PHOTOBIOLOGICAL HYDROGEN PRODUCTION

A SEMINAR REPORT

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Certificate

This is to certify that DHEERAJ SURATE, ATHARV JADHAV, VIRAJ GUPTA has successfully completed the **SEMINAR** work entitled **"PHOTOBIOLOGICAL HYDROGEN PRODUCTION"** during the prescribed period in the academic year 2021-2022. This Seminar Report is submitted in the partial fulfillment of the requirement for the Award of Degree of

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CONTENT

ABSTRACT

Hydrogen is the simplest element in the periodic table with one electron, one proton and one neutron. But as simple it seems; it has vast range of application in numerous fields. Hydrogen is considered as the cleanest fuel as it produces carbon free energy. Being the cleanest fuel, clean production of hydrogen is still questionable. Presently the largescale production of hydrogen is done by steam methane refining which is not just limited to hydrogen production but also leads to formation of carbon monoxide and a small amount of carbon dioxide which are the green house gases. Researches have found that carbon free hydrogen could be produced by photobiological means. Certain algae and cyanobacteria produces hydrogen under sunlight to get red of excess energy before starting photosynthesis. A enzyme named hydrogenase is responsible for production of hydrogen in these algae and cyanobacteria. Optimising the conditions, we can produce adequate amount of hydrogen gas. Photobiological production of hydrogen has potential to be one of the most cost-effective ways to produce hydrogen from renewable energy.

Keywords: Greenhouse gases, cyanobacteria, photosynthesis, hydrogenase, nitrogenase.

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CHAPTER 1

INTRODUCTION

Hydrogen is a versatile energy carrier that can release energy through a number of different processes such as direct combustion, catalytic combustion, steam production, and fuel cell operations. Hydrogen is essentially an energy carrier rather than a primary fuel. Although the most abundant element, it cannot be mined. It rarely exists in elemental form, instead being bound up in water or other compounds. It does, however, sometimes exist in nature, most notably within certain algae and photosynthetic bacteria that produce it as a way to dissipate excess energy. If we can effectively manipulate those microorganism processes, we can capture solar energy directly in a form that is an ideal transportation fuel. In terms of environmental impact, hydrogen may be the best alternative to fossil fuels because it drastically reduces the release of climate-changing gases and compounds harmful to human health.

Hydrogen is a versatile energy carrier that can release energy through a number of different processes such as direct combustion, catalytic combustion, steam production, and fuel cell operations. In terms of environmental impact, hydrogen may be the best alternative to fossil fuels because it drastically reduces the release of climate-changing gases and compounds harmful to human health. Photobiological hydrogen production is an attractive option to generating hydrogen by photoautotrophic organisms from sunlight and water. This process is most effective and important for avoiding using fossil fuel. Photobiological hydrogen production consumes naturally occurring carbon dioxide gas to produce oxygen and biomass; hence, it is renewable and sustainable. Microorganisms such as green algae, cyanobacteria, purple non-sulfur bacteria, and dark fermentative bacteria are used to generate biohydrogen. In the light, solar energy is converted into adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) as a source of energy and reductants, respectively. In the dark, organic compound is synthesized from CO_2 and H_2O.

Hydrogen use in fuel cells produces only water vapor and electricity at the point of use. Also, hydrogen can be stored to match energy production to energy demand. These make the use of hydrogen highly attractive as an energy carrier in addition to, or instead of, electricity. As an energy carrier rather than a basic energy source, however, hydrogen is only as “good” as the energy used to make it. Nearly all current U.S. and most of the world’s hydrogen production involves steam reformation of natural gas. This, of course, uses an increasingly scarce fossil fuel that can be used directly for electrical production to meet peak demand and also has home heating as a high-priority use. Hydrogen could also be made by reforming gasified biomass or using renewably generated energy to power water electrolysis. But in the long run, the highest efficiencies should be achieved with technologies such as photoelectrochemistry or photobiochemistry, which can produce hydrogen directly from solar energy.

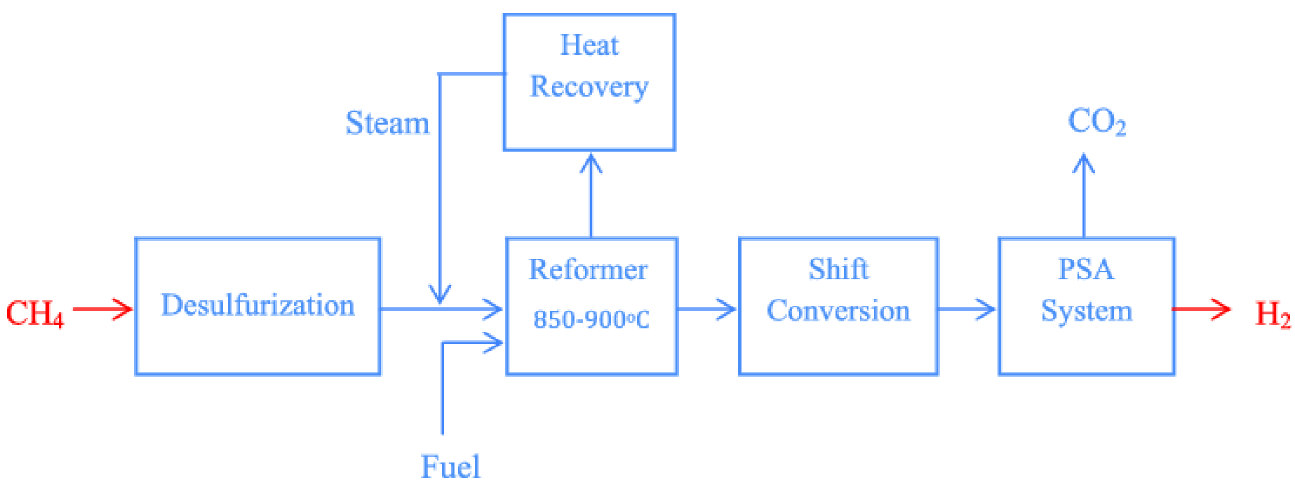


Fig.1 Steam Methane Refining

CHAPTER 2
LITERATURE REVIEW

- Photobiological Production of Hydrogen - A Solar Energy Conversion Option by Paul Weaver, Stephen Lien, Michael Seibert a Solar Energy Research Institute (1999) explains A promising alternative biological process for solar energy conversion has been coined "biophotolysis" by L. O. Krampitz. The term first appeared a number of years ago (NSF/NASA Solar Energy Panel, 1973) and was defined as the formation of H2 gas from water using the photosynthetic apparatus of green plants and algae. Unfortunately, there is confusion as to the precise definition since photo-svnthetic bacteria can also produce H_2. However, photosynthetic bacteria do not use water as the primary reductant, and consequently, bacterial photoproduction of H2 is not strictly included in Krampitz's definition of biophotolysis (though many investigators use the term for both processes). Confusion from terminology aside, the purpose of this report is to review the field of photobiological H2 production and to assess its potential for applied conversion systems. Included is a brief history of the field, the enzymology and biochemistry of H2 production, H2 evolution systems and rates, technical problems, and future prospects.

- Roshan Sharma Poudyal, Indira Tiwari, Agni Raj Koirala, Hajime Masukawa in the article Hydrogen Production Using Photobiological Methods (July 2015) states and explains the photobiological hydrogen production concerning the different methods used to produce hydrogen from green algae and cyanobacteria. In their report the internal processes taking place inside a algae and cyanobacteria are demonstrated. The role of hydrogenase and nitrogenase in the hydrogen synthesis is further demonstrated. The different pathways and phases which the bacteria and the algae undergoes in elaborated.

- Photobiological Hydrogen Production - Prospects and Challenges (June 2009) a article presented by Carrie Eckert and Maria Ghirardi. The article summarises Cyanobacteria and green algae produce hydrogen gas in the dark fermentatively and in the light under photosynthetic conditions. Hydrogenase enzymes catalyze hydrogen production in these phototrophs; the algal hydrogenase has an iron-iron cluster at its catalyticsite, while the cyanobacteria enzyme contains a nickel-iron cluster. Key technical challenges include overcoming oxygen sensitivity of hydrogenase enzymes, outcompeting other metabolic pathways for photosynthetic reductants, dissipating the proton gradients across the photosynthetic membrane, and ensuring adequate efficiency when capturing and converting solar energy. Despite technical challenges, abundant solar energy and water provide incentives for developing large-scale means to produce hydrogen photobiologically.

- National Renewable Energy Laboratory (NREL) presents a paper on Photobiological Hydrogen Production. Stating Certain algae and cyanobacteria photoproduce hydrogen for short times as a way to get rid of excess energy before starting up the photosynthetic carbon fixation process. NREL researchers have successfully developed a bacterial system for synthesis of a key enzyme hydrogenase—that is responsible for photosynthetic hydrogen evolution in green algae. They are now focusing on enzyme engineering to block the access of oxygen, which can stop hydrogen production, to the catalytic site of the hydrogenase. Photobiological production of hydrogen has potential to be one of the most cost effective ways to produce hydrogen from renewable energy.

- Photobiological Hydrogen Production report by Journal Bioscience And Bio Engineering (1999) by Yasuo Asada and Jun Miyake states The principles and recent progress in the research and development of photobiological hydrogen production are reviewed. Cyanobacteria produce hydrogen gas using nitrogenase and/or hydrogenase. Hydrogen production mediated by native hydrogenases in cyanobacteria occurs under in the dark under anaerobic conditions by degradation of intracellular glycogen. In vitro and in vivo coupling of the cyanobacterial photosynthetic system with a clostridial hydrogenase via cyanobacterial ferredoxin was demonstrated in the presence of light. Genetic transformation of Synechococcus PCC7942 with the hydrogenase gene from Clostridium pasteurianum was successful; the active enzyme was expressed in PCC7942. The strong hydrogen producers among photosynthetic bacteria were isolated and characterized. Coculture of Rhodobacter and Clostriudium was applied for hydrogen production from glucose. A mutant strain of Rhodobacter sphaeroides RV whose light-harvesting proteins were altered was obtained by UV irradiation. Hydrogen productivity by the mutant was improved when irradiated with monochromatic light of some wavelengths. The development of photobioreactors for hydrogen production is also reviewed.

- Photobiological Hydrogen Production: Recent advantages and states of art (Sept 2011) demonstrates Photobiological hydrogen production has advanced significantly in recent years, and on the way to becoming a mature technology. A variety of photosynthetic and non-photosynthetic microorganisms, including unicellular green algae, cyanobacteria, anoxygenic photosynthetic bacteria, obligate anaerobic, and nitrogen-fixing bacteria are endowed with genes and proteins for H_2-production. Enzymes, mechanisms, and the underlying biochemistry may vary among these systems; however, they are all promising catalysts in hydrogen production. Integration of hydrogen production among these organisms and enzymatic systems is a recent concept and a rather interesting development in the field, as it may minimize feedstock utilization and lower the associated costs, while improving yields of hydrogen production. Photobioreactor development and genetic manipulation of the hydrogen-producing microorganisms is also outlined in this review, as these contribute to improvement in the yield of the respective processes.

- International Journal Of Hydrogen Energy (Vol 27, Dec 2002) summarises Biological production of hydrogen can be carried out by photoautotrophic or photoheterotrophic organisms. Here, the photosystems of both processes are described. The main drawback of the photoautotrophic hydrogen production process is oxygen inhibition. The few efficiencies reported on the conversion of light energy into hydrogen energy are low, less than 1.5% on a solar spectrum basis. However, these can be increased to 3–10%, by the immediate removal of produced oxygen. The photochemical efficiency of hydrogen production can be calculated theoretically, and is estimated to be 10% (on solar spectrum basis) for the photoheterotrophic process. With use of the theoretical photochemical efficiency, and the climatic data on sunlight irradiance at a certain location at a certain moment of the year, the theoretical maximum hydrogen production can be estimated. Data on H_2 yields and photochemical efficiency from experiments reported in the literature are summarized. Photochemical efficiencies, essentially based on artificial light, can reach 10% or even more, but only at low light intensities, with associated low-H_2 production rates. Some reflections on possible photobioreactors lead to two types of (modified) photobioreactors that might be successful for a large-scale biological hydrogen production.

CHAPTER 3

PHOTO BIOLOGICAL HYDROGEN PRODUCTION

I. PRINCIPLE

The photobiological hydrogen production process uses microorganisms and sunlight to turn water, and sometimes organic matter, into hydrogen. This is a longer-term technology pathway in the early stages of research that has a long-term potential for sustainable hydrogen production with low environmental impact.

Microorganisms such as green algae, cyanobacteria, purple nonsulfur bacteria, and dark fermentative bacteria are used to generate biohydrogen. In the light, solar energy is converted into adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) as a source of energy and reductants, respectively. In the dark, organic compound is synthesized from CO2 and H2O.

In photolytic biological systems, microorganisms—such as green microalgae or cyanobacteria—use sunlight to split water into oxygen and hydrogen ions. The hydrogen ions can be combined through direct or indirect routes and released as hydrogen gas. Challenges for this pathway include low rates of hydrogen production and the fact that splitting water also produces oxygen, which quickly inhibits the hydrogen production reaction and can be a safety issue when mixed with hydrogen in certain concentrations. Researchers are working to develop methods to allow the microbes to produce hydrogen for longer periods of time and to increase the rate of hydrogen production.

Some photosynthetic microbes use sunlight as the driver to break down organic matter, releasing hydrogen. This is known as photofermentative hydrogen production. Some of the major challenges of this pathway include a very low hydrogen production rate and low solar-to-hydrogen efficiency, making it a commercially unviable pathway for hydrogen production at this time.

Researchers are looking at ways to make the microbes better at collecting and using energy to make more available for hydrogen production, and to change their normal biological pathways to increase the rate of hydrogen production.

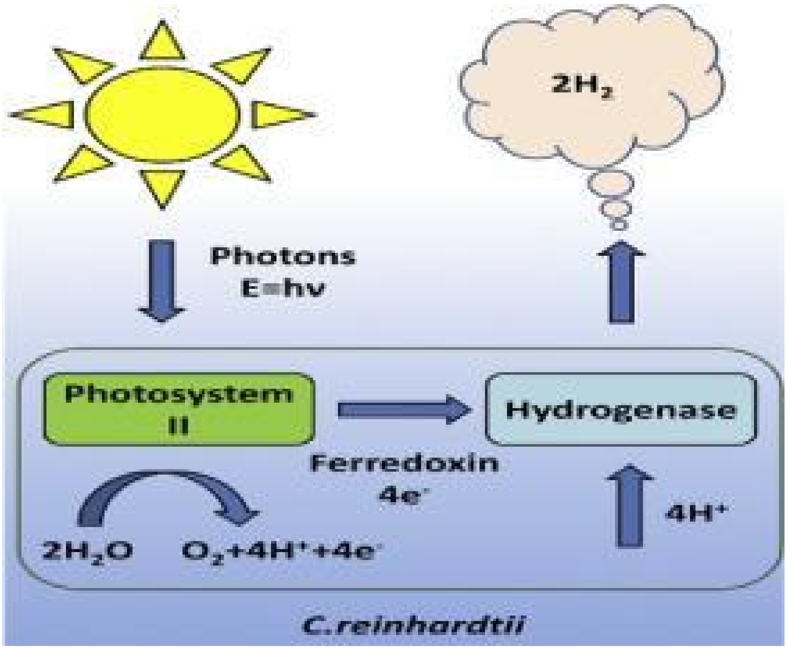
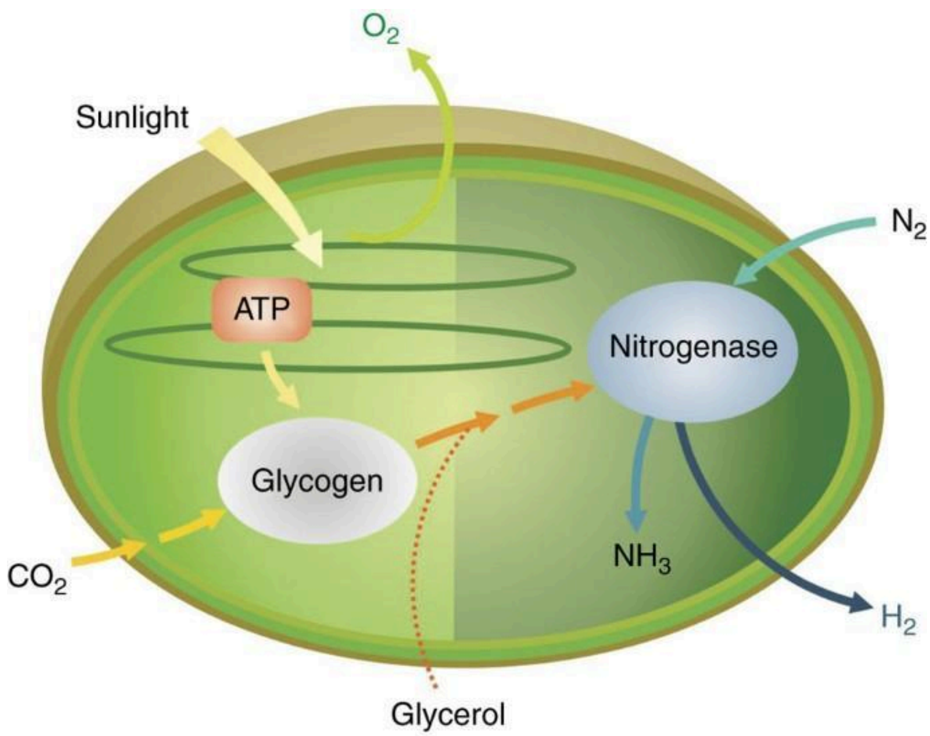


Fig 2. Hydrogen production overview in Chlamydomonas Reinhardtii.



$$N_2 + 8H^+ + 8e^- + 16ATP \longrightarrow 2NH_3 + H_2 + 16ADP + P_i$$

Fig 3. Hydrogen Production overview using nitrogenase.

II. Photohydrogen Production In Algae

As early as 1940 Gaffron observed that the green algae Scenedesmus undergoes an "adaptive" process when incubated anaerobically in the presense of H_2 for several hours in the dark. Adapted cells take up H_2 when CO_2 is present. Similar observations were reported for other species of green, red, and brown algae (Frenkel and Rieger, 1951). If the CO_2 is replaced by a fermentable substrate such as glucose~ the cells evolve H_2 (Gaffron and Rubin, 1942).

Both H_2 uptake and evolution are enhanced by light. Nevertheless, if the light intensity is increased beyond even moderate levels (>500 lux), activity ceases and normal oxygenic photosynthesis resumes. Photoevolution of H_2 is distinguishable from dark fermentative H_2 metabolism from the action of dinitrophenol, which· completely inhibits dark H_2 production though it stimulates H_2 photoevolution. Recently identified numerous species of algae, mostly in the "chlorophycaea class, which also photoevolve H_2 under anaerobic conditions.

Apparently hydrogenase alone mediates H_2 metabolism in these organisms since they have never been shown to fix N_2, and their H_2 exchange reactions are CO-sensitive, as are those of other hydrogenases.

III. Photohydrogen Production In Cyanobacteria

Certain isolates of cyanobacteria (or blue-green algae as they were formerly designated) also exhibit H_2 exchange reactions. Frenkel and Rieger (1951) noted that some strains, though not all, take up H_2 in a manner similar to that observed. in algae. However, Benemann and Weare (1974) demonstrated recently that Anabaena cylindrica can also undergo a CO-insensitive photoproduction of H_2 in the absence of N_2. It is quite probable that this latter reaction is mediated by nitrogenase, which has long been known in certain species of this group, particularly those of the Anabaena genus.

CHAPTER 4

ENZYMES

I. Hydrogenase

Hydrogenases encompass a number of different proteins whose function in vivo can be summarized by the reaction sequence indicated. If we further stipulate that H_2 must be the main and natural product or substrate of the enzyme activity, then nitrogenases are excluded since they produce H_2 only in the absence of N_2. It is difficult to envisage a natural environment where N_2 would not be present in sufficient concentrations to inhibit hydrogen evolution.

Hydrogenase is usually detected by observing H_2 evolution or uptake using either manometric or amperometric techniques. In these instances the H_2 exchange is often coupled to physiological substrates; to non-physiological electron acceptors such as oxidized methylene blue; or to electron donors such as dithionite plus reduced methyl viologen. Colorimetric quantitations can be made on redox dyecoupled systems as well. Finally, isotopic exchange assays using tritium have been employed. This method has advantage that the kinetic data obtained are independent of the slow electron transport reactions in coupled assays. For example, H_2 activation, which this method assays, is probably not the rate-limiting step in the consumption of H_2 coupled to CO_2 fixation. The amperometric technique for measuring hydrogen exchange developed by Wang et al. (1971) has the advantages of speed and sensitivity. Similarly, simultaneous measurements of H_2 and O_2 exchanges are possible using the apparatus described by Jones and Bishop (1976). However, these electrode systems are not appropriate when gases such as H_2S or CO are present (they interfere with the electrode) or for long-term experiments (the concentrations of dissolved gases in the medium reach equilibria). Since nitrogenase is also capable of evolving H_2, assays monitoring the release of H_2 must exclude any contribution from nitrogenase. Hydrogenase activity is strongly inhibited by CO (Haberman and Rittenberg, 1943; Peck et al., 1956) whereas H_2 evolution via nitrogenase is not. Thus, CO is a means for discriminating between the two (except in amperometric assays since CO is an interfering gas). There are other tests to distinguish between hydrogenase and nitrogenase activity (such as the ATP requirement of the latter), but in practice they are more difficult to use.

Table 1: Representative Organisms Exhibiting Hydrogenase Activity

Sr. no	Organism type	Organism name
1	Photosynthetic bacteria	Rhodospirillum rubrum Rhodopseudomonas capsulata Rhodomicrobium vannielii Chromatium vinosum Thiocapsa roseopersicina Chlorobium thiosulfatophilum
2	Cyanobacteria	Anabaena cylindrica Synechococcus elongatus Synechocystis sp. Nostoc muscorum
3	Euglenoid	Euglena gracilis
4	Green Algae	Chlamydomonas reinhardtii Chlorella fusca Scenedesmus obliquus Ulva lactuca
5	Red Algae	Porphyra umbilicalis Porphyriduim cruentum
6	Brown Algae	Ascophyllum nodosum
7	Nonphotosynthetic Bacteria	Escherichia coli Klebsiella pncumonioe Alcaligines eutrophus Dcoulfovibrio vulgaris Clostiidium pasteurianum Methanobacterium sp. Rhizobium leguminosarum Azotobacter vinelandii

FeFe-Hydrogenases:

Green algal hydrogenases belong to the class of FeFe-hydrogenases, which are also found in strict anaerobes, fungi, and protists. So far hydrogenase genes have been characterized in diverse green algal species including Scenedesmus obliquus, Chlamydomonas reinhardtii, Chlorella fusca, and Chlamydomonas moewusii. Each of these genes encodes a protein of about 48 kDa, with about 50% sequence similarities. The monomeric hydrogenase protein harbors a metallo-catalytic site, the H-cluster. The FeFe-hydrogenases contain only iron as a metal within their catalytic sites. The H-cluster consists of a [4Fe-4S] cubane linked through a protein cysteine residue to a 2Fe subcluster. The iron atoms of the [4Fe-4S] center bind to the protein structure by three additional cysteine residues. Except for the bridging cysteine, the iron atoms of the 2Fe center are coordinated to carbon monoxide (CO) and cyanide (CN) ligands.

Expression of the hydrogenase gene is tightly regulated in green algae. In C. reinhardtii, for example, anaerobiosis induces transcription of the two hydrogenase structural genes, as well as two maturation genes involved in the biosynthesis and assembly of the H-cluster. The FeFe-hydrogenases link to ferredoxin as an electron donor, and typically produce hydrogen. Oxygen irreversibly inactivates the algal hydrogenase H-cluster, with a half-life of a few seconds.

The chemical nature of the species bound to the H-cluster after exposure to oxygen is not known. However, the distal Fe appears to be oxidized when

the enzyme is exposed to oxygen.

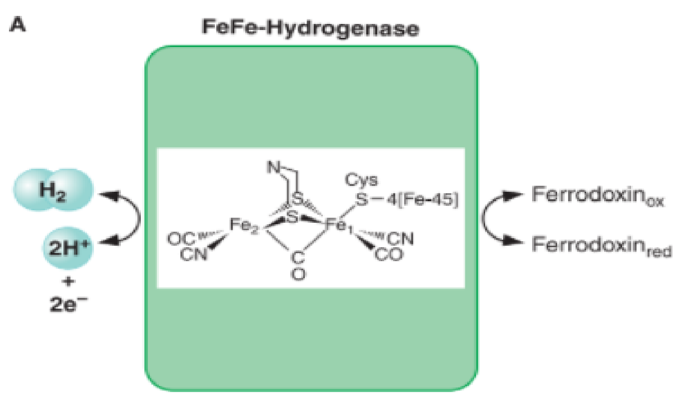


Fig 4. FeFe-Hydrogenase

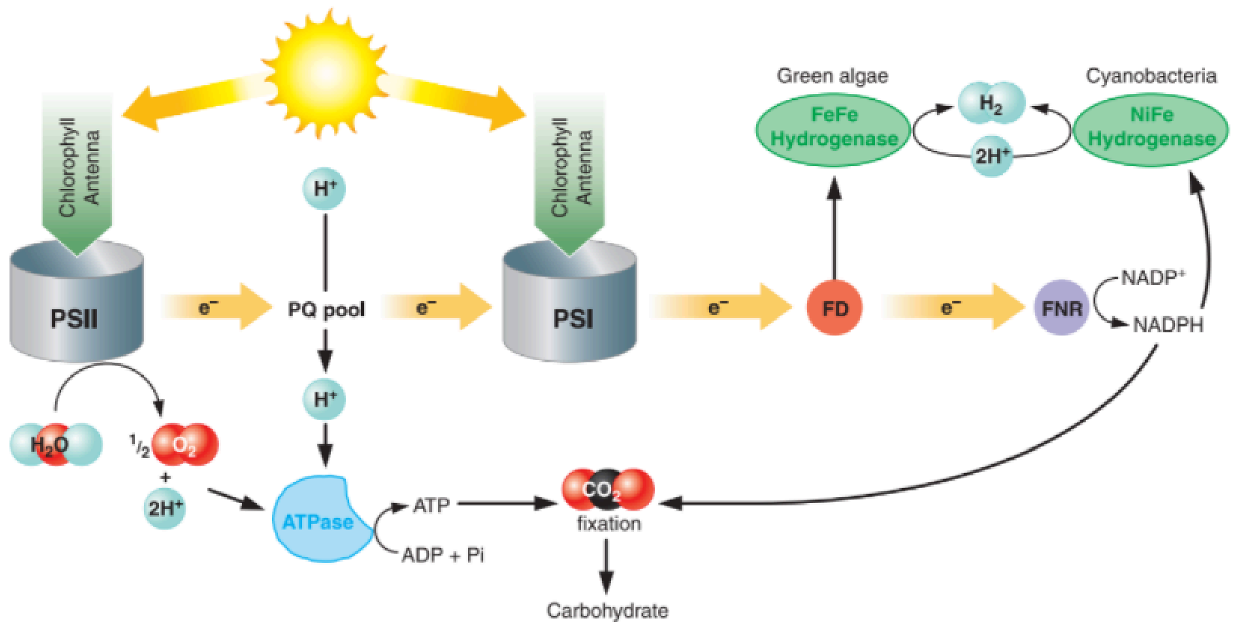


Fig 5. Photosynthetic pathway of H2 production in green algae (employing FeFe-hydrogenase) and in cyanobacteria (employing NiFe-hydrogenase). PS: photosystem; PQ: plastoquinone; FD: ferredoxin; FNR: ferredoxin-NADP oxidoreductase.

II. NYTROGENASE

The availability of combined nitrogenous compounds (e.g., ammonia, nitrate, and protein) is essential for biosynthetic reactions and cell growth. Most life forms must be fertilized or fed with these compounds. However, a few types of organisms--all procaryotes (bacteria and cyanobacteria) are able to reduce ("fix") atmospheric N_2 to the level of ammonia and are thus an essential link in the biosphere's nitrogen cycle. The process is carried out by a repressible enzyme complex, called nitrogenase, which functions at the most reducing end of the redox potential scale in biological systems. While the presence of the enzyme creates unique capacities for the host, it also poses problems for the cell as well as for the investigators seeking to understand the processes it catalyzes.

Of prime importance to this review is the fact that nitogenase in the absence of its physiological substrate or product (N_2 or NH_4^+, respectively) reduces protons and thereby evolves H_2. In fact, it was the observation of H_2 photoproduction in a culture of rubrum that led Kamen and Gest (1949) to discover that photosynthetic bacteria can fix N_2. This section describes briefly the distribution and properties of nitrogenase and indicates the feasibility of employing the enzyme in radiant energy conversion systems. Numerous comprehensive reviews on nitrogenase have recently appeared in the literature.

A culture that increases in cell mass for long periods of time in the absence of combined nitrogen (but in the presense of N_2 gas) probably contains nitrogenase, especially if concomitant increases in cellular nitrogen are observed. However, care must be taken to ensure that the organism is.in pure culture before N_2 fixation capacities are assigned. In complex symbiotic systems such as those found in the root nodules of legumes, though, this is not always feasible in practice. Fortunately, more direct assays for nitrogenase activity have been perfected. Burris (1972) has described many of these techniques which are applicable to both whole cell and cell-free systems.
Mass spectrometric analysis was the first rapid, definitive assay employed, and the technique involves following the incorporation of 1 N_2 into cellular constitutents. The isotope is a specific and sensitive tracer for nitrogenase activity. Dilworth (1966) subsequently recognized that nitrogenase can also catalyze the reduction of acetylene to ethylene, and this has provided the basis for a quick and simple assay. The ethylene evolved is detected by gas chromatography. The assay is now the method of choice for quantitatively measuring nitrogenase activity. Manometric or amperometric determinations of H evolution can also be used as an assay for nitrogenase activity provided that contributions from hydrogenase are suppressed or otherwise corrected for. Nitrogenase-mediated ammonia production can be measured by a modified Conway microdiffusion technique with Nessler's reagent (Bulen, 1965), but the commercial availability of ammonia-specific electrodes has recently supplanted this type of measurement. Nevertheless, accurate determinations of ammonia production from crude· extracts are difficult or impossible if ammonia assimilatory enzymes are also present. Regardless of the assay method used, nitrogenase activity in cell-free extracts is strictly dependent upon the presence of both a strong reductant, usually dithionite, and Mg-ATP (Burns and Bulen, 1965). Thus, the H_2 evolution activity of the ATP-dependent, CO-insensitive

nitrogenase can be readily discriminated from that of the ATP-independent, CO-sensitive hydrogenase.

The primary reaction catalyzed by nitrogenase is summarized as follows:

$$N_2 + 6e^- + 6H^+ + nATP \longrightarrow 2NH_3 + nADP + nPi$$

$$2H^+ + 2e^- + mATP \longrightarrow H_2 + mADP + mPi$$

Table 2: Representative Organisms Exhibiting Nitrogenase Activity

Sr. no	Organism	Organism name
1	Photosynthetic Bacteria	Rhodospirillum rubrum Rhodopseudomonas capsulata Rhodomicrobium vannielii Chromatium sp. Thiocapsa roseopersicina Chlorobium sp.
2	Cyanobacteria	Anabaena cylindrica Nostoc muscorum Gleothece sp. Plectonema boryanum
3	Nonphotosynthetic Bacteria	Klebsiella pneumoniae Clostridium pasteurianum Rhizobium japonicum Azotobacter vinelandii Desulfovihrto sp.

CHAPTER 5
MECHANISM

I. Phases

Two phases of photobiological hydrogen production in microalgae have been reported previously: aerobic and anaerobic. These two phases have different reactions to the contribution of hydrogen. The overall reactions are described as;

$$H_2O \longrightarrow 2H^+ + 2e^- + (1/2)O_2 \quad \text{(Aerobic Phase)}$$

$$2H^+ + 2e^- \longrightarrow 2H_2 \quad \text{(Anaerobic Phase)}$$

During the aerobic phase in microalgae, the H_2O is split into protons (H^+), electrons (e^-), and oxygen (O_2). Hence, the H^+ and e^- generated by water in PSII are stored as different metabolic products, such as proteins and carbohydrates, and these H^+ and e^- are essential precursors for H_2 production. During the anaerobic phase, H_2 is produced by hydrogenase. Mostly [FeFe] hydrogenase is rapidly activated under anaerobic conditions that catalyze the reduction of H^+ to H_2 using ferredoxin as an e^- donor. There, PSII contributes direct and indirect pathways for the source of e. In a direct pathway, e driven from the water-splitting activity of PSII is delivered into the electron transport chain (ETC) during state transition and finally reaches [FeFe] hydrogenase, which reduces H^+ to H_2. However, in an indirect pathway, proteins, carbohydrates, or starches that are stored during the aerobic phase are subsequently fermented and enter into an ETC. The thylakoid membrane of algae and cyanobacteria contains a photosystem with a light-harvesting protein complex, reaction center, and chlorophyll. It has the ability to capture light energy, which facilitates water oxidation to release protons (H_2) and electrons (e). These electrons are transported via the ETC, and iron-sulfur protein ferredoxin (PetF) or NAD(P)H via FNR (ferredoxin/NAD(P) H oxidoreductase) acts as an e donor to [FeFe] or [NiFe] hydrogenase, respectively.

In algae, ferredoxin directly linked to synthesize H_2 via the ETC and in cyanobacteria NAD(P)H donates e to [NiFe] bidirectional hydrogenase to synthesize H_2. Adenosine triphosphate and NADPH generated by light reaction are used to fix CO_2 during a dark reaction. Based on current progress, three pathways have been employed for biohydrogen production in cyanobacteria: (1) The first is

light-driven ETC from water via PSII to PSI, ferredoxin (Fd) to ferredoxin: NADPþ oxidoreduction (FNR). Finally, H2 is produced by [NiFe] bidirectional hydrogenase from the electrons supplied by reduced NADP. (2) Hydrogen is produced by the degradation of lipids, starches, and carbohydrates.

In this pathway, NAD(P)H is oxidized and electrons are transported through plastoquinone (PQ) for H_2 production. (3) The last is cyanobacterial nitrogen fixation by nitrogenase, in which H_2 is produced as a byproduct under anaerobic conditions or in heterocysts that provide a microaerobic environment.

II. Pathways

Direct biophotolysis means the production of H2 gas under the illumination of light in biological organisms. In the chloroplast of algae and cyanobacteria, the thylakoid membranes consist of chlorophyll pigments in both photosystems, i.e., photosystem I (PSI) and photosystem II (PSII). The light energy absorbed by these pigments raises the energy level of electrons from water oxidation to PSII to PSI to ferredoxin, where a portion of the light energy is directly stored in hydrogen gas. Direct photobiological H2 production from water using solar energy is a good example of massive (large-scale) production of hydrogen gas by photosynthesis, in which solar energy is used to split water into H2 gas. Microorganisms include single-cell cyanobacteria (Syne-chocystis), multicellular cyanobacteria (Nostoc sp.), and green algae (Chlamydomonas sp.). Overall, the reaction of direct biophotolysis can be described as;

$$2H_2O + \text{Light} \longrightarrow 2H_2 + O_2$$

However, indirect biophotolysis refers to the production of H2 from intracellular energy reserves including carbohydrates such as starch and glycogen in microalgae and cyanobacteria. Hence, this process is composed of two stages: carbohydrates synthesis in the light and dark fermentation of carbohydrates for H2 production. Hence, indirect biophotolysis can be described as the following reactions:

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

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

$$2CH_3COOH + 4H_2O + \text{light} \longrightarrow 8H_2 + 4CO_2$$

In overall:

$$12H_2O + \text{light} \longrightarrow 12H_2 + 6O_2$$

CHAPTER 6

PHOTOBIOLOGICAL REACTOR

After the identification of suitable strains of algae and cyanobacteria, various bioreactors can be used for photobiological H_2 production by microorganisms in which light energy is converted into biochemical energy. Some factors such as areaevolume ratio, temperature, agitation, and gas exchange also influence the performance of bioreactors. Bioreactors can be used to harvest light energy more easily and minimize energy loss. A few decades ago, Sathiyamoorthy and Shanmugasundaran (1994) developed a polypropylene bag that was a simple and inexpensive culture
vessel on which to grow cyanobacteria. Later, tubular, flat panel, and bubble column types of major bioreactors had been designed for photobiological H_2 production (Akkerman et al., 2002). Several types of photobioreactors have been designed; a typical dual photobioreactor that uses sunlight to generate electricity and produce H_2.

Based on the growth conditions of microorganisms, bioreactors are classified as two types: one-phase and two-phase. In a one-phase bioreactor, H_2 is produced by PSII turnover and the activity of HydA enzyme (chloroplast hydrogenase enzyme) under microoxic condition. The microoxic condition does not inhibit hydrogenase for H_2 production. Similarly, the two-phase bioreactor consists of both aerobic and anaerobic conditions for H_2 production. In the two-phase type, cells are grown under aerobic conditions to accumulate more biomass, and then cells are transferred into sulfur-deprived medium to induce H_2 production (Hankamer et al., 2007). The quality and quantity of light are also important for growing phototrophic microorganisms within the bioreactor. To achieve this goal, a tank bioreactor and high surface area volume ratio bioreactor were developed. Owing to the shading effects of light, tank bioreactors have been considered less effective.

In addition, based on the effective production of biomass and H_2 by microorganisms, bioreactors are classified into two types: open system and closed system (Dasgupta et al., 2010). The open system bioreactor is a simple and traditional system in which algae and cyanobacteria can grow under natural conditions such as small ponds, lakes, or artificial containers for biomass production. However, the efficiency and collection of H_2 are inconvenient. Hence, closed-system bioreactors are considered safe reactors for improving the biomass and H_2 production efficiency of microorganisms in a modern way, because photobioreactors have been designed to multiply rapidly with a high density of microalgae biomass production. Some important points for designing bioreactors for photobiological H_2 production are summarized by;

1. Temperature control and an agitation system in photobioreactors
2. Use of mixed microorganisms for better employment of solar energy
3. More research on genetic and metabolic engineering of microorganisms to improve better yield for overall performance of photobioreactors
4. Bioreactor performance with the same volume but different areaevolume ratio for a given microorganism for H_2 production.

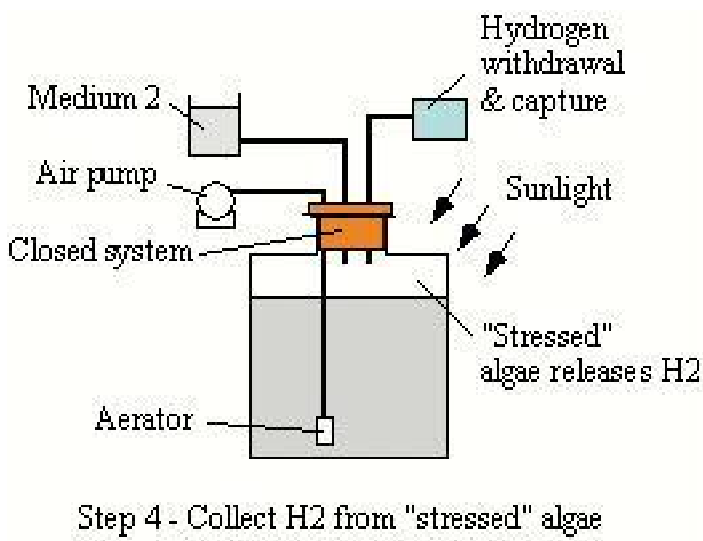


Fig 6. Generalised design of the Photobioreactor.

In addition, the genetic study of microorganisms and the design of an effective photobioreactor for the long-term cultivation of microorganisms are essential because tocdate, photobioreactors have been developed only for laboratory purposes. Apart from this, two-stage culture strategies have been developed to induce the productivity of microalgae such as Chlorophyta and Scenedesmaceae. These processes consist of fast growth induction in stage 1 followed by lipid induction in stage 2. With this strategy, overall productivity was improved and a better quality of biodiesel was obtained (Xia, Ge, Zhou, Zhang, & Hu, 2013). Comparatively, two-stage culture strategies were more effective than biomass production by microalgae in open ponds or vertical tank bioreactors.

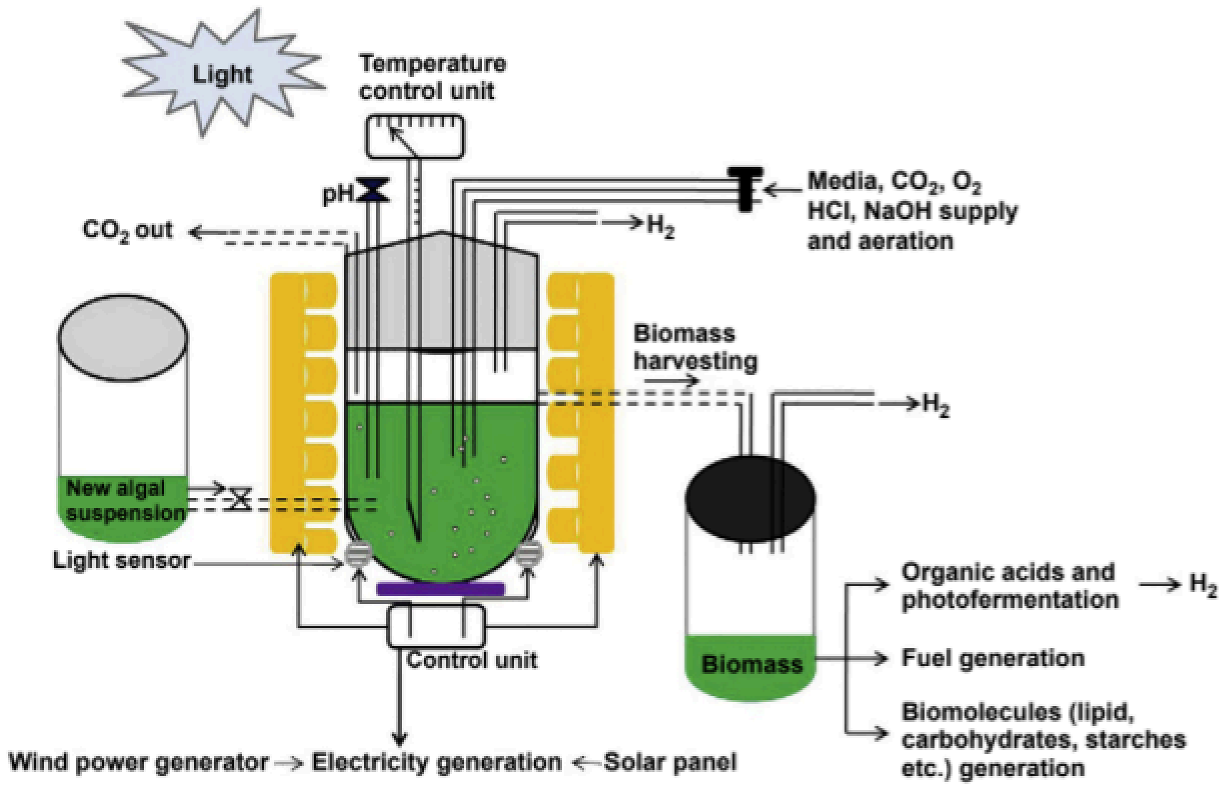


Fig 7. Detailed view of Photobioreactor.

CHAPTER 7:
NECESSARY CONDITIONS FOR ACTIVATION OF HYDROGENASE

I. Anaerobiosis

Algal photosynthesis and hydrogen production are sister processes. Both start with the same solar-energy-activated splitting of water to oxygen, electrons, and protons; protons and electrons then go to a second enzymatic reaction. In one case, the “normal” second reaction fixes carbon dioxide to produce sugar, and in the other, an alternative reaction produces hydrogen molecules. Picture a population of algae at the bottom of a pond at night. The organisms have been respiring and not photosynthesizing, so conditions have become largely anaerobic. When light first hits, the water-splitting reaction starts up right away. But there is a lag time of a few minutes in the carbon-fixing reaction before light activates the enzymes for carbon fixation.

Electrons produced by the water splitting would harm or destroy the organism if the excess energy were not somehow dissipated. The algae evolved an alternative second reaction that combines the protons and electrons to form hydrogen molecules, thus getting rid of the excess energy. It is this temporary alternative process—which normally only lasts for a few minutes—that we would like to tap. Once enough oxygen builds up from the water-splitting reaction, the algae are forced to shut off hydrogen production and go to carbon fixation to the starch that provides their food source. Thus, algal hydrogen production is naturally inhibited by the presence of oxygen.

Overcoming that inhibition is a major focus of photobiological hydrogen production research. The algal hydrogen-production process is driven by enzymes known as [Fe-Fe] hydrogenase, because of the presence of a unique 2Fe2S (iron and sulfur) metallocluster in the catalytic center of the core of the proteins. Researchers have found that the shut-off of hydrogen production is due to inhibition of the enzyme by oxygen. The inhibition depends on oxygen physically diffusing into the enzyme's catalytic center and irreversibly binding to it, thus halting further catalytic activity. scientists and research partners have built computer models of the [Fe-Fe]-hydrogenase from the bacterium Clostridium pasteurianum (not photosynthetic, but with a structure very similar to that of the hydrogenase of the green alga Chlamydomonas reinhardtii upon which research centers: Clostridium's crystal structure is known; Chlamydomonas' structure has not yet been solved). This model allowed them to conduct highly sophisticated simulations of gas movement in and out of the enzyme. They found that, although hydrogen may move out of the enzyme by additional pathways, there are just two main ones by which oxygen molecules could make their way into the enzyme. Visualization of the oxygen-diffusion channels (red and yellow ribbons) in the hydrogenase enzyme structure. The oxygen-sensitive FeS catalytic centers are indicated in green, yellow and red, and the backbone of the protein is shown in gray. If we can effectively manipulate those microorganism processes, we can capture solar energy directly in a form that is an ideal transportation fuel. With this knowledge, researchers were able to model amino-acid substitutions and other potential mutations and combinations of mutations along the pathways to identify promising ones for preventing oxygen from reaching the enzyme center. They then proceeded to actually enzyme engineer some of those mutations, express them in the industrial bacterium E. coli (again, not itself photosynthetic) and then test the oxygen sensitivity and hydrogen productivity of the recombinant enzymes. Thus far, although one of many attempted mutations along one enzyme pathway yielded modest improvement, others did not, proving detrimental for both oxygen sensitivity and hydrogen productivity. Hydrogenase engineering efforts for the enzyme continue, as this holds great promise as the key to overcoming oxygen inhibition. An analysis projects that this could lead to hydrogen production with as high as 10% efficiency in the conversion of solar energy.

II. Inactivating Algal Photosynthesis

An alternative approach to sustaining algal hydrogen production artificially is to partially inactivate the normal photosynthetic process. scientists have been able to do this by depriving an algal culture of sulfate, which is necessary for protein synthesis and particularly for the production of a key enzyme for photosynthesis that has an especially fast turnover time. As the sulfate is used up, photosynthesis slows. After about a day, photosynthesis produces less oxygen than respiration consumes, and the culture becomes anaerobic and switches from carbon fixation to a combination of hydrogen-production and starch degradation. Starch degradation supports the consumption of the low amount of oxygen that is still derived from residual photosynthesis and contributes reductants to hydrogen production. So, if the culture is present in a sealed reactor, it starts accumulating hydrogen gas. ’s development of this technique has already progressed through several advances. Initial operation with the algae in simple suspension could be maintained for 3-4 days. The cycle was repeated at least three times by alternating with periods of providing sulfate for normal photosynthesis to allow the algae to provide energy for themselves and avoid other problems from sulfur deprivation. The next level of development used a chemostat-based system that cultivated the algae with adequate nutrients in one reactor. The algae were then transferred to a second, sulfate-deprived reactor in which they produced hydrogen. Finally, in the continuous flow system, the algae are used as feedstock for fermentative processes or gasified. This system cut costs to one-third as much as simple batch processing and was run continuously for 6 months. The next improvement came with immobilization of the algal cultures on either glass fibers or alginate films. Because the immobilized algae were no longer swimming around or performing other functions, they needed less energy (algae live partially off stored starch while in the hydrogen-producing state) and the culture could be maintained for 25 days in batch operation. An alternate version of the immobilization technique that provides enough nutrients for minimal starch production, but not enough to shut off the hydrogen production, was operated continuously for 3 months. Because nutrient deprivation inherently limits the productivity of the algae, this technology is likely to achieve 1% to 2% solar energy capture at best, hence our recent emphasis on engineering the hydrogenase enzyme. Nevertheless nutrient deprivation has proved highly valuable to researchers in developing an understanding of algal hydrogen production metabolism and potential methods for cultivation.

III. Sulphur starvation and other necessary conditions

- H_2 evolution in laboratory-cultivated algae occurs only under a stringent set of conditions.
- Anaerobiosis is required for the activation of hydrogenase and the partial pressure of CO_2 also must be kept low i.e. less than 40Pa.
- The process is easily inhibited by light intensity considerably below that required to saturate photosynthesis.
- Furthermore, even under these rather restrictive laboratory conditions, H_2 production remains a highly transitory event, lasting from a few minutes to several hours.
- In green algae, sulfur-starvation exerts a specific inhibitory effect on the rate of oxygenic photosynthesis without affecting the rate of mitochondrial respiration.
- Within 24 h of sulfur-starvation, photosynthesis declines to an activity level lower than that of respiration. In consequence, illuminated but sealed cultures become anaerobic within 24 h, leading to the expression of the hydrogenase and to hydrogen photo-evolution.

- Electrons for the hydrogenase are provided both by a residual H_2O-oxidation activity (primary source) and by endogenous substrate catabolism.
- Such physiological attenuation of the photosynthesis-respiration ratio permits a continuous hydrogen evolution, which in the experiments lasted for 4–5 days.
- Under such conditions, it was possible to photoproduce and to accumulate significant volumes of hydrogen, using the green alga C. reinhardtii, in a sustainable process that could be employed continuously for several days.
- The discovery of the sulfur-starvation technique in *C.* reinhardtii represented an effective model for examining the biotechnology of algal hydrogen photoproduction.
- With this organism it was possible to sustain the hydrogen production process for a long enough time to optimize the culture conditions in order to maximize hydrogen gas production.

CHAPTER 8

INFERENCE

I. INFERENCE

- In Chlamydomonas Reinhardtii , hydrogen can be produced both by direct (via PSII) and indirect process (through the fermentation of starch accumulated during the aerobic phase).

- The contribution of the indirect pathway to hydrogen can be estimated. This contribution depends mainly on experimental conditions and on the phase of the sulfur starvation process.
- It has generally found that the PSII contribution covers 70%–80% of the total hydrogen production.
- When grown on a mass scale, green algae and cyanobacteria are inefficient in their use of high-intensity light because their large light absorbing antennae cause a saturation of electron transport at less than 10% full sunlight (light absorption is faster than the rate of electron transport at sunlight intensities above 10%). The excess absorbed light is therefore dissipated as heat or fluorescence. Moreover, since the top layer of cells in the reactor capture most of the incident photons, the remaining cells will be shaded and will not contribute to hydrogen photoproduction, which will result in overall low light conversion efficiencies per reactor.
- All these efforts to overcome the natural short-term nature of algal hydrogen production are still in early research stages. Many challenges face any industrial production process, but these have promise for producing an ideal transportation fuel in a renewable and non-polluting way.
- None of the currently identified photobiological H_2 producing systems have reached the development point at which pilot plant operations would be of benefit. However, progress during the past few years has turned a laboratory curiosity into a phenomenon that may be applicable in future solar energy conversion applications. Conversion efficiencies in whole-cell biophotolysis systems (algae and cyanobacteria) might approach 4% to 5% while those in cell free systems could reach 10%. Conversion efficiencies in H_2 photoproduction systems utilizing photosynthetic bacteria might be much higher since both the substrates and sunlight contribute energy to the process. In comparison, biomass production schemes presently exhibit maximal solar conversion efficiencies of 1% to 1.5% (on an annual basis) and the product (wood, etc.) is not as convenient a fuel or chemical as H_2.

II. Advantages

- Photobiological H_2 production uses microorganisms to convert solar energy into hydrogen gas.
- Photobiological H_2 production, especially by photosynthetic microorganisms, has several advantages because it requires simple techniques and low-cost energy (natural sunlight) compared with electrochemical H_2 production based on water splitting. Hence, these methods only use sunlight and water as renewable sources of energy.
- Photobiological Hydrogen Production is the cleanest way of hydrogen production.
- No Green House gas is Produced.
- No Carbon Compounds are produced.
- Sufficient amount of hydrogen can be produced.
- Photobiological H_2 production does not emit environmentally polluting gases and toxic compounds.
- Green algae, cyanobacteria, and photosynthetic bacteria are abundant everywhere and we can easily grow them under suitable artificial conditions. Most of these microorganisms are not environmentally harmful. Hence, we can easily grow such microorganisms to fulfill our goal.
- During photobiological H_2 production, many photosynthetic bacteria can use wide-spectrum light energy and organic waste.
- Photobiological H_2 production by microorganisms under anaerobic conditions produces valuable metabolites such as lactic acid, butyric acid, and acetic acid as byproducts.
- The photon conversion efficiency to produce H_2 from sunlight is high: 10-60%
- This method is useful for carbon sequestration. Solar-powered H_2 production by microorganisms has a unique process for CO_2 sequester. In the aerobic phase, CO_2 is converted into biomass; in the anaerobic phase H_2 is subsequently produced.
- Biohydrogen production by photosynthetic microorganisms requires the use of a simple solar reactor such as a transparent closed box with a low energy requirement.
- Hydrogen production by sunlight is cheap compared with the current fossil fuel system and the synthetic fossil fuel system for H_2 production.

CHAPTER 9

FUTURE

I. Photobiological Hydrogen Production Future Scope

Photobiological H_2 production using photosynthetic microorganisms such as bacteria, algae, and cyanobacteria is an exciting topic for research. Currently, H_2 is considered a fuel for the future as a renewable source of energy that does not create greenhouse gases. In addition, the method of photobiological H_2 production using photosynthetic microorganisms shows promise and great interest for generating carbon-free, clean, and pure H_2 from abundant natural resources such as water and sunlight. However, feasible and commercial exploration for better yield of H_2 is required. Further exploration is needed to improve photosynthetic microorganisms by either metabolic or genetic engineering. There are some ideas for improving photobiological H_2 production:

(1) identifying the most suitable microorganisms and incorporating hydrogenase and nitrogenase into selected microorganisms

(2) optimizing culture conditions

(3) stabilizing photobioreactors

(4) improving feedstocks

(5) performing comparative analyses of photobiological H_2 production

(6) improving H_2 yield using cheap or cost-effective raw materials

(7) exploring novel species of photosynthetic microorganisms that use better solar energy to improve H_2 production

(8) improving photobioreactors for better propagation of microalgae for effective H_2 production.

Long-range applications should emphasize cell-free systems based on the H_2 photoproduction pathway found in green algae. Green algae employ a rather direct electron pathway between water and H_2 and do not have the metabolic complications associated with N_2 fixation. Furthermore, they represent an energetically feasible model system and use water as a substrate. Specific research directions in this area should emphasize. Biochemical and biophysical studies of water splitting and reaction centre mechanisms that may give insight into the development of chemical analogues.

II. Work to do in semester VI

- As we have studied and researched one of the ways of hydrogen production; we are planning to optimise the hydrogen production conditions through photobiological means. Doing so we will acquire more knowledge regarding the photobiological hydrogen production.
- Our aim for semester VI is to emphasise the research on hydrogen production in efficient quantity.
- Study the photobiological hydrogen production plant on industrial level.
- Designing a fuel cell assisted by a assured hydrogen source.

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