Indo Global Journal of Pharmaceutical Sciences, 2015; 5(1): 66-89

INDO GLOBAL JOURNAL OF PHARMACEUTICAL SCIENCES
ISSN 2249- 1023

Hydrogen Production by Photosynthetic Organisms with Special Reference to Bioreactor Technology

Ahmed A. Issaa [*1], Idress Hamad Attitalla [2,5], Ramadan A. Alhendawi [3], Saber E. Mansour [4], Rajeev K Singla [6]

[1] *Botany Department, Faculty of Science, Assiut University, Assiut 71516, Egypt*

[2] *Department of Microbiology, Faculty of Science, Omar Al-Mukhtar University, Box 919, Al-Bayda, Libya*

[3] *Faculty of Natural Resources, University of Omar Al-Mukhtar, Box 919, Al-Bayda, Libya*

[4] *Agricultural Research Centre, Al-Bayda, Libya*

[5] *Department of Chemistry, Faculty of Science, Omar Al-Mukhtar University,* Box 919, Al-Bayda, Libya

[6] *Division of Biotechnology, Netaji Subhas Institute of Technology, Sector-3, Dwarka, New Delhi-110078, India*

Address for Correspondance: Ahmed A. Issaa, aaissaeg@yahoo.com

ABSTRACT: The review deals with photosynthetic H_2 production by various organisms, paying a special attention to bioreactor technology. It includes a general characterization of the catalyzing enzymes (hydrogenase and nitrogenase), quantum efficiency, the kinetics and mechanism of H_2 photoevolution, the distribution and activity of H_2 photoproducers (bacteria, cyanobacteria & 33 genera of eukaryotic algae) , physiological functions of this process as well as recent development in photobiological hydrogen technology. Hydrogen gas is a potential carrier of energy. For that reason used to bring space shuttles into their orbit .In this case, a fuel cell can generate electricity from hydrogen and oxygen. Its high energy content makes hydrogen gas an interesting energy carrier. An environmental friendly way is to use solar energy. In that particular case, we are talking about photohydrogen. Environmental parameters and physiological factors, which may be of use to optimize algae and cyanobacterial hydrogen generation, are summarizing. These parameters include: light intensity, gas atmosphere (Co_2, N_2 and O_2), temperature, pH, carbohydrate substrates, metal ions, H_2 uptake systems, age of cyanobacterial culture, cell density, and immobilization of cells. Nitrogenase is a major catalytic enzyme of hydrogen production in cyanobacteria, which can express three distinct nitrogenases: molybdenum nitrogenase, vanadium nitrogenase and iron nitrogenase. Cyanobacterial and algal hydrogenase is an enzyme that catalyzes both hydrogen evolution and hydrogen uptake. Today, several parameters are computer controllable in the photobioreactors. Already the photobioreactor to 20 L and its application for cultivation of various photosynthetic cells, *Chlorella, Chlamydomonas, Scenedesmus, Spirulina and Anabaena*, scaled up.

KEYWORDS: Hydrogen Gas; Cyanobacteria; Photobioreactor.

INTRODUCTION

The majority of earthly life forms are based on a bioenergetic cycle of photosynthesis and / or respiration described by the following equation:

$$2H_2O \xrightleftharpoons{8hv} O_2 + 4H^+ + 4e^-$$

$$\{ G \sim 470 \text{ kJ}(\sim 2.46\text{ev})\}$$

Hydrogen is be considered an environmentally desirable fuel since its combustion product (water) is non-polluting and it can produced in renewable energy systems. There are currently several industrial methods for production of H_2 mostly from natural gas ,oil, coal and water. Nearly 90% of H_2 is obtained by reaction of natural gas or light oil with steam at high temperature (reforming).Coal gasification and electrolysis of water are other industrial methods for H_2 production. These industrial methods mainly consume fossil energy sources and sometimes hydroelectricity [1].

Considerable researches have done on the utilization of solar energy for H_2 production. A number of reviews have been published in the past on the hydrogen production by water splitting using photoelectrochemical, photochemical and photobiological methods [2]. Photochemical methods use a photosensitizer to promote water photolysis, but the yields are poor. Photoelectrochemical methods apply semi- conductor electrodes for light absorption and charge separation .The problem here is to fined suitable electrode materials with a high enough band gap to generate the photovoltage required for water splitting.Photobiological H_2 production has a number of advantages and capitalizes on the fact that microbial species produce molecular hydrogen. It has been suggested that the most suitable candidates for the development of an environmentally acceptable technology for hydrogen production are cyanobacteria and green algae [3-5]. This is because cyanobacteria and green algae are unique in their ability to produce hydrogen using water as their ultimate electron substrate and solar energy as energy source.Simultaneously green algae and cyanobacteria consume CO_2 from air with H_2 evolution [6,7].

The design, optimization and practical demonstration of computer –controlled photobioreactors in which solar energy is used for hydrogen production by photosynthetic organisms would be an important step toward an advanced hydrogen production technology. Development of photobioreactors is a rapidly developing branch of environmental biotechnology based on the utilization of light energy and wasted CO_2 [8-10] The aim with the recent state of the art is to discuss the recent studies of physiological, biochemical and genetic characteristics of photosynthetic organisms in relation to practical beneficial application of these organisms in photoreactors for hydrogen production.

Distribution of hydrogen photoproducers

Many photosynthetic organisms have the capacity to photoproduce molecular hydrogen. According to Boichenko and Hoffmann [11], these include several hundred species belonging to at least 50 genera of prokaryotes and 33 genera of eukaryotes. It is worthy to mention that the numbers of H_2-metabolizing phototrophes are incapable of H_2 photo-evolution, although they carry out other hydrogenase mediated reactions.

1- Photosynthetic bacteria

Purple photosynthetic bacteria, a biochemically very flexible and adaptable group of microorganisms, contains only one photosystem generate ATP *via* a cyclic electron flow, but incapable of direct photochemical reduction of ferredoxin. Although these bacteria contain at least two types of hydrogenases [12-14], H_2 photoproduction is mediated only by nitrogenase when both ATP and low potential electrons from ferredoxin, (reduced *via* a dark ATP-linked process) are available Fig. (1). Since the nitrogenase system operates with a very low rate under saturating irradiances, N_2-fixing purple bacteria synthesize large amounts of the enzyme in nitrogen-starved cells reaching up to 25% of total soluble proteins. The increase in nitrogenase content of the cells is be stimulated by the increase of photophosphorylation rate, and ensures high capacity to H_2 photoproduction. In green photosynthetic bacteria, however, H_2 evolution utilizes inorganic sulphur compounds as electron donors. Unlike purple bacteria, the photogenerated potential of reaction centres in green bacteria is sufficiently low for a direct reduction of ferredoxin. The halophilic archeobacterium *Halobacterium halobium*, which carries out a unique type of anaerobic photosynthesis by bacteriorhodopsin-mediated light-driven H^+-pump, is incapable to H_2 photoproduction [15].

2 – Cyanobacteria

Cyanobacteria (blue- green algae), like all bacteria, lack nuclei, mitochondria and chloroplasts .However, there O_2-evolving and CO_2-consuming photosynthesis comprises two photosystem that generate reductants from water in mechanisms similar to those of green plants. Morphologically cyanobacteria are divided into unicellular and filamentous forms. The latter include a group of heterocystous species, containing distinct specialized cells (heterocysts),which fix nitrogen. Also some unicellular cyanobacteria are capable of N_2 reduction and H_2 evolution mediated by nitrogenase [5]. The net H_2 evolution by cyanobacteria is thus the sum of H_2 production catalysed by the nitrogenase and H_2 consumption catalysed by the uptake and probably by the reversible hydrogenase [16]. The uptake hydrogenase is a thylakoid-bound enzyme, whereas the reversible hydrogenase is

associated with cytoplasmic membranes, and in filamentous cyanobacteria, both enzymes are present in heterocysts as well as in vegetative cells.

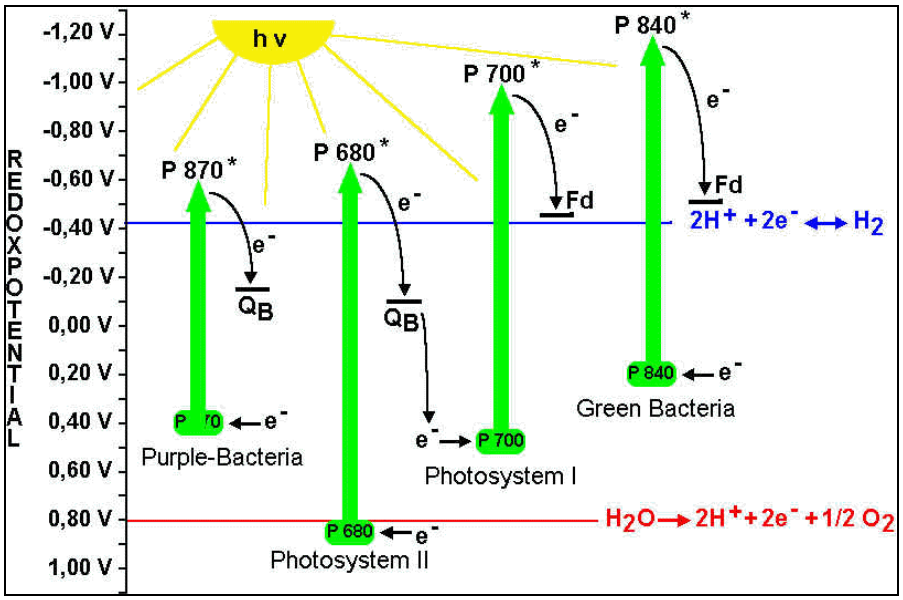
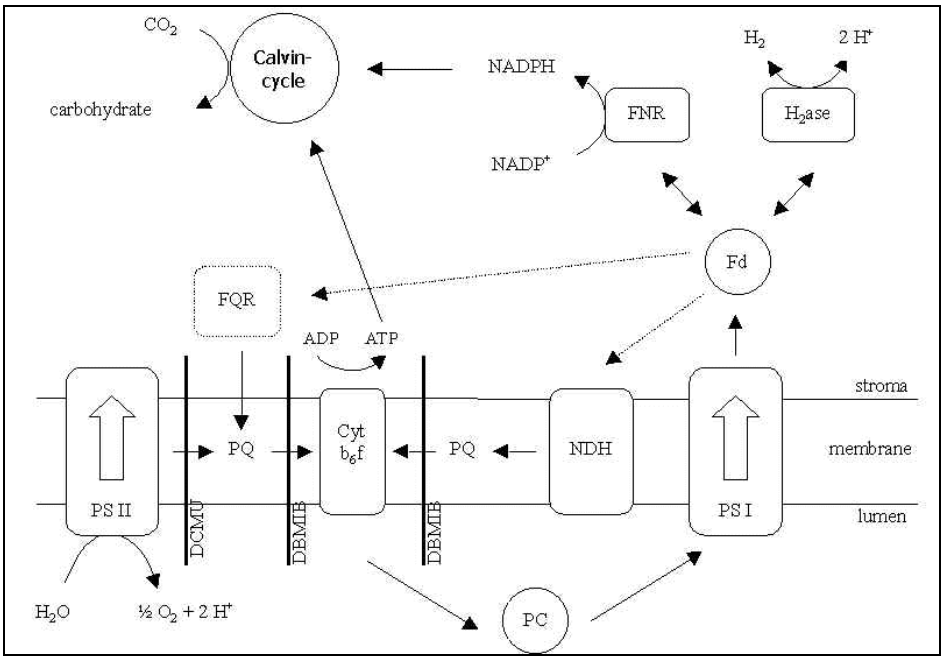


Figure (1): The schematic representation of hydrogen production by photosynthetic organisms via photosynthetic phosphorelation [7].



Table(1): Genera of photosynthetic prokaryotes having the capacity to hydrogen photoproduction (according to Kessler [17-19], Lambert and Smith [20], Gogotov [13]). *Nitrogen fixing cyanobacteria (Berchtold and Bachofen [21], Markov *et al*. [5, 22] probably capable of H_2 photoproduction.

Purple sulphur bacteria	**Cyanobacteria(cont.)**	*Microcystis*
Chromatium	Calothrix	*Myxosarcina*
Ectothiorhoodospira	*Chamaesiphon*	*Nostoc**
Thiocapsa	*Chlorogloea*	*Nodularia**
Thiocystis	*Chroococcus*	*Oscillatoria*
Purple-nonsulphur bacteria	*Chroococcidiopsis*	*Plectonema*
Rhodobacter	*Coccochloris*	*Pleurocapsa**
Rhodomicrobium	*Cyanothece*	*Pseudanabaena**
Rhodopseudomonas	*Cylindrospermum*	*Rivularia**
Rhodospirillum	*Dermocarpa**	*Schizothrix**
Green bacteria	*Dichothrix**	*Scytonema*
Chlorobium	*Fischerella*	*Sphaeronostoc**
Pelodictyon	*Gloeobacter*	*Spirulina*
Cyanobacteria	*Gloeocapsa*	*Stigonema*
*Amorphonostoc**	*Gloeotheca*	*Stratonostoc**
*Anabaena**	*Gloeotrichia**	*Synechocystis*
*Anabaenopsis**	*Hapalosiphon**	*Synechocooccus*
*Aphanizomenon**	*Hyella**	*Tolypothrix*
Aphanocapsa	*Lyngbya*	*Trichodesmium**
*Aphanothece**	*Mastigocladus*	*Westiellopsis**
*Aulosira**	*Microcoleus*	*Xenococcus**

Cyanobacteria are the best candidates for hydrogen production because:

* Cyanobacteria are photosynthetic prokaryotes lacking cell organelles like chloroplasts and mitochondria so that all electron transport reactions have to carry out within the same thylakoid membrane system. The electron supply is be substantially maintained under *in vivo* conditions by the interaction of various electron transport systems possibly sharing the same redox components.
* Cyanobacteria can be easily grown for a long time as immobilized cultures, which are more hydrogen evolving than the free-living ones.
* Cyanobacteria are highly adaptive to wide variations of environmental conditions and, subsequently, they can survive under extremely stressing conditions due to their evolutionary history.

Among all photosynthetic organisms, only some cyanobacteria are capable to H_2 photoproduction under aerobic conditions inspite of the oxygen sensitivity of nitrogenase. This is be achieved by operation of some protective mechanisms within the cells [23] that may be promising for biotechnological applications. Data on hydrogen metabolism in a particular prokaryote *Prochloron* having chloroplast-like organization of thylakoid membranes and light-harvesting chlorophyll a/b-protein complexes are still lacking.

3 - Algae

The ability for H_2 photoproduction have been recorded in 30 genera of green algae , two species of yellow green algae, and one species of diatoms, in most cases in unicellular organisms and three primitive multicellular algae, filamentous *Tribonema*, *Ulothrix* and *Volvox*. This ability was never observed in green, red, and brown macroalgae or in some widely used unicellular algae as *Porphyridium cruentum*, *Euglena gracilis*, *Dunaliella salina* which belong to the hydrogenase - containing species. All these findings indicate variations of H_2-metabolizing pathoways in eukaryotes, because of different properties of distinct hydrogenases, similar to those of prokaryotes, or due to different compartmentation of a uniform enzyme in the cells [24-26].

Various species of algae differ extremely in the H_2 photoproduction capacities, similar to different strains of the same species [27, 28] or different populations of the same strain under various growth and adaptation conditions. This may reflect modulation of amounts and activity of the inducible hydrogenase(s). Steady state rates of H_2 photoproduction in green algae usually do not exceed 0.6 - 5.6 mmol kg^{-1} (Chl) S^{-1}, that is comparable with the rate of dark H_2 production in the most active strains. The main reason for this low steady state rate of H_2 photoproduction is the stimulation of a competitive ferredoxin - mediated cyclic electron flow around PS I, since the turnover time of electron transfer from plastoquinone pool to the cytochrome b_6f complex is comparable to the turnover time of hydrogenase [29].

4 - Mosses:

According to Ben-Amotz *et al.* [30], all five species of tested mosses exhibited uptake hydrogenase activity but were incapable of H_2 photoproduction.

5 - Higher plants:

There are several reports on a hydrogenase activity in germinating seeds and roots as well as in leaves [31, 32], isolated leaf cells, isolated chloroplosts and even in subchloroplast PS II preparations [29]. In comparative studies of H_2 photoproduction in *Chlorella* and leaf discs of higher plants, Efimtsev *et al.* [31] found that the polarographic signal attributed to evolved H_2 in the latter was at least 1000-fold smaller than that in the algae. Furthermore, Benemann et al. [33] and Moller and Lin [34] observed the hydrogenase activity in calluses, roots and hypocotyls, but not in leaves. Thus, it seems that even if the phonomenon of H_2 exchange in photosynthetic tissues of higher plants exists, then it is of a marginal significance for their metabolism. Also, the nature of evolved H_2in subchloroplast PS II particles [29] is obscure, and may be, possibly, a result of a nonenzymaic reaction .Nevertheless, the problem needs further careful studies [35].

Mechanism of hydrogen metabolism:

The basic requirements for hydrogen metabolism can be simplified into an enzyme system and an electron source.

A) Enzyme system*:*

In cyanobacteria, there are two enzymes can be involved in hydrogen metabolism:

- Nitrogenases that produce hydrogen during nitrogen fixation.
- Hydrogenases that catalyze reversible or unidirectional evolution of molecular hydrogen.

Bacillariophyta	**Chlorophyta(cont.)**	**Chlorophyta(cont.)**
Nitzschia	*Chlorella*	*Neochloris*
Xanthophyta	*Chlorococcum*	*Oocystis*
Pleurochloris	*Chlorosarcinopsis*	*Pandorina*
Tribonema	*Chodatella*	*Pediastrum*
Chlorophyta	*Coccomyxa*	*Pseudospongiococcum*
Ankistrodesmus	*Dictyosphaerium*	*Scenedesmus*
Bulbochaeta	*Eudorina*	*Scotiella*
Carteria	*Golenkinia*	*Selenastrum*
Coelastrum	*Gonium*	*Tetraedron*
Dictyococcus	*Haematococcum*	*Ulothrix*
Chlamydomonas	*Halochlorococcum*	*Volvox*
Chlamydobotrys	*Kirchneriella*	

Table (2): Genera of photosynthetic eukaryotes having the capacity to hydrogen photoproduction (From Bishop *et al.* [36], Greenbaum [37-40], Boiechenko *et al.* [25,26]).

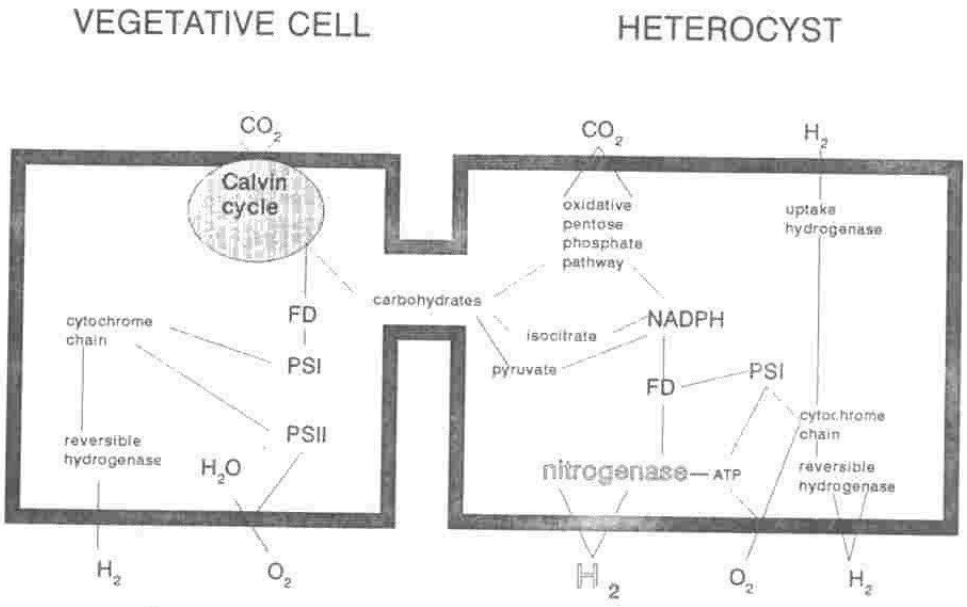


Figure (2): Digramatic relationship between hydrogen production and cell metabolism in heterocystous cyanobacteria [22].

1 - Nitrogenase-Catalyzed Hydrogen Evolution:

As an inherent property of the enzyme mechanism, a side reaction of nitrogenase is the evolution of hydrogen, according to either reaction of the following:

*) ***In the presence of nitrogen***

$N_2 + 8H^+ + 8Fd_{red} + 16$ Mg-ATP $\rightarrow 2NH_3 + H_2 + 8Fd_{ox} +$ 16 Mg- ADP + 16 Pi

*) **In the absence of nitrogen**

$2H^+ + 2\ Fd_{red} + 4$ Mg-ATP $\rightarrow H_2 + 2Fd_{ox} + 4$ Mg-ADP + 4 Pi

Cyanobacteria with nitrogenase- catalysed hydrogen production can be classified into three groups based on their morphological and physiological characterisitic.These are: heterocystous, nonhterocystous filamentous and nonhterocystoous unicellular species.

***) Heterocystous Cyanobacteria :**

In these species, heterocysts,are the site of nitrogenase reactions under aerobic growth conditions.Many heterocystous strains have been studied for

hydrogen production. Among these are:*Anabaena cylinderica, A.azollae, A. variabilis , A. flos-aquae, Chlorogloeopsis fritschii, Mastigocladus laminosus, M.thermophilus, Nostoc muscorum, Nostoc sp.* [16, 41]. Heterocysts possess a number of morphological and biochemical modifications designed to protect nitrogenase from oxygen inactivation. They are lacking both photosynthetic carbon dioxide fixation and oxygen evolution. On the other hand, they possess all the necessary photosystem I components and are capable of photophosphorylation with synthesis of ATP.

Some reductants can serve as sources of electrons to ferredoxin in heterocysts such as:-

-NADH generated by the glycoloic pathway.

-isocetrate by means of isocitrate dehydrogenase.

-pyruvate may provide reduced ferredoxin *via* the enzyme pyruvate:ferredoxin oxidoreductase.

-Moleucular hydrogen donates electrons by means of uptake hydrogenase to ferredoxin via the photosynthetic electron transfer chain in light or the respiratory chain in heterocyst [22].

Nitrogenase activity has also been detected in vegetative cells under anaerobic or microaerobic conditions.However,to date, hydrogen production by vegetative cells has not been observed [42].

***) Nonheterocystous Unicellular Cyanobacteria :**

Hydrogen production in nonheterocystous unicellular strains is always under the influence of O_2, produced during photosynthesis. There is no universal system for the oxygen protection of nitrogenase- catalysed hydrogen production in nonheterocystous cynobacteria. Most unicellular cyanobacteria can produce hydrogen under anaerobic or microaerobic conditions. On the other hand, only a few such cyanobacteria were shown to be capable of aerobic hydrogen production [8].

***) Nonheterocystous Filamentous Cyanobacteria:**

Hydrogen photoproduction by nonheterocystous filamentous strains has been intensively studied in marine cyanobacteria *Lyngbya sp.* isolate N.108, *Oscillatoria miami* BG7 , and *Phormidium valderianum* .Greater amount of hydrogen photoproduction was shown in *Oscillatoria sp* compared to the heterocystous cyanobacterium *A. cylindrica*. *Oscillatoria miami* was shown to exhibit sustained and high rates of hydrogen photoproduction *via* a two steps process of aerobic photosynthesis and anaerobic hydrogen photoproduction [43].

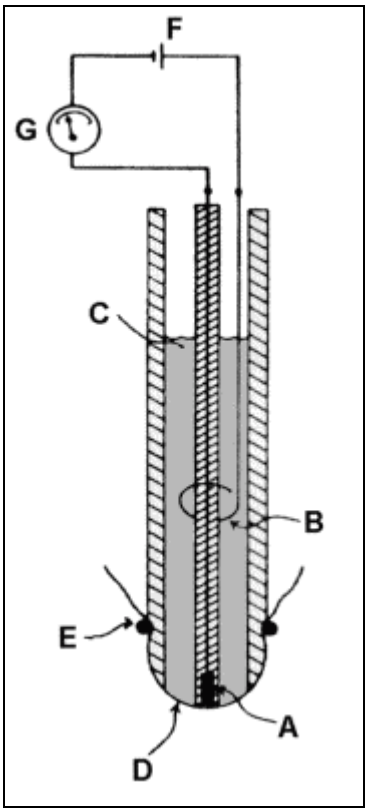
The nitrogenase enzyme system consists of two metalloproteins, a MoFe-protein (properly dinitrogenase) of 240 KDa, and a Fe-protein (dinitrogenase reductase) of 60 KDa. The MoFe-protein is a tetramer of two similar subunits, heterodimers, which contain MoFe-cofactor composed of culsters (4 Fe : 3S) and (Mo : 3Fe : 3S), binding substrates, and P-cluster pair of two (4 Fe : 4 S), facilitating electron transfer from the (4 Fe : 4 S) cluster of Fe-protein to the MoFe-cofactor [44].

2-Hydrogenase – Catalyzed Hydrogen Evolution

Hydrogenases are a heterogeneous group of enzymes now known to be widespread in prokaryotes and eukaryotes [45]. They catalyze consumption or evolution of hydrogen and thus they are be subdivided into "uptake" and "bidirectional" hydrogenases. Both contain iron and nickel in their active centers, so-called (NiFe) hydrogenases. Uptake hydrogenases are located at the thylakoid membrane of heterocysts from filamentous cyanobacteria. They are mainly active in recycling hydrogen molecules that are be evolved during nitrogen fixation and referred to as uptake hydrogenase. They are membrane bound enzymes re-oxidizing hydrogen molecules and feeding electrons thus produced into the electron transport chain *via* the quinone pool of the thylakoid membrane which finally donated to O_2 in the dark [46, 47]. This reaction is sensitive to CO and CN^- and is be coupled to oxidative phosphorylation [48].

The second enzyme, the so-called bidirectional hydrogenase is, active at least in *vitro* not only in hydrogen uptake, but also in the evolution of the gas. It can be found in both heterocysts and vegetative cells as well as in unicellular strains. It is monomeric soluble enzyme containing a catalytic center termed the H-cluster and ferredoxin. It also catalyzes hydrogen oxidation (the same as the uptake enzyme). The physiological function of bidirectional hydrogenase is still obscure. This enzyme has been purified from the unicellular strain *Anacystis nidulans* SAUG 1402.1 (*Synechococcus* PCC 6301) as well as from the filamentous nitrogen-fixing *Anabaena variabilis* .Mutant construction and analysis is be performed to elucidate the physiological function of the enzyme [49, 50].

Measurements of hydrogenase activity were recorded using a hydrogen electrode. Native and SDA - PAGE used in combination with Western immunoblots in order to verify the occurrence and to identify hydrogenases in organisms grown under different external conditions (Serebryakova *et al*. 1999,2000).To measure hydrogen gas , qualitatively and quantitatively two prominent techniques are available : Clark-type electrodes and gas chromatography. The Clark-type electrode (figure ,3) is a sensitive instrument for studying hydrogen metabolism and allows measurements in the gas phase as well as in aqueous solution.



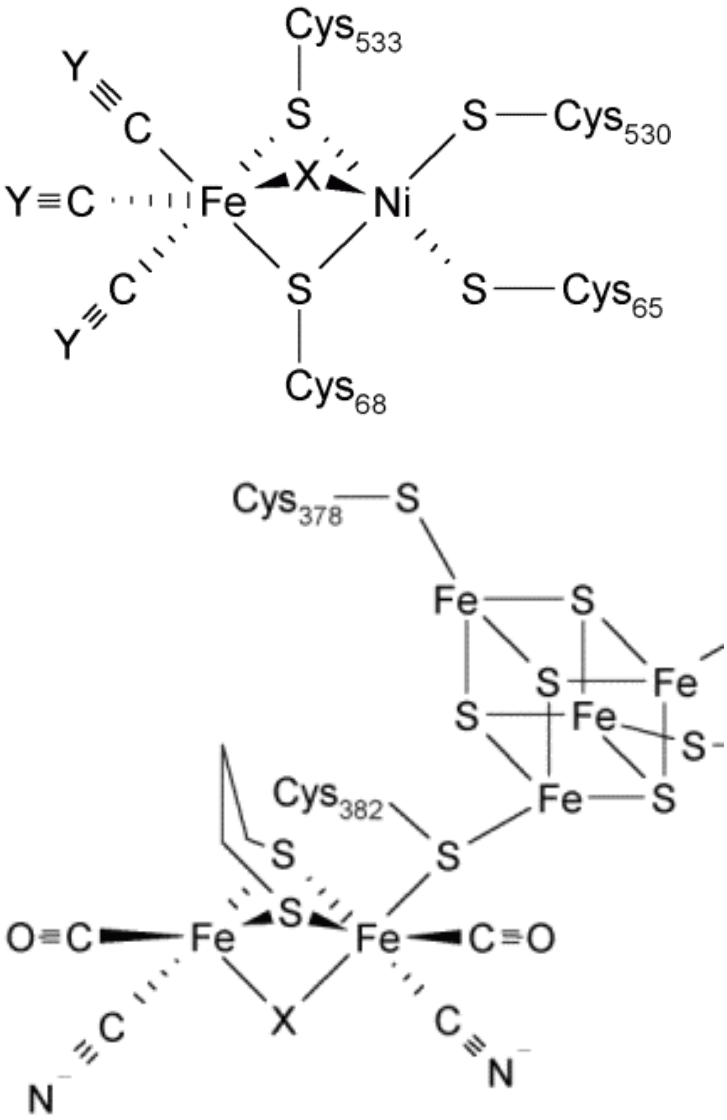
Figure(3):The Clark-type electrode consists of a Pt- (A) and a reference Ag/AgCl-electrode (B) covered by a film of half-saturated KCl electrolyte (C) enclosed within a Teflon membrane (D) which is held in place by a rubber ring (E). Originally developed for measuring oxygen gas, it is only a matter of polarity, whether the electrode senses hydrogen or oxygen gas. For hydrogen measurements 600 mV (F) are supplied ,and the electrodes output (G).

Molecular hydrogen is a key intermediate in the metabolism of bacteria algal hydrogen metabolism, is really a curiosity! Thus all information on hydrogenases is derived from investigations with bacteria. Hydrogenases catalyze the simplest of chemical reactions, e.g. the interconversion of the neutral molecule hydrogen and its elementary constituents: (two protons and two electrons). It was recognized that hydrogenases contain iron and nickel. Hence, two basic types of hydrogenases exist, those that contain only iron and those that additionally contain nickel. In the end-1990s, a third type of hydrogenases is being discovered in archaebacteria, which contains no metal at all. NiFe-hydrogenases are typically heterodimers with the active site in the larger subunit. As we now know from the crystal structure of the NiFe-hydrogenase from the sulfur-reducing bacterium *Desulfovibrio gigas* the active site looks like that:

French researchers resolved the crystal structure in 1995. It took a bit longer to get the structure from a Fe-hydrogenase. However, in 1998 it was resolved from the bacterium *Clostridium pasteuranium* by Meyer and Gagnon [51] and Peters *et al* [52].

It is interesting that hydrogenases contain carbon monoxide (CO) and cyanide (CN) in their active site. Both compounds are generally highly toxic [53].

The following scheme shows how the hydrogenase is believe to be connecting to the photosystems in *Scenedesmus obliquus* [7, 54].



The physiological role of reversible hydrogenase remained unclear untill recently .There is now evidence that reversible hydrogenase play a role in dark anaerobic degradation of carbon reserves with hydrogen being produce as an electron sink [8, 55].The unicellular *Cyanobacterium cyanothece* 7822 ,is capable of hydrogenase –catalyzed hydrogen production *in vivo* under anaerobic condition in the dark without the addition of an artifical reductant such as methyl viologen [56, 57]. In the light hydrogenase mediated hydrogen production occur in the nonheterocystous filamentous cyanobacterium ,*Oscillatoria limentica* .However when *O. limentica* is inhibited in the presence of sulphide ,photosynthetic oxygen evolution is inhibited and adaptive changes occur .This allows transfer of electron from sulphide to photosystem I- dependent reaction including hydrogen evolution [58]. The requirement for illumination during growth in order to exhibit hydrogenase activity probably reflects an energy requirement for the cell metabolism (protein synthesis).

The hydrogenase is constitutively present, no matter whether the organism faces anaerobic conditions or not. Thus, one can suppose a crucial role of the enzyme in algal photosynthesis. As shown in the scheme, (figure 4) ferredoxin (Fd) is the natural electron donor for the hydrogenase. Thus the hydrogenase takes over electrons from photosystem I. The electrons are coming from the splitting of water at photosystem II. For algae facing anaerobic stress caused by darkness and respiration or the environment, this reaction would give algae the ability to release excess electrons from the linear photosynthetic electron transport chain during a switch from dark to light intensities. The hydrogenase-mediated release of excess electrons from ferredoxin would also be consistent with the need of supplying the Calvin cycle with ATP. In the dark there is only little ATP in the chloroplast available [59]. Coming into the light the Calvin cycle thus cannot use the reductive power of NADPH because of the lack of energy. The proton consuming hydrogenase reaction would enhance the formation of a proton gradient over the thylakoid membrane, supporting ATP synthesis. This theory is consistent the findings by Stuart and Gaffron (1940) that uncoupling photophosphorylation in *Scenedesmus* causes an apparent enhancement of photohydrogen production. What is the benefit for the algae? If algae encounter light stress photosystem I reduces more ferredoxin than can be oxidized by other metabolic processes like the Calvin cycle. Thus, the electrons would stuck in the photosystems and the light energy would be hazardous due to radical generation. In the presence of oxygen, that situation will hardly appear: ferredoxin can be oxidize by oxygen in the so-called Mehler reaction. But what happens under anaerobic conditions? In such case, the hydrogenase could substitute for the oxygen dependent Mehler reaction [55, 60, 61].

B) Electron sources:

Photosynthesis, respiration and fermentation act as possible sources of electrons for proton reduction into molecular hydrogen. Kinetic studies indicated that ATP hydrolysis proceeds electron transfer in the Fe-protein/MoFe-protein complex. Dissociation or structural reorganization of this complex is the rate-limiting step in the catalytic cycle of nitorgenase which operates with a low rate of 5 turnovers per s. Artificial electron donors (e.g. methyl viologen or reduced DCPIP) efficiently supplement electrons also for H_2 evolution through saturation of redox components under non-

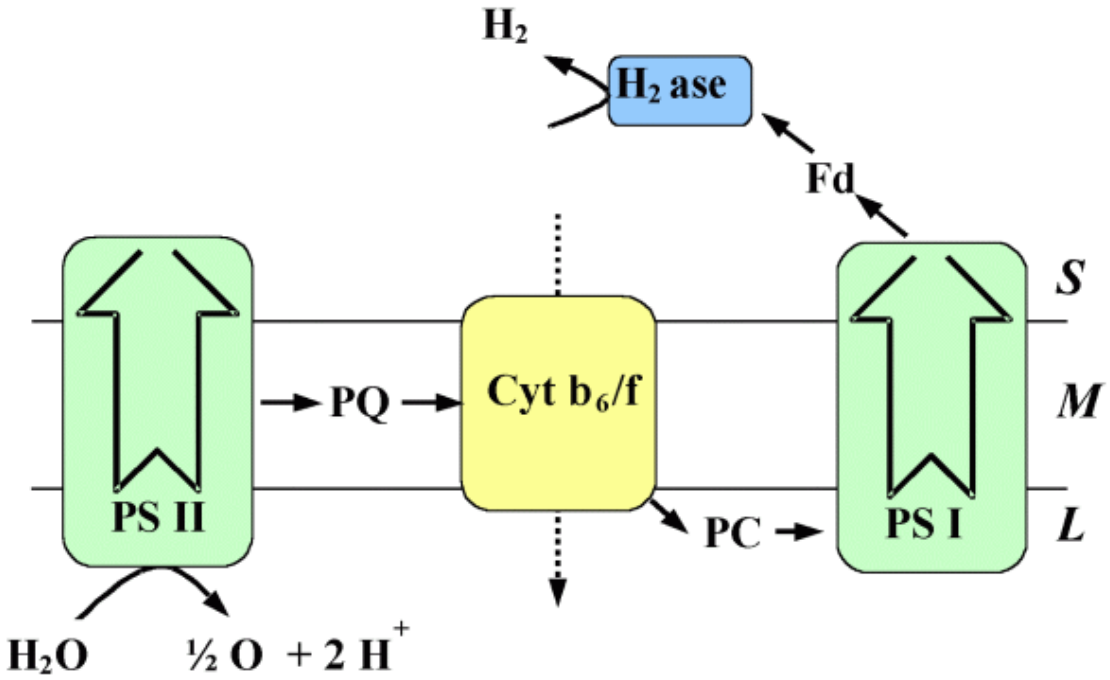
photosynthetic conditions (e.g. inhibited PS II). With respect to hydrogen production being finally mediate by ferrodoxin, it is generally accept that PS II and I function separately. Some aspects of hydrogen evolution mechanism are being illustrated in Figs. (1, 2, 3 & 4).

1-Photosystem I reactions:

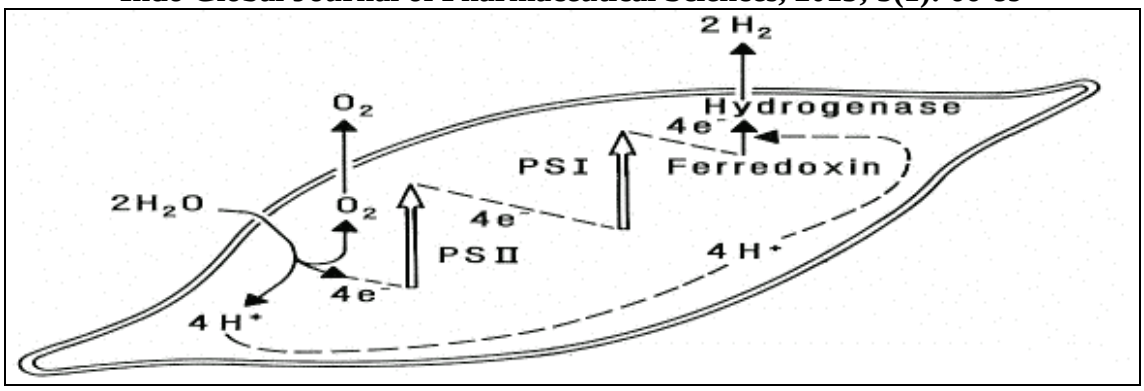
Earlier studies using inhibitors, mutants and monochromatic radiation have led to the conclusion that H_2 photoproduction depends on the activity of photosystem I (PS I). The PSI-driven H_2 production proceeds with a very high maximum quantum efficiency [62]. In green algae the quantum yield of H_2 reaches 20-25%, close to the theoretical value for two-electron reaction of H_2 production with the PS I / PS II ratio of I : I. Compared with the hydrogenase - mediated reaction of PS I, ATP dependent.. H_2-evolution *via* nitrogenase in photosynthetic bacteria is characterized by an about 4-fold decreased quantum efficiency in relation to number of photochemical centers. Under certain conditions H_2 photoproduction represents a one-electron reaction of semi-reduced ground state of hydrogenase, which is be achieved *via* a dark ATP-dependent electron flow:

$$NADPH + H^+ \rightarrow Fd \rightarrow \text{hydrogenase.}$$

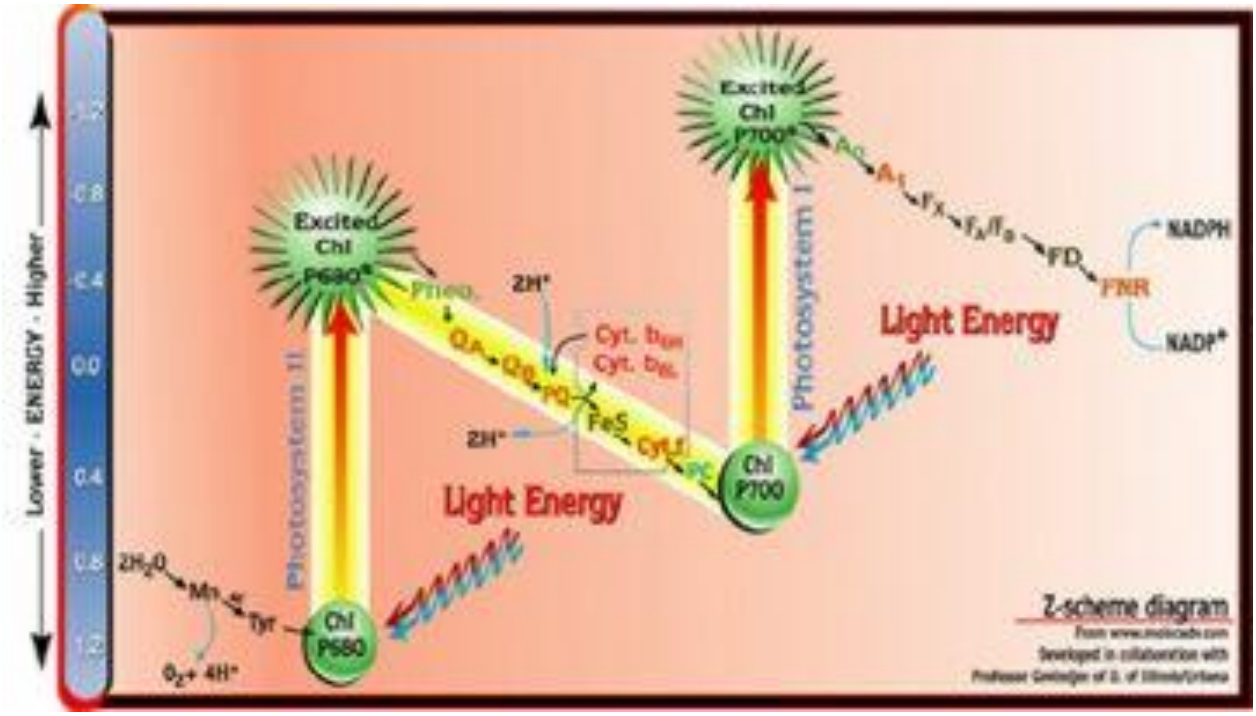
The immediate source of electrons for PS I-driven H_2 photoproduction is a pool of reduced carriers between the two photosystems, electron equivalents, belong to cyt b_6f/ plastocyanin and plastoquinone sub pools or two fractions of PS I complexes. Depending on irradiance, the PS I donor pool is be competitively reproduced by an electron flow from fermentative metabolism *via* the NADH-plastoquinone oxido-reductase and chloroplastic succinate dehydrogenase or by the PS II-driven electron flux from the water oxidising system [30, 38]. Although some PS I-deficient mutants have reported to be capable of evolving molecular hydrogen [63] careful examination of these mutants revealed that PS I-contaminate. Besides H_2 and O_2 evolution, an operation of associated redox reactions also documented by measurements of chlorophyll fluorescence induction [24] and NADH content in the cells [55, 64, 65].



Figure(4):Diagrammatic relationship between hydrogen production and two photosystems in *Scenedesmus obliquus*.



Figure(5):Hydrogen production and two photosystems in *Scenedesmus obliquus* cell.



Figure(6): Z-Scheme diagram of light reactions in green plants and algae.

2-Photosystem II reactions:

In the absence of alternative electron acceptors (CO_2, NO_2, NO_3), a prolonged steady-state rate of H_2 photoproduction in PS I is sustained mainly by the electron flux from water-splitting reaction of PS II [38, 40, 66]. However, the light-saturated rates of simultaneous evolution of H_2 and O_2 are rather low, possibly in consequence of a negative feedback of intracellular O_2 concentration on hydrogenase as a sink of electrons. Beside the long-range electron flow from PS II to hydrogenase via PS I, there is a theoretical possibility of a short circuit in PS II-driven electron flow to low potential acceptors via photoreduced pheophytin. Boichenko and Litvin [67] and Ball *et al* [68] found that some PS I-lacking mutants of Chlamydomonas were capable of high radiant energy saturated rates of H_2 photoproduction (11 - 22 mmol kg-1 (Chl) S^{-1}) with turnover time of 23 ms for corresponding reaction centers. However, the quantum yield of this H_2 production was very low (0.3 - 0.7%) indicating the participation of a small amount of functional photosynthetic units. The spectral analysis does not exclude the possibility of presence in the tested PS I-less mutants of a minor amount of PS I complex that can be not detected by other methods. Moreover, other "true" PSI-lacking mutants of *Chlamydomonas* with the normal dark hydrogenase activity but incapable of H_2 photoproduction have been found [48, 69].

In *Oscillatoria chalybia*, however, no decrease in the light-induced hydrogen evolution (m/e = 2) is be recorded with the light induction of the dark inactive Calvin cycle [70]. Thus, carbon dioxide and protons do not compete for photosynthetic electrons [63].

Metronidazole is selectively toxic to anaerobic bacteria and shows very little effect on aerobic microorganisms. The precise mechanisms of the metronidazole inhibitions are not fully understand, but here is a good evidence that it interacts with low potential electron carriers (ferredoxin, flavodoxin) in the pyruvate synthase or hydrogenase reactions present in

anaerobic bacteria. Metronidazole is also assumed to inhibit the ferredoxin dependent reactions but unaffected other photoreductions in chloroplasts of higher plants [70-72].

Factors Affecting Hydrogen production

Various factors may have an influence on cyanobacterial hydrogen production e.g light intensity, gas atmosphere, temperature, composition of the growth medium .immobilization, etc.

Species and Strains: Rates of hydrogen production can vary greatly in different species. Screening of cyanobacteria from different ecosystems may provide suitable H_2 producers. Thus many researchs have been undertaken with heterocystous cyanobacteria and it was found that the nonheterocystous marine cyanobacterium *Oscillatoria* sp. shows higher rates of hydrogen photoproduction than heterocystous *A . cylindrica* [73-75].

Light and Dark Condition: Light is an essential factor for hydrogen evolution by algae and cyanobacteria since hydrogen evolution depend directly or indirectly on the rate of photosynthetic reactions. Hydrogen production usually increases with increasing light intensity. However, at high light intensities hydrogen production is associated with high oxygen production rates and is rapidly inhibited [76].The relationship between hydrogen production and light intensity is dependent on the culture age, gas phase and density of culture [5]. At the later stages of growth .the efficiency of the light conversion to hydrogen production decreased. Hydrogen evolution also depends on light quality [77]. There are some indications that the dark –light illumination can increase hydrogen production compared to continuous illumination in cyanobacteria.

Age of Cyanobacterial Culture and Cell Density: The hydrogen production rate depends on the age of the culture with the maximum rate of hydrogen photoproduction being observed at the beginning of the stationary phase [76]. Hydrogen production decreased in older cultures .In contrast, the oxygen -evolution capacity and photosynthetic pigment decreased steadily with time.

Temperature and pH: The optimum temperature for hydrogen production varied considerably with the organism. Temperature conditions that are optimal for growth of cyanobacteria may not necessarily be optimal for hydrogen production. Hydrogen photoproduction did not occur at pH values below 6.5 or above 10 in cyanobacteria [9].

Culture Medium

CO2: Like all phototrophic organisms, cyanobacteria use carbon dioxide for photosynthesis. Cyanobacterial cultures grown under limiting CO_2 conditions have hydrogen production rates proportional to their growth rates.In nonheterocystous cyanobacteria, CO_2 inhibits nitrogenase probably by competing for ATP and reductant [77, 78].

N2: Molecular nitrogen ,which is the substrate for nitrogenase ,inhibits nitrogenase catalysed hydrogen production in some cyanobacteria .Inhibitory effect of nitrogen or hydrogen production by *A. cylindrica* is relieved by low concentration of carbon monoxide (an inhibitor for all nitrogenase reactions except the hydrogen-producing reaction of nitrogenase) and acetylene (an inhibitor of hydrogenase) [79].

Fixed Nitrogen (Nitrate.Ammonium,etc): Nitrogenase catalyzed hydrogen evolution is inhibited by the presence of fixed nitrogen (ammonium, NO_3, NO_2 and urea) in the growth medium [80].

Physiological Active Compounds and Carbohydrates: Photohydrogen production in *A. variabilis* was stimulated up to 7-fold by the addition of a cell extract of the water fern, *Azolla caroliniana* to the medium [81]. Nitrogenase activity and hydrogen photoproduction by cyanobacteria can be enhanced in the presence of exogenous carbohydrate. Nguen proposed that exogenous carbohydrates protect the nitogenase from oxygen [82].

Metal ions Vanadium Sulphide: Hydrogenase activity is stimulated by the divalent cations Zn^{2+} , Ni^{2+}, Mn^{2+} , Mg^{2+}, CO^{2+} and Fe^{2+} (Asada *et al*. 1992,1998).Nickel is involved in several biological processes and low concentrations are required for the synthesis of active hydrogenase, hydrogen production by cyanobacteria depends on the supplying of growing cultures with iron . Hydrogen photoproduction in *A. variabilis* catalyzed by vanadium nitrogenase was 4 times higher than hydrogen photoproduction catalyzed by molybdenum nitrogenase (Asada *et al.* 2000).

Molecular Hydrogen: Molecular Hydrogen in high concentration (up to the 50% in the gas phase) inhibits nitrogenase activity and photosynthesis in cyanobacteria [83].This can lead to the inhibition of hydrogen photoproduction as well.

H_2 Uptake: Most heterocystous and some nonheterocystous cyanobacteria possess an active H_2 uptake system [84]. Maximization of net hydrogen productionby some heterocystous cyanobacteria includes minimization of hydrogen consumption catalyzed by the so-called uptake hydrogenase or/and reversible hydrogenase. H_2 consumption in the dark depends on O_2 uptake according to the equation:

$$H_2 + 0.5O_2 = H_2O \text{ (oxyhydrogen reaction)}$$

Immobilization of Cells: Cyanobacteria, when immobilized in matrices such as calcium ginate ,agar,cotton,polyurethane or polyvinylfoams,hollow fibres or glass beads produce hydrogen for weeks and months [22]. Little is known about the mechanisms which induce changes in hydrogen production when cells are immobilized [85].

Biochemistry and Genetics of Hydrogen production.

The genetics of algae and cyanobacterial hydrogen production has received little investigation because most attention has been directed on the role of nitrogenase and/or hydrogenase in hydrogen production. They include methods for induction and

selection of mutants, methods for introduction of DNA into cells, and methods for selection and analysis of complemented mutants and recombinants. According to Markov *et al*. [22], Seibert *et al.* [86] and Elsen *et al.* [15] the possible work of objectives of genetic work related to cyanobacterial hydrogen photoproduction include:

*Investigation of genes controlling the proportion of cells that differentiates to heterocysts.
*Investigation of hydrogenase genes aimed at deletion of, uptake, hydrogenase activity.
*Optimization of photosynthetic conversion efficiency for hydrogen production.
*Obtaining mutants detective in alternative electron sinks than hydrogen.

Nitrogenase genes (*nif)*

Both nitrogenase and hydrogenase are complex enzymes .Their synthesis required the action of large number of accessory genes and whose expression is regulated by products of several regulatory genes. .In addition, the three-nitrogenase systems (Mo, V, Fe) are genetically distinct, encoded by different structural genes [87]. Nitrogenase genes can be divided into three categories according to the classification presented above and describe the relation between cyanobacterial nitrogenase and molecular oxygen. A number of genes that are turned on or off in *Anabaena* heterocysts have been cloned and sequenced. Most attention has focused on the three-gene *nifH*, *nifD* and *nifK*. The *nifH* gene codes for the structural units of dinitrogenase reductase, and *nifD,nifK* for the structural units of dinitrogenase. In contrast to the other microorganisms, in the vegetative cells of cyanobacteria a large segment of DNA separates the *nif* gene from *nifHD* genes .It seems that the DNA separating *nifK* and *nifHD* does not contain *nif* structural genes [88]. During heterocyst differentiation, this DNA segment is removed and nitrogenase activity initiated [89].

Hydrogenase genes

Genetic investigations of hydrogenase have only just begun. Recently the nucleotide sequence of the gene proposed to encode the small subunit of the reversible hydrogenase of the thermophylic unicellular *Synechococcus* PCC 6716 and the heterocystous *A. cylindrica* has been isolated. Major aim of genetic work with uptake hydrogenase is to produce hydrogenase–deficient (*hup*-) strains of cyanobacteria.

Hybridization DNA from *A. cylindrica* and three plasmids containing cloned hydrogenase genes from the bacterium *Bradyrhizobium japonicum* has made [90]. Studies on mutant organisms containing hydrogenases that are able to operate at

higher O_2 concentration [13, 91, 92], suggested that the enzyme is amenable to manipulation that may affect its O_2 tolerate. This observation led to investigatation of several classical genetic approaches to generate and isolate *Chlamydomonas reinhardtii* mutant that can produce H_2 in the presence of O_2. They involved using random mutagensis, followed by application of selective pressures under gradually increasing O_2 concentration .The two selective pressure [15, 93-97] were based on the reversible activity of algal hydrogenase, e.g., H_2-production and H_2 –uptake. Due to the lake of specificity of the selective pressure, a chemochromic sensor also developed to allow quickly screening the survivors of the selective pressures for H_2-producing clones using the combination of mutagenensis, selection and screening. This led to isolation of two generations of H_2-production mutants, 76Dd4 and 141F2, with respectively 4 and 9 times higher tolerance to oxygen compared to *WT*. Sequences of bi-directional hydrogenase from several different cyanobacteria is now available. All of them show a high similarity to the soluble NAD-reducing hydrogenase of *Aalcaligenes eutrophus* [57, 98-99]. Surprisingly, the respective gene clusters contain additional genes that are homologous to peripheral subunits of NADH: ubiquinone oxidoreductase, also known as complex I, of the respiratory electron transport. This discovery led to a structural model of the bi-directional hydrogenase associated with this large membrane complex [99-101]. Genes encoding other homologues of these subunits could not found outside the hydrogenase gene cluster in the complete genomic sequence of *Synechocystis sp*. PCC6803 . In addition, Appel *et al.* [102] stated that, the activity of bi-directional hydrogenase of the cyanobacterial *Synechocystis sp*. PCC6803 was found not to be regulating in parallel to respiration but to photosynthesis. A mutant with a deletion in large hydrogenase was impaired in the oxidation of photosystem I (PSI) which excites either PSI alone or both photosystems. PSII of the mutant was higher than that of *WT* cells .The transcript level of the photosynthetic genes *psbA, psaA* and *petB* was found to be different in hydrogenase –free mutant cells compared to wild – type cells *WT* which indicate that the hydrogenase has an effect on the regulation of these genes [103]. Collectively, these results suggest the functions of the bi-directional hydrogenase as a valve for low potential electrons generated during the light reaction of the photosynthesis, thus preventing a slowing down of electron transport [104-107].

Enzyme	Hydrogenase	Nitrogenase
H2-Production	Yes	Yes
H2-Uptake	Yes	No
Reaction-energy dependent ATP	No	Yes
Oxygen sensitive	Yes	Yes
Subunits	1-3	6
Catalytic	High	Low
Present in prokaryotes	Yes	Yes
Present in eukaryotes	Yes	No

Table 4. Hydrogenases Vs. Nitrogenases

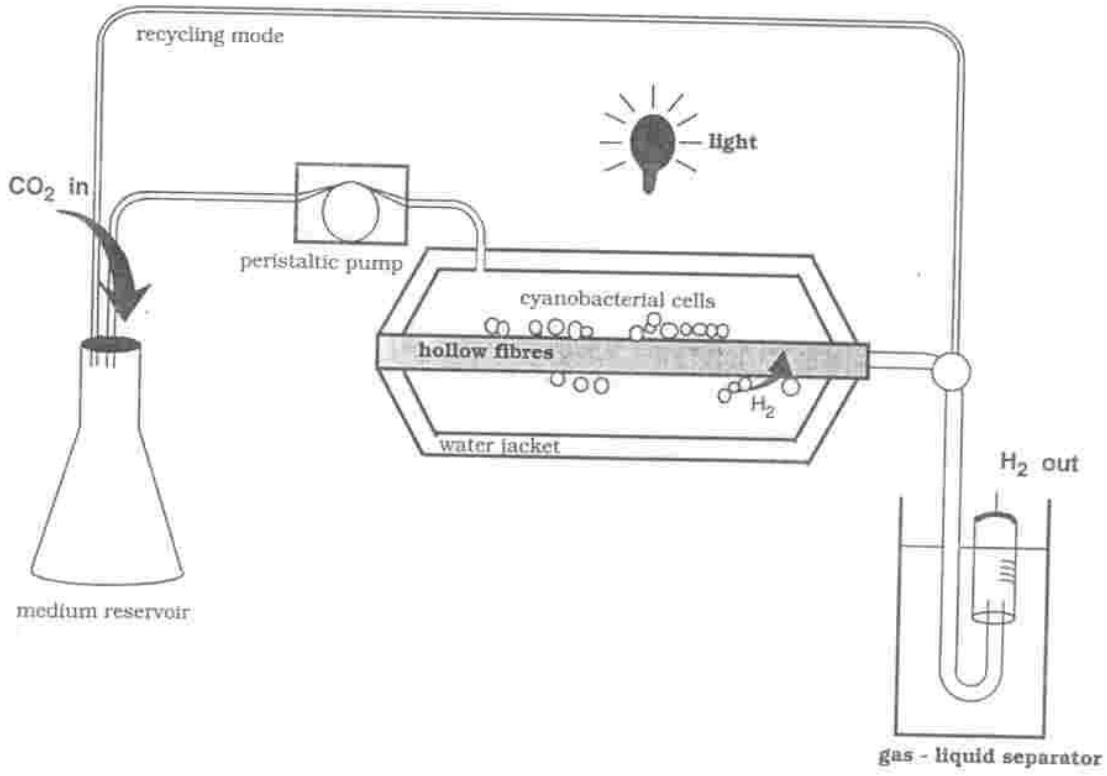


Figure (7): Schematic digram of hollow fibre photobioreactor for continuous production of hydrogen by immobilized cyanobacteria.

Photobioreactors

Photosynthetic microorganisms can be engineered to produce pharmaceuticals, chemical intermediates , and clean energy (e.g., hydrogen) They also fix atmospheric carbon dioxide an important consideration as increased levels of carbon dioxide are linked to global warming .It is expected that, in the future, photosynthetic microorganisms will play a larger role than higher plants in photosynthetic carbon dioxide fixation because they have higher photosynthetic rates per unit biomass and, if optimized, can be cultivated in a compact space [108, 109].

To produce algae derived materials at competitive prices, efficient large-scale photobioreactors must have designed. The combination of control and large scale is the key to success as well as to exploit the potentials of photosynthetic cells. Photobioreactors are sophisticated type of continuous

culture with the uptake of carbon dioxide. Many closed photobioreactors have proposed for the cultivation of microalgae. The most common are vertical or horizontal tubular, helical (serpentine), and inclined or horizontal thin-panel photobioreactors. Some of the photobioreactors that work well in the laboratory may not work as well when scaled up because the surface-to-volume ratio decreases, causing poor light distribution inside the reactor. Figure (7) shows a schematic diagram of a photobioreactor for continuous production of H_2 by immobilized blue green algae on hydrophilic and hydrophobic cellulosic hollow fibers was greater than to the hydrophobic polysulphone fibers [110-112].

A two-phase photobioreactor can run continuously for a period of several months with a blue green algal suspension.

CO_2 uptake phase:

$CO_2 + H_2O \rightarrow$ photosynthetic products + O_2

Maximum CO_2 consumption rate = 150 - 170 mlg^{-1} dry wt h^{-1}

H_2 photoproduction phase:

Photosynthetic products $\rightarrow H_2$
Maximum H_2 production rate = 20 ml g-1 dry wt h^{-1}

In the CO_2 uptake phase, the cells take up CO_2 from the gas phase and synthesize the products that subsequently be used for H_2 photoproduction in the H_2 production phase. Such two-stage system of photosynthetic accumulation of starch followed by anaerobic dark fermentation with H_2 production in algae as well as in mixed cultures of algae and photosynthetic bacteria demonstrated a stable but rather moderate yield. Use of previously fixed carbon (carbohydrates) through the oxidative pentose phosphate pathway (which generates the reductant for nitrogenase and hence H_2 production) occurs in blue green algae with the release of CO_2. Improvement of this system is limit also by ATP dependence of the dark H_2 production. The addition of N_2 to this system is essential for long-term H_2 production because N_2 fixation is required to maintain cell metabolism. In addition, it is significant to operate the photobioreactor at higher CO_2 concentration than the normal level in air. The immobilization materials (cellulosic fibers) are relatively cheap because they are make from waste products of cotton industry [113-114].

Incorporating heterocystous blue green algae that possess an active H_2 uptake system can operated under a partial vacuum and with continuous flow of medium through the system, thus avoiding H_2 consumption.

Light inside the photobioreactor:

Various light parameters have been used to assess light conditions inside photobioreactors, and the modeling of photosynthetic cell growth has often been based on parameters such as the incident and average light intensities. The concept of mean light intensity is an improvement over the incident light intensity but does not consider light distribution within the photobioreactor.

According to Einstein's low of photochemical equivalence, the photosynthesis rate (and hence the cell growth rate) should be proportional to the rate of light energy absorbed by the cells. During the linear growth phase, the cell concentration in the photobioreactor is high, so depending on the depth of the reactor, the growing cells absorb almost all the supplied light energy. Total light energy supplied per unit volume of photobioreactor (E_t/V), therefore, would a better measure of photobioreactor performance than the incident or the average light intensities [114].

Growth index

One important step in designing and optimizing photobioreactors is the mathematical modeling of photosynthetic cell growth. Classic models such as that of Monod are base on the specific growth rate of the cell [115]. In most of the growth kinetics and photosynthetic cell growth models, specific rates during the exponential growth phase are use as growth parameters [25]. However, during high-cell density batch cultivation of photosynthetic cells, there are distinct sequential growth phases. The cell growth rate during a growth phase, which has an overwhelming influence on culture productivity, would be a good index for process design and optimization.

As a first step in the photobioreactor design, the relative significance of the exponential and the linear growth rates during light-limited batch cultivation of photosynthetic cells using various types and sizes of photobioreactors. The results indicated that there was negative correlation between the specific growth rates and the linear growth rates or between the specific growth rates and the final cell concentrations during the cultivation of *Chlorella pyrenoidosa* C-212 and *Spirulina platensis* M-135 cells. However, regardless of the type and size of the photobioreactor, There is a positive correlation between the linear growth rates and the final cell concentrations for *C. pyrenoidosa* and *S. platensis* [89, 113, 116-120]. A mathematical model that explain the existence of the various growth phases during the light-limited batch cultivation, predicts that the linear growth phase is longer than the exponential growth phase under various conditions. At a given Et/V, the linear growth rates decreased with an increase in depth of the photobioreactors, indicating that the light distribution inside the reactor must considered in the rational design and scale-up of photosynthetic processes. Compared with the fairly homogenous distribution of light inside a very shallow photobioreactor,distinct spatial heterogeneity of light intensities inside the deep photobioreactors. When a photobioreactor containing a high cell concentration is illuminate from the surface, the cells at the surface absorb light rapidly, and light intensity decreases sharply into the center of the reactor. As a result, the photobioreactor can divided into illuminated and non-illuminated volume fractions. The concept of a light distribution coefficient (Kiv) defined as the cell concentration at which 50% of the photobioreactor volume receives enough light for photosynthetic growth. The

higher the Kiv, the more uniformly light is distributed within the photobioreactor.

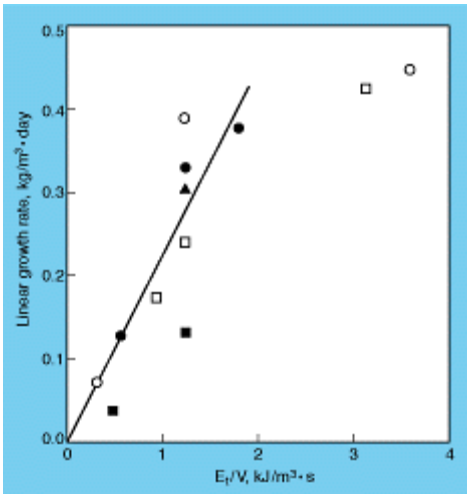


Figure (8): The effects of light energy supplied per uni t volume of photobioreactor (Et/V) on the linear growth rates of *Chlorella pyrenoidosa* in cuboidal photobioreactors. The depths of the photobioreactors were 0.02 m, 0.04 m, 0.06 m, 0.08 m, and 0.16 m.

The significance of this coefficient can seen in the comparison of the Kiv values of two 0.02-m-deep cuboidal photobioreactors (Figure 9). Photobioreactor A is illuminate from one surface with an incident light intensity of 325µmol/m^2s, and B is illuminated from two surfaces at incident light intensities of 162.5 µmol/m^2s. By assuming a critical light intensity of 7.65 µmol/m^2s and a light extinction coefficient of 200 m^2/kg [116], the effect of cell concentration on the illuminated volume fraction was calculated. Although the Et/V in the two photobioreactors is the same, photobioreactor B is more uniformly illuminated; consequently, the Kiv value for B (3.1 kg/m^3) is higher than that for A (1.9 kg/m^3).

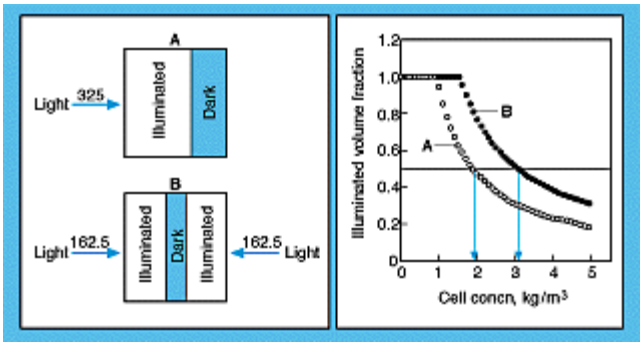


Figure (9): The effects of cell concentration on the illuminated volume fraction of a cuboidal photobioreactor illuminated from one surface (A) or from two surfaces (B). Although the light energy supplied per unit volume (in µmol/m2s) is the same in the two photobioreactors, the calculated light distribution coefficients were 1.9 kg/m3 and 3.1 kg/m3 for photobioreactors A and B, respectively.

Ogbonna *et al*. [113] found that the linear growth rates increased with an increase in Kiv. However, as in the case of Et/V, the data were scattered, show that Kiv alone is not a sufficient index of light supply efficiency of photobioreactors. At a constant Kiv, however, a linear relationship observed between the linear growth rate and the Et/V. Similarly, when the Et/V held constant, there was a good correlation between the Kiv and the linear growth rate.

On the basis of the results of the recent work [6, 55], the light supply coefficient defined as the product of the light energy supplied per unit volume and the light distribution coefficient (Et/V Kiv)--as an index of the light supply efficiency in photobioreactors. There was a linear relationship between the light supply coefficient and the linear growth rates of *C. pyrenoidosa* and *S. platensis* in cuboidal photobioreactors of various sizes. When other internally illuminated and externally illuminated cylindrical photobioreactors were used , Ogbonna *et al*. [117] found a posative correlation between the linear growth rates of *C. pyrenoidosa* and the light supply coefficient, indicating that the proposed light supply coefficient can be used to quantitatively evaluate light condition inside the photobioreactor, regardless of the cell type, reactor type, or size.

Scale-up parameters

Most microbial fermentation processes significantly affected by the degree of mixing within the bioreactor. Consequently, parameters that directly or indirectly describe mixing behavior in the bioreactor have used as bioreactor design criteria. The volumetric mass transfer coefficient is the most widely used index for bioreactor design and scale-up today. Ogbonna *et al* [113] and Fang and Liu [54]) stated that the effect of volumetric mass transfer coefficient (k_La) on the photoautotrophic cultivation (using only light and inorganic material) of *C. pyrenoidosa* and the variation of k_La between 6 and 145 h-1 had no significant effect on the linear growth rate (Figure 10). This shows that, unlike its use to scale-up processes with many heterotrophic (i.e., requiring organic carbon sources) microorganisms, k_La is not a good parameter for the scale-up of photosynthetic processes.

Because of the rapid light attenuation inside photobioreactors, spatial heterogeneity of light intensities occurs inside the photobioreactor. Thus, even when light energy is available in the entire photobioreactor, limitation of light energy is the most commonly encountered problem in large-scale cultures of photosynthetic cells. Conventionally, illumination surface area per unit volume is used as a photobioreactor design criterion. An efficient photobioreactor has a high surface area-to-volume ratio. In a cultivation pond, this is achieved by keeping the pond as shallow as possible. Tubular photobioreactors and thin panels are the most widely investigated closed systems for photosynthetic cell cultivation, because they have high surface area-to-volume ratios. However, many economic and technological problems in the scale-up of such photobioreactors result from the need to keep the surface area-to-volume ratio high, limiting tube diameters and panel depths [121].

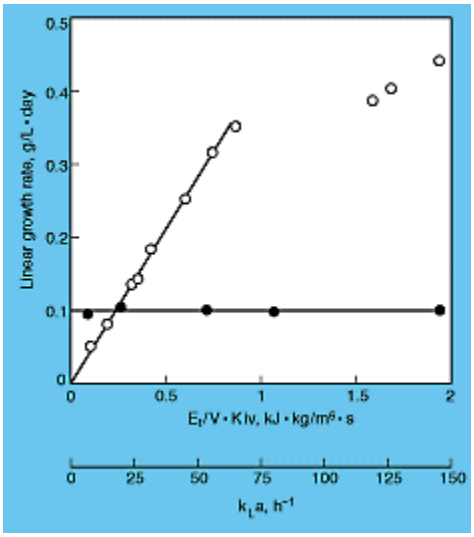


Figure (10) : The effects of the light supply coefficient (Et /VKiv;) and the volumetric oxygen transfer coefficient (k $_L$a;) on the photoautotrophic linear growth rate of *C. pyrenoidosa.*

The effects of Et/V Kiv on the linear growth rates of *C. pyrenoidosa* in various types and sizes of photobioreactors are also show in Figure 10. The inflection observed at high Et/V Kiv values implies a decrease in the photosynthetic efficiency in response to excessive supply of light energy. The relationship between the light supply coefficient and the linear growth rate of *S. platensis* in cuboidal photobioreactors of various sizes was also linear. Therefore, this coefficient is a good index of light supply efficiency of various types of photobioreactors. Because photobioreactor performance determined by the availability of light energy, so the light supply coefficient can used not only to evaluate various types of photobioreactors but also for rational design and scale-up of photobioreactors. When a photobioreactor with the optimal light supply coefficient for a target process is design, it should efficiently scale up by keeping this coefficient at its optimum [6].

Large - scale processes

Many photobioreactors that have been proposed work well in the laboratory are extremely difficult to implement. Commercial, scale-up potentials should be a primary design criterion for photobioreactors. A successful scale-up will achieved only if the data obtained with a small-scale reactor can reproduce with large-scale reactors. To achieve this, the factors that affect cell growth and productivity must maintained within the optimal range as the reactor scaled up from the laboratory to the industrial scale [6, 55].

Like other microorganisms, the growth and productivity of photosynthetic cells are affected by many factors, including media components, temperature, mass transfer characteristics, pH, and concentrations of O_2 and CO_2 in the reactor. However, as shown in Figure 10, the light supply is more important than mass transfer rate during autotrophic cultivation of photosynthetic cells. If the intensity of the light-distributing object is constant, then the light supply coefficient of a unit decreases with an increase in the size of the unit.Therefore at a constant light intensity, there is an optimal unit size for a given cell and process. An optimal unit size for a process is determined firstly, and the photobioreactor scaled up by increasing the number of this unit in three dimensions. In this way, the optimal light supply coefficient of the reactor maintained during the scale-up [122].

Usually, one bioreactor used to cultivate various types of cells and produce various metabolites. These processes can do by using the appropriate substrate and controlling the temperature, aeration, pH, and other factors as desired. Because the difference between the ordinary bioreactor and the photobioreactor is the presence or absence of light, it is reasonable to consider light as a part of the photobioreactor. The light requirements of cells and processes vary greatly; consequently, the optimal light supply coefficient depends on the cell type and the process. Thus, each cell and process requires a different photobioreactor [6, 55, 118, 119].For economic reasons, it is desirable to use the same photobioreactor for several processes; therefore, it should be possible to change the light supply coefficient of the photobioreactor to suit the process. The light supply coefficient of a photobioreactor is a function of the size of each unit (the distance between two light-distributing objects) and the light intensity. It is technically easier to change the light intensity than the distance between the light-distributing objects. A photobioreactor can be used for various processes by changing the intensity of the light-distributing objects, either by using a light source with controllable intensity or by changing the light source altogether. In this way, the light supply coefficient of the photobioreactor can changed, even during cell cultivation. This design allows for starting with low light intensity at the initial stages of growth when the cell concentration is still low and then increasing the light intensity as the cultivation progresses [113].

Hydrodynamic stress

Mixing is very important in photobioreactors. It helps to keep the cells in suspension, distribute the nutrients and the generate heat within the reactor, improve CO_2 transfer into the reactor, degas the photosynthetically produced O_2, improve mass transfer between the cells and the liquid broth, and facilitate the movement of cells in and out of the illuminated part of the photobioreactor. However, because the growth rates of most photosynthetic cells are very low, only a very low degree of mixing is required to achieve most of these objectives [116]. Furthermore, at high light distribution coefficients, the variation in light intensity within the reactor is minimal, so there is little advantage in moving the cells toward and away from the light source [123]. Many photosynthetic cells have no cell wall, and some are mobile or filamentous, making them fragile and sensitive to shear stress. Therefore, it is desirable to keep the hydrodynamic stress as low as possible.

Cultivation under a sterile condition

The risk of contamination by heterotrophic microorganisms is low when there is no organic carbon source in the medium.

However, at facilities where many other photoautotrophic cells are cultivated, contamination by other photoautotrophs can be a serious problem. Thus, the new photobioreactor should be able to withstand sterilization procedures [96].

Productivity versus efficiency

The photobioreactor consisting of a cylindrical glass vessel and a fluorescent lamp that illuminated from the center to determine the optimal unit size. The light intensity of the fluorescent lamp was constant, but it is possible to induce variation in the light supply coefficient of the reactor by using glass vessels of different diameters. The effects of unit size (diameters) on the light supply coefficients, the linear growth rate, and yield coefficient during the cultivation of *C. pyrenoidosa* are shown in Figure (11).

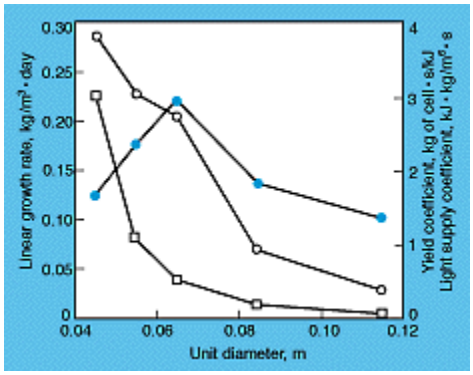


Figure (11): The effects of unit diameter on linear growth rate, yield coefficient, and light supply coefficient of C. pyrenoidosa. Each unit was internally illuminated by a 4-W fluorescent lamp producing a light intensity of 163 µmol/m2s.

The light supply coefficient and thus the linear growth rate decreased with increasing unit diameter. The highest productivity (in this case, the linear growth rate) obtained with very narrow units corresponding to high light supply coefficients. However, under such conditions, more light supplied than the cells can use efficiently because of photoinhibition or energy loss in the form of heat. This process leads to low yield coefficients. The yield coefficient increases with increasing unit diameter, because at relatively low light supply coefficients, most of the supplied light energy is absorbed and efficiently used for cell growth and product formation. However, with large unit sizes, the light supply coefficient decreases and the illuminated volume fraction of the unit is low (i.e., a large portion of the unit is dark). This increases maintenance energy and decreases productivity and the yield coefficient [116, 117, 123].

For a light source of constant intensity, the optimal unit size depends on the cell and the process economy. If light represents a significant percentage of the total production cost, then greater importance should attached to the efficiency of light use, and the unit size giving the highest yield coefficient should selected. However, if the cost of light is relatively cheap (such as the case of solar energy), the design criterion should obtain the highest productivity. A high light supply coefficient is desirable; provide that the light intensity is not high enough to cause photoinhibition. In most cases, a compromise made between productivity and the yield coefficient [124].

A photobioreactor for *C. pyrenoidosa* cultivation

Prototype photobioreactor consisting of four units built with 0.5-cm-thick transparent Pyrex glass for the cultivation of *C. pyrenoidosa* (Figure 12). Each unit was equipped with a centrally fixed glass tube into which the light source inserted. Transparent glass tubes were used as housings for the lamps, so the reactor illuminated by simply inserting the lamps into the glass tubes (no mechanical fixing). Any light source could used. 4-W either fluorescent or halogen lamps with controllable light intensity were used as the illuminating system. Because the lamps are not mechanically fixed and can easily be replaced, the same reactor can be used for efficient cultivation of various cells by using a light source with controllable light intensity or by simply replacing the light source with one that gives the desired light intensity [6, 55].

For mixing, an impeller modified in shape so that it did not touch the glass-housing unit during rotation used. This impeller has low shear stress and high mixing capacity. With this impeller, a kLa value of 100 h-1 achieved at an aeration rate (with air) of 0.5 V/Vmin and an agitation speed of 250 rpm. This value is enough to prevent cell sedimentation, achieve a sufficient rate of CO_2 transfer to the culture, and prevent O_2 build-up within the reactor [116, 117]. Aeration was done through a 5.5-cm-diameter ring sparger with four 1-mm-diameter holes. The glass housing units serve as baffle plates in breaking the gas bubbles, thus increasing the kLa. When a higher gas transfer rate is required, a sparger with numerous smaller diameter holes should used. One benefit of this design is that only the reactor is heat sterilized. The lamps are inserted after cooling, thus making it possible to cultivate under sterile conditions [116]. The production of *C. pyrenoidosa* biomass in this photobioreactor using 4-W fluorescent lamps, which give a light supply coefficient of 0.374 kJkg/m^2s. The linear growth rate (0.164 kg/m3day) was consistent with the light supply coefficient, and the final cell concentration was 1.37 g/L. This concentration is relatively low and would result in high cost of downstream processing. When the light supply coefficient was increased to 0.692 kJkg/m^2 s, the linear growth rate and the final cell concentration increased relative to the light supply coefficient but the efficiency of the cell's use of the supplied light energy decreased.

Chlorella and some other algae can metabolize organic carbon sources, so heterotrophic cultivation can used to achieve high cell concentrations. Under such conditions the photosynthetically derived products are not accumulate and the main advantage of photosynthetic cell cultures is lost. Experiments adapted by Ogbonna *et al*. [113] showed that the protein and chlorophyll contents of *Chlorella* biomass produced from heterotrophic cultures were much lower than those of the photoautotrophic cultures. In some other species (e.g., *Euglena)*, chlorophyll synthesis was completely inhibited under heterotrophic conditions.

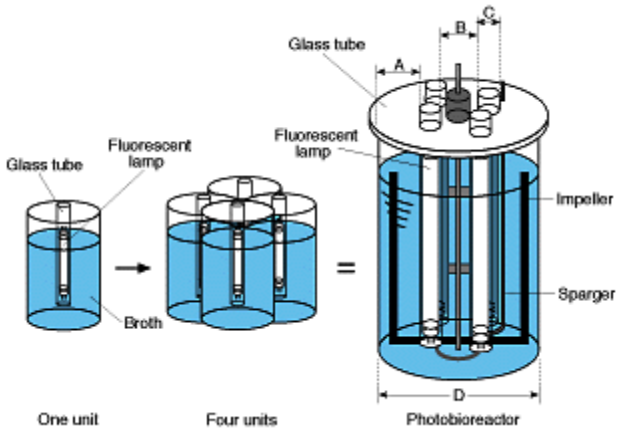


Figure (12): The novel photobioreactor. A: The distance from the reactor wall to the glass tube (2.3 cm). B: The distance between the opposite glass tubes (4.6 cm). C: The diamater of the glass tube housing the fluorescent lamp (2.4 cm). D: The diameter of the reactor (14 cm). Source: Adapted from Reference 21.

Also, they investigated a sequential heterotrophic-photoautotrophic cultivation system to produce high concentration of *C. pyrenoidosa* biomass with high cellular protein and chlorophyll contents. This method involves passing a high concentration of monoalgal biomass from a heterotrophic culture through a photobioreactor for accumulation of protein and chlorophyll. This system composed of the conventional mini-jar fermentor for the heterotrophic phase and the new photobioreactor for the photoautotrophic phase. The exhaust gas from the heterotrophic phase used for aeration of the photoautotrophic phase to reduce CO_2 emission (Figure 13). The mini-jar fermentor and the photobioreactor were filled with the media and inoculated with the preculture of *C. pyrenoidosa*. The lamps of the photobioreactor turned off, and both reactors wrapped with aluminum foil to shut out the light. The cultures then grown heterotrophically using glucose as the carbon source. When the glucose completely consumed, the photobioreactor lamps turned on; continuous feeding of fresh medium into the mini-jar fermentor started; and the effluent continuously passed into the photobioreactor for protein and chlorophyll accumulation. Changes in the cell concentration as well as chlorophyll and protein contents of the cells in the photobioreactor shown in Figure 14. It was possible to produce high *C. pyrenoidosa* biomass concentration (14 g/L) containing 63.5% protein and 2.5% chlorophyll continuously for >600 h. During the steady state, the CO_2 concentration in the exhaust gas was reduced by 15% and the cell productivity was 4 g/L day. This productivity is much higher than the values reported to date in photoautotrophic cultures.

Solar energy

Because the cost of electricity is high, only few algae-derived products can produced at competitive prices with artificial light sources. Use of solar energy is obviously desirable, because it is abundant and free. However, an appropriate method for harvesting the solar energy and distributing the light inside the photobioreactor is required. Optical fibers can used. A light collection device consisting of Fresnel lenses used to collect the solar light, which then distributed inside the reactor through the optical fibers (Figure 15). Because the position of the sun changes continuously, the device is equipped with a light-tracking sensor so that the lenses rotate with the position of the sun. Because of diurnal and seasonal changes in the sunlight intensity high volumetric productivity is difficult to achieve if only solar energy used for reactor illumination. At night the cells metabolize their intracellularly stored carbohydrates for cell maintenance, resulting in decreased productivity [113, 117]. For maximum productivity, therefore, the solar light should be supplemented with an artificial light source at night and on cloudy days. By combining light sources, an optimal amount of light can supplied continuously to the photobioreactor during the process at reduced cost.

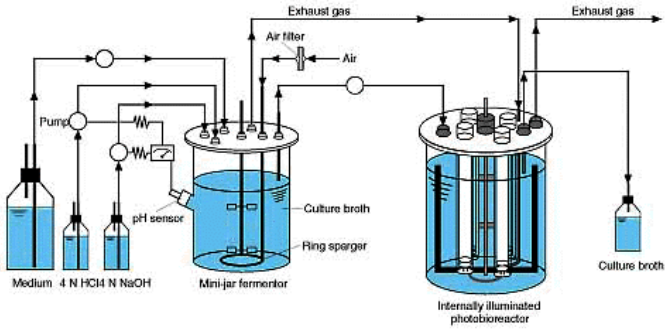


Figure (13): System used for the continuous sequential heterotrophic-photoautotrophic cultivation of *C. pyrenoidosa*. The working volumes of the mini-jar and the photobioreactors were 2.0 and 3.0 L, respectively.

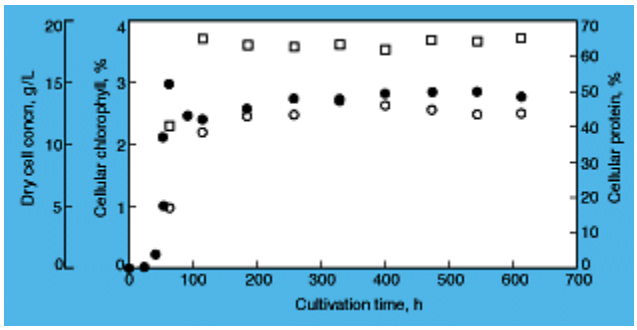


Figure (14): Changes in cell concentration, chlorophyll concentration, and protein concentration of *C. pyrenoidosa* cells in the photobioreactor during the continuous sequential heterotrophic-photoautotrophic cultivation.

A major problem of this system that the efficiency of light collection and transmission very low (about 7%), so a more efficient system still needed. Already scaled up the photobioreactor to 20 L, and its application for cultivation of various photosynthetic cells is currently being investigated [6, 55, 123]

.

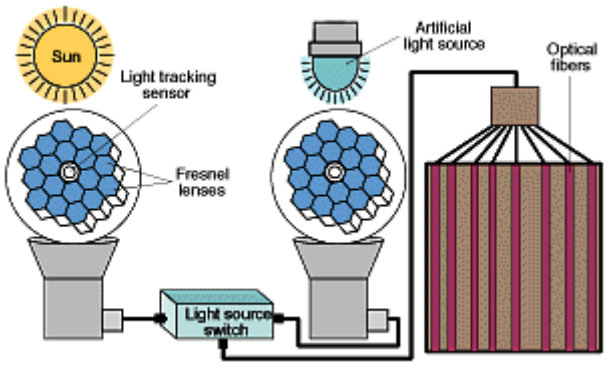


Figure (15): System used for light collection and distribution through the optical fibers. An artifical light source supplements solar energy during the night or on cloudy days.

Table (5): List of Hydrogen –Producing Cyanobacteria(after, Tamagnini *et al*. [90])

Organism	**Methods**	**Measurment**	**M moles H2/h**
Anabaena cylindrica	Closed system ,GC	hours	0.49
Anabaena cylindrica	Closed system ,GC	hours	.0.7
Anabaena cylindrica	Closed system ,GC	hours	0.17
Anabaena cylindrica	Closed system ,GC	hours	0.1
Anabaena cylindrica	Inert gas GC	Days	1.4
Anabaena cylindrica	Inert gas GC	minutes	0.04
Anabaena cylindrica	Inert gas GC	Days	0.58
Anabaena cylindrica	Closed system ,GC	days	0.2
Anabaena flos-aquae	Closed system ,GC	minutes	0.17
Anabaena sp.	Closed system ,GC	hours	0.043
Anabaena 7120	Closed system ,GC	hours	42
Calothrix	Closed system ,GC	Days	0.13
Calothrix scopulorum	Closed system ,GC	hours	0.13
Mastigocladus Laminosus	Inert gas GC	Days	0.29-0.59
Mastigocladus	Closed system ,GC	hours	71
Mastigocladus laminosus	Closed system ,GC	Days	0.17
Nostoc muscorum	Closed system ,GC	hours	0.37
Nostoc muscorum	Closed system ,GC	hours	12
Oscillatoria brevis	Closed system ,GC	hours	0.17
Oscillatoria miami BG 7	Closed system ,GC	Days	0.18,230
Oscillatoria miami BG 7	Closed system ,GC	Days	0.29,260
Oscillatoria miami BG 7	Closed system ,GC	days	0.38
Plectonema boryanum	Closed system ,GC	hours	268
Synechococcus-sp.BG 043511			2
Tolypothrix	Inert gas GC	hours	0.46

Physiological significance

All green photosynthetic plants - including algae - consume carbon dioxide in the presence of light to build tissue, respiring oxygen as a waste product. "But because hydrogenase shuts down in the presence of oxygen, it doesn't function during photosynthesis. It is only works during darkness, when photosynthesis is not occurring.

Because plant functions are at low ebb during darkness, the amount of hydrogen produced is minimal. But to solve the problem, by imposing a nutrient stress to the algae. First they grow out the algae, fattened it under normal photosynthetic conditions. Then withhold sulfur. Sulfur is critical for the completion of normal photosynthesis. In the absence of the element, the algae ceased emitting oxygen and stopped storing energy as carbohydrates, protein and fats. Instead, the algal cells began using an alternative metabolic pathway to exploit stored energy reserves anaerobically - in the absence of oxygen. The hydrogenase activated, splitting large amounts of hydrogen gas from water and releasing it as a byproduct.

The significant thing is that the plant is using the energy of the sunlight to produce hydrogen, not oxygen. Without sulfur, it produces a great deal more hydrogen in the presence of light than it does under normal circumstances in the dark. The algae ultimately would die if the nutrient stress were maintained for more than a few days, but they can be "fattened" again with sulfur and sunlight, allowing for repetitions of the process and continued harvesting of hydrogen gas. Eventually, the process used for the production of huge quantities of hydrogen. Hydrogen burns clean and hot, and it constitutes one-third of the water found in the Earth's oceans, rivers, lakes and atmosphere.

The functions of hydrogenase(s) in cell metabolism are altogether debatable. It is getting more complicated in non-heterocystous blue green algae where the enzyme exists in oxygen-evolving cells while all known hydrogenases are sensitive to oxygen (oxygen insensitive hydrogenases are scarcely reported and genetically studies are running to clone oxygen insensitive hydrogenases [94, 96]. In heterocysts, there is most probably very little opportunity for oxygen-related inhibition of hydrogenases. The question why such enzymes exist in green photosynthetic cells (seemingly with no physiological contribution and is subjected to oxygen-related inhibition) urges a molecular comparison between heterocystous- and green cell hydrogenases. Nevertheless, several postulations have proposed for physiological importance of hydrogenase, some of them are:

After prolonged periods of anaerobiosis, endogenous donors over reduce photosynthetic electron transport chain from fermentative metabolism. Moreover, the process is probably even activating by hydrogenase. Simultaneously, anaerobic conditions inhibit the functioning of water-oxidizing system that sometimes cannot remove by external oxidants. In this non-fundamental state, PS II possibly mediates a cyclic electron flow with the participation of cyt b-559 and reduced plastoquinone pool (*via* Q_a and Q_b). H_2 photoproduction in hydrogenase containing – algae promotes rapid reoxidation of carriers between the two photosystems and increases the ATP level due to coupled photophosphorylation. It indirectly stimulates substrate phosphorylation level that triggers CO_2 fixation and the mechanism of positive feedback of evolving O_2 on operation of water-splitting system. Obviously, this important function of hydrogenase/PSI couple gives to algae a selective ecological advantage to survive and grow under natural environment with occasional anaerobic conditions (for example, during a mass multiplication of accompanying heterotrophic microorganisms). In addition, the ability of reversible hydrogenase to derive reductants without participation of PS II by directing the available radiant energy into PS I-dependent cyclic phosphorylation could prove beneficial in light-limiting anaerobic environments. An unidentified phycobilisome-bound hydrogenase interacts with a protein kinase, regulating the distribution of excitation energy between the two photosystems. Therefore, some workers postulated that hydrogen metabolism simulates a safety valve at either direction (H_2 oxidation or proton reduction) depending on the energy status of the cell.

There is an intriguing correlation between hydrogenase content in green algae and the growth enhancement and chlorophyll synthesis under unfavorable conditions.

Hydrogenases cooperate with nitrogenase complex, recycling the H_2 lost during the N_2-fixing cycle, and/or protecting the latter against oxygen inactivation by an oxy hydrogen reaction.

A light modulation of the dark fermentative H_2 production in greening mutants of *Chlorella* through competition of hydrogenase with NADPH-photochlorophyllide photoreductase and an unidentified photoreductase of Mg-protochlorophyrin for common electron donors.

Only blue green algae and algae are net energy producers from the viewpoint of H_2 photoproduction since at least 4 mol ATP per mol evolved H_2 are consumed in the reaction of blue green algal nitrogenase, but not in the hydrogenase-mediated reaction.

Biological hydrogen production is the most challenging area of biotechnology with respect to environmental problems. The future of biological hydrogen production depends not only on research advances, i.e.improvement in efficiency through genetically engineering microorganisms and/or the development of bioreactors, but also on economic considerations (the cost of fossil fuels), social acceptance, and the development of hydrogen energy systems.

Cars already have been developed that run on hydrogen-powered devices known as fuel cells. These vehicles are virtually pollution-free; the only substance emitted from the tailpipe is water vapor. They do not release carbon dioxide or other heat-trapping gases, which are widely considered the primary culprits in global warming. Fuel cells big enough to

power electrical generating plants could also be built. long-term goal is to develop strains of algae that we would grow in mass cultures to produce enormous quantities of hydrogen gas. However, at this point, they have to improve the production performance.

REFERENCES

[1] Melis, A., Happe, T. Hydrogen production .Green algae as asource of energy. Plant Physiol., 2001; 127:740-748.

[2] Veziroglu, T.N., Takahashi, P.K. Hydrogen Energy Progress VIII.Proceeding of the 8th World Hydrogen Energy Conf. Honolulu and Waikoloa,Hawaii, USA Pergamon, New York , 1990, pp. 1594.

[3] Mitsui, A., Kumazawa, S., Takahashi, A., Ikemoto, H., Suda, S., Hanagata, N., Domeier, M., Komatsu, M., Benkert, J. Overview on the biological hydrogen photoproduction research.In: Hydrogen Photoproduction Workshop IV.Waikoloa,Hawaii, 1990, pp. 171.

[4] Gogotov, I.N., Troshina, O.Y. Biotechnological foundation of hydrogen production by employing phototrophic microorganisms .In :Hydrogen Photoproduction Workshop IV. Waikoloa, Hawaii, 1990, pp. 123.

[5] Markov, S.A., Lichtl, K.K., Pao, D.O. A holow fibre photobioreactor for continuous production of hydrogen. J. Hydrogen Energy, 1993; 18: 901-906.

[6] Harris, E.H. *Chlamydomonas* as a Model Organism. Annu. Rev. Plant Physiol. Plant.Mol. Biol., 2001; 52: 363-406.

[7] Wuenschiers, R., Schulz, R., Senger, H. Electron pathways involved in H_2-metabolism in the green alga *Scenedesmus obliquus*. Biochim. Biophys. Acta, 2001; 1503:271-278.

[8] Happe, T., Schuetz, K., Boehme, H. Transcriptional and mutational analysis of the uptake hydrogenase of the filamentous cyanobacterium *Anabaena variabilis* ATCC 29413. J. Bact., 2000; 182 :1624-1631.

[9] Matthies, C., Kuhner, C.H., Acker, G., Drake, H. *Clostridium uliginosun* sp. Nov.,a novel acid-tolerant,anaerobic bacteria with connecting filaments.I .J. System. Evolu. Microbiol., 2001; 51: 1119-1125.

[10] Fang, H.H.P., Zhang, T., Liu, H. Microbial diversity of a mesophilic hydrogen-producing sludge. Applied Microbiol . Biotechnol., 2002; 58:112-118.

[11] Boichenko, V.A., Hoffmann, P. Photosynthetic hydrogen production in Prokaryotes and Eukaryotes: Occurrence, mechanism and functions. Photosynthetica, 1994; 30: 527-552.

[12] Adams, M.W.W., Mortenson, L.E., Chen, J.S. Hydrogenase. Biochim. Biophys. Acta, 1981; 594: 105-176.

[13] Gogotov, I.N. Hydrogenases of phototrophic microorganisms. Biochemie, 1986; 68: 181-187.

[14] Schnackenberg, H., Ikemoto, S., Myachi. Relationship between oxygen-evolution and hydrogen-evolution in a *Chlorococcum* strain.J. photochem. Photobiol., 1995; 28: 171-174.

[15] Elsen, S., Dischert, W., Colbeau, A., Bauer, C-E. Expreession of the uptake hydrogenase and molybdenum nitrogenase in Rhodobacter capsulatus is coregulated by the Reg B-RegA two-component regulatory system. J. Bact., 2000; 182: 2831-2837.

[16] Hall, D.O., Markov, S.A., Watanabe, Y., Rao, K.K. The potential applications of cyanobacterial photosynthesis for clean technologies. Photosynth. Res., 1995; 46: 159-167.

[17] Kessler, E. Effect of anaerobiosis on photosynthetic reactions and nitrogen metabolism of algae with and without hydrogenase. Arch. Microbiol., 1973; 93: 91-100.

[18] Kessler, E. Hydrogenase, photoreduction and anaerobic growth of algae. In Algal Physiology and Biochemistry. Blackwell, Oxford, 1974; pp 454-473.

[19] Kessler, E. Hydrogen metabolism of eukaryotic organisms. In HG Schlegel, G Gottschalk, N Pfennig, eds, Microbial Production and Utilization of Gases. Göttingen, Germany, 1976; pp 247-254.

[20] Lambret, G.R., Smith, G.D. The hydrogen metabolism of cyanobacteria (blue-green algae) . Biol. Rev., 1981; 56: 589 - 660.

[21] Berchtold, M., Bachofen, R. Hydrogen formation by cyanobacterial cultures selected for nitrogen fixation. Arch. Microbiol., 1979; 123: 227-232.

[22] Markov, S.A., Bazin, M.J., Hall, D.O. The potential of using cyanobacteria in photobioreactors for hydrogen production.Advanced in Biotechnol. Engin., 1995; 52: 60-86.

[23] Hill, S. How is nitrogenase regulated by oxygen? FEMS Microbiol. Rev., 1988; 54: 111-130.

[24] Boichenko, V.A., Arkhipov, V.N., Litvin, F.F. Simultaneous measurements of fluorescence induction and hydrogen photoproduction in *Chlorella vulgaris* under anaerobic conditions. Biofizika, 1983; 28: 976-979.

[25] Boichenko, V.A., Greenbaum, E., Seibert, M. Hydrogen production by photosynthetic microorganisms. In MD Archer, J Barber, eds, Photoconversion of Solar Energy: Molecular to Global Photosynthesis, Vol. 2. Imperial College Press, London, 1999.

[26] Boichenko, V.A., Ladygin, V.G., Litvin, F.F. Structural and funcational organization of photosynthetic units in the cells of *Chlamydomonas reinhardtii* mutants. Mol .Biol., 1989; 23:107-118.

[27] Hallenbeck, P.C., Benemann, J.R. Hydrogen from algae. In J Barber, ed, Photosynthesis in Relation to Model Systems. Elsevier/North-Holland Biomedical Press, New York, 1979, pp 331-364.

[28] Brand, J.J., Wright, J.N., Lien, S. Hydrogen production by eukaryotic algae. Biotech. Bioeng., 1989; 33: 1482-1488.

[29] Allakhverdiev, S.I., Mal'tsev, S.V., Klimov, V.V. $NADP^+$ photoreduction and hydrogen photoevolution by photosystem II particles. Photosynth. Res., 1992; 43:139-142.

[30] Ben-Amotz, A., Erbes, D.L., Riederer-Handerson, A.M., Peavey, D.G., Gibbs, M. H_2 metabolism in photosynthetic organisms.I. Darke hydrogen uptake by algae and mosses.Plant Physiol., 1975; 56:72-77.

[31] Efimtsev, E.I., Boichenko, V.A., Litvin, F.F. Photoinduced evolution of hydrogen by bacteria, algae, and higher plants. Dokl. Akad. Nauk SSSR, 1975; 220: 986-989.

[32] Efimtsev, E.I., Boichenko, V.A., Litvin, F.F. Action spectra for photosynthesis and hydrogen evolution in bacteria, algae and higher plants. Dokl. Akad. Nauk SSSR, 1975; 220: 1238-1240.

[33] Benemann, J.R., Berenson, J.A., Kaplan, N.O., Kamen, M.D. Hydrogen evolution by a chloroplast-ferredoxin-hydrogenase system. Proc Natl Acad Sci USA, 1973; 70: 2317-2320.

[34] Moller, I.M., Lin, W. Membrane-bound NAD(P) H dehydrogenases in higher plant cells. Annu. Rev. Plant Physiol., 1986; 37: 309-334

[35] Melis, A., Happe, T. Hydrogen production .Green algae as a source of energy. Plant Physiol., 2001; 127:740-748.

[36] Bishop, N.I., Frick, M., Jones, L.W. Photohydrogen production in green algae: water serves as the primary substrate for hydrogen and oxygen production. In A Mitsui, S Miyachi, A San Pietro, S., 1977.

[37] Greenbaum, E. The tumover times and pool sizes of photosynthetic hydrogen production by green algae. Solar Energy, 1979; 23: 315-320.

[38] Greenbaum, E. Simultaneous photoproduction of hydrogen and oxygen by photosynthesis. Biotechnol. Bioeng., 1980; 22 : 1-13.

[39] Greenbaum, E. Photosynthetic hydrogen and oxygen production: kinetic studies. Science, 1982; 196: 879-880.

[40] Greenbaum, E. Energetic efficiency of hydrogen photoevolution by algal water splitting. Biophys. J., 1988; 54: 365-368.

[41] Benemann, J.R., Weare, N.M. Hydrogen evolution by nitrogen-fixing *Anabaena cylindrica* cultures. Science, 1974; 184: 174-175.

[42] Matthies, C., Kuhner, C.H., Acker, G., Drake, H. *Clostridium uliginosun* sp. Nov.,a novel acid-tolerant,anaerobic bacteria with connecting filaments.I .J. System. Evolu. Microbiol., 2001; 51: 1119-1125.

[43] Asada, Y., Miyake, M. Photobiological hydrogen production. J. Biosci.Bioen., 1999; 88: 1-6.

[44] Harris, E.H. *Chlamydomonas* as a Model Organism. Annu. Rev. Plant Physiol. Plant.Mol. Biol., 2001; 52: 363-406.

[45] Schulz, R. Hydrogenases and hydrogen production in eukaryotic organisms and cyanobacteria. J. Mar. Biotechnol., 1996; 4:16-22.

[46] Roessler, P.G., Lien, S. Activation and de novo synthesis of hydrogenase in Schlegel HG, Schneider K (1978) In HG Schlegel, K Schneider, eds, Hydrogenases: Their Catalytic Activity, Structure and Function. Göttingen, Germany, 1984, pp 15-44.

[47] Boichenko, V.A., Hoffmann, P. Photosynthetic hydrogen production in Prokaryotes and Eukaryotes: Occurrence, mechanism and functions. Photosynthetica, 1994; 30: 527-552.

[48] Itoh, T., Asada, H., Tobioka, K., Kkodera, Y., Matsushima, A., Hiroto, M.N.H., Kamachi, T., Okura, I., Inada, Y. Hydrogen gas evolution and carbon dioxide fixation with visible light by chlorophyllin coupled with polyethylene glycol. Bioconj. Chemim., 2000; 11: 8-13.

[49] Dawar, S., Mohanty, P., Behera, B.K. Sustainable hydrogen production in the cyanobacterium *Nostoc sp* ARM 411 grown in fructose –and magnesium sulphate- enriched culture. W. J. Microbiol. Biotech., 1999; 15 :289-292.

[50] Schnacken, J., Miyake, M.M.J., Zorin, N.A., Asada, Y. *In vitro* and *vivo* coupling of *Thiocapsa* hydrogenases with cyanobacterial and algal electron mediators. J. Biosc. Bioeneg., 1999; 88:30-34.

[51] Meyer, J., Gagnon, J. Primary structure of hydrogenase I from *Clostridium pasteuranium*. Biochem., 1991; 30: 9697-9704.

[52] Peters, J.W., Lanzilotta, W.N., Lemon, B.J., Seefeldt, L.C. X-ray crystal structure of the Fe-only hydrogenase (CpI) from *Clostridium pasteuranium* to 1.8 angstrom resolution. Science, 1998; 282:1853-1858.

[53] Happe, T., Mosler, B., Naber, J.D. Induction, localization and metal content of hydrogenase in the green alga *Chlamydomonas reinhardtii*. Eur. J. Biochem., 1994; 222: 769-774.

[54] Fang, H.H.P., Liu, H. Effect of pH on hydrogen production from glucose by a mixed culture. Bioresource Technol., 2002; 82: 87-93.

[55] Wuenschiers, R., Schulz, R., Senger, H. Electron pathways involved in H_2-metabolism in the green alga *Scenedesmus obliquus*. Biochim. Biophys. Acta, 2001; 1503:271-278.

[56] Albracht, S.P.J. Nickel hydrogenases: in search of the active site. Biochim. Biophys. Acta, 1994; 1188: 167-204.

[57] Bioson, G., Bothe, H., Schmitz, O. (2000) Transcription analysis of hydrogenase genes in cyanobacteria *Aanacystis nidulans* and *Anabaena variabilis* monitored by RT-PCR. Current Microbiol. 2000; 40:315-321.

[58] Weaver, P.F., Lien, S., Seibert, M. Photobiological production of hydrogen. Sol. Energy, 1980; 24: 3-45.

[59] Redding, K., Cournac, L., Vassiliev, I.R., Golbeck, J.H., Peltier, G., Rochaix, J-D. Photosystem I is indispensable for photoautotrophic growth, CO2 fixation, and H_2 photoproduction in Chlamydomonas reinhardtii. J. Biol. Chem., 1999; 274: 10466-10473.

[60] Benemann, J.R. Hydrogen Biotechnology: Progress and Prospects. Nature Biotechnol., 1996; 14: 1101-1103.

[61] Wykoff, D.D., Davies, J.P., Melis, A., Grossman, A.R. The regulation of photosynthetic electron transport during nutrient deprivation in *Chlamydomonas reinhardtii*. Plant Physiol., 1998; 117:129-139.

[62] Christoph, R., Senger, H. Participation of the two photosystems in light. –Photochem. Photobiol., 1985; 2: 553-557.

[63] Greenbaum, E., Lee, J.W., Tevault, C.V., Blankinship, S.L., Mets, L.J. CO_2-fixation and photoevolution of H_2 and O_2 in a mutant of *Chlamydomonas* lacking photosystem I. Nature, 1995; 376: 438-441.

[64] Melis, A. Spectroscopic methods in photosynthesis: photosystem stoichiometry and chlorophyll antenna size. Phil. Trans. R. Soc. Lond. B, 1989; 323: 397-409.

[65] Melis, A. Dynamics of photosynthetic membrane composition and function. Biochim. Biophys. Acta, 1991; 1058: 87-106.

[66] Gibbs, M., Gfeller, R.P., Chen, C. Fermentative metabolism of *Chlamydomonas reinhardtii*: II.

Photoassimilation of acetate. Plant Physiol., 1986; 82: 160-166.

[67] Boichenko, V.A., Litvin, F.F. Effective cross-section of two photochemical reactions of photosynthesis and the discrete character of the variations in sizes of the photosynthetic units in green algae. Dokl. Akad. Nauk. SSSR, 1986; 286: 733-737.

[68] Ball, S.G., Dirick, L., Decq, A., Martiat, J-C., Matagne, R.F. Physiology of starch storage in the monocellular alga *Chlamydomonas reinhardtii*. Plant Science, 1990; 66: 1-9.

[69] Reeves, M.E., Greenbaum, E. Long-term endurance and selection studies in hydrogen and oxygen photoproduction by Chlamydomonas reinhardtii. Enzyme Microb .Technol., 1985; 10: 169-174.

[70] Bader, K.P., Schmid, G.H. Cooperative binding of oxygen to the water spliting enzyme in the filamentous cyanobacterium *Oscillataria chalybea*. Biochim. Biophys. Acta, 2000; 1456: 108-120.

[71] Edwards, D.F., Mathison, G.E., Platt, D.J. Metronidazole – an antimicrobial drug which inhibits photosynthesis. Z. flanzenphysiol., 1974; 71: 424-427.

[72] Eisbrenner, G., Bothe, H. Modes of electron transfer from molecular hydrogen in *Anabaena cylindrica* Arch. Microbiol., 1979; 123: 37-45.

[73] Mitsui, A., Kumazawa, S., Takahashi, A., Ikemoto, H., Suda, S., Hanagata, N., Domeier, M., Komatsu, M., Benkert, J. Overview on the biological hydrogen photoproduction research.In: Hydrogen Photoproduction Workshop IV.Waikoloa,Hawaii, 1990, pp 171.

[74] Serebryakova, L.T., Sheremetiva, M.E., Lindbald, P. Hydrogenase activity of the unicellular cyanobacterium *Gloeocapsa alpicola* CALU 743 under conditions of nitrogen starvatiion. Mikrobiologia, 1999; 68:293-298.

[75] Serebryakova, L.T., Sheremetiva, M.E., Lindbald, P. H2 – uptake and evolution in the unicellular cyanobacterium *Chroococcidiopsis thermalis* CALU 758. Plant Physiol. Biochem., 2000; 38: 525-530.

[76] Agar, J., Suda, S., Takeyama, H., Lee, W., Mitsui, A. Hydrogen and oxygen photoproduction by marine unicellular cyanobacterium under high light intensities equivalent to mid-day intensities. In: Abstract 2nd Int.Marine Biotechnol.Conf.Baltimore, USA, 13-16 1991, pp 75.

[77] Kumazawa, S., Suda, S., Mitsui, A. Effect of cell densities to the hydrogen photoproduction by marine cyanobacteria, Synechococcus sp. Miami BG4351 and *Anabaena* sp. TU37-1. In: Abstract 91st General Metting of Amer.Soc.Microbiol., 1991, pp 222

[78] Taiz, L., Zeiger, E. Plant Physiology, 2nd ed. Sinauer Associates Inc, USA, 1998.

[79] Lambret, G.R., Smith, G.D. The hydrogen metabolism of cyanobacteria (blue-green algae). Biol. Rev., 1981; 56 : 589 - 660.

[80] Issa, A.A. Aspects of growth and nitrogenase activity of the cyanobacterium *Nostoc muscorum* in continuous culture. Cryptogamie Algologie, 1995; 16: 247-253.

[81] Miura, Y. Hydrogen production by biophotolysis based on microalgal photosynthesis. Proc. Biochem., 1995; 30: 1-7.

[82] Nguen, T.H. Condition of nitrogen fixation in cyanobacteria *Plectonema boryanum* and *Gloeothece* .Summary of Ph D thesis, Moscow State University, Moscow, 1985.

[83] Shi, D-J. Energy metabolism and structure of the immobilized cyanobacterium *Anabaena azollae.* Ph D thesis.King College London, University of London, London, 1987.

[84] Serebryakova, L.T., Sheremetiva, M.E., Lindbald, P. H2 – uptake and evolution in the unicellular cyanobacterium *Chroococcidiopsis thermalis* CALU 758 .Plant Physiol. Biochem., 2000; 38: 525-530.

[85] Benemann, J.R. Hydrogen production by microalgae. J. Appl. Phycol., 2000; 12:291-300.

[86] Seibert, M., Flynn, T., Benson, D., Tracy, E., Ghirardi, M.L. Development of selection and screening procedures for rapid identification of H_2-producing algal mutants with increased O_2 tolerance. In O Zaborsky, ed, Biohydrogen. Plenum Press, New York, 1998, pp 227-234.

[87] Davies, J.P., Yildiz, F., Grossman, A.R. Mutants of *Chlamydomonas* with aberrant responses to sulfur deprivation. Plant Cell, 1994; 6: 53-63.

[88] Hazelkorn, R. Genetic system in cyanobacteria .In:Miller JH (ed) Methods in Enzymology.Academic Press, Harcourt Brace Jovanovich, San Diego, Vol 204, 1991, pp 418.

[89] Shah, V., Garg, N., Madamwar, D. Ultrastructure of the fresh water cyanobacterium *Anabaena variabilis* SPU033 and its application for oxygen-free hydrogen production. FEMS Microbiol. Letters, 2001; 194:71-75.

[90] Tamagnini, P., Costa, J-L., Almeida, L., Oliveira, M-J., Salema, R., Lindbald, P. Diversity of cyanobacterial hydrogenases, a molecular approach. Current Microbiol., 2000; 40: 356-361.

[91] Maione, T.E., Gibbs, M. Hydrogenase-mediated activities in isolated chloroplasts of *Chlamydomonas reinhadtii*. Plant Physiol., 1986; 80: 360-368

[92] Maness, P-C., Weaver, P.F. Biological H2 from fuel gases and from H2O . In Proceeding of the 1999 U.S.DOE Hydrogen Program Review.1999: 111-124, NREL/CP-570-26938.

[93] McBride, A.C., Lien, S., Togasaki, R.K., San, P.A. Mutational analysis of *Chlamydomonas reinhardtii* application to biological solar energy conversion. In A Mitsui, S Miyachi, A San Pietro, S Tamura, eds, Biological Solar Energy Conversion. Academic Press, New York, 1977, pp 77-86

[94] Ghirardi, M.L., Flynn, T., Foresiter, M., Seibert, M. Development of an efficient algal hydrogen- producing system. In Proceeding of the 1998 U.S.DOE Hydrogen Program Review. 1999: 16-29, NREL/CP-570-26938.

[95] Ghirardi, M.L., Togasaki, R.K., Seibert, M. Oxygen sensitivity of algal H2-production. Appl. Biochem. Biotech., 1997; 63: 141-151.

[96] Ghirardi, M.L., Zhang, L., Lee, J.W., Flynn, T., Seibert, M., Greenbaum, E., Melis, A. Microalgae: A green source of renewable H_2 .Trends in Biotechnol., 2000;18:506-510.

[97] Flynn, T., Ghirardi, M.L., Seibert, M. Isolation of *Chlamydomonas* mutant with improved oxygen-tolerance. In Division of fuel chemistry, ACS Meeting, New Orleans, LA, 1999; 44:846-850.

[98] Schmitz, H.B. $NAD(P)^+$ - dependent hydrogenase activity in extracts from the cyanobacterium *Anacystis nidulans*: FEMS Microbiol., 1996; 135: 97-101.

[99] Appel, J., Schulz, R. Hydrogen metabolism in organisms with oxygenic photosynthesis: hydrogenases as important

regulatory devices for a proper redox poising? J. Photochem. Photobiol., 1998; 47: 1-11.

[100] Voordouw, G., Brenner, S. Nucleotide sequence of the gene encoding the hydrogenase from Desulfovibrio vulgaris. Eur. J. Biochem., 1985; 148: 515-520.

[101] Voordouw, G., Strang, J.D., Wilson, F.R. Organization of the genes encoding [Fe] hydrogenase in Desulfovibrio vulgaris. J. Bacteriol., 1989; 171: 3881-889.

[102] Appel, J., Saranya, P., Klaus, S. The bidirectional hydrogenase of Synechocystis sp. PCC 6803 works an electron valve during photosynthesis. Arch. Microbiol., 2000; 173:333-338.

[103] Sveshnikova, D.A., Sveshnikova, N.V., Rao, K.K., Hall, D.O. Hydrogen metabolism for mutant forms of *Anabaena variabilis*. –FEMS Microbiol. Lett., 1997; 147: 297-301.

[104] Melis, A., Murakami, A., Nemson, J.A., Aizawa, K., Ohki, K., Fujita, Y. Chromatic regulation in *Chlamydomonas reinhardtii* alters photosystem stoichiometry and improves the quantum efficiency of photosynthesis. Photosynth. Res., 1996; 47: 253-265.

[105] Melis, A., Neidhardt, J., Benemann, J.R. *Dunaliella salina* (Chlorophyta) with small chlorophyll antenna sizes exhibit higher photosynthetic productivities and photon use efficiencies than normally pigmented cells. J .Appl. Phycol., 1999; 10: 515-525.

[106] Melis, A., Zhang, L., Forestier, M., Ghirardi, M.L., Seibert, M. Sustained Photobiological Hydrogen Gas Production upon Reversible Inactivation of Oxygen Evolution in the Green Alga *Chlamydomonas reinhardtii*. Plant Physiol., 2000; 122:127-135.

[107] Richard, D.J., Sawers, G., Sargent, F., McWalter, L., Boxer, D.H. Transcriptional regulation in response to oxygen and nitrate of the operons encoding the (NiFe) hydrogenases 1 and 2 of *Escherichia coli*. Microbiol., 1999; 145: 2903-2910.

[108] Chand, R.L., Darshan, K.H., Helmut, M.F., Johannas, S.C. Services of algae to the environment. J.Microbiol.Biotechnol., 2000; 10:119-136.

[109] Issa, A.A., El-Enany, A.E., Abdel-Basset, R. Modulation of the photosynthetic source :sink relationship in cultures of the cyanobacterium *Nostoc rivulare*. Biologia Plant., 2002; 45:212-225.

[110] Ogbonna, J.C., Yada, H., Masui, H., Tanaka, H. Loofa (*Loofa cylindrica*) sponge as a carrier for microbial cell immobilization. J. Ferment. Bioeng., 1994; 78: 437-442.

[111] Miyak, J., Miyak, M., Asada, Y. Biotechnological hydrogen production: Research for efficient light energy conversion. J.Biotechnol., 1999; 70: 89-101.

[112] Kawaguchi, H., Hashimoto, K., Hirata, K., Miyamoto, K. H_2 production from algal biomass by a mixed culture of *Rhodobium marinum* A-501 and *Lactobacillus amylovorus*. J. Bioscien. Bioengin., 2001; 91:277-282.

[113] Ogbonna, J.C., Yada, H., Masui, H., Tanaka, H. Cyclic autotrophic/ heterotrophic cultivation of photosynthetic cells-A method of achieving continuous cell growth under light/dark cycles. Bioresource Technol., 1998; 56: 65-72.

[114] Nohke, T., Mizuno, O. Hydrogen fermentation of organic municipal wastes. Water Science Technol., 2000; 42:155-162.

[115] Adams, M.W.W. The structure and mechanism of iron-hydrogenases. Biochim. Biophys. Acta, 1990; 1020: 115-145.

[116] Ogbonna, J.C., Yada, H., Masui, H., Tanaka, H. Light supply coefficient-A newengineering parameter for photobioreactor design. J. Ferment. Bioeng., 1995; 80: 369-376.

[117] Ogbonna, J.C., Yada, H., Masui, H., Tanaka, H. A novel internally illuminated stirred tank photobioreactor for large-scale cultivation of photosynthetic cells. J. Ferment. Bioeng., 1996; 82: 6-67.

[118] Ogbonna, J.C., Yada, H., Masui, H., Tanaka, H. Industrial-size photobioreactors. CHEMTECH, 1997; 27: 43-49.

[119] Ogbonna, J.C., Yada, H., Masui, H., Tanaka, H. Sequential heterotophic /autotrophic cultivation- An efficient method for producing Chlorella biomass for health food and animal feed. J. Appl. Phycol., 1997; 9:359-366.

[120] Aoyama, K., Uemura, I., Miyake, J., Asada, Y. Fermentative metabolism to produce hydrogen gas and organic compounds in a cyanobacterium, *Spirulina platensis*. J. Ferment. Bioenerg., 1997; 83: 17-20.

[121] Yildiz, F.H., Davies, J.P., Grossman, A.R. Characterization of sulfate transport in *Chlamydomonas reinhardtii* during sulfur-limited and sulfur-sufficient growth. Plant Physiol., 1994; 104: 981-987.

[122] Dent, R.M., Han, M., Niyogi, K.K. Functional Genomics of Plant Photosynthesis in the Fast Lane Using *Chlamydomonas reinhardtii*. Trends in Plant Sci., 2000; 8: 364-371.

[123] Lee, T.H., Park, J-Y., Park, S. Growth of Rhodopseudomonas palustris under phototrophic and non-phototrophic. Biotechnol. Lett., 2002; 24: 91-96.

[124] Liu, J-G., Hall, D.O., Rao, K.K., Tsygankov, A.A., Svenhnikov. H_2 production by *Anabaena variabilis* mutant in computer controlled two-stage air-lift tubular photobioreactor. Chinese J. Oceanol. Limnol., 2000; 18:126-131.

Indo Global Journal of Pharmaceutical Sciences(ISSN 2249 1023 ; CODEN- IGJPAI; NLM ID: 101610675) indexed and abstracted in EMBASE(Elsevier), SCIRUS(Elsevier),CABI, CAB Abstracts, Chemical Abstract Services(CAS), American Chemical Society(ACS), Index Copernicus, EBSCO, DOAJ, Google Scholar and many more. For further details, visit http://iglobaljournal.com