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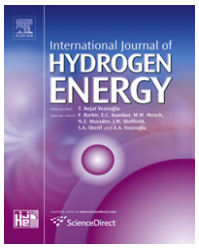

Review

Recent trends on the development of photobiological processes and photobioreactors for the improvement of hydrogen production

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ABSTRACT

Hydrogen production through biological routes is promising because they are environmentally friendly. Hydrogen production through biophotolysis or photofermentation is usually a two stage process. In the first stage CO_2 is utilized for biomass production which is followed by hydrogen production in the second stage in anaerobic/sulfur-deprived conditions. In addition, one-stage photobiological hydrogen production process can be achieved using selected cyanobacterial strains. The major challenges confronting the large scale production of biomass/hydrogen are limited not only on the performance of the photobioreactors in which light penetration in dense cultures is a major bottleneck but also on the characteristics of the organisms. Other dependable factors include area/volume (A/V) ratio, mode of agitation, temperature and gas exchange. Photobioreactors of different geometries are reported for biohydrogen production: Tubular, Flat plate, Fermentor type etc. Every reactor has its own advantages and disadvantages. Airlift, helical tubular and flat plate reactors are found most suitable with respect to biomass production. These bioreactors may be employed for hydrogen production with necessary modifications to overcome the existing bottlenecks like gas hold up, oxygen toxicity and poor agitation. This review article attempts to focus on existing photobioreactors with respect to biomass generation and hydrogen production and the steps taken to improve its performance through engineering innovation that definitely help in the future design and construction of photobioreactors.

1. Introduction

Presently, fossil fuel is considered to be a major source of energy. Because of its ever-increasing demand at one end as well as its limited availability and non-renewable reservoir at the other it is obvious that we need an alternative source of energy. Photobiological hydrogen production has shown promise during the last decades. Gaffron and Rubin [1] and

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later Spruit [2] reported the simultaneous light-activated photo-production of hydrogen and oxygen using a photobiological process. The Research Institute of Innovative Technology for the Earth (RITE) has developed novel hydrogen production technologies for efficient production of hydrogen utilizing solar energy and photosynthetic microorganisms [3]. These processes can be beneficial in two ways: firstly by removing the green house gases (mostly CO_2) which are responsible for global-warming and production of non-polluting, renewable potential energy carrier (biophotolysis by green algae and cyanobacteria) and secondly using waste materials that potentially create ecological hazards, as substrate (photofermentation by photosynthetic bacteria).

Photobiological hydrogen production has several limitations and the yield is very low compared to thermochemically and electrochemically produced hydrogen. Theoretically, during direct biophotolysis 2 mol of hydrogen should be produced from 2 mol of water, in indirect biophotolysis 12 mol of hydrogen is expected from 1 mol of glucose and in photofermentation 4 mol of hydrogen is expected from 1 mol of acetic acid. The actual yields are much lower than the theoretical maximum value because the enzymes (hydrogenase, nitrogenase) involved in the production of hydrogen in algae and cyanobacteria are rapidly inhibited by evolved oxygen. An advantage of the hydrogen production by purple photosynthetic bacteria is the lack of O_2 evolving activity. However the presence of uptake hydrogenases reduces the overall yield of the process [4,5].

Various photobioreactors have been used for biomass and hydrogen production from photosynthetic microorganisms. In photobioreactor light energy is converted into biochemical energy. The fundamental factors that differentiate photobioreactors from normal reactor for chemotrophs are i) it is transparent and allows maximum penetration of light, ii) the energy source is instantaneous, cannot be stored in the reactor, and iii) self shading of cells occur. Self shading results in loss of excess absorbed energy via fluorescence and heat resulting in temperature rise necessitating additional cooling systems for bioreactors. A major issue limiting the large-scale production of hydrogen is the restricted light penetration into the deeper regions of the reactor. Other factors limiting the performance of the bioreactors include area/volume (A/V) ratio, agitation, temperature and gas exchange. Different genetically engineered strains are also being used to improve the hydrogen yield [6]. Many laboratory scale bioreactors have been constructed with improved features, novel characteristics and well defined culture conditions [7–9]. The thicknesses of the reactors are usually small to increase the A/V ratio and to avoid shading effects. External illumination (e.g. sunlight) can be taken into the deeper regions of the reactor through fiber optic cables and distribute the light evenly [10,11]. Diffused light plays a critical role in hydrogen production. Based on this, induced and diffused photobioreactors were developed [12,13].

Several types of bioreactors have been designed to improve the above mentioned factors for biomass production and with a little modification for hydrogen production. Among them the tubular, flat panel and vertical-column type of photobioreactors are widely used for hydrogen production. There are several merits and demerits of these reactors. Large illumination area can be achieved in tubular type but is limited to scale up due to higher concentrations of photosynthetically generated dissolved oxygen and high input energies for pumping (2000 W/m^2) [14]. The illumination area is small in vertical-column photobioreactors, but still they are widely used for microalgae and photosynthetic bacteria owing to their compactness, low cost, ease of operation and low shear stress in airlift and bubble column method of agitation [15–17]. High photosynthetic efficiencies and effective control of gas pressure can be achieved in flat-plate photobioreactors [18,19] and has been found more economic compared to other bioreactors [20] but difficulty arises to maintain the culture temperature and suitable agitation system during hydrogen production.

Development of a suitable photobioreactors is still challenging. With a brief introduction to the microbiology, biochemistry and molecular biology of photosynthetic hydrogen production, the scope of this paper is to give a clear idea of the existing photobioreactors with respect to biomass generation and hydrogen production and the steps taken to improve its performance through engineering innovation and genetic modifications of the organisms.

2. Microbiology

Three different groups of microorganisms that are involved in biological hydrogen production processes: aerobic green algae (eukaryotes), cyanobacteria (blue-green algae), and anaerobic photosynthetic bacteria (mostly Gram negative prokaryotes). These are called phototrophs as they use light as their energy source. Different hydrogen producing organisms and their hydrogen yield are shown in Table 1. Gaffron and his co-researchers first observed that under aerobic condition unicellular green algae, *Scenedesmus obliquus*, is able to generate hydrogen in presence of light [1]. Later, Bishop and Gaffron indicated that light-dependent evolution of hydrogen appeared to require both photosystems (PSII and PSI) [21]. Green algae and cyanobacteria have both PSII and PSI, structurally and functionally similar to the higher eukaryotes. In some filamentous cyanobacteria, selected vegetative cells are differentiated into specialized structures called heterocysts, which provide an anaerobic environment for the oxygen labile nitrogenase through compartmentalization.

With few exceptions purple and some green photosynthetic bacteria are anaerobic Gram negative prokaryotes. Some of these organisms may contain specialized light-harvesting structures called Chlorosomes, as is the case for example among green sulfur bacteria. The non-sulfur bacteria can use organic acids from industrial organic wastes and waste products of dark fermentation to produce hydrogen. Although these bacteria may contain hydrogenases [22], the anoxygenic hydrogen evolution is mostly observed by nitrogenase when ATP and electrons are available [23].

The type of light harvesting pigments, photosystems, source of reducing power and enzyme systems involved in various phototrophic hydrogen producing organisms are summarized in Table 2.

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Table 1 – Different hydrogen producing microorganisms and their optimal physiochemical condition for achieving maximum hydrogen production rate.

Organism	Strains	Optimum physiochemical conditions				Hydrogen production rate			References
		pH	T °C	Light Intensity	Carbon source	(ml L^{-1}_{cult} h^{-1})	μmol/mg dw/h	μmol/mg chl a (protein)/h	
Green algae	*Chlamydomonas reinhardtii*[a]	7	–	100 μE/m^2/s	Acetate (17 mM)	2.1	–	–	[24]
	Chlamydomonas MGA 161	8	30	25 W/m^2	5% CO_2	4.48	–	–	[25]
	Chlorella sorokiniana Ce[a]	–	–	120 μE/m^2/s	Acetate	1.35	–	–	[26]
	Platymonas subcordiformis[a]	–	–	22 W/m^2	–	0.048	–		[27]
Cyanobacteria									
Heterocystous	*Anabaena cylindrica* B 629	–	–	7000 lux	5% CO_2	–	0.103	–	[28]
	Anabaena variabilis ATCC 29413	–	30	150 μE/m^2/s	1% CO_2	–	0.25	–	[29]
	Anabaena azollae	–	–	140 μE/m^2/s	2% CO_2	13		38	[30]
	Aphanocapsa montana	–	–	4–6 W/m^2/s	Air	–	0.4		[31]
	Gleobacter PCC 7421	–	–	20 μE/m^2/s	CO	–		1.38	[32]
Non-heterocystous	*Aphanothece halophytico*	–	–	–	–	–	–		
	Chroococcidiopsis thermalis CALU 758	–	–	70 μE/m^2/s	1% CO_2	–	–	0.7	[33]
	Spirulina platensis	–	–	8 W/m^2		4.032	–	–	[34]
	Microcystis PCC 7820	–		20 μE/m^2/s	CO	–	–	0.16	[32]
	Oscillotoria limosa	–	20	1200 lux	CO_2	–		0.83	[35]
	Oscillotoria miami BG7	7.75	35	100 μE/m^2/s	CO_2	–	–	0.25	[36]
	Synechococcus PCC 602	–	–	20 μE/m^2/s	CO	–	0.66	–	[31]
	Synechocystis sp. PCC 6803	–	–	50 μE/m^2/s	$NaHCO_3$	–	–	0.81	[37]
Photosynthetic Bacteria									
Purple sulfur	*Thiocapsa roseopersicina*	–	–	–	–	–	–	–	[38]
	Chromatium sp. miami PSB 1071	–	–	140 μE/m^2/s	Succinate, thiosulphate	–	–	6	[39]
Purple non-sulfur	*Rhodobacter capsulatus* ST410	–	–	66 W/m^2	Malate	100	–	–	[40]
	Rhodobacter sphaeroides RV	–	–	155 W/m^2	–	131	–	–	[41]
	R. sphaeroides S	–	–	35 W/m^2	–	19	–	–	[42]
	R. sphaeroides O.U. 001	–	–	200 W/m^2	Malate	20	–	–	[43]
	Rhodopseudomonas palustris	–	–	434 W/m^2	Acetate, propionate, butyrate, ethanol	7.6	–	–	[44]
	Rhodospirillum rubnum	–	–	400 W/m^2	Lactate, glutamate	180	–	–	[45]
Green sulfur	*Cholobium limicola*	–	–	–	–	–	–	–	[38]
Green gliding	*Chloroflexu aurantiacus*	–	–	–	–	–	–	–	[38]

a sulfur-deprived conditions.

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Table 2 – Some features of hydrogen producing phototrophs.

Micro-organism	Types	Light-harvesting pigments	Presence of photosystem	Source of reducing power	Enzyme majorly involved in hydrogen production
Green Algae (Chlorophyta)	Aerobic, oxygenic, eukaryotes	Chlorophyll *a*, *b* Carotenoids	PSI & PSII	H_2O and/or organic substrate	Hydrogenase
Blue-green algae (Cyanophyta)	Aerobic, oxygenic, Gram + ve prokaryotes	Chlorophyll *a* Carotenoids Phycobilisome	PSI & PSII	H_2O and/or organic substrate	Bidirectional hydrogenase and/or Nitrogenase
Purple sulfur bacteria	Anaerobic, anoxygenic, Gram −ve prokaryotes	Bacterio-chlorophyll a/b Carotenoids	Single photosystemsimilar to PSII	H_2S, S^0, $S_2O_3^{-2}$	Nitrogenase
Purple non-sulfur bacteria	Anaerobic, anoxygenic, Gram −ve prokaryotes	Bacterio-chlorophyll a/b Carotenoids	Single photosystem similar to PSII	Organic acids	Nitrogenase and/or Bidirectional hydrogenase
Green sulfur bacteria	Anaerobic, anoxygenic, Gram −ve prokaryotes	Chlorosomes, that contain either Bchl c, d, or e in addition to Bchl *a*	Single photosystem similar to PSI	H_2S, S^0, $S_2O_3^{-2}$	Hydrogenase
Green gliding bacteria	Anaerobic, anoxygenic, Gram −ve prokaryotes	Chlorosomes with Bchl c/d + Bchl *a*	Single photosystem similar to PSII	–	Hydrogenase

3. Biochemistry

Phototrophic microorganisms have very diverse physiology and metabolism; therefore there are also different pathways to generate hydrogen [Fig. 1]. The advantages and disadvantages of different processes of hydrogen production are summarized in Table 3. The processes can be distinguished as follows:

- Hydrogen evolution by green algae and cyanobacteria
  Direct biophotolysis
  Indirect biophotolysis
- Anoxygenic hydrogen evolution by photosynthetic bacteria.

3.1. *Hydrogen evolution by green algae and cyanobacteria*

3.1.1. *Direct biophotolysis*

Hydrogen production through direct biophotolysis is a biological process that splits water to produce hydrogen and oxygen by utilizing solar energy. Green algae contain Photosystem II and Photosystem I for capturing light energy and exhibit oxygenic photosynthesis like higher plants. In absence of oxygen, electrons (e^-) from reduced ferredoxin (Fd) can also be used by the hydrogenase to reduce protons (H^+) and evolve hydrogen (H_2) (eqn. (1)).

$$2H_2O + h\nu \rightarrow O_2\uparrow + 4H^+ + Fd\ (red)\ (4e^-) \rightarrow Fd\ (red)$$
$$(4e^-) + 4H^+ \rightarrow Fd\ (ox) + 2H_2 \quad (1)$$

Partial inhibition of PSII can generate anaerobic condition for the cell within a photobioreactor, as there is less water-oxidation activity to evolve O_2 and the residual O_2 is used by respiration [46]. In a ground breaking work of Melis and his coworkers it was found that sulfur deprivation inhibits PSII activity that led to anaerobic conditions within a photobioreactor [47,48]. Kyle et al. showed that photoinhibition is accompanied by selective loss of a 32-kDa protein (later identified as the PSII reaction centre protein D1) followed by activation of the reaction centre through rapid inbuilt repair mechanism [49]. In sulfur deprivation, re-biosynthesis of the D1 protein after loss is inhibited due to the scarcity of cysteine and methionine. Anaerobiosis induces the expression of [FeFe]-hydrogenase in algal cells [50,51] and sustained hydrogen production can be achieved [47,52]. The overall process is depicted in Fig. 2. Sulfate permease mutant can evolve H_2 without depleting sulphate in the culture media [53]. Some photosystem II inhibitors have also been used to inhibit water oxidation activity [54]. Instead of acetate, which is normally used in the media as a carbon source, CO_2 emitted in industries can be coupled to the system as it will be less expensive in large scale H_2 production as well as helpful for CO_2 removal [55].

3.1.2. *Indirect biophotolysis*

Indirect biophotolysis is a very efficient process to separate O_2 and H_2 evolution phases (eqn. (2) and (3) mostly observed in cyanobacteria [Fig. 1]. The stored carbohydrate is oxidized to produce H_2. The general reaction is as follows:

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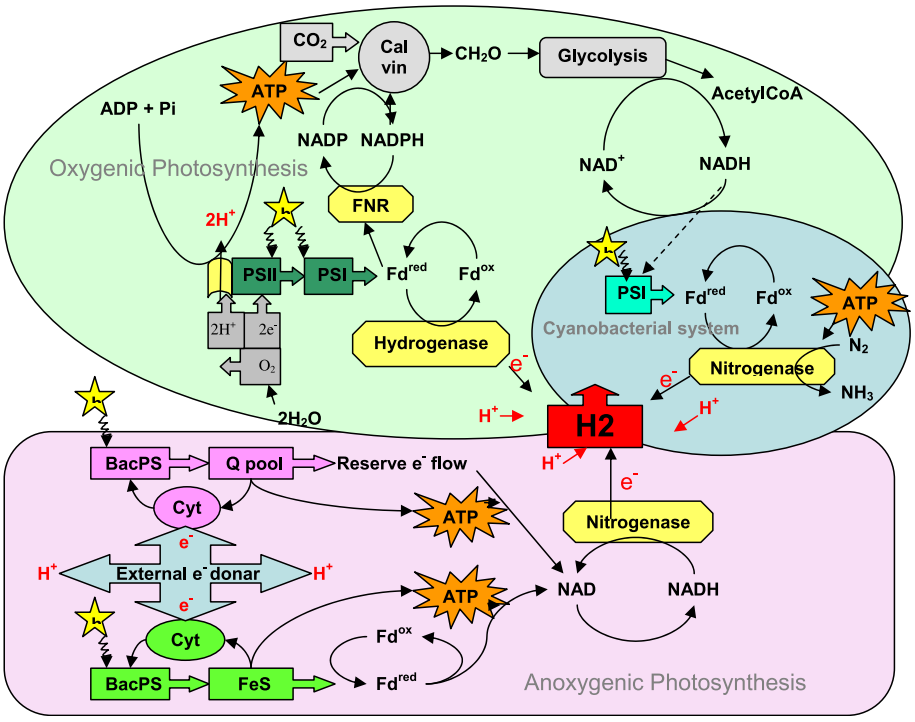

Fig. 1 – Light-dependent different electron transport pathways for hydrogen production. Showing the oxygenic hydrogen production in green algae through hydrogenase. Specially showing that, how blue-green algae (N_2 fixing) produced hydrogen through nitrogenase, driving electrons from photosynthetically produced reserve carbon source. O_2 evolution separated from H_2 evolution either by heterocyst or by temporal separation. Showing the anoxygenic hydrogen production in photosynthetic bacteria through nitrogenase. Purple bacteria. Green bacteria.

$$12H_2O + 6\,CO_2 \rightarrow C_6H_{12}O_6 + 6\,O_2 \quad (2)$$

$$C_6H_{12}O_6 + 12H_2O \rightarrow 12H_2 + 6\,CO_2 \quad (3)$$

In anaerobic dark conditions, pyruvate ferredoxin oxidoreductase (PFOR) responsible for decarboxylation (CO_2 evolution) of pyruvate to acetyl-CoA is linked to H_2 production via reduction of ferredoxin. In presence of light, ferredoxin is reduced by NADH produced during catabolism of pyruvate by the pyruvate dehydrogenase (PDH). The N_2-fixing cyanobacteria produce hydrogen mainly by nitrogenase (fixing N_2 to NH_3) instead of bidirectional hydrogenase, however in several non-N_2-fixing cyanobacteria, H_2 evolution is also observed through bidirectional hydrogenase [4,5].

In filamentous cyanobacteria, nitrogenase is located in the heterocycts with a functional PSI (no PSII activity). The electrons are donated to PSI in the heterocyst come from reserve carbon transported from the neighbor vegetative cell. However, the hydrogen production is energetically burden due to the biosynthesis and maintenance of the heterocysts and the significant ATP requirement of nitrogenase (eqn. (4) and (5).

$$N_2 + 8\,H^+ + Fd\,(red)\,(8e^-) + 16\,ATP \rightarrow 2\,NH_3 + H_2 + Fd\,(ox) + 16\,ADP + Pi \quad (4)$$

$$8\,H^+ + 8\,e- + 16\,ATP \leftrightarrow 4H_2 + 16\,ADP + 16\,Pi \quad (5)$$

Heterocyst provides spatial separation of O_2 and H_2 evolution. Non-heterocystous cyanobacteria can separate O_2 and H_2 production in time (temporal separation). It has been found that nitrogenase can be converted from active to inactive form after a sudden and short-term exposure to high oxygen concentrations [56,57].

3.2. *Anoxygenic hydrogen evolution by photosynthetic bacteria*

Twoprincipal classesof photosynthetic bacteria, thepurple-bacteria and the green bacteria, carry outphotosynthesiswith a single photosystem. Green bacteria have the PSI type reaction centre. Inorganic/organic substrates are oxidized to donate electrons to reduce ferredoxins via FeS proteins. Reduced ferredoxin serves directly as electron donor for the dark reaction (fixation of CO_2) as well as for the H_2 production.

In contrast,purple bacteria contain a PSII like reaction centre thus is incapable of reducing ferredoxin, but can generate ATP via cyclic electron flow. The electrons desired for nitrogenase mediated hydrogen evolution is driven from inorganic/organic substrate, which proceeds to the "quinone pool" through reaction centre bacteriochlorophyll (P_{870}). However, the energy potential of quinone is insufficiently negative to reduce NAD^+ directly. Therefore, the electrons from the quinone pool are forced backward to reduce NAD^+ to NADH. This energy requiring process is called reversed

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Table 3 – Advantages and disadvantages of different light-dependent hydrogen production processes.

Micro-organism involved in hydrogen production	Preferable light-dependent metabolic pathway for H_2 generation	Advantages	Disadvantages
Green algae	Direct and indirect biophotolysis through bidirectional hydrogenase	This method (biophotolysis) can be established as the best potential method to satisfy the future energy demand • Hydrogen-economy strategy based on a virtually limitless and renewable source. • Energy cycle is carbon-free • Also the theoretical energy efficiency is much higher for hydrogen production from biophotolysis (40%)(Prince and Kheshgi 2005)compared to hydrogen production from biomass (1%).	Simultaneous production of O_2 and H_2 • Inhibition of hydrogenase by oxygen.
Blue-green algae	Indirect biophotolysis through nitrogenase	H_2 evolution is separated from O_2 evolution • Compartmentalization in heterocystous N_2 fixing cyanobacteria • Temporal separation (light/dark) in non-heterocystous cyanobacteria	Energetically burdensome • Biosynthesis and maintenance of heterocysts • Significant ATP requirement of nitrogenase. Presence of uptake hydrogenase • Re-oxidize the produced molecular hydrogen
Photosynthetic bacteria	Photofermentation through nitrogenase	Helping in removal of environmental pollutants • Use of industrial waste. • Use of organic acids produced from dark fermentation.	Need N_2 limited condition Need pretreatment of industrial effluent as it may be toxic and opaque in nature Presence of uptake hydrogenase • Re-oxidize the produced molecular hydrogen

electron flow. There is no evolution of oxygen in this process. The net amount of hydrogen produced is influenced by the activity of uptake hydrogenase [58].

Photosynthetic purple bacteria have been considered the best for photobiological hydrogen production [59] as it can utilize industrial wastes [60] and the byproduct (organic acids) of dark fermentation (eqn. (6)).

$$CH_3COOH + 2H_2O \rightarrow 4H_2 + 2\,CO_2 \quad (6)$$

4. Enzyme responsible for hydrogen production

In broad sense two types of enzymes, hydrogenases and nitrogenases are catalyzing the reduction of protons (H^+) to produce molecular hydrogen (H_2). Both these enzymes are metalloproteins (contain metals in their active sites).

4.1. *Hydrogenase*

Phylogenetic analyses, based on sequence alignments of catalytic subunits of hydrogenases, have led to the identification of three phylogenetically distinct classes of enzymes, [NiFe]-hydrogenases, [FeFe]-hydrogenases and Fe–S free hydrogenases, initially called metal free but later renamed [Fe]-hydrogenases, each characterized by a distinctive functional core that is conserved within each class [61]. Turnover for hydrogen production is much higher for [FeFe]-hydrogenases whereas [NiFe]-hydrogenases are more oxygen tolerant. [Fe]-hydrogenase is not involved in hydrogen production. This hydrogenasecatalyzethe reduction of CO_2 by H_2 to methane, and the bacteria that rely on such enzymes are mostly methanogens.

4.2. *Nitrogenase*

The bacterial enzyme nitrogenase has agronomic importance as it is responsible for natural fertilization of soil by fixing N_2

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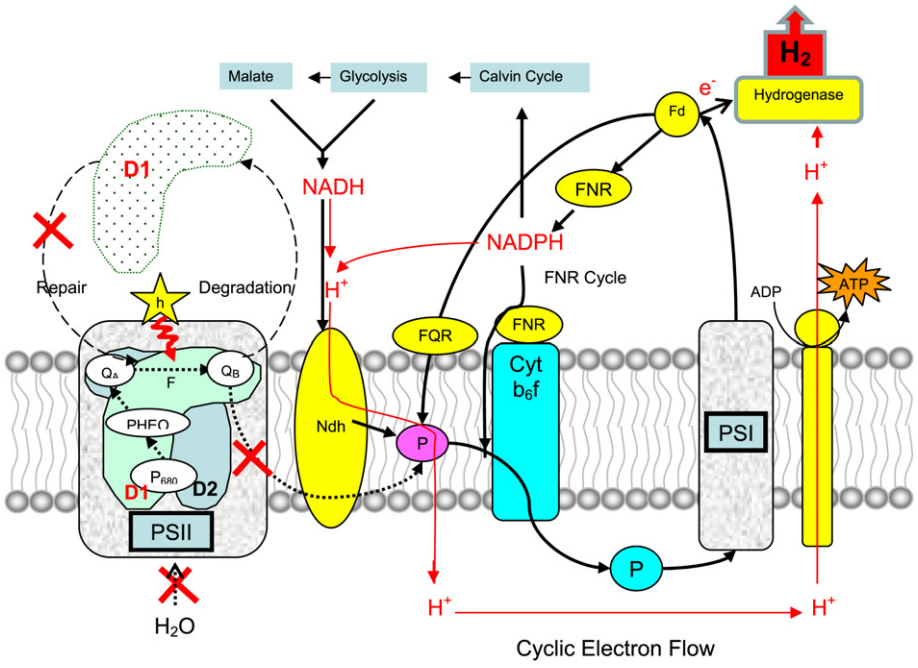

Fig. 2 – Sulfur deprivation produce anoxic condition for induction of hydrogenase. PSII is partially inhibited and electrons are mostly derived from reserve carbon source via plastoquinon.

into NH_3. Though, the nitrogenase is mainly involved in N_2-fixation but it also capable of simultaneous H_2 production. Researchers are exploiting this capability of nitrogenase to produce higher amount H_2 in N_2 limited condition [62].

4.3. *Limitations of enzymes involved in hydrogen production*

If harnessed properly, hydrogenase containing microorganisms could be used to supply the next generation economic fuel. However, the major challenges that researchers face in this transit is to overcome the sensitivity of hydrogenase to oxygen. Continuous production of H_2 is difficult in the light since oxygen is one of the obligatory products of water oxidation. Isolation of hydrogenase from cells and its subsequent analysis is also very difficult. To date only a few hydrogenases are known to display tolerance to oxygen and various strategies have been adopted to generate oxygen tolerant mutants. Hydrogenase from extremophiles which are naturally more tolerant to oxygen may provide further inspiration for the development of such technologies. *Aquifex aeolicus* hydrogenase is a good candidate for biotechnological applications due to their high resistance to aerobic and thermal inactivation [63]. It was observed that algal hydrogenase which are the smallest and the simplest of hydrogenases, encoding only the H-cluster where the active domain is located, are much more sensitive to oxygen inactivation. It was believed that F-cluster domain might provide additional protection to the active site from oxygen inactivation. On the other hand, the conserved amino acid sequence of the H-cluster or the active site suggests that the amino acid composition of this domain is critical to the protection of the active site from oxygen. A recent overview is made on green algal *hydA* genes, including the demonstration of the transcription of three genes with characteristics of hydrogenase in a single green algal strain [64].

Nitrogenases are sensitive to oxygen, but are relatively less than hydrogenases. Nitrogenases are mainly present not only in anaerobic prokaryotes but also in cyanobacteria where the anaerobic hydrogen production is separated from the oxygenic photosynthesis through special (heterocyst) or temporal (light/darkness). Hydrogen production is favored under nitrogen limiting conditions and is a highly energy consuming process.

5. Genetic and metabolic engineering for enhanced hydrogen production

Genetic and metabolic modification can be a very effective and promising method to optimize and redirect the flow of reducing equivalents (electrons) to the enzyme and also the accumulation of protons (H^+), to achieve a satisfactory amount of hydrogen (H_2) evolution in large scale. The various genetic and metabolic engineering strategies for overcoming the bottlenecks of hydrogen production are summarized in Table 4.

For enhanced photo-production of hydrogen, photosynthetic efficiency can be increased by modifying the light-harvesting antenna complexes responsible for capturing the solar energy. Large antenna complexes of cyanobacteria and green algae help them to grow in low light condition, but

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Table 4 – Genetic and metabolic engineering strategies for overcoming the bottlenecks of hydrogen production.

Strategies	Advantages	Microorganisms	References
Pigment reduction	Increasing the photosynthetic efficiency	Green algae (*Chlamydomonas reinhardtii, Dunaliella salina*)	[66,68]
		Synechocystis PCC 6803	[67]
		Rhodobactersphaeroides (mutant MTP4 & P3)	[69,70]
Generating anaerobic environment	Activating hydrogen producing enzyme	*C. reinhardtii* (*arp* mutant)	[71,72]
		C. reinhardtii (D1 protein mutant)	[73]
Oxygen-tolerant enzyme	Producing hydrogen in presence of oxygen	*Chlamydomonas* sp.	[53]
		Synechococcus sp. PCC7942	[74]
Eliminating competitive inhibition by other e^- acceptor	Redirecting the e^- flux towards the hydrogen producing enzyme	*C. reinhardtii* 137c	[75]
Introducing foreign efficient hydrogen producing enzyme	Enhancing hydrogen production in that particular micro-organism, that may be efficient in other criteria.	*Synechococcus elongates*	[77,78]
		Rhodospirillum rubrum	[76]
Eleminating uptake hydrogenase activity	Inhibiting re-oxidation of produced hydrogen	*Thiocapsa roseopersicina* (*hypF*-deficient mutant)	[79]
		Rhodospirillum rubrum (*hupL*-deficient mutant)	[80]
		R. sphaeroides (*hupSL*–deficient mutant)	[81]
Enhancing the capacity of deriving e^- from carbohydrates	Contributing in hydrogen production in anoxic dark condition	*C. reinhardtii* (Stm6 strain)	[84,85]
Inhibiting or over-expressing the crucial metabolic enzymes	Redirecting the e^- flux towards hydrogen producing enzyme	*Synechocystis* (mutant M55), *Rhodobacter capsulatus* (IR4)	[86]

results in dissipation and loss of excess photons as fluorescence or heat at saturating light conditions. It is debatable whether the LHCIIb (major peripheral antenna), or CP26 and CP29 (minor antenna) or another component is critical for non-photochemical quenching (NPQ) that protects the PSII in *Chlamydomonas reinharditii* [65].Reduction in pigment content can lead to better penetration of light inside the reactor and reduce the wastage of light energy. It has been observed that hydrogen production could be increased by a mutant algal strain having reduced chlorophyll antenna [66]. It was also expected that cyanobacteria deficient in phycobilisomes, which implies uncoupling of electron transport and preferable excitation of PSI by illumination, can enhance the rate of hydrogen production [67]. Another promising technology, the RNA interference (RNAi) technology has also been used to down regulate the entire family of light harvesting complexes (LHC) in *Chlamydomonas reinhardtii* [68]. Using a *Rhodobacter sphaeroides* mutant MTP4 within the plate-type reactor, 50% more hydrogen is produced than its wild type counterpart *R. sphaeroides* RV [69]. Another mutant (P3 mutant) with 2.7 folds decrease in core antennal (LH1) content and 1.6 folds increases in peripheral antennal (LH2) content has given accelerated H_2 production compared to wild type [70]. A differential strategy is necessary to sort out the pigments that are produced specifically during hydrogen production stage and its contribution towards the photosynthetic efficiency.

One of the major problems for producing hydrogen using biological systems, is the extreme sensitivity of hydrogen producing enzymes to oxygen. Cyanobacteria and green algae produce oxygen due to the water splitting in PSII which inhibits the hydrogenase activities. Under normal growth conditions, the photosynthetic rate is 4–7 folds higher than the respiration rate. By using attenuated photosynthesis/respiration ratio (P/R ratio) mutants (*apr* mutants) of *Chlamydomonas reinhardtii,* the P/R ratio drops below one, thereby establishing anaerobic conditions which mimic the physiological status of sulfur-deprived cells [71]. The sulfur-deprivation method that may cause cell growth inhibition and death can be avoided [72]. It has also been reported that a sulfur-deprived *C. reinhardtii* D1 mutant that carried a double amino acid substitution, is superior to the wild type for hydrogen production. The leucine residue L159 in the D1 protein was replaced by isoleucine, and the asparagine N230 was replaced by tyrosine (L159I-N230Y). This strain is very efficient for prolonged H_2 production and also having lower chlorophyll content and higher respiration rate that contributes in net yield [73].

Significant research has been performed to increase the oxygen tolerance of hydrogen producing enzymes, especially hydrogenases [74]. By introducing random and site directed mutagenesis in *Chlamydomonas*, strains with 10 fold more oxygen tolerance has been obtained [53].

Lee and Greenbaum described that there is a competitive inhibition of hydrogen production by CO_2 fixation. CO_2 fixation can be inhibited if ATP requirements are not fulfilled. Genetic insertion of a polypeptide protein channel with a hydrogenase promoter can reduce the proton gradient across the thylakoid membrane by avoiding the ATPase channel where ATP is produced during proton transfer in algal cells [75].

Efficiency of the enzymes is another important factor affecting the rate of hydrogen photo-production. In photosynthetic bacteria, net hydrogen production can be increased by improving the efficiency of nitrogenase. By knocking out *glnB* and *glnK*, genes encoding P_{II}-like proteins, the problem of repression of nitrogenase by ammonium ions has been overcome in *R. sphaeroides* [76]. Another possibility is heterologous over expression of an efficient enzyme in the cells. Cyanobacteria have a bidirectional [NiFe]-hydrogenase which may be inefficient for hydrogen production and hydrogen is mostly produced by nitrogenase. Interestingly an [FeFe]-hydrogenase (*hydA*) from *Clostridium pasteurianum* was introduced, expressed and functional, without the co-expression

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of maturation proteins, in the cyanobacterium *Synechococcus elongatus* [77,78]. In addition, clostridial *hydA* has been cloned into *Rhodospirillum rubrum* and the native hydrogenase of *R. rubrum* (*hydC*) has been over expressed. In both cases pyruvate is the electron donor for hydrogen production [76].

To achieve the theoretical maximum value for hydrogen production, there is an additional obstacle in cyanobacteria and photosynthetic bacteria. The produced hydrogen could be re-oxidized again by another enzyme known as the uptake hydrogenase (NiFe-hydrogenase). Mutants, deficient of uptake hydrogenase activity dramatically increases the net production of hydrogen. Inactivation of all hydrogenases, including all uptake activities, in *Thiocapsa roseopersicina* (*hypF*-deficient mutant) under nitrogen-fixing condition, caused a significant increase in the hydrogen evolution capacity [79]. Ruiyan and co-researchers also reported that a *R. rubrum* mutant deleted of *hupL* encoding the large subunit of uptake hydrogenase produced increased amount of H_2 [80]. To date significant research has been performed to inhibit the uptake hydrogenase activity using different approaches. Gokhan and co-researchers used a suicide vector for site directed mutagenesis of uptake hydrogenase (*hupSL*) in *R. sphaeroides*. They obtained 20% more H_2 production than wild type [81].

Along with external electron donors like H_2O for cyanobacteria and green algae, and sulfur containing inorganic compounds or organic acids for photosynthetic bacteria, reducing equivalents can be derived from either reserve carbon source produced during biomass formation. In cyanobacteria and green algae, this alternative metabolic process can produce hydrogen via fermentative reactions and/or reducing plastoquinon (PQ) by Ndh to maintain the NAD/NADH balance and ATP supply [82,83]. After random gene insertion in *C. reinhardtii*, a strain named Stm6 with modified respiratory metabolism was isolated. This strain is able to accumulate large amount of starch in the cells, and has low dissolved oxygen concentration. It can produce 5–13 times more hydrogen than the wild type [84]. Supplying a carbon source like acetate or glucose externally, hydrogen production increase more compared to inorganic media. Species of *Chlorella* has a hexose uptake protein that is involved in transferring external carbohydrate to the cell. Recently, the HUP1 (hexose uptake protein) from *Chlorella kessleri* was introduced into *C. reinhardtii* to increase the supply of external glucose into the cell. Hydrogen production capacity was increased about 150% by supplying 1 mM glucose to a strain of Stm6 where HUP1 has been inserted [85].

Some modifications have also been made in the metabolic pathways to redirect the electron flux towards the hydrogen producing enzyme. Thus NADPH-dehydrogenase deficient mutant M55 of *Synechocystis*, a non-nitrogen fixing cyanobacteria, accumulate NADPH and evolve significant amounts H_2 by the bidirectional NADP-dependent hydrogenase. By improving the synthesis of D-malic enzyme and using D-malate as the sole carbon source in strain IR4 of *Rhodobacter capsulatus*, a 50% higher hydrogen production has been demonstrated [86]. Metabolic engineering by genetic modification could be a stable solution for efficient hydrogen production. This approach could rationalize different other problems and parameters for producing hydrogen in a large scale with a pilot reactor.

6. Photobiological reactors for the improvement of hydrogen production

6.1. Effect of physiochemical parameters

The performance of a photobioreactor in terms of hydrogen production is not just limited by physical parameters, like for example the quantity of light penetrating into the reactor, but also depends on the physiochemical parameters influencing the various biochemical pathways towards hydrogen production.

The major physiochemical parameters affecting hydrogen production are:

- pH
- Temperature
- Light intensity
- Dissolved oxygen
- Dissolved CO_2
- Shear due to agitation
- Carbon and nitrogen sources, and their specific ratios

Parameters for biomass and hydrogen production are different. For example, a carbon to nitrogen (C:N) ratio of 7.5:10 induces biomass formation while 15:2 induces hydrogen production in *R. sphaeroides* [87]. Apart from C:Nratio, the source of nitrogen-ammonia, glutamate or yeast extract can also affect the process as nitrogenases are reversibly inhibited by ammonia [88]. When nitrate was added in fed-batch mode, an increase in carbon fixation rate of 56.6% for green algae and 68.8% for cyanobacteria was observed owing to the extension of exponential growth phase by more than 3 days [89]. Optimum pH of the medium is usually around 7, except for *Chlorella* and *Spirulina* which require higher pH around 10 for biomass production. Temperature requirements are in the range of 25–35 °C. In green algae, growth can be either autotrophic (using CO_2 from the atmosphere) or photoheterotrophic (using an organic substrate). However, hydrogen production by indirect biophotolysis usually requires that the entire cells are exposed to anaerobic conditions. By optimizing concentrations of key nutrients in the media of *Synechocystis* sp. PCC 6803, a 150 folds increased H_2 production was achieved mainly due to a 44 folds increase in glycogen concentration [37]. Light intensity in the range of 50–200 $\mu E/m^2/s$ was generally found to be optimal and intensities greater than 200 $\mu E/m^2/s$ may cause inhibitory effects. It was shown that the light conversion efficiency to hydrogen decreased with increasing light intensity [90]. The mode of operation, batch, continuous or fed-batch, also influences the yield mainly due to substrate or product inhibition. Suitable physiochemical parameters required for hydrogen production are summarized in Table 1.

6.2. Physical parameters

The physical factors affecting the performance of a photo bioreactor are:

- Light penetration
- High area to volume ratio

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- Temperature control
- Transparency and durability of the material of construction
- Gas exchange
- Agitation system

6.3. *Types of existing photobioreactors*

Based on the mode of operation the bioreactors used for biomass production and/or hydrogen production can be broadly classified into batch, continuous and fed-batch. There are various types of photobioreactors designed and tested for biomass production and few have been successful in large scale operation.

The photobioreactors can be broadly classified into two major types:

i) Open system: raceway ponds, lakes etc.
ii) Closed system: Tubular, flat plate, conical, pyramidal, fermentor etc.

The schematic representation of the various photobioreactor configurations are shown in Fig. 3.

6.3.1. *Closed system*

Open pond systems are suitable only for biomass production. They cannot provide the anaerobic conditions required in case of hydrogen production, and offer poor possibilities to control parameters like temperature, pH, and nutrient concentrations. It has been found that in certain closed systems the amount of biomass produced was about three times of that obtained with open systems [91,92] which reduces harvesting costs. The degree of control in closed systems is very high and it is possible to control crucial parameters that influence the culture to produce either biomass or hydrogen. The various types of closed system geometries are compared in Table 5.

6.3.1.1. *Tubular reactors*. The tubular reactors are of different configurations falling under the following major categories [92]: i) simple airlift and bubble column (vertical type) agitated by bubbling CO_2, ii) horizontal tubular reactor in which the light harvesting and gas exchange units are separated, iii) helical tubular reactor in which the material of construction is transparent and flexible and is coiled in a desired fashion, and iv) α-shaped reactors.

6.3.1.1.1. *Vertical tubular reactors (VTR)*. The airlift and bubble column reactors fall under this category. It consists of vertical transparent tubes (polyethylene or glass tubes) in which the agitation is achieved with the help of bubbling at the bottom. Airlift bioreactor possesses good mixing properties while bubble column configuration has efficient aeration without any internal constructions. In few bioreactors the bubbling is done by sparging from the sides. Supply of CO_2 and removal of O_2 is very efficient in this type of reactors. The advantages include low material cost, high transparency, high area to volume ratio, biomass productivity and low contamination risk. Usage of such systems with various capacities and modifications to overcome practical difficulties for biomass production have been reported [93,94]. Miron et al. studied the hydrodynamics and mass transfer in bubble column, split

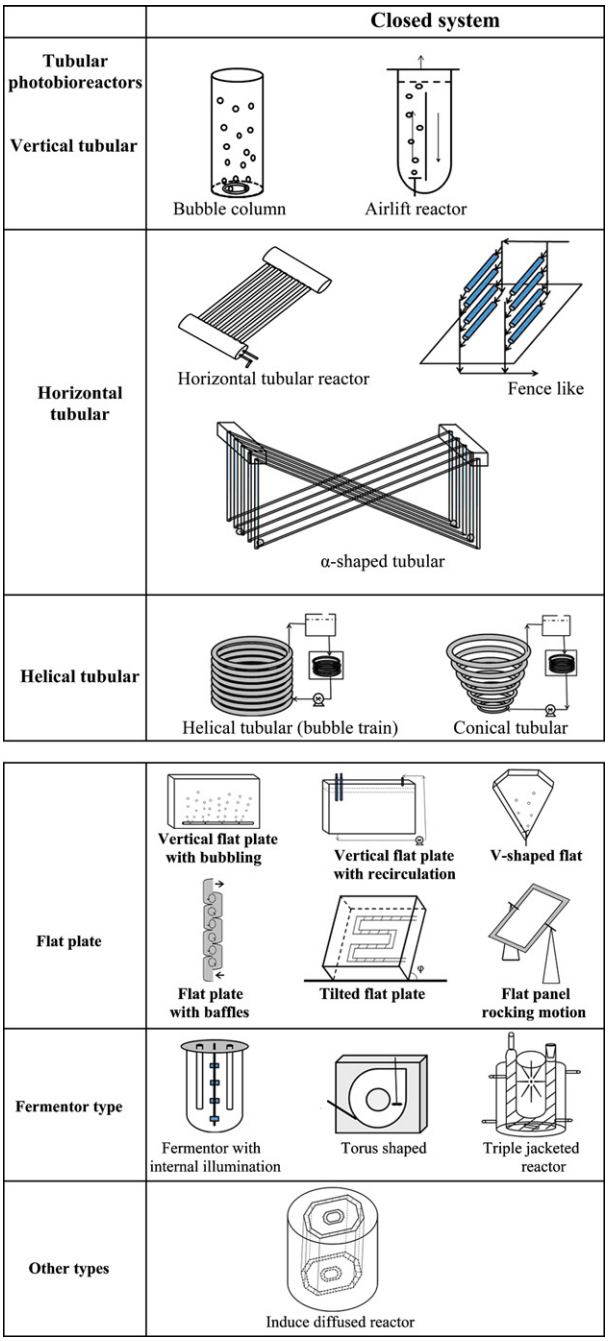

Fig. 3 – Schematic representation of the different photobioreactors for biomass and hydrogen production.

cylinder airlift and concentric draft tube sparged airlift reactors for *Phaeodactylum tricornutum*. In all the three bioreactors a biomass concentration of 4 kg m^{-3} was achieved with a specific growth rate of 0.022 h^{-1} [95].

The major drawbacks in scaling up are fragility, gas transfer at the top regions of the reactors temperature control and gas holdup. Cells are carried along with the bubbles and face high shear when the bubble bursts [96]. Use of vertical tubular reactors for the hydrogen production stage is challenged by the type of agitation system, in which the bubbling of an inert gas will dilute the stream of hydrogen. With

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continuous argon circulation a light conversion efficiency of 1.1% was reached in a column photobioreactor operated in semicontinuous mode [43]. Although recirculation of hydrogen produced is a suggested solution [97] but the effect of activation of uptake hydrogenase and feedback inhibition of hydrogen which affect the net yield and thus the efficiency of the system should be taken into account.

6.3.1.1.2. *Horizontal tubular reactors (HTR)*. Horizontal tubular reactors are widely used for their orientation towards sunlight that results in high light conversion efficiency. The gas is introduced into the tube connection or via a dedicated gas exchange unit. The various configuration of HTR are long tubular with parallel sets of tubes, looped tubes and near horizontal tubular reactors (NHTR) of which NHTR has been reported for hydrogen production.

The major drawback of this type of reactor is temperature control [98]. Methods adapted for cooling are described in the temperature control section (below). Oxygen buildup due to photosynthetic activity results in photobleaching and thus reduced photosynthetic efficiency [17].

6.3.1.1.3. *Near horizontal tubular reactor (NHTR)*. The NHTR designed by Tredici consists of parallel tubes made of plexiglass connected at the top and bottom ends by tubular plexiglass manifolds and tilted at 5° from the surface [99]. The elevation helps in reducing gas holdup and improves oxygen removal. With *Arthrospira platensis* a volumetric productivity of 1.26 g L^{-1} d^{-1} was achieved. With *R. capsulatus* the hydrogen production rate has been found to be around 3.3 ml L^{-1} h^{-1} [100].

6.3.1.1.4. *Helical tubular reactors*. Helical tubular reactors are constructed by coiling straight tubes made of flexible plastic into three dimensional helical frameworks with a desired inclination. It is externally coupled with a gas exchanger and a heat exchanger. A centrifugal pump is used to drive the feed through the tubes in an ascending fashion. Due to its high area to volume (A/V) ratio, it is possible to achieve photosynthetic efficiency (PE) of up to 6.6% with a volumetric productivity of 0.9 g L^{-1} d^{-1} with *A. platensis* [101].

A helical photo bioreactor with A/V = 200 m^{-1} was used in two staged hydrogen production using *Anabaena azollae* with rates of up to 13 ml $L^{-1}h^{-1}$ hydrogen. Polyurethane foam balls were used to prevent the deposition of the culture in the inner walls (which is known as biofouling) [30].

Many modifications of helical framework have been proposed to improve the design and light distribution. It was reported that for a given area 60° cone angle of conical helical layout had the maximal photo-receiving area and photosynthetic efficiency of 6.84% [102].

6.3.1.1.5. *α-shaped reactors*. The α-shaped reactor is another type of tubular photo bioreactor designed and constructed based on algal physiology and sunlight [103]. In this reactor, the culture is lifted 5 m by air to a receiver tank and flows down an inclined PVC tube (2.5 cm ID × 25 m) to reach another set of air riser tubes and the process repeated for the next set of tubes. The unidirectional and high liquid flow rate occur at relatively low air flow rates and resulted in biomass concentration of about 10 gDW L^{-1}. This reactor design comprises of all the basic requirements for hydrogen production. If needed, hydrogen production in large scale can be achieved by replacing air with an inert gas in the system.

6.3.1.2. *Flat plate reactors*. Flat plate reactors are of special interest due to their high A/V ratio when the thickness is minimal [92]. The orientation of the plates is either vertical (transparent sides facing east-west), tilted (north-south), or horizontal. In large scale production, several plates are arranged parallel over an area. The flat plate reactors are characterized by an open gas transfer area thus reducing the need for a dedicated degassing unit. In a comparison between the different types of outdoor reactors by Tredici and Zittelli [101], it was hypothesized that the better performance of the flat plate reactors (1.93 g L^{-1} d^{-1} and 5.30% PE) was due to lack of susceptibility to the orthogonal rays during midday that cause light saturation effects on the culture. Vertical flat plate reactors can be divided into three generations. The first generation comprised of transparent sheets sandwiching a frame while the second generation was distinguished by alveolar panels. The flat plate airlift reactor of Subitec GmbH (http://www.subitec.com/technik.html) consisting of two deep-drawing film half-shells welded together to form internal static mixers is considered to be the third generation. The mixing is usually achieved by bubbling air from the nozzles at the bottom or from the sides. The rate of mixing exerts little influence on the productivity and photosynthetic efficiency in low density cultures while it has great impact in high density cultures for efficient utilization of light. It should be noted that when the mixing rate is too low maximum utilization of light is affected while higher rates result in cell damage [104]. A V-shaped flat panel reactor has a very high mixing rate and very low shear stress owing to its engineering features eliminating escape corners, providing low shear and lack of cell adhesion to the walls of the reactor [7]. It was a design based on a model on fluidized bed reactors. A polysaccharide concentration of 25.96 g L^{-1} was achieved with *P. cruentum*.

In the case of these bioreactors being used in hydrogen production, mixing is achieved by recirculation of the evolved gas [97,105].

A 6.5 L capacity flat plate solar bioreactor with temperature control, tilted 30° to the horizontal and facing south was used for hydrogen production in outdoor [106]. A maximum rate of 10 ml H_2 L^{-1} h^{-1} was achieved in outdoor illumination. Another vertical flat plate photo bioreactor of Zhang et al. consists of baffles to improve the agitation [107].

6.3.1.3. *Alveolar panels*. The modular flat panel photo bioreactor (vertical alveolar plates) with A/V ratio of 80 m^{-1} was reported for mass cultivation of *A. azollae* and *Spirulina platensis* [108–110]. These bioreactors are constructed with transparent PVC or polycarbonate sheets that are internally portioned to form rectangular channels called alveoli. The major disadvantage of this system is the oxygen buildup due to high photosynthetic activity. The use of alveolar panels with internal or external gas exchange has also been mentioned for hydrogen production [98]. Unlike tubular reactors the alveolar plates do not achieve light dilution (unless placed at high

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Table 5 – Types of photo-bioreactor with their optimal features.

Type of photo bioreactor	S/V ratio	Agitation system	Temperature Control	Gas exchange	Advantages	Disadvantages	References
Tubular reactors							
Vertical tubular	Small	Airlift, bubble column		Open gas exchange at headspace	Good mixing, efficient CO_2 supply and O_2 removal	Scale up is limited, major light is reflected due to angle	[93, 4]
Horizontal tubular	Large	Recirculation with diaphragm/ mechanical pumps	Shading, overlapping, water spraying	Injection into feed, and dedicated degassing units	Adequate angle towards sunlight,	High shear due to pumps, risks of O_2 buildup, biofouling, separate gas exchange unit required	[17,98,99]
Helical tubular	Large	Centrifugal pumps	Heat exchanger	-do-	High S/V, easy scale up by increasing the number of units	O_2 buildup, separate gas exchange, pumps exert more shear, cell debris accumulate inside	[30,102]
α-shaped reactor	Large	Airlift	-do-	Injection in the vertical units and degassed at top	High unidirectional flow rate with low air flow rate, high S/V	Foam formation due to high cell density	[103]
Flat plate reactors							
Flat panel bubbled at bottom	Medium	Bubbling at bottom or from sides, recirculation	Heat exchange coils	Bubbling	Open gas transfer avoids O_2 buildup	Shear due to entrainment of cells till bubbles burst	[101,104]
V-shaped panel	Medium	Bubbling	-do-	-do-	Very high mixing rate, low shear	Agitation system can dilute H_2 formed	[7]
Alveolar panel	Large	Bubbling	water circulation in lower row	-do-	High S/V due to alveolar panels, uniform distribution of light	O_2 buildup, high air flow rates required to move across the channels	[99,108,109]
Flat panel pivoted at centre	Medium	Pulsating motion	Heat exchange coils	Degasser	Good mixing, low shear	Scale up is difficult	Unpublished
Floating type bioreactor	Medium	Sea saw motion	No cooling required	-do-	Low energy for operation, good agitation, can be installed on lakes and sea floor		[44]
Fermentor type with internal/external lighting	Small	Impellers	Heat exchange coils	By sparger	high degree of control of various parameters	Light conversion efficiency is less	[111]
Torus shaped reactor	Medium	Marine Impeller	Cooling fans	CO_2 inlet after impeller, outlet at top	Good mixing conditions owing to shape avoiding dead zones,		[8,112]

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Annular triple jacketed with lighting from innermost chamber	Medium	Magnetic stirrer	Outer water jacket	Open gas exchange	Good S/V and temperature control, open gas exchange	Scaling up is difficult, Biofouling	[9]	
Induced diffused PBR	Large	Not required	–	–	Greater thickness achievable due to	Material costs	[12,13]	

inclination to the horizontal), thus cultures suffer from light saturation and from photoinhibition.

6.3.1.4. *Flat plate reactors with rocking motion*. The use of flat plate reactors for hydrogen production is limited by the poor agitation achieved with recirculation systems. To overcome the problem of agitation the flat plate reactor has been mounted on a stand and gave it a rocking motion with the help of motor with eccentric cam motion. 2 rpm can provide sufficient agitation (unpublished work of the authors). The type of agitation in the system is found to a pulsation motion at greater tilting angles. The system is completely balanced and hence requires very low energy for the movement of the entire reactor.

A floating type of reactor which consists of a moving bed on a triangular roof and having see-saw type of motion constructed on sea or lakes and agitated by the motion of the waves was reported for hydrogen production using *Rhodopseudomonas palustris* strain R-1 [44]. Although the light conversion efficiency is low (0.308%), this reactor is very innovative and the water surrounding provides the temperature control.

6.3.1.5. *Fermentor type of reactors*. Commercial bioreactors for heterotrophic organisms have been modified and used as photobioreactors. In controlled bioreactors, every parameter can be monitored and controlled precisely with higher degree compared to other reactor types. It is mainly used for optimization studies and their scaling up is limited due to low A/V ratio. For very large volumes, illumination is provided internally [111]. Agitation is provided with the help of an impeller (marine or ribbon type) or a magnetic stirrer (in smaller units). It offers open gas exchange but have many restrictions towards scaling up.

6.3.1.6. *Other reactors geometries*.

6.3.1.6.1. *Torus shaped reactor*. The torus shaped reactor build at Legrand's lab is an innovative tool for methodical optimization of H_2 production using photosynthetic microorganisms. It consists of a marine impeller that produces a three dimensional swirling action. It is fully automated and can be operated in batch and continuous modes. The light is provided externally by tubes placed parallel to the illuminated surface [8,112].

6.3.1.6.2. *Annular triple jacketed reactor*. The annular triple jacketed reactor consists of three concentric chambers. Fluorescent bulbs are inserted in the innermost chamber [9]. Surrounding this is the culture chamber agitated with the help of a magnetic stirrer. Temperature is controlled by circulating water in the outermost chamber. The reactor has a high A/V ratio and a light conversion efficiency of 3.7% was achieved for hydrogen production using *R. sphaeroides* O.U.001. Similar annular reactor involving *Anabaena variabilis* PK84 resulted in a light conversion efficiency of 1% [113].

6.3.1.6.3. *Induced diffused photobioreactor*. The main design criterion of the photobioreactors is to maximize the A/V ratio. Due to this, the thickness of the reactor is small and hence economically not feasible. Reda et al. [12] introduced the

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concept of induced and diffused photo bioreactor (IDPBR) for distributing the light homogenously inside the bioreactor. It consists of two parts, first a diffusion plate made of two transparent polyacrylate (PMMA) sheets. One of the sheets is treated with dot printing on one side for diffusion of light. A reflection sheet (PET) is placed on the printed surface and fastened tightly between the two plates to make up the diffusion plate. A maximum light energy conversion to H_2 of about 9.23% has been reported for this type of photobioreactors [12,13].

6.4. Design criterion

6.4.1. Surface area to volume (A/V) ratio

The A/V ratio is the major concern which determines the amount of light (which is the limiting factor) entering the system per unit volume of the reactor. The higher the A/V ratio, the higher the cell concentration and greater will be the volumetric productivity. Reactors with same volume but different A/V ratios were evaluated for biomass productivity [100]. It was seen that the photosynthetic photon flux density (PPFD) at the surface of the culture was significantly lower for reactors with higher A/V ratios as the impinging radiation was distributed over greater surface area. This resulted in 18% higher volumetric productivity in flat reactor than the curved chamber.

6.4.2. Mass transfer of CO_2

Algae and cyanobacteria require inorganic carbon for biomass production. In aqueous environment inorganic carbon exists as CO_2 (aq), H_2CO_3, HCO_3^- and CO_3^- depending on pH and temperature [114]. Although HCO_3^- is easily absorbed by the cells, it is a poor carbon source compared to CO_2 [115]. When CO_2 is injected into the reactor, mass transfer takes place, the rate of which is proportional to the concentration gradient. The limiting factor in this process is the resistance to diffusion of CO_2 across the gas-liquid film and the liquid film at the microbial surface. Mathematically the rate of mass transfer CO_2 (N_{CO_2}) is given by

$$N_{CO_2} = k_L a\left(C^*_{CO_2} - C_{CO_2}\right) \quad (7)$$

where k_L is the liquid-phase mass transfer coefficient, a is the specific area available for mass transfer, $C_{CO2}{}^*$ is the saturation concentration of CO_2 in the liquid phase, and C_{CO2} is the concentration of CO_2 in the bulk of culture medium. The mass transfer coefficient k_La is influenced by parameters like metabolite profile, bubble size, gas and liquid flow rate and the concentration of CO_2 (% v/v) in the sparged gas. The value of k_La for different reactors for a given superficial gas velocity and % v/v CO_2 and their interrelationship are available [116–120].

6.4.3. O_2 removal

Oxygen evolution during photosynthesis causes toxic effects like photobleaching and even inhibition of photosynthesis. Hence it is necessary that an efficient degassing system should be present. Accumulation of O_2 is a serious problem in reactors with high A/V ratio. It is necessary to have a separate degassing unit in which the distance between the entrance and exit is such that even smallest bubbles can disengage. O_2 removal is not of major concern in flat plate, airlift and bubble column reactors. A high performance gas-liquid transmission device described for enclosed flat plate *Spirulina* culture system claims to satisfy the demand for carbon supplement and excessive oxygen removal [121]. In reactors during hydrogen production, degassing is only necessary when the O_2 production (due to basal activity of PSII) is higher than respiration.

6.4.4. Mixing

Mixing ensure the homogenous distribution of nutrients and avoids light and temperature gradients across the bioreactor. The flashing light effect provided by movement between light to dark zones has been found to be essential for higher biomass productivity [122]. Inadequate mixing will result in settling of biomass, stagnant or dead zones, cell aggregation and formation of multiphase system thus affecting the mass transfer rate. The different types of mixing systems are pumping, mechanical stirring and airlift type. The pumps used for recirculation cause larger shear forces which are lethal to the cells. It is learnt that the degree of stress in the centrifugal and rotary displacement pumps is proportional to the rotational speed [105]. In mechanical stirring, shear is maximum in the area surrounding the impeller. Bubble column reactors offer the minimum stress. The cell damage associated with bubbling are: (i) cell interactions with bubble generation at the sparger, (ii) cell interactions with bubbles coalescing and breaking up in the region of bubble rise; and (iii) cell interactions with bubbles at the air–medium interface [96,123].

Mixing can be improved by using horizontal and vertical baffles in flat plate airlift photobioreactors [107]. The baffles create a defined circulation path exposing the cells to intermittent light and thus creating a flash light effect. Good mixing is achieved by giving a simple rocking action to the flat panel reactor mounted on a stand (unpublished).

6.4.5. Temperature control

Temperature control is of major concern in closed photobioreactors due to probable overheating. Various methods to keep the temperature low are:

- Overlapping of tubes,
- Evaporative cooling by spraying water on the surface of the tubes,
- Placing the light harvesting unit inside a pool of water, the temperature of which is controlled,
- Regulating the temperature of feed or recirculation stream

The most effective and economical method is when water is sprayed only during overheating and then recycled at the bottom after cooling. It is better suggested to go for thermo tolerant species to avoid problems of overheating.

6.4.6. Material of construction

The materials used for construction of photobioreactors play a major role in deciding the establishment and maintenance costs. The property of the materials should be such that it is stable and long lasting. The materials used should have the following characteristics:

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- High transparency
- Flexible and durable
- Non toxic
- Resistance to chemicals and metabolites produced by the microorganisms
- Resistance to weathering
- Low cost

It was found that only PMMA and Teflon retained their transparency over months when exposed to natural climatic conditions [124]. By comparing the properties of the different types of materials (Table 6), it is found that PMMA and PET havemore advantages for bioreactor construction with still PVC remaining an alternate when chemical resistance is of more concern than transparency. It is seen that materials like natural rubber, silicon rubber and polypropylene are found to have toxic effects on S-deprived algal cells [125].

6.5. *Comparison of the performance of the photobioreactors*

The performances of the different photobioreactors for biomass production and hydrogen production are compared in the Tables 7 and 8. Airlift systems remains as to provide a promising method of agitation as it provides the minimum shear compared to the other types. CO_2 enriched stream is used in the biomass production stage and recirculation of the evolved gases in airlift mode is effective in a hydrogen production stage. With *P. tricornutum*, hydrogen and biomass productivities were 1.2 g L^{-1} d^{-1} and 20 g m^{-2} d^{-1} respectively [126]. It was seen that although the alveolar panels achieved high volumetric productivity of 1.45 g L^{-1} d^{-1}, the photosynthetic efficiency is low due to oxygen toxicity at high photosynthetic rate [106]. A helical tubular reactor offers the highest A/V ratio (200 m^{-1}) and is considered to be the most effective, but also possesses some operational difficulties. Biomass productivity of 0.9 g L^{-1} d^{-1} for *A. platensis* [101] and a hydrogen production rate of 13 ml L^{-1} h^{-1} for *A. azollae* [30] are reported for helical tubular reactor. Flat panel reactors are reported to give the greater light conversion efficiency which reduces drastically on increase in thickness in the direction of light. With an A/V ratio of 40 m^{-1}, a biomass productivity of 1.09 g L^{-1} d^{-1} was achieved using *A. platensis*. Induced diffused reactors take advantage of the spatial distribution of the light in the deeper regions of the reactor and hence greater reactor thickness are achievable. The efficiency of light conversion to hydrogen is found to be maximum (9.23%) for this bioreactor [13]. Fermentor type of

Table 6 – Materials for construction of photo bioreactors.

Material	Properties	Advantages	Disadvantages
Glass	Density 2.35–2.52 g/cm^3 Transparency 95%	High transparency, chemical stability, durability	Fragile, installation costs are high
Polymethyl methyl acrylate (PMMA)	Mol. Formula $(C_5O_2H_8)_n$ Density 1.19 g/cm^3 Melting point 130–140 °C Tranparency 92%	Good impact strength than PC & PS, high transmittance, excellent environmental stability	Not autoclavable, easily scratched, poor resistance to many solvents
Polycarbonate (PC)	Mol. Formula $(C_{16}H_{14}O_3)_n$ Density 1.22 g/cm^3 Melting point 267 °C Tranparency 92%	Autoclavable, good impact resistance and optical properties, UV absorbing	Diffusible to gases due to low molecular mass, affected by solvents, ammonia, NaOH and concentrated acids
Polyethylene (PE)	Mol. Formula $(C_2H_4)_n$ Density 0.91–0.97 g/cm^3 Melting point 115, 135 °C Tranparency 80–85%	Chemically inert, high resistance to acids, alkalis and solvents	Loss of strength and tear resistance on exposure to light and oxygen
Polypropylene (PP)	Mol. Formula $(C_3H_6)_n$ Density 0.85–0.94 g/cm^3 Melting point 160 °C Tranparency 80%	Autoclavable, good resistance to fatigue, resistance to corrosion and chemical leaching,	Yellowing and loss of transparency on exposure to natural environment
Polyvinyl Chloride (PVC)	Mol. Formula $(C_2H_3Cl)_n$ Density 1.2–1.34 g/cm^3 Melting point 80 °C Tranparency 80%	Excellent resistance to acids and alkalis, low permeability to gases, high tensile strength, UV resistant	Poor resistance to aldehydes, esters, aromatic and halogenated hydrocarbons and ketones, transmittance is low
Polystyrene	Mol. Formula $(C_8H_8)_n$ Density 1.05 g/cm^3 Melting point 240 °C	Thermo plastic,	
Polyethylene terephthalate (PET)	Mol. Formula $(C_{10}H_8O_4)_n$ Density 1.37 g/cm^3 Melting point 260 °C	Strong and impact resistant, low permeability to gases,	Hygroscopic
Polyurethane (transparent)	Mol. Formula $(C_{25}H_{42}N_2O_6)$ Density 1.340 g/cm^3 Melting point 80 °C	High optical transmittance, excellent UV stability (non yellowing)	Cost is high

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Table 7 – Comparison of performance of reactors with respect to hydrogen production.

Reactor type	Organism	Surface to volume ratio	Volumetric productivity ($g\ L^{-1}\ d^{-1}$)	Areal productivity ($g\ m^{-2}\ d^{-1}$)	Photosynthetic efficiencies %	References
Airlift	*Phaeodactylum tricornutum* UTEX 640	–	1.2	20	–	[126]
Flat plate reactor	*Arthrospira platensis*	40	1.09	–	4.84	[101]
Alveolar	*Nannochloropsis* sp.	80	1.45	–	0.48	[108]
Helical tubular	*A.rthrospira platensis*	53	0.9	–	6.6	[101]
Near horizontal tubular	*A.rthrospira platensis*	70	1.4	28	5.6	[101]
Conical[a] tubular	*Chlorella*		1.01	28.3	6.84	[102]

a For 60 °C cone angle.

reactors offers the advantage of precise control of physico-chemical parameters. Hydrogen production rate was comparatively high with *Rhodospirulum ruburum* in bioreactors with internal lighting [127].

6.6. Energy analysis

The net energy ratio (NER) of a system is defined as the ratio of the total energy produced (in terms of biomass and hydrogen) over the energy required for plant operations like mixing, pumping, aeration and cooling.

$$\mathrm{NER} = \frac{\sum \text{Energy Produced (biomass/hydrogen)}}{\sum \text{Energy input(mixing, aeration, pumping, cooling etc.)}} \quad (8)$$

Open pond systems require the minimal energy input only during harvesting while raceway ponds consume energy for the paddle wheels for mixing. Tubular reactors involve pumps for recirculation of media and compressed gas for aeration and additional cooling system. Bubble column and airlift reactor systems are advantageous in mixing only by sparging compressed air while temperature control is achieved by regulating the temperature of the inlet gas. Tubular systems are estimated to have NER > 1 by Burgess and Fernandez-Velasco [129]. Only raceways ponds are reported to have an NER > 1 by Huesemann and Beneman [130]. Rodolfi et al. reported the NER > 1 for flat plate reactor (alveolar panels) [131]. In a revised study it is estimated that the NER for biomass production for second generation

Table 8 – Comparison of the hydrogen yield and light conversion efficiency to hydrogen in different photobioreactors.

Type of photo bioreactor	Organism	H_2 yield	Light conversion efficiency (%)	References
Vertical tubular reactor	*Rhodobacter sphaeroides* O.U.001	20 ml $L^{-1}\ h^{-1}$ [a]	1.1	[43]
Annular triple jacketed	*R. sphaeroides* O. U.001	6.5 ml $L^{-1}\ h^{-1}$	3.7	[9]
Helical tubular reactor (also called as bubble train)	*Anabaena azollae*	13 ml $L^{-1}\ h^{-1}$	–	[30]
Near Horizontal tubular reactor	*Rhodobacter capsulatus* (DSM 155)	3.3 ml $L^{-1}\ h^{-1}$	–	[100]
Flat plate tilted solar bioreactor	*R. sphaeroides* O. U.001	10 ml $L^{-1}\ h^{-1}$ [b]	–	[106]
Flat panel with gas recirculation	*Rhodopseudomonas* sp. HCC 2037	25 ml $H_2\ l^{-1}\ h^{-1}$	–	[97]
Fermentor type	*Rhodospirillum rubrum*		8.67	[127]
Induced diffuse PBR	*R. sphaeroides* RV	5.03 mM H_2/mM lactate	9.23	[41]
Floating type PBR	*Rhodopseudomonas palustris* R-1	10–12 ml $L^{-1}\ h^{-1}$	0.308	[44]

a Calculated by Akkerman [128].
b Maximum rate of hydrogen production.

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flat-plate bioreactor (alveolar panels) is 4.51, 8.34 for raceway ponds and 0.20 for horizontal tubular reactor [132].

Burgess and Fernandez-Velasco estimated the NER of tubular photobioreactor for production of H_2 by microalgae [129]. For a hypothetical microalgal H_2 generation efficiency of 5% the estimated NER is ~6 for low density polyethylene film and glass. The floating type of reactor requires no external energy for agitation [44]. Hence, it is expected to have an NER > 1 for hydrogen production if the light conversion efficiency to hydrogen can be improved.

7. Conclusion

Hydrogen production using photobiological systems has the potential to become an effective method of gaseous energy recovery. To establish biohydrogen as a future clean energy carrier more innovative research needs to be performed to improve the efficiency of the organisms, as well as to provide optimum culture conditions by improving the effectiveness of the photobioreactors.

Photobiological hydrogen production can among other factors become limited by the light penetration which influences the photo-conversion efficiency. While a major focus is on increasing the A/V ratio, much importance is also given to reduce the pigment and chlorophyll content of the organisms in order to tackle the issue. A helical tubular reactor offers the highest A/V ratio which is reported to be an important parameter for the improvement of light conversion efficiency. Flat panel reactors are reported to give the maximum light conversion efficiencies. Induced diffused reactors take advantage of the spatial distribution of the light in the deeper regions of the reactor. Marine type impellers were also shown to offer good agitation owing to the geometry of reactors such as the torus shaped one. The organisms used for hydrogen production are highly temperature sensitive. Out of the various methods for temperature control, evaporative cooling and recirculation of water are found to be effective and economical in large scale operation. Another factor affecting the performance of the reactors is the type of gas exchange systems that can remove the oxygen buildup and allows for collection of the evolving gases. Thereby whatever the organism, method and technique, the ultimate goal is to produce hydrogen in large scale that should be cost effective and economic to compete with fossil fuels.

It is evident that the airlift system in combination with other bioreactors (helical and flat plate) is most suitable for achieving both high CO_2 sequestration and hydrogen production.

8. Future scope

In order to make the photobiological hydrogen production process more economic the following points require immediate attention:

i) Feasible temperature control and agitation system in photobioreactor.
ii) Use of mixed microbial consortium for better utilization of solar spectrum,
iii) More exploration on metabolic engineering of the organisms that improves light penetration and yield which influences the overall performance of the photobioreactor.
iv) Reactor performances with same volume but different A/V ratios for a given organism for hydrogen production may be explored.

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