only technology competitive in electrical supply systems that have excess capacity or stable demand for new power. The EIA's analysis includes the transmission and integration costs imposed by intermittent technologies deployed by the companies and agencies in Table 2.

If the economic potential of biologically produced hydrogen energy resources can be realized, it would represent an enormous source of energy production with the capacity to produce 14,830.8 billion MWh of energy annually, or three times more than present global energy needs. Therefore, this energy technology could not only reduce greenhouse gas emissions, it could also increase profitability by lowering the cost of energy production. With rapid increases in global population and continuing environmental problems, developing alternative sources of energy offers solutions. The capability of individual nations to produce

Table 2. Comparative costs of electrical energy generation (cents/kWh).

PG&E	SCE	SDG&E	Average
Biologically H_2	**5.94**	**6.82**	**6.98**
Geothermal	7.19	6.75	7.03
Wind	8.40	9.77	8.68
Small Hydro	8.72	8.91	8.66
Solar Thermal	14.23	13.48	13.52
Solar PV	15.18	11.90	13.96
UOG Solar PV	16.21	47.00	21.65

hydrogen would eliminate oligopolies in the fossil fuel industries and energy price volatility. Hydrogen produced by cyanobacteria offers the promise of a cleaner renewable energy resource.

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