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Hydrogen production by biological processes: a survey of literature

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Abstract

Hydrogen is the fuel of the future mainly due to its high conversion efficiency, recyclability and nonpolluting nature. Biological hydrogen production processes are found to be more environment friendly and less energy intensive as compared to thermochemical and electrochemical processes. They are mostly controlled by either photosynthetic or fermentative organisms. Till today, more emphasis has been given on the former processes. Nitrogenase and hydrogenase play very important role. Genetic manipulation of cyanobacteria (hydrogenase negative gene) improves the hydrogen generation. The paper presents a survey of biological hydrogen production processes. The microorganisms and biochemical pathways involved in hydrogen generation processes are presented in some detail. Several developmental works are discussed. Immobilized system is found suitable for the continuous hydrogen production. About 28% of energy can be recovered in the form of hydrogen using sucrose as substrate. Fermentative hydrogen production processes have some edge over the other biological processes.

Keywords: Hydrogen; Photosynthesis; Fermentative; Hybrid bioreactions

1. Introduction

Today global energy requirements are mostly dependent on fossil fuels (about 80% of the present world energy demand). This will eventually lead to the foreseeable depletion of limited fossil energy resources. Presently, the utilization of fossil fuels are causing global climate change mainly due to the emission of pollutants like CO_x, NO_x, SO_x, C_xH_x, soot, ash, droplets of tars and other organic compounds, which are released into the atmosphere as a result of their combustion. In order to remedy the depletion of fossil fuels and their environmental misdeeds hydrogen has been suggested as the energy carrier of the future. It is not a primary energy source, but rather serves as a medium through which primary energy sources (such as nuclear and/or solar energy) can be stored, transmitted and utilized to fulfil our energy needs.

Hydrogen is the most plentiful element in the universe, making up about three-quarters of all the matter. The atmosphere contains about 0.07% hydrogen, while the earth's surface contains about 0.14% hydrogen. Hydrogen is the lightest element. The mass of 1 l of hydrogen is 0.09 g, while the mass of 1 l of air is about 1.2 g. The higher heating value of hydrogen is 3042 cals/m^3 (considering water as a product). In combustion, water is the main product, thus, hydrogen is regarded as a clean non-polluting fuel. As compared to other gaseous fuels like water gas, hydrogen is harmless to humans and the environment [1,2].

Today environmental pollution is a great concern to the world, mainly due to rapid industrialization and urbanization. So, increasing focus is being placed on clean energy alternatives for satisfying growing energy demand. Hydrogen has various other uses [3–5], which can be broadly divided into the following categories:

(a) As a reactant in hydrogenation processes: hydrogen is used to produce lower molecular weight compounds, saturate compounds, crack hydrocarbons or remove sulfur and nitrogen compounds.

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Nomenclature

ADP	adenosine diphosphate
ATP	adenosine triphosphate
CoA	coenzyme A (β-mercaptoethylamine + pantothenic acid + ADP with 3′-phosphate group)
Cyt	cytochrome
Da	dalton (a non-SI unit of atomic and molecular mass)
DCMU	3-(3,4 dichlorophenyl)-1,1dimethyl urea
FAD	flavine adenine dinucleotide
FADH	flavine adenine dinucleotide (reduced form)
Fd(ox)	ferredoxin (oxidised form)
Fd(red)	ferredoxin (reduced form)
GS	glutamine synthetase
K_i	inhibition constant $(g/l)^{-1}$
k_S	saturation constant (g/l)
K_m	Michaelis–Menten constant (g/l)
LP	Leudeking–Piret
MY	malt extract and yeast extract
NAD^+	nicotinamide adenine dinucleotide
NADH	nicotinamide adenine dinucleotide (reduced form)
NADP	nicotinamide adenine dinucleotide phosphate
NADPH	nicotinamide adenine dinucleotide phosphate (reduced form)
PC	plastocyanin
PQ	plastoquinone
PSI	photosystem-I
PSII	photosystem-II
PVA	polyvinyl alcohol
S	substrate concentration (g/l)
$Y_{x/S}$	yield coefficient (g cell mass/g glucose degraded)

Greek letters

α	growth-associated coefficient (dimensionless)
β	non-growth associated coefficient (h^{-1})
v	specific product formation rate (h^{-1})
μ	specific growth rate (h^{-1})
μ_{max}	maximum specific growth rate (h^{-1})

(b) As an O_2 scavenger: hydrogen is used to chemically remove trace amount of O_2 to prevent oxidation and corrosion.
(c) As a fuel in rocket engines.
(d) As a coolant in electrical generators to take advantage of its unique physical properties.

The above stated areas of hydrogen utilization is equivalent to 3% of the energy consumption today, and is expected to grow significantly in the years to come.

At present hydrogen is produced mainly from fossil fuels, biomass and water. The methods of hydrogen production from fossil fuels are

(a) Steam reforming of natural gas.
(b) Thermal cracking of natural gas.
(c) Partial oxidation of heavier than naphtha hydrocarbons.
(d) Coal gassification.

Methods of hydrogen production from biomass are

(e) Pyrolysis or gassification (which produces a mixture of gases, i.e., H_2, CH_4, CO_2, CO, N_2).

Methods of hydrogen production from water are

(f) Electrolysis.
(g) Photolysis.
(h) Thermochemical process.
(i) Direct thermal decomposition or thermolysis.
(j) Biological production.

Out of the above listed processes, nearly 90% of hydrogen is produced by the reactions of natural gas or light oil fractions with steam at high temperatures (steam reforming). Coal gassification and electrolysis of water are other

industrial methods for hydrogen production. These industrial methods mainly consume fossil fuel as energy source, and sometimes hydroelectricity [6–10]. However, both thermochemical and electrochemical hydrogen generation processes are energy intensive and not always environment friendly. On the other hand, biological hydrogen production processes are mostly operated at ambient temperatures and pressures, thus less energy intensive. These processes are not only environment friendly, but also they lead to open a new avenue for the utilization of renewable energy resources which are inexhaustible [11–15]. In addition, they can also use various waste materials, which facilitates waste recycling. The objective of this paper is to review literature on different biological hydrogenation processes and make a comparative analysis.

2. Biological hydrogen production processes

Biological hydrogen production processes can be classified as follows:

2.1 Biophotolysis of water using algae and cyanobacteria.
2.2 Photodecomposition of organic compounds by photosynthetic bacteria.
2.3 Fermentative hydrogen production from organic compounds, and
2.4 Hybrid systems using photosynthetic and fermentative bacteria.

2.1. Biophotolysis of water using algae and cyanobacteria

This method uses the same processes found in plants and algal photosynthesis, but adapts them for the generation of hydrogen gas instead of carbon containing biomass. Photosynthesis involves the absorption of light by two distinct photosynthetic systems operating in series: a water splitting and O_2 evolving system ("photosystem II" or PSII) and a second photosystem (PSI), which generates the reductant used for CO_2 reduction. In this coupled process, two photons (one per photosystem) are used for each electron removed from water and used in CO_2 reduction or H_2 formation. In green plants only CO_2 reduction takes place, as the enzymes that catalyze hydrogen formation, the hydrogenases, are absent [4]. Microalgae, both eucaryotic (such as the green algae) and procaryotes (the cyanobacteria or blue–green algae), have hydrogenase enzymes, and can produce hydrogen under certain conditions [11].

Hydrogen photo evolution by unicellular algae was first demonstrated by Gaffron and Rubin [16]. Hydrogen production by microalgae has been reviewed by several researchers [17–20]. Bonemann et al. [21] studied the effect of oxygen on the water-splitting reaction. They established that in a direct biophotolysis reaction, electrons flow from water through the two photosystems (PSII and PSI) of plant photosynthesis, to the hydrogen evolving enzyme hydrogenase via electron carrier (Ferredoxin Fd), as follows:

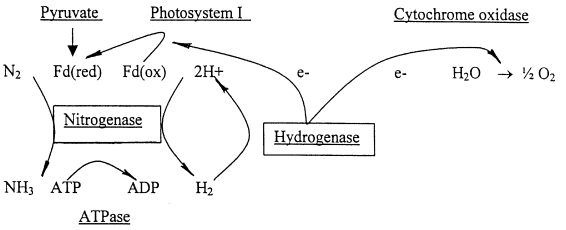

Fig. 1. Nitrogenase-catalyzed hydrogen formation and hydrogenase-catalyzed H_2 uptake of cyanobacteria [23].

$$H_2O \rightarrow PSII \rightarrow PSI \rightarrow Fd \rightarrow \text{Hydrogenase} \rightarrow H_2.$$
$$\downarrow$$
$$O_2$$

However, the rate of hydrogen production was lower than typical rate for CO_2 reduction. Small amount of O_2 inhibits the hydrogenase activity during biophotolysis reaction which reduces hydrogen evolution [21]. Many microalgae, in particular species classified as "green algae", produce hydrogen after a period of anaerobic conditions in the dark, during which the hydrogenase enzyme is activated and synthesized, and small amounts of hydrogen production are observed. When such "anaerobically adapted" algae are returned to light (but still under anaerobic conditions), H_2 evolution rates often increase dramatically, but cease once normal photosynthesis (O_2 evolution, CO_2 fixation) is re-established.

Cyanobacteria or blue–green algae are gram positive bacteria with same type of photosynthesis as higher plants and they exist in marine environment as well as in different soils/ecosystems [22]. These are called nitrogen-fixing bacteria and they are capable of biophotolysis, the light-driven splitting of water into hydrogen and oxygen, in reactions which involve the nitrogenase and hydrogenase [23]. The relationship between nitrogenase-catalyzed hydrogen formation and hydrogenase-catalyzed hydrogen uptake in cyanobacteria are depicted in Fig. 1. Hydrogen production through nitrogenase or hydrogenase — both by diazotrophic cyanobacteria — has been suggested as a good biological system for photoharvesting hydrogen from water [24–30]. One of the major obstacles in obtaining sustained H_2 photoproduction is the photosynthetically generated O_2 which irreversibly inactivates H_2 producing systems and supports O_2-dependent H_2-uptaking activity [24,31–33]. Cyanobacteria are able to fix atmospheric nitrogen via ATP-dependent nitrogenase activity. Nitrogenase located in the heterocysts provides O_2 protection for the enzyme. Under normal physiological conditions, the nitrogen fixed in the heterocysts is transported to the adjacent vegetative cells as glutamine, which then enters into the cellular metabolic pool. The conversion of ammonium to glutamine is catalyzed by glutamine synthetase (GS) also located in the heterocysts. It is possible to get NH_4^+ in the medium using GS inhibitor [34].

The two schemes of biochemical pathways for hydrogen forming and nitrogen-fixing cyanobacteria are shown as follows:

Heterocystous nitrogen-fixing bacteria:

$$\begin{array}{lllllll} H_2O & \rightarrow \text{Photosystems (vegetative cells)} & \rightarrow [CH_2O]_2 & \rightarrow \text{Ferredoxin} & \rightarrow \text{Nitrogenase} \\ \downarrow & & \uparrow & \downarrow \text{NADPH} & \downarrow \\ O_2 & & CO_2 \text{ Recycle} & CO_2 & H_2 \end{array}$$

Nonheterocystous nitrogen-fixing bacteria:

$$\begin{array}{lllllll} H_2O & \rightarrow \text{Photosystems (vegetative cells)} & \rightarrow [CH_2O]_2 & \rightarrow \text{Ferredoxin} & \rightarrow \text{Nitrogenase} \\ \downarrow & & \uparrow & \downarrow \text{NADPH} & \text{or Hydrogenase} \\ O_2 & & CO_2 \text{ Recycle} & CO_2 & \uparrow \quad \downarrow \\ & & & \text{PS-I} \rightarrow & \text{ATP} \quad H_2 \end{array}$$

According to Lee et al. [18], green algae are probably better for hydrogen production than cyanobacteria whereas the latter uses more energy intensive enzymes, ATP-requiring nitrogenase for the production of H_2.

2.2. Photo-decomposition of organic compounds by photosynthetic bacteria

Phototrophic bacteria are indicated in the current literature as the most promising microbial system for the biological production of hydrogen [35–39]. The major benefits are noted below:

(1) high theoretical conversion yields,
(2) lack of O_2-evolving activity, which causes problem of O_2 inactivation of different biological systems,
(3) ability to use wide spectrum of light, and
(4) ability to consume organic substrates derivable from wastes and then, for their potential to be used in association with wastewater treatment.

The overall biochemical pathways for the photo fermentation process can be expressed as follows:

$$\begin{array}{lll} (CH_2O\}_2 & \rightarrow \text{Ferredoxin} & \rightarrow \text{Nitrogenase} \rightarrow H_2. \\ & \uparrow \text{ATP} & \uparrow \text{ATP} \end{array}$$

Carbon monoxide can also be used for the production of hydrogen using microbial shift reaction by the photosynthetic bacteria [40] as follows:

$$CO + H_2O \rightarrow CO_2 + H_2. \quad (1)$$

2.3. Fermentative hydrogen production from organic compounds

Hydrogen evolution by fermentation has been treated with little attention, while hydrogen evolution by photosynthetic microorganisms has been extensively studied. The evolution of hydrogen by fermentation has, however, several advantages for industrial production, such as:

(a) Fermentative bacteria have very high evolution rate of hydrogen.
(b) They can produce hydrogen constantly through day and night from organic substrates.
(c) They can have growth rate good for supply of microorganisms to the production system.

Therefore, the fermentative evolution is more advantageous than photochemical evolution for mass production of hydrogen by microorganisms [15,41–45]. Fermentative hydrogen production can be maximized through the effective coupling of the following factors:

(1) An accessible and rich source of electron and biochemical electron pump.
(2) An active hydrogenase.

2.3.1. Fermentative route of electron generation

In heterotrophic organisms, the anaerobic mode of growth poses special problems for the cell with respect to the disposition of electrons from energy-yielding oxidation reaction. This is particularly so when the overall reduced power requirement for biosynthetic activity can be satisfied only by degradation of a relatively large quantity of an organic compound that serves as the energy source. Accordingly, various kinds of specific controls are necessary to regulate electron flow in the metabolism of strict and facultative anaerobe. One of these is reflected by the ability of many such organisms to dispose off "excess" electrons (e-) in the form of molecular hydrogen (H_2) through the activity of hydrogenase.

It is known that bacteria which undergoes, mixed acid fermentation or butane 2,3 diol fermentation evolves hydrogen through formate decomposition as

$$HCOOH \rightarrow H_2 + CO_2. \quad (2)$$

The pathway of pyruvate decomposition through acetyl-CoA produces this formate as follows:

$$CH_3CO\ COOH + H\ CoA \rightarrow CH_3\ CO\ CoA + HCOOH. \quad (3)$$

The acetyl CoA gives rise to either acetate or ethanol, viz.

$$CH_3\ CO\ CoA + H_2O \rightarrow CH_3\ COOH + H\ CoA, \quad (4)$$

$$CH_3\ CO\ CoA + 2NADH + 2H^+ \rightarrow\ CH_3CH_2OH + H.CoA + 2NAD^+. \quad (5)$$

Besides, there is another pathway for hydrogen evolution called the NADH pathway. In this pathway hydrogen is evolved by the re-oxidation of NADH as follows [46].

$$NADH + H^+ \rightarrow\ H_2 + NAD^+. \quad (6)$$

This NADH is made during the fermentative conversion of glucose to pyruvate called glycolysis. The pathway could be represented by the following overall reaction:

$$C_6H_{12}O_6 + 2NAD^+ \rightarrow 2CH_3CO\ COOH + 2NADH + 2H^+. \quad (7)$$

The hexose monophosphate pathway allows the cells to derive more reducing power from glucose at the expense of producing more carbon dioxide. The availability of CO_2 determines the production of succinate and formate as follows:

$$CH_3COCOOH + CO_2 + NADH + H^+ \rightarrow 2HCOOH + H_2O + NAD^+, \quad (8)$$

$$CH_3COCOOH + CO_2 + 2NADH + 2H^+ \rightarrow 2CH_3COOH + H_2O + 2NAD^+. \quad (9)$$

Again, it has been found that

$$\text{residual NADH} = (\text{produced NADH}) - (\text{used NADH}) = 2 \times \text{acetate} + 2 \times \text{butyrate} + \text{Butanediol} + 4 \times \text{Acetone} - \text{succinate} - \text{Formate}. \quad (10)$$

From the above equation, it is easily understood that the production of acetate, butyrate, butanediol and acetone should be increased, and the production of succinate and formate should be decreased by some means to obtain high yield of hydrogen. Thus, if CO_2 is removed compulsorily from the culture liquid, the amounts of formate and succinate should be reduced in the products. This will no doubt increase the hydrogen production due to more availability of NADH.

2.3.2. *Active hydrogenase*

Hydrogenase enzymes are playing important role in the fermentative hydrogen production [47]. Woodward et al. extensively studied the effect of hydrogenase and glucose dehydrogenase for the hydrogen production from glucose. These enzymes were used separately in vitro, and the contribution of these enzymes for hydrogen and organic acid production in a fermentation system has been justified [48].

2.4. *Hybrid system using photosynthetic and fermentative bacteria*

Hybrid system comprises of non-photosynthetic and photosynthetic bacteria. It can enhance the hydrogen production. Variety of carbohydrates can be digested *by C. butyricum*.

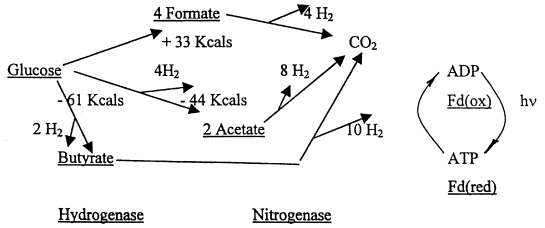

Fig. 2. Mode of biochemical decomposition of glucose by photosynthetic and anaerobic bacteria in a hybrid biological hydrogen generation system [49].

This bacterium produces hydrogen with the degradation of carbohydrates without using light. Resulting organic acids could be sources for photosynthetic bacteria to produce hydrogen [49–52]. Fig. 2 illustrates the energetic view of hydrogen production by anaerobic and photosynthetic bacteria. Anaerobic bacteria decompose carbohydrates to obtain both energy and electron. Because reaction only with negative free energy could be possible, organic acids formed by the anaerobic digestion could not be decomposed to hydrogen any more. Complete degradation of glucose to hydrogen and carbon dioxide is impossible by anaerobic digestion. Photosynthetic bacteria could use light energy to overcome the positive free energy reaction (bacteria can utilize organic acids for hydrogen production). The combination of the both kinds of bacteria not only reduces the light energy demand of photosynthetic bacteria but also increases hydrogen production [49].

3. Microbiology

Different microorganisms participate in the biological hydrogen generation system such as green algae, cyanobacteria (or blue–green algae), photosynthetic bacteria and fermentative bacteria, which are tabulated in Table 1. About 50 years ago Gaffron et al. discovered that the eucaryotic unicellular green algae, *Scenedesmus obliquus*, is able to evolve molecular hydrogen by means of a hydrogenase in the light under anaerobic conditions [16]. This is called direct biophotolysis. It is possible to maintain the direct biophotolysis reaction for longer periods, by removing the oxygen as it is produced by circulating inert gas or using glucose and glucose oxidase. Indirect biophotolysis processes are the paths followed by cyanobacteria. In this system, photosynthesis (O_2 evolution and CO_2 fixation) and N_2-fixation (thus H_2 production) are either spatially or temporally separated from each other. The spatial separation is achieved by differentiation of two cell type "vegetative" cells, which carry out normal photosynthesis and provide the nitrogen-fixing "heterocysts" with the reductant (carbohydrate) required by nitrogenase. In the heterocyst, nitrogenase is protected from O_2 by a heavy cell wall, that reduces O_2 diffusion, and by high rates of respiration, absorbing any residual oxygen [73]. Importance of hydrogenase present in fermentative bacteria for

Table 1
Microorganisms used for hydrogen generation

Broad classification	Name of the microorganisms	References
Green algae	*Scenedesmus obliquus*	[53]
	Chlamydomonas reinhardii	[12]
	C. moewusii	[12]
Cyanobacteria		
Heterocystous	*Anabaena azollae*	[55,56]
	Anabaena CA	[26]
	A. variabilis	[57,58]
	A. cylindrica	[23,54]
	Nostoc muscorum	[63]
	N. spongiaeforme	[58]
	Westiellopsis prolifica	[58]
Nonheterocystous	*Plectonema boryanum*	[24]
	Oscillotoria Miami BG7	[19]
	O. limnetica	[61]
	Synechococcus sp.	[62]
	Aphanothece halophytico	[61]
	Mastidocladus laminosus	[64]
	Phormidium valderianum	[65]
Photosynthetic bacteria	*Rhodobater sphaeroides*	[37,68]
	R. capsulatus	[67]
	R. sulidophilus	[69]
	Rhodopseudomonas sphaeroides	[59]
	R. palustris	[39]
	R. capsulata	[66]
	Rhodospirillum rubnum	[70]
	Chromatium sp. Miami PSB 1071	[60]
	Chlorobium limicola	[71]
	Chloroflexu aurantiacus	[71]
	Thiocapsa roseopersicina	[71]
	Halobacterium halobium	[72]
Fermentative bacteria	*Enterobacter aerogenes*	[15]
	E. cloacae	[44]
	Clostridium butyricum	[41]
	C. pasteurianum	[42]
	Desulfovibrio vulgaris	[64]
	Magashaera elsdenii	[64]
	Citrobacter intermedius	[42]
	Escherichia coli	[41]

H_2 generation has been pointed out by several researchers [47,48,74].

4. Major enzymes

There are three fundamentally different hydrogen producing and metabolizing enzymes found in algae and cyanobacteria:

(1) the reversible or classical hydrogenases,
(2) the membrane-bound uptake hydrogenases, and
(3) the nitrogenase enzymes.

4.1. The reversible or classical hydrogenases

These oxidize ferredoxin or other low redox electron carriers, both natural and artificial, in a readily reversible reaction. The hydrogen evolution reaction in green algae, first described in 1942 [53,75], is due to such a reversible hydrogenase.

4.2. The membrane-bound uptake hydrogenases

These are able to take up hydrogen at low partial pressures, reducing a relatively high-potential electron acceptor (at the level of the NAD/NADH couple, or even FAD/FADH), but producing little or no measurable hydrogen.

4.3. The nitrogenase enzymes

These normally reduce N_2 to ammonia, but can also evolve hydrogen, particularly in the absence of N_2 gas. Among the algae, only the blue–green algae (cyanobacteria) have these enzymes. Hydrogen evolution by these enzymes is an irreversible reaction coupled to the hydrolysis of at least four ATP/H_2 produced, making this reaction energy inefficient. Microalgae can have one, two or all three types of these enzymes present and active simultaneously, and their activities can change by large factors with relatively minor changes in the growth condition. Further, several forms of each of the enzymes are known, distinguished primarily by the metal content (e.g., Ni, Fe for hydrogenase; Mo, V or Fe for nitrogenase) and are sometimes present simultaneously in the same organism. This can lead to some difficulty in the interpretation of any net hydrogen evolution or uptake observed [11]. The presence of nitrogenase and hydrogenase have been found in photosystems and fermentation bacteria, respectively.

5. Genetic manipulation of microorganisms

Cyanobacteria in general, possess three hydrogen metabolizing enzymes: nitrogenase, membrane-bound hydrogenase, and soluble hydrogenase. Hydrogenases are mostly involved to utilize hydrogen. So, attempts have been made to maximize the amount of hydrogen production by manipulation of metabolic scheme, namely by maximizing the hydrogen-producing nitrogenase and minimizing that consumed by the so called hydrogenase. So, hydrogenase negative gene has been found to be useful for the hydrogen generation [23].

6. Theoretical considerations

Little information is available on the kinetics of biological hydrogen production processes. However, Kumar et al. studied the cell growth and substrate degradation kinetics of *Enterobacter cloacae* IIT-BT 08 with the help of Monod model [76]. Substrate and biomass concentration profiles of the experimental and simulated data are significantly different from each other. This might be due to substrate and/or product inhibition. Since in the hydrogen generating system the product is gaseous hydrogen which escapes and is collected in a gas collector, product inhibition cannot take

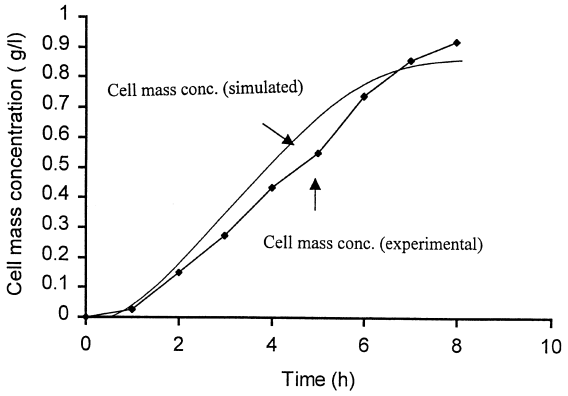

Fig. 3. Comparison between experimental and simulated cell concentration profiles using substrate inhibition model [76].

place. The following Andrew's equation is commonly used to explain substrate inhibition in the biological system:

$$\mu = (\mu_{max} S)/(k_s + S + K_i S^2), \quad (11)$$

where μ, μ_{max}, K_i, k_S and S are specific growth rate (h^{-1}), maximum specific growth rate (h^{-1}), inhibition constant (g/l), saturation constant (g/l), substrate concentration (g/l), respectively. However, this was found unsuitable for the fermentative hydrogen production using *E. cloacae* IIT-BT 08. The following modified by Andrew's was suggested and found suitable for the biological hydrogen production process.

$$\mu = (\mu_{max} S)/(k_s + S - K_i S^2). \quad (12)$$

The cell mass concentration profile calculated from this equation had good agreement with the experimental values (Fig. 3).

Leudeking–Piret (LP) model was considered to find out the mode of hydrogen generation. The plot of specific hydrogen production rate (v) vs. specific growth rate (μ) indicates that the hydrogen is a purely growth associated product. It was observed that hydrogen production profile obtained from the LP equation has good agreement with the experimental results [76] (Fig. 4).

$$v = \alpha\mu + \beta, \quad (13)$$

where α and β are growth associated coefficient (dimensionless) and non-growth associated coefficient (h^{-1}), respectively.

7. Typical results obtained from the biological hydrogenation processes

The purpose of biological hydrogen studies is to develop commercially practical hydrogen production processes by exploiting hydrogen producing ability of microorganisms through modern biotechnology. Attempts have already

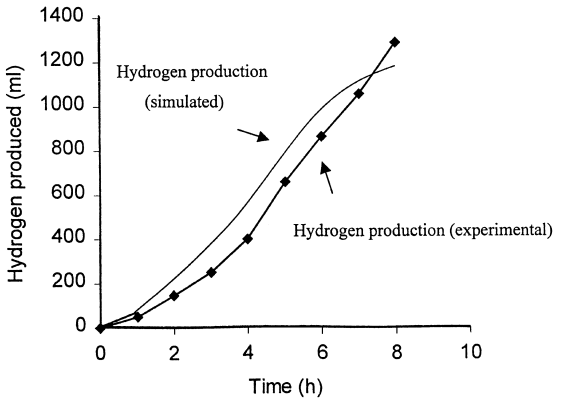

Fig. 4. Comparison between experimental and simulated hydrogen production Profile [76].

been made by several researchers to find out the suitability of different biological processes. Some important research works are discussed herebelow in order to understand present-state-of-art.

7.1. Effect of physico-chemical parameters

Several catalytic properties of the hydrogenase from *Scenedesmus obliquus* have been examined to optimize the purification condition. The K_m-value (Michaelis-Menten constant) for H_2 evolution in the presence of the most effective mediator methylviologen is 0.66 mM. The pH-optima was 6.3, the temperature-optima was 50°C and the energy of activation was 38.4 ± 2 KJ/mol. Molecular weight of hydrogenase was reported as 55 and 36 kDa as the enzyme consisted of two subunits [53]. Benemann [11] suggested that two stage indirect biophotolysis concept uses open ponds for microalgal CO_2 fixation into storage carbohydrates, followed by transfer of the culture to a dark, anaerobic fermentation vessel, in which the hydrogenase would be activated and/or induced. Lee et al. [18] proposed the two-light reaction Z-scheme of photosynthesis. In this scheme, PSII can split water and reduce the plastoquinone (PQ) pool, the cytochrome (Cyt) b/f complex, and plastocyanin (PC), while PSI can reduce ferredoxin (Fd)/nicotinamide adenine dinucleotide phosphate ($NADP^+$) and oxidise PC, the Cyt b/f complex, and the PQ pool. Based on the absolute light absorption of visible polychromatic illumination in the low-intensity region of the light saturation curve, the conversion efficiencies of 6–24% were obtained [12].

Hydrogen photoproduction by *Oscillatoria* sp. Miami BG7 was studied on its relationship to nitrogen nutrient in the culture medium. When a combined nitrogen sufficient culture was inoculated into a combined nitrogen-limited medium, cellular content of chlorophyll and protein decreased, whereas the carbohydrate content increased significantly during the culture period. Accompanying this change of cellular composition, the oxygen photoproduction capability decreased and hydrogen photoproduction capability increased dramatically (260 μmol/mg chlorophyll/h). Hydrogen production has been found to be strictly light dependent under anaerobic conditions [19]. *Plectinema boryanum* photoproduced H_2 when cells grown aerobically in nitrate were transferred to microaerobic or anaerobic conditions without nitrate. It was observed that H_2 photoproducing cells simultaneously excreted ammonium in the medium, which was possibly due to phycocyanin degradation in comparison to aerobic nitrate-grown cells [24]. In the case of *Anabaena azolla*, combined nitrogen sources (NH_4Cl/KNO_3) stimulated H_2 production [55]. Addition of 3-(3,4 dichlorophenyl)-1,1dimethyl urea (DCMU) inhibited hydrogen uptake in *A. variabilis, W. prolifica and Nostoc* sp. but not in *N. spongiaeforme* [58]. Mo, V, Fe nitrogenases were induced in *Anabaena variabilis*. Cultures expressing V-nitrogenase exhibited the highest resistance to alkaline pH and grew even at pH 10. H_2 evolution was practically independent of the culture at pH 7–9. But it was reverse in case of Mo and Fe-nitrogenase [57]. *Anabaena cylindrica* possesses an integral membrane hydrogenase and a soluble hydrogenase, both of which are nickel dependent. The release of nitrogenase-catalyzed hydrogen can be controlled chemically and also by manipulating nickel levels in the growth medium, suggesting that selection of uptake hydrogenase-deficient mutants would be an important objective for maximizing biophotolysis [23].

A simplified system with enhanced rates of hydrogen production was obtained by polyethylene glycol mediated fusion of *Halobacterium halobium* MMT_{22} and *Escherichia coli*. A continuous evolution of hydrogen was observed at the rate of 60.2 μmol/mg cell/min for about 2 weeks by fusion product [72]. Four strains of non-sulfur photosynthetic bacteria were isolated from root zone associations of aquatic plants like *Azolla*, *Salvinia*, and *Eichhornia*, as well as the deep-water rice. Based on the gross cell morphology and pigmentation, the isolates resembled *Rhodopseudomonas* sp. and was designated as BHU 1–4 strains. The BHU strains 1 and 4 selected as the most active thermostable hydrogen producing strains of local origin as far as Indian tropical climate was concerned [59]. The doubling time of *Chromatium sp*. Miami PBS 1071 was 1.75 h which is one of the fastest rates observed for marine photosynthetic bacteria. This strain could not utilize carbohydrate for growth, but could employ various other carbon and nitrogen compounds [60]. Photosynthetic bacteria have been found to be capable to utilize different organic compounds. *Rhodobacter sphaeroides* RV cells were cultivated on lactate containing solution and produced hydrogen [36]. Early stationary phase of growth of the organism was found suitable for hydrogen production. pH and glutamate concentration have significant affect on hydrogen production [68]. The effect of light intensity on the biomass and hydrogen production are shown in Figs. 5 and 6. For both biomass growth and photoproduction of hydrogen, light intensity higher than optimum,

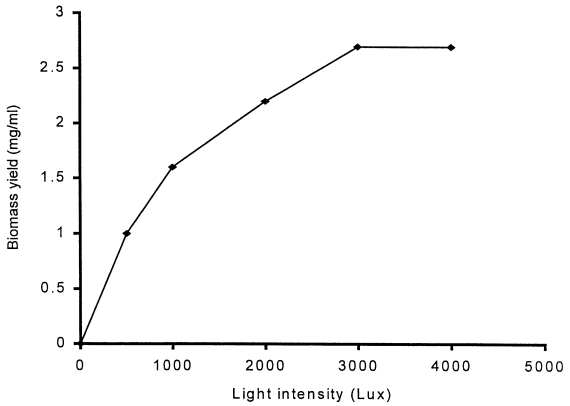

Fig. 5. Effect of light intensity on the biomass yield of *R. sphaeroides* O.U. 001 [77].

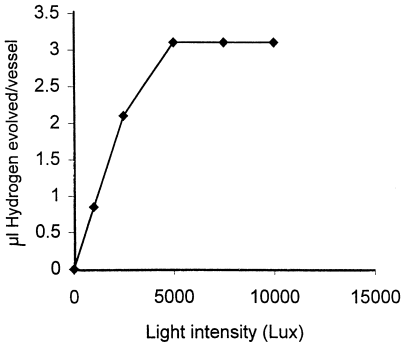

Fig. 6. The effect of light intensity on the photoproduction of hydrogen by *R. sphaeroides* O.U. 001 [77].

however, did not cause inhibition unlike the algal hydrogen evolution [77]. Roychowdhury et al. [81] found that solid wastes and digested sewage sludge have the potential to produce large amounts of hydrogen by suppressing the production of methane, by introducing a low volt electricity into the sewage sludge.

Tanisho et al. [78] observed that the removal of CO_2 from the culture liquid of *Enterobacter aerogenes* E.82005 affected the promotion of the yield of hydrogen. The pH of the culture was found to have important influence on the hydrogen-evolving activity. The most vigorous growth of the culture was found to occur at pH 7.0 [41]. A gram-negative hydrogen producing facultative anaerobe was isolated and characterized as *Enterobacter cloacae* IIT-BT 08. Hydrogen yields by using this microorganism varied from substrate to substrate (2.2 mol/mol glucose, 6 mol/mol of sucrose and 5.4 mol/mol cellobiose, considering 1% w/v substrate in MY medium). The maximum rate of hydrogen production (29.63 mmol/g dry cell h) achieved at 36°C and initial pH 6.0. The pH profiles of the

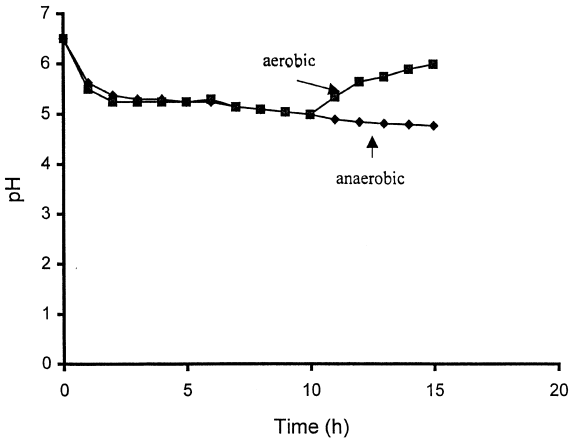

Fig. 7. pH profiles of the medium of *E. cloacae* IIT-BT 08 in aerobic and anaerobic conditions [44].

fermentation broth under aerobic and anaerobic conditions were monitored and found to differ from each other particularly beyond the pH of 4.8 (Fig. 7). About 28.34% of substrate energy recovered in the form of hydrogen from sucrose as a substrate [44]. This organism was also found to produce both α-amylase and hydrogen in a batch system using soluble starch as a substrate [45]. The values of μ_{max}, k_S and $Y_{x/S}$ of the cell were 0.568/h, 3.658 g/l and 0.0837 g biomass/g substrate, respectively [76].

7.2. Waste utilization

Different photosynthetic and fermentative bacteria can utilize waste materials like municipal solid wastes, industrial effluents, sewage sludge etc. [36,45,52,79–81]. Fascetti et al. [36] reported the work on the photosynthetic hydrogen evolution from municipal solid wastes. Batch-wise and continuous experiments showed that the acidic aqueous stream obtained from such refuse was good substrate for the growth of *R. spaeroides* RV. The substrate from the acidogenesis of fruit and vegetable market wastes gives higher hydrogen evolution rate as compared to synthetic medium (100 vs 35 ml H_2/g dry weight h). Waste water from a distillery was used as the electron donor for the photoproduction of hydrogen by *R sphaeroides* O.U. 001 [52]. Immobilized photosynthetic hydrogen producing bacteria were found suitable for the treatment of both organic and inorganic wastes [79]. Miyake [49] proposed that mixed culture of photosynthetic and anaerobic bacteria provided a method of utilization of a variety of resources for hydrogen production. Hydrogen production from whey by phototrophic bacteria like *R. rubrum* and *R. capsulatus* was discussed by Venkataraman et al. [80]. Roychowdhury et al. [81] discussed the hydrogen generation from the fermentative bacteria. Kumar et al. [45] reported the suitability of starch based residues for hydrogen production.

Table 2
Unit cost of energy obtained by different processes

Type of energy	Conversion efficiency (%)	Unit cost of energy content of fuel (US $/MBTU)	References
Photobiological hydrogen	~ 10	~ 10	[11]
Fermentative hydrogen	~ 10	~ 40	[85]
H_2 from coal, biomass	—	4	[2]
H_2 from advanced electrolysis	—	10	[2]
H_2 from thermal decomposition steam cyclical	—	13	[2]
H_2 from photochemical	—	21	[2]
Fermentative ethanol	15–30	~ 31.5	[85]
Gasoline	—	6	[2]

7.3. Immobilized whole cell systems

Immobilized whole cell systems have several advantages as compared to suspended cells systems. One important advantage is to reuse the system repeatedly. Continuous and stable hydrogen production was achieved by immobilizing the combined system of *Phormidium valderianum*, *Halobacterium halobium* and *Escherichia coli* in a PVA-alginate film. The intermittent supply of nitrogen was found to be essential to retain cellular metabolic activities, which in turn showed prolonged production of hydrogen [65]. Substrate like glucose can be utilized by this system and, therefore, it may have a potential significance in removing organic materials from the wastewater and simultaneously producing hydrogen. *Rhodobacter sphaeroides* O.U. 001 was immobilized in calcium alginate beads and this was used for continuous hydrogen production. It enhanced hydrogen production two to three folds. Hydrogen production from the wastewater of a tofu factory was examined by using *Rhodobacter sphaeroides* immobilized in agar gels. The maximum rate of hydrogen production observed from the wastewater was 2.1 l/h/m [82]. *Enterobacter cloacae* IIT-BT 08 was immobilized on environmentally friendly solid matrices such as lignocellulosic materials by the adsorption technique. Maximum rate of hydrogen production in the case of carrier SM-A was 44 mmol/l h at a dilution rate of 0.93/h with glucose conversion efficiency of 53% [83].

7.4. Use of different bioreactor configurations

In the case of microalgae strain, higher biomass was produced in a large pond than that produced in a tubular photobioreactor [11]. However, hydrogen production was found to be more in the tubular photobioreactor. Gas hold up is the major problem of immobilized whole cell system, because it reduces the working volume of the bioreactor. The tapered and rhomboid bioreactors were found to give better results as compared to tubular bioreactor mainly due to reduction of gas hold up. The gas hold up in tubular reactor was reduced by 67% by using a rhomboid bioreactor [83]. A low-cost closed tubular glass photobioreactor allowing axenic cultivation of phototrophic microorganisms was constructed. This bioreactor was used for the cultivation of *Rhodobacter sphaeroides* and *Rhodospirillum rubnum*, the oxygenic phototrophic cyanobacterium *Synechocystis* sp. Strain PCC6803 and microalga *Chlorella* sp. [84]. The cell growth was found to differ from each other with respect to different organisms.

8. Energy analysis

The yield of hydrogen from sucrose is 6.0 mol/mol sucrose. Assuming overall fuel cell efficiency as 80% [85], Gibb's free energy of hydrogen as 56.7 kcal/mol, the lower heating values of hydrogen and sucrose as 58.3 and 1234 kcal/mol, respectively, the following energy analysis was done:

$$\begin{aligned}&\text{energy recovery from substrate}\\&\quad=\text{lower heating value of hydrogen}\\&\quad\times H_2\text{ yield/lower heating value of sucrose}\\&\quad=58.3\times 6.0/1234=28.34\%,\end{aligned}\tag{14}$$

$$\begin{aligned}&\text{final conversion efficiency}\\&\quad=\text{Gibb's free energy for } H_2\times H_2\text{ yield}\\&\quad\times\text{overall efficiency of fuel cell/}\\&\qquad\text{lowerheating value of sucrose}\\&\quad=56.7\times 6.0\times 0.8/1234\\&\quad=22.05\%.\end{aligned}\tag{15}$$

Tanisho [85], Benemann [11] and Bockris [2] calculated the energy cost by different biological as well as other hydrogen generation processes as compared to conventional fuels (Table 2). Hydrogen from coal and biomass is the cheapest when compared with other processes. Ten per cent of

Table 3
Comparison of different biological hydrogen production processes

Organisms used	Raw materials used	Doubling time (h)	Maximum rate of H_2 production (mmol H_2/ l h)	Maximum rate of H_2 production (mmol H_2/ g dry cell h)	Major products of the process	References
Photosynthetic bacteria Double photosystem		7–25				
Oscillatoria sp. Miami BG7	Medium-A except NH_4Cl		0.4	0.3	$H_2/CO_2/O_2$= 6:3:1, biomass	[19,86]
Anabaena cylindrica	Nitrogen-starved medium[a]	25	1.2	1.3	H_2, O_2, biomass	[87]
Anabaena variables	Allen and Arnon medium[a]			0.6	H_2, O_2, biomass	[88]
Anabaena CA	ASP-2 medium[a]			2.14	H_2, O_2, biomass	[26]
Single photosystem		2.2–9				
Rhodopseudomonas capsulata	Lactate with other nitrogen source		5.3	5.3	H_2, CO_2, O_2. small amount of fatty acids, biomass	[89,85]
Rhodopseudomonas capsulata	Fermented cow dung			0.3	H_2, CO_2, O_2. small amount of fatty acids, biomass	[37]
Rhodopseudomonas sp.	Vegetable starch			1.3	H_2, CO_2, O_2. small amount of fatty acids biomass	[66]
Rhodopseudomonas sp	Sugarcane juice			2.0	H_2, CO_2, O_2. small amount of fatty acids biomass	[50]
Rhodopseudomonas sp	Sugarcane wastewater			15[b]	H_2, CO_2, O_2. small amount of fatty acids biomass	[16]
Rhodopseudomonas sp	Whey			1.11	H_2, CO_2, O_2. small amount of fatty acids biomass	[50]
Rhodopseudomonas sp	Dairy wastewater			16[b]	H_2, CO_2, O_2. small amount of fatty acids biomass	[16]
Rhodopseudomonas sphaeroides	Orange processing effluent			133[b]	H_2, CO_2, O_2. small amount of fatty acids biomass	[90]
Rhodopseudomonas palustris	Straw paper mill effluent			1.2	H_2, CO_2, O_2. small amount of fatty acids biomass	[39]
Rhodopseudomonas palustris	Sugar refinery waste			1.2	H_2, CO_2, O_2. small amount of fatty acids biomass	[39]

Table 3 (Continued.)

Organisms used	Raw materials used	Doubling time (h)	Maximum rate of H_2 production (mmol H_2/l h)	Maximum rate of H_2 production (mmol H_2/g dry cell h)	Major products of the process	References
Rhodobacter sphaeroides	Lactic acid ferm. waste			5.9	H_2, CO_2, O_2. small amount of fatty acids biomass	[52]
Rhodobacter sphaeroide	Distillery wastewater			0.46	H_2, CO_2, O_2. small amount of fatty acids biomass	[91]
Rhodobacter sphaeroide	Lactate from MSW			0.05	H_2, CO_2, O_2. small amount of fatty acids biomass	[37]
Rhodobacter sphaeroide	Lactate liquor MSW			4.7	H_2, CO_2, O_2. small amount of fatty acids biomass	[37]
Rhodospirillium rubrum	Organic compounds		3.0	2.5	H_2, CO_2, O_2. small amount of fatty acids biomass	[92,85]
Mixed microorganismsa[a] containing *Phormidium valderianum*, *Halobacterium halobium*, *Escherichia coli* in 1:1:1 proportion	ASNIII medium[a] devoid of combined nitrogen in TES buffer			19[c]	H_2, CO_2, O_2. small amount of fatty acids biomass	[93]
Fermentative bacteria Strict anaerobe		0.16-2				
Clostridium butyricum	Glucose containing medium[a]		—	7.3	H_2, CO_2, high conc. of fatty acids, biomass	[94]
Facultative anaerobe						
Citrobacter intermedius	Cellulose, starch, glucose		11	9.5	H_2, CO_2, high conc. of fatty acids, biomass	[42]
C. freundii	Stillage			1.8	H_2, CO_2, high conc. of fatty acids, biomass	[95]
Enterobacter aerogens E82005	Sugar cane	0.25	11.36	17	H_2, CO_2, high conc. of fatty acids, biomass	[85]
Enterobacter cloacae IIT BT-08	Sucrose containing mediuma[a]	0.32	37.03	29.63	H_2, CO_2, high conc. of fatty acids, biomass $H_2/CO_2 = 9$	[44]

[a]Medium contains different components.
[b]Expressed as ml H_2/g Bchlorophyll-a h.
[c]Expressed in mmol/mg protein h.

conversion efficiency of the fermentative hydrogen production process was found less attractive, when compared with the conventional fuels. Kumar et al. [44] found out that it is possible to increase the conversion efficiency to 28.34%. Therefore, it is obvious that this process may be economically attractive by improving the efficiency further by adopting an immobilized system [83]. Two stage photobiological hydrogen production process was found attractive [11].

Table 4
Merits and demerits of different biological processes for hydrogen production

Type of microorganism	Merits	Demerits
Green algae	Can produce hydrogen from water Solar conversion energy increased by 10 folds as compared to trees, crops	Require light for hydrogen production. O_2 can be dangerous for the system
Cyanobacteria	Can produce hydrogen from water Nirogenase enzyme mainly produces H_2 Has the ability to fix N_2 from the atmosphere	Uptake hydrogenase enzymes are to be removed to stop the degradation of H_2. Require sun light About 30% O_2 present in the gas mixture with H_2 O_2 has inhibitory effect on nitrogenase CO_2 present in the gas
Photosynthetic bacteria	Can use different waste materials like, whey, distillery effluents, etc. Can use wide spectrum of light	Require light for the hydrogen production Fermented broth will cause water pollution problem CO_2 present in the gas
Fermentative bacteria	It can produce hydrogen all day long without light It can utilize different carbon sources like, starch, cellobiose, sucrose, xylose, etc. and so different types of raw materials can be used It produces valuable metabolites such as butyric acid, lactic acid, acetic acid, etc. as by products It is anaerobic process, so there is no oxygen limitation problems	The fermented broth is required to undergo further treatment before disposal otherwise it will create water pollution problem CO_2 present in the gas

9. Comparative studies

So far, as microbial hydrogen production is concerned, Table 3 has been prepared to give the comparative studies on the different microbial hydrogen-producing systems. It is clear that the rate of fermentative hydrogen production is always faster than that of the photosynthetic hydrogen production. The merits and demerits of the different biological processes are presented in Table 4.It has been found that most of the biological processes are operated at an ambient temperature (30–40°C) and normal pressure. So, these processes are not energy intensive. In addition, fermentative hydrogen production processes produce some useful fatty acids, such as lactic acid, acetic acid, butyric acid, etc. These acids have to be separated, otherwise, they will pose water pollution problems. A photosynthetic bacterium on the other hand produces low concentration of fatty acids.

10. Purification of hydrogen

The gases produced by biological processes mostly contain hydrogen (60–90% v/v). However, different impurities like CO_2 and O_2 are present in the gas mixtures. CO_2 acts as fire extinguisher. This is sparingly soluble in water. Scrubbers can be used to separate CO_2. Fifty percent w/v KOH solution is a good CO_2 absorbent. So, it can be used for CO_2 removal. The presence of O_2 in the gas may cause a fire hazard. Water solubility of O_2 is less as compared to that of CO_2. Alkaline pyrogallol solution can be used for the removal of O_2 from the gas mixture. Another important problem is the presence of moisture in the gas mixture. It must be reduced, otherwise, the heating value of the fuel will be decreased. This can be achieved by passing the mixture through either a dryer or a chilling unit (by condensing out the vapor in the form of water).

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