Biohydrogen Production: Integrated Approaches to Improve the Process Efficiency

Chandrasekhar Kuppam, Soumya Pandit, Abudukeremu Kadier, Chakradhar Dasagrandhi, and Jeevitha Velpuri

Abstract In recent years, hydrogen (H_2) has emerged as a clean and attractive substitute fuel since it can be produced from renewable energy sources. Upon combustion of hydrogen, it generates only water as a major by-product. In hydrogen and fuel cell technology, hydrogen can be applied in fuel cell technology; it produces only water as a major by-product with high energy yield, hold great potential for meeting in a quite unique way by empowering the so-called hydrogen-based economy. To make hydrogen-based economy viable, it is crucial to use renewable resources in place of fossil fuels to produce hydrogen. In this direction, by considering attractive and renewable characteristics of hydrogen led us to improve a variety of biological processes for the production of hydrogen. Nonetheless, commercialization of the biological process depends on improvements in process

C. Kuppam (✉)
School of Applied Bioscience, Agriculture Department, Kyungpook National University, Building-1, Dong-Daegu, South Korea

Bio-Engineering and Environmental Science (BEES), CSIR-IICT, Hyderabad, India
e-mail: chanduibt@gmail.com

S. Pandit
Department of Desalination and Water Treatment, Ben-Gurion University of the Negev, Beersheba, Israel

Department of Biotechnology, IIT-Kharagpur, Kharagpur, India
e-mail: sounip@gmail.com

A. Kadier
Department of Chemical and Process Engineering, Faculty of Engineering & Built Environment, National University of Malaysia, Selangor, Malaysia
e-mail: abudoukeremu@163.com

C. Dasagrandhi
School of Food Science and Biotechnology, Kyungpook National University, Dong-Daegu, South Korea
e-mail: microchakri2001@yahoo.com

J. Velpuri
Department of Environmental Science and Technology, Jawaharlal Nehru Technological University, Hyderabad, Telangana, India
e-mail: jeevithavelpuri@gmail.com

V.C. Kalia, P. Kumar (eds.), *Microbial Applications Vol.1*,
DOI 10.1007/978-3-319-52666-9_9

design along with an understanding of the nature of hydrogen producing communities and process optimization. Thus, this chapter highlights the major factors involved towards the improvement of biohydrogen production processes. Environmental impact of hydrogen as carbon-neutral energy carrier is also discussed. This also includes a technical and economic analysis of the biohydrogen and its role in the proposed hydrogen economy coupled with fuel cell and in transport application. Technological advancements based on hydrogen-based fuel cell designs and process integration approaches are also discussed.

Keywords Biohydrogen • Biomethane • Bioenergy • Dark fermentation • Photofermentation • Microbial electrolysis cells • Biopolymers • Algae

1 Introduction

The rising gap between the world's energy need and inadequate supply has resulted in a sharp rise in fossil fuel usage. This led us to encounter severe limitations forced by an alarming escalation in global pollution levels as well as fossil fuel depletion. In addition, an ever-increasing level of greenhouse gas releases after the combustion of fossil-based fuels in turn aggravated the complications of global warming. At present, carbon dioxide level is exceeding 350 ppm by volume where it can potentially increase the greenhouse effect by raising the global temperature (Venkata Mohan and Pandey 2013; Chandrasekhar et al. 2015a). During the past decades, total carbon (organic) released via human accomplishments is corresponding to that gathered over millions of years. Therefore, concerns about global climate change due to GHG emissions and depletion of fossil fuel reserves have driven obvious attention to the study and improvement of carbon-neutral and renewable energy substitutes to fulfill the mounting energy requirements (Lee et al. 2013). Hence, divergence of energy and fuel selections is one of the primary necessities in the present global energy consequence (Chandrasekhar et al. 2015a). In this regard, bioenergy presents a sustainable and hopeful alternative for fossil fuels, which can defend in contrast to an energy crunch and defend the world from the verge of environmental disaster.

2 Biohydrogen: A Zero-Carbon Fuel

In recent years, global attention has been paid to biohydrogen as one among the carbon-neutral energy sources. Hydrogen gas is perhaps a multipurpose energy source that can change the usage of hydrocarbon-based fossil fuels since it shows the higher energy yield (122 kJ/g per unit mass), which is 2.75-fold greater than that of currently using fossil fuels (Christopher and Dimitrios 2012) and after its combustion with oxygen yields H_2O (water) as the only product, which is evidently favorable for the fall of greenhouse gas emissions. In specific, in the frame of

energy systems, hydrogen gas is outstanding choice of an energy transporter, more related to electrical energy than fossil fuels (Elam et al. 2003). The great electrochemical reactivity makes hydrogen an ideal for fuel cell technology in the field of suitable catalysts along with other ways to use hydrogen for electricity generation and also for energy storage (Kumar et al. 2015a). The current "merchant" and "captive" markets for hydrogen are well established in many industries (e.g., oil refinery, metal treatment, food production, and fertilizer manufacture) with the main demand in the petroleum refinery and ammonia production industries. Thus, applications such as a fuel for transportation and an energy carrier in the stationary power plant lead the hydrogen market to a rising field with huge future potential.

Currently, molecular hydrogen has been mainly generated from fossil fuel-based resources. The worldwide hydrogen production at present surpasses one billion m^3/day, of which 48%, 30%, and 18% is generated from natural gas, oil source, and coal source, respectively, and the outstanding 4% by water electrolysis (water-splitting) (Venkata Mohan and Pandey 2013). However, the production of hydrogen gas from fossil fuel-based resources is concomitant with GHG emission (Venkata Mohan et al. 2011; Venkateswar Reddy et al. 2011b). Alternatively, hydrogen gas production through biological routes from biomass is one of the rising technologies due to its eco-friendly and sustainable nature. A scientometric investigation using data available in the ISI Web of Knowledge (since 2001) indicated that the number of research articles published on biohydrogen accounts for 2635 records with a considerable number of citations (60,472), average citations for each item (22.95), and H-index (102). As illustrated in Fig. 1, literatures associated with biohydrogen research exhibited a steep increase after 2003, reached maximum records of 224 in 2008 (overall citations: 2883), and documented increasing trend till 2015 (records, 343) with marginal variations (Fig. 1). Average citation per year also revealed an increasing trend year by year, evidently indicating that prompt and promising research is under way to make the biohydrogen production process technologically viable.

Various organisms are known to yield hydrogen under definite conditions, including cyanobacteria that usually use carbohydrates to accumulate energy from photosynthesis mechanism to generate hydrogen from H_2O and microalgae that exploit solar energy to split H_2O for hydrogen production (Chandrasekhar et al. 2015a). Even with the striking advantageous features, low hydrogen production rates, less substrate conversion efficiency, and acid-rich intermediate metabolites (volatile fatty acids; VFA), production and accumulation are practical difficulties to overcome. In view of this, many researches on biological hydrogen production processes are in advancement, and various novel approaches and technologies are being investigated to overcome a few of the existing troubles and furthermore to enhance the overall process efficiency. Toward these aims, a number of sophisticated technologies for high molar hydrogen yields through metabolic engineering by providing metabolic energy to cross thermodynamic limitations, enhancing electron flux for proton (H^+) reduction, rerouting metabolic pathways to improve substrate consumption by expressing heterologous proteins, and so on have been well described (Hallenbeck 2012; Chandrasekhar et al. 2015a). In this chapter, we

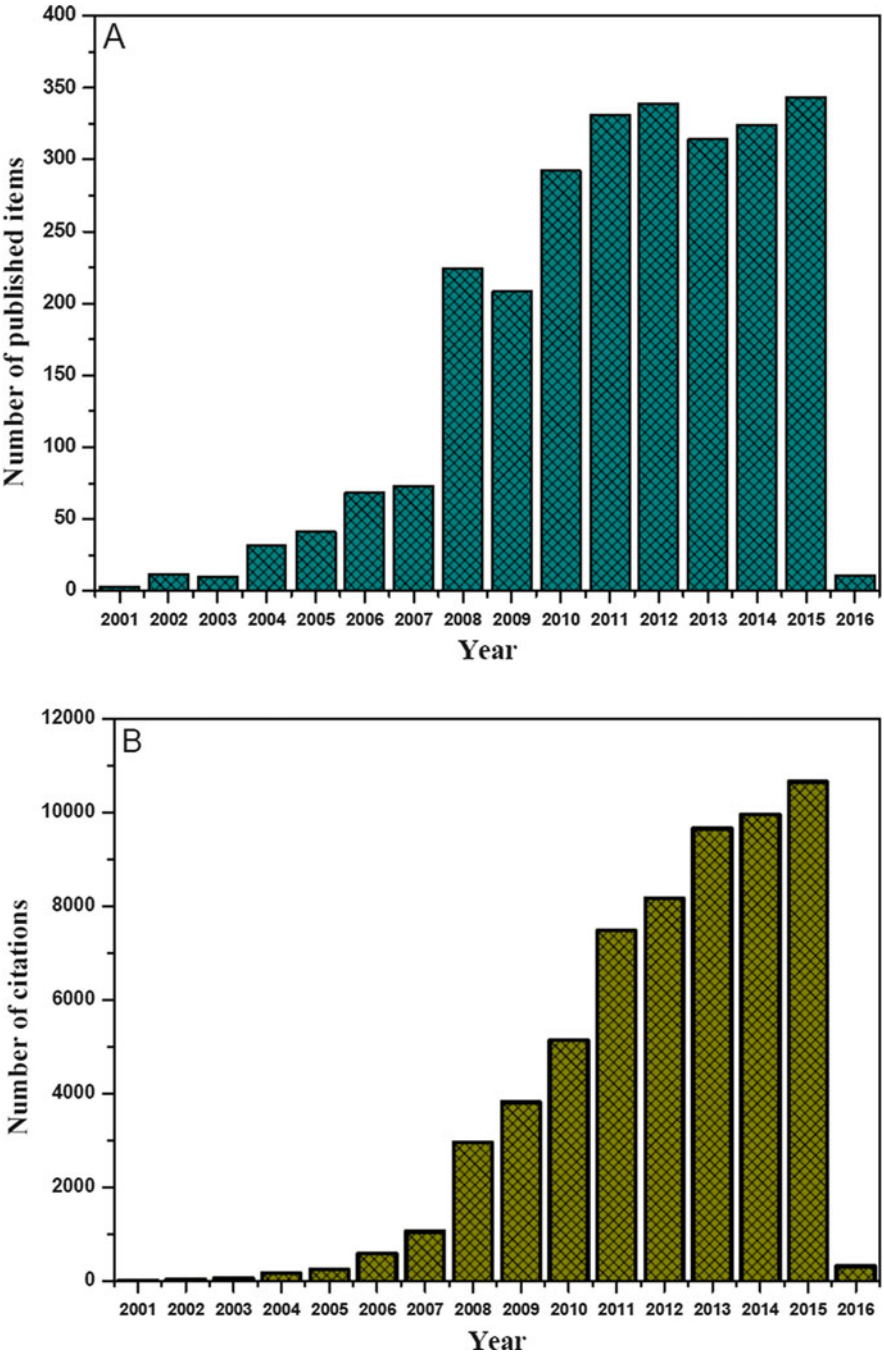

Fig. 1 Scientometric analysis on biohydrogen. Number of published items (**a**) and number of citations (**b**) in each year (from 2001 to 2016)

focus on the biological processes/routes for hydrogen production to evaluate the efficiency and practical applicability of those processes with respect to operation factors and delineate some potentially limiting factors. In addition, alternate choices such as integration approaches and electro-fermentation to enhance process efficiency are conversed.

3 Diversity of Hydrogen Producers

In the environment, wide diversity of microorganisms including archaea, cyanobacteria, bacteria (facultative aerobic and anaerobic), and lower eukaryotes are reported as hydrogen producers (Boichenko et al. 2004; Kalia and Purohit 2008; Kumar et al. 2013, 2015b; Chandrasekhar et al. 2015a), which may act individually or as consortia of same types or as mixed cultures (Patel et al. 2012a, 2014; Chandrasekhar et al. 2015a) (Fig. 2). Concurrently, hydrogen consuming organisms with syntrophic association with hydrogen producing organisms contribute to balancing Earth's hydrogen. It has been noted that the molecular adaptation events may help a variety of microorganisms survive in their ecological habitats even in extreme conditions (harsh environment) such as complete darkness, extreme temperature, and presence or absence of oxygen (O_2). For example, typical extremophilic archaea living in extreme conditions such as salt lakes and hot springs are phototrophs, lithotrophs, or organotrophs, evolving hydrogen through their specific machineries, which is different from the bacterial hydrogen fuel cycle. Nonetheless, major hydrogen producing organisms are characteristic heterotrophs during their metabolic process. Few dark fermentative microorganisms do not necessitate sunlight as an energy source and can grow under anaerobic circumstances. These organisms are considered as obligate anaerobes, which are additionally categorized depending on their growth temperature and oxygen tolerance (Fig. 2). In practical aspects, cultivation of facultative anaerobes is more viable over obligate anaerobic microorganisms. However, by considering microbial growth temperature, they may be further subclassified into thermophiles, mesophiles, and psychrohiles. Even though the thermophiles grow at high temperatures where very intensive energy is required (Chandrasekhar et al. 2015a), their overall hydrogen yield is very closer to the theoretical yield by devastating the thermodynamic barrier. Several photo-fermentative microorganisms necessitate solar/light energy to evolve hydrogen in anoxygenic circumstances. Under anaerobic conditions, photoautotrophs including green algae and cyanobacteria can produce hydrogen through biophotolysis, by taking advantage of their specific metabolic route under specific conditions (Chandrasekhar et al. 2015a).

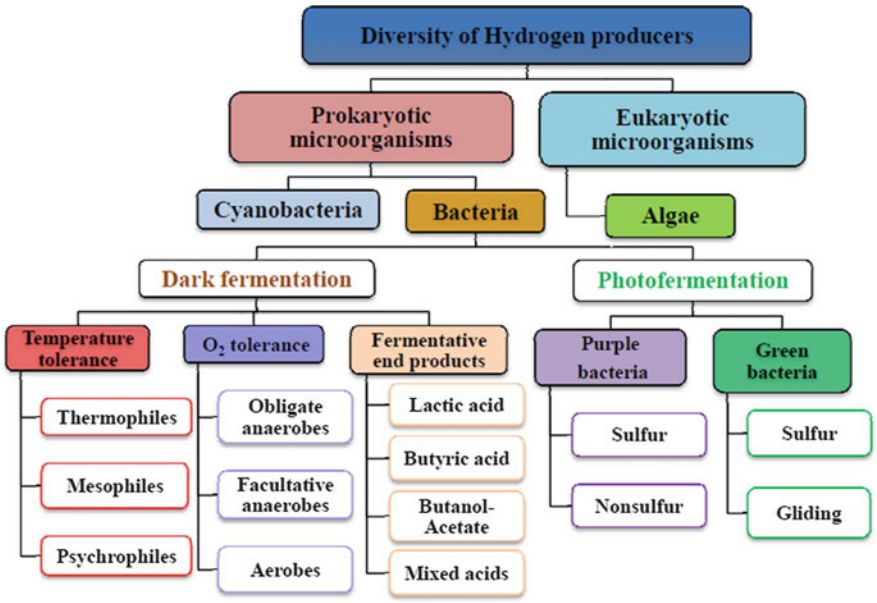

Fig. 2 Schematic illustration of the diversity of hydrogen producers

4 Biohydrogen Production Processes

Biohydrogen production by either biological routes or thermochemical treatment of biomass can be described as biohydrogen. Similarly, thermochemically produced hydrogen gas is also named as biohydrogen due to the usage of biomass as a potential feedstock. However, different biological routes are available for the production of biohydrogen pertaining to anaerobic, photobiological, microbial electrolysis cell (MEC)/electro-fermentation, and enzymatic routes. The research society around the globe showed significance interest upon biological routes of hydrogen production in recent years. The passing decades above all illustrated remarkable research on both applied and basic fields.

On the basis of hydrogen evolving systems, a wide variety of diverse biological processes can be classified as follows: (1) water-splitting photosynthesis mechanism; (2) photo-fermentation mechanism; (3) dark fermentation mechanism; and (4) electro-fermentation/microbial electrolysis process (Fig. 3). Each process has its own advantages and disadvantages over other methods with respect to practicability and energy efficiency. Therefore, choice of suitable biocatalyst and/or inoculum is major subject, which is in a straight line interrelated with hydrogen production process. As expected, hydrogen can be produced by applying a single microbial type or by mixed consortia, of which few of them are hydrogen producers, whereas the rest of them utilize hydrogen for their energy supplies. Early researches on hydrogen production were typically restricted to the usage of single cultures by

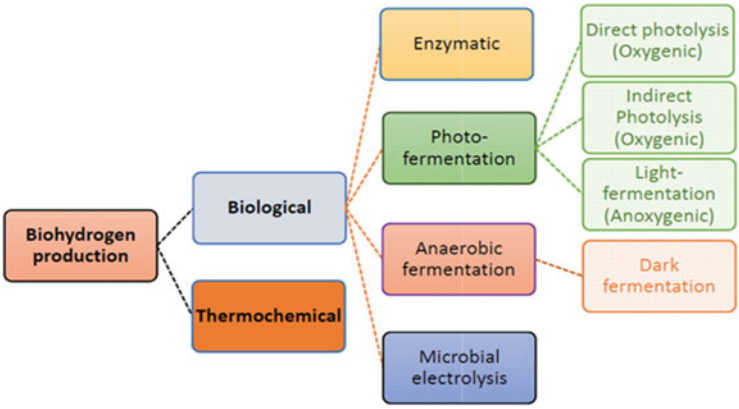

Fig. 3 Schematic illustration concerning different routes of biohydrogen production

feeding with a defined substrate. On the other hand, when carbon-rich wastewater is used as the carbon source, mixed culture might be favorable and practically significant for the production of hydrogen at large scale (Venkateswar Reddy et al. 2011a; Mullai et al. 2013; Roy and Das 2016). In addition, mixed microbial populations are typically preferred due to operational simplicity, stability, different biochemical pathways, and also an opportunity of using a wide variety of substrates as energy source, as well as to avoid needless sterilization process (Wang and Wan 2009; Venkata Mohan et al. 2013). As a result, for the practicability of microbial hydrogen production in future, an appropriate choice of biohydrogen systems jointly with deep knowledge of their biological and physicochemical functions is a decisive factor.

4.1 Biophotolysis (Water-Splitting Photosynthesis)

At present, the highly desirable hydrogen production mechanism is biophotolysis process. The oxygen producing photosynthetic biocatalysts (e.g., cyanobacteria and green microalgae) used for this process require only water and sunlight/light energy. This process has amazing theoretical advantage of an essentially unlimited supply of substrate and, potentially, the availability of incredible total energy. In green algae, a [FeFe]-hydrogenase drives the hydrogen production, whereas in the case of heterocystous cyanobacteria, nitrogenase is responsible for this process. However, this process is additionally separated as direct biophotolysis and indirect biophotolysis processes (Fig. 3). As illustrated in Fig. 4, in direct biophotolysis process, e^- resulting from water-splitting mechanism are transported through PS II and PS I to ferredoxin (Fd) as an e^- transporter, and consequently the reduced form of Fd(Fd_{re}) reduces an enzyme hydrogenase which plays a key role in hydrogen production (Melis et al. 2000):

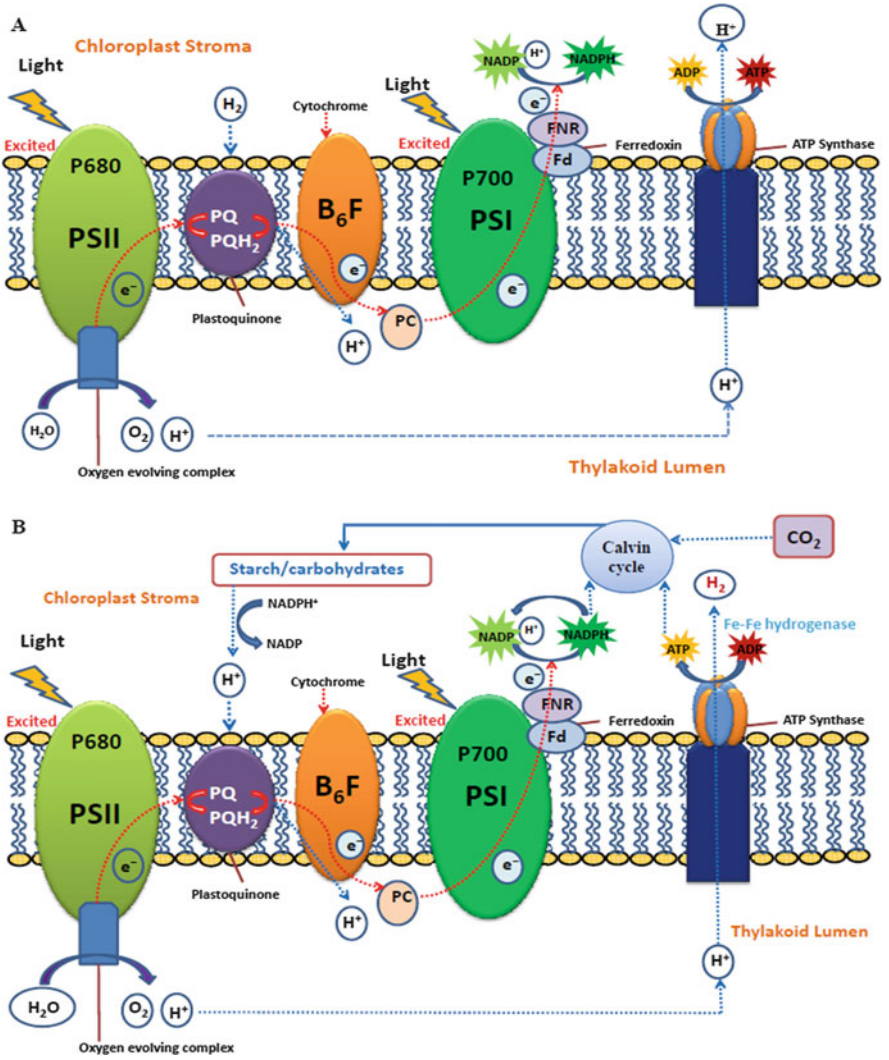

Fig. 4 Schematic representation of biohydrogen evolution through (**a**) direct biophotolysis and (**b**) indirect biophotolysis

$$2H^+ + 2 \text{ reduced ferredoxin } (Fd_{re}) \leftrightarrow H_2 + 2 \text{ oxidized ferredoxin } (Fd_{ox}) \quad (1)$$

whereas in the case of indirect biophotolysis process, solar/light energy converts to chemical energy (and stored in the form of carbohydrate molecules), which are further reused to produce biohydrogen. So far, these hydrogen producing systems have been intensively explored using green algae and cyanobacteria (Hallenbeck 2012). Since the biohydrogen production by cyanobacteria takes place in the

heterocyst and oxygen evolving photosynthesis as a microscopic-indirect biophotolysis process, associated with carbon dioxide fixation, extremely oxygen-sensitive nitrogenase can be protected, which leads to the formation of hydrogen.

$$\begin{aligned}&\text{Nitrogen } (N_2) + 8H^+ + 8e^- + 16ATP \\ &\quad \rightarrow \text{hydrogen } (H_2) + 2NH_3 + 16ADP + 16Pi \end{aligned} \tag{2}$$

On the other hand, hydrogen evolution by [FeFe]-hydrogenase and oxygen evolving photosynthesis mechanism cannot take place at the same time in green algae. Therefore, in order to attain sustainable hydrogen production, elemental sulfur deficiency, which caused a rigorous (≈90%) decrement in photosynthesis process, was subjected to microorganisms grown on acetate as carbon source, consequential in a severe decline in oxygen evolution rate together with enhanced respiration due to the presence of leftover acetate. This situation guides the microbes to grow in anaerobic environment to produce biohydrogen via utilization of few e^- from the leftover H_2O-splitting process (direct biophotolysis mechanism) and reserved carbon molecules (indirect biophotolysis mechanism) (Tekucheva and Tsygankov 2012).

In addition, persistent hydrogen production by single-cell, non-heterocystous cyanobacterium *Cyanothece* has been reported by growing in the glycerol-supplemented medium allowing for respiratory protection (Bandyopadhyay et al. 2010) or by replacing evolved oxygen with Argon gas during photosynthesis (Venkata Mohan and Pandey 2013). Very recently, alternative strategies to improving hydrogen production have been studied, together with a reducing the antenna size (Kosourov et al. 2011), downregulation of photosystem II proteins (Scoma et al. 2012), and alterations in operating parameters (Esquível et al. 2011).

4.2 Anoxygenic Photo-fermentation

This process similarly involves the transformation of solar/light energy in the direction of biomass with hydrogen and carbon dioxide. For this process, PNS photosynthetic bacteria as well as *Rhodobacter* species can be used to change organic acids (e.g., CH_3COOH, $C_3H_6O_3$, and $C_4H_8O_2$) to hydrogen and carbon dioxide in defined conditions such as anoxic and anaerobic environment. Indeed, under ammonium ions deficiency, these microorganisms capture light energy to change carbon-rich organic acids into hydrogen using nitrogenases (Azwar et al. 2014). On the other hand, oxygen-sensitive nitrogenase is not an issue for this mechanism since the PNS bacteria used to carry out this process have non-oxygenic photosynthesis mechanism (Chandrasekhar et al. 2015a). Nonetheless, nitrogenases also have numerous defects for hydrogen production due to inhibition of their expressions by NH_4, low catalytic action, and less photochemical efficiency (Brentner et al. 2010). In hypothesis, the photo-fermentation process is capable of completing conversion of organic compounds into hydrogen, even touching a

moderately high hydrogen partial pressure; meanwhile, hydrogen production is driven by nitrogenase and leads to the formation of ATP through the capture of light energy through a photosynthesis.

4.3 Dark Fermentation

Dark or heterotrophic fermentation by anaerobic microorganisms as well as some microalgae (such as green algae) can produce hydrogen by utilizing carbon-rich substrate in the absence of sunlight/solar energy under anaerobic environment (Figs. 5 and 6). So far, numerous studies on biological hydrogen production through this mechanism have been achieved by employing facultative and obligate anaerobes (Chandrasekhar and Venkata Mohan 2014a, b). This process happens at a higher reaction rate than bio-photolysis and photo-fermentation process. On the other hand, low hydrogen yield on substrate due to the production and accumulation of numerous acid intermediates is considered to be a major drawback. The dark fermentation mechanism aids to produce NAD(P)H and FADH as reducing powers during metabolism, which are followed by sequential re-oxidation by terminal e^- acceptor (TEA), which leads to the formation of ATP. In the case of aerobic respiration process, oxygen acts as a potential TEA that aids in generating ATP as energy-rich molecule via simultaneous regeneration of reducing powers (Chandrasekhar et al. 2015a). On the other hand, anaerobic respiration process utilizes a diversity of organic and inorganic compounds (e.g., SO_4^{2-}, NO^{3-}, etc.) as TEA (Fig. 6). Therefore, glycolysis is considered as vital metabolic process where glucose/substrate can be converted to pyruvate (CH_3COCOO^-), an essential intermediately metabolite (Fig. 5).

Further, CH_3COCOO^- in the acidogenic metabolic pathway together with hydrogen production under anaerobic circumstances leads to the formation of acid intermediates such as CH_3COOH, C_2H_5COOH, C_3CH_7COOH, $C_4H_6O_5$, and so on, collectively called as volatile fatty acids (Eqs. 3–7).

$$\text{Glucose} + 2H_2O \rightarrow 2CH_3COOH + 2CO_2(\uparrow) + 4H_2(\uparrow) \quad (3)$$

$$\text{Glucose} + 2H_2 \rightarrow 2C_2H_5COOH + 2H_2O \quad (4)$$

$$\text{Glucose} \rightarrow C_3CH_7COOH + 2CO_2(\uparrow) + 2H_2(\uparrow) \quad (5)$$

$$\text{Glucose} + 2H_2 \rightarrow C_4H_6O_5 + CO_2(\uparrow) \quad (6)$$

$$\text{Glucose} \rightarrow C_2H_5OH + CO_2(\uparrow) \quad (7)$$

As affirmed on top, both facultative and obligate microorganisms can produce hydrogen with a wide range of substrates (Chandrasekhar et al. 2015a). Facultative anaerobes convert CH_3COCOO^- to acetyl-CoA and further into $HCOO^-$ (formate) by pyruvate formate lyase (PFL) and then yield hydrogen by the catalysis of formate hydrogen lyase (FHL).

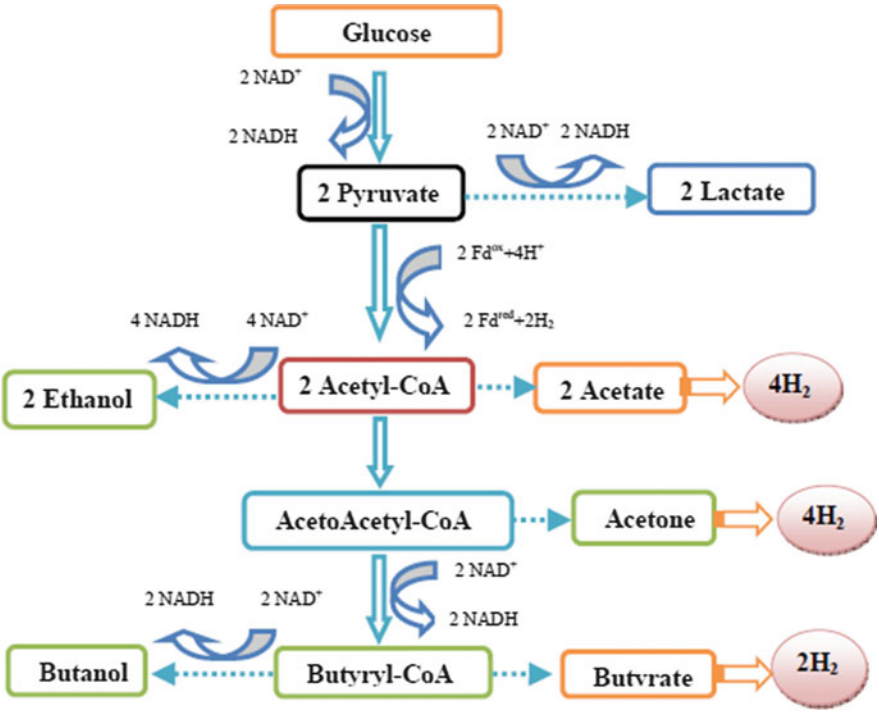

Fig. 5 Schematic illustration of anaerobic fermentation pathways for the breakdown of glucose and production of various metabolites

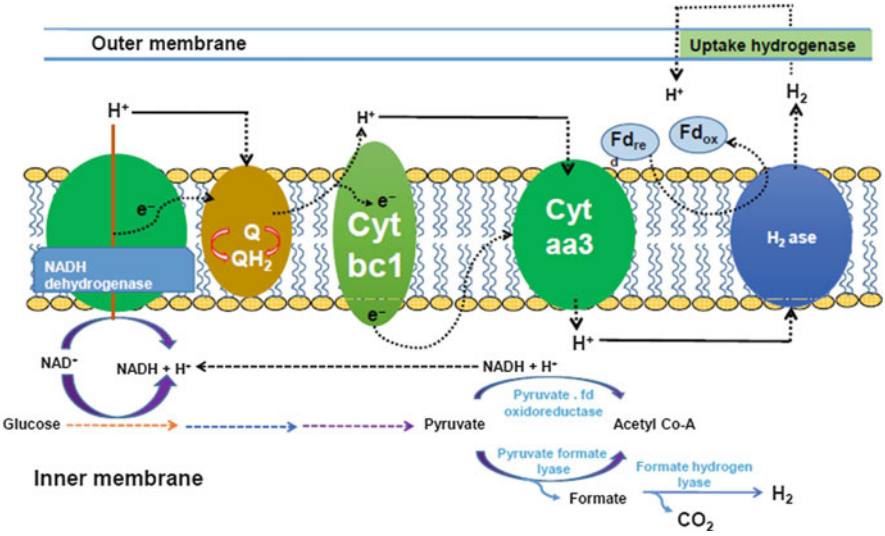

Fig. 6 Schematic representation of hydrogen evolution through dark fermentation

4.4 Microbial Electrolysis Cells (MECs)

MECs, an innovative and rapid method for producing hydrogen from different types of organic substrates, have been in very hasty progress over the last few years (Kadier et al. 2015, 2016). These MEC systems are fundamentally modified microbial fuel cells (MFCs) (Chandrasekhar and Venkata Mohan 2012; Kiran Kumar et al. 2012; Chandrasekhar et al. 2015b; Deval et al. 2016), which have been under investigation for decades. Applying MEC technology as an alternate electrically driven hydrogen production technology allows the conversion of organic substrates into hydrogen. Therefore, this technology is also termed as an electro-fermentation technology. Electrochemically active microorganisms produce carbon dioxide, e^-, and H^+ at the anode by oxidizing substrate (Fig. 7). The MEC technology moderately resembles an MFC (Venkata Mohan and Chandrasekhar 2011a, b), where the major difference exists with the requirement of a small input of external voltage. MECs can achieve more than 90% of hydrogen production efficiency, where reasonably less (33% of hydrogen) energy recovery is possible with the dark fermentation process (Chandrasekhar et al. 2015a).

The type of raw materials used and the high yield of hydrogen make the MEC technology a promising and economically viable technology. Its novelty lies in the fact that hydrogen can be efficiently produced from organic waste substrates like wastewater. With increasing concerns about climate change, limited availability of fossil fuels, and security of energy supply, hydrogen as the clean energy carrier for the future has drawn intense global interests due to its unique properties. Biohydrogen from MEC is predictable to play a vital role in a non-fossil fuel based future economy. MEC is becoming popular because microorganisms can be used as catalyst and wastewater can be effectively utilized as potential substrate for hydrogen production (Chandrasekhar et al. 2015a).

5 Economic Feasibility and Technical Challenges

In recent years, several researchers made an effort to make the biohydrogen production methods economically viable (Chandrasekhar et al. 2015a). Nonetheless, few vital technical obstacles endure (Table 1), and if these technical problems are overcome, the overall biohydrogen production efficacy will rise through the biological processes. However, these technical challenges might be overcome with an effective bioreactor design, process amendments, assortment of suitable substrate, and with the choice of appropriate and competent biocatalyst. During biohydrogen production, by-products produced by the microorganism compete with hydrogen evolving metabolic pathways and this rerouting reduces overall hydrogen yields (Pandit et al. 2014). Henceforth, numerous investigators are endeavoring to redirect the metabolic pathways to decrease the formation of the low-end metabolic products (Roy et al. 2015, 2016). To overcome the

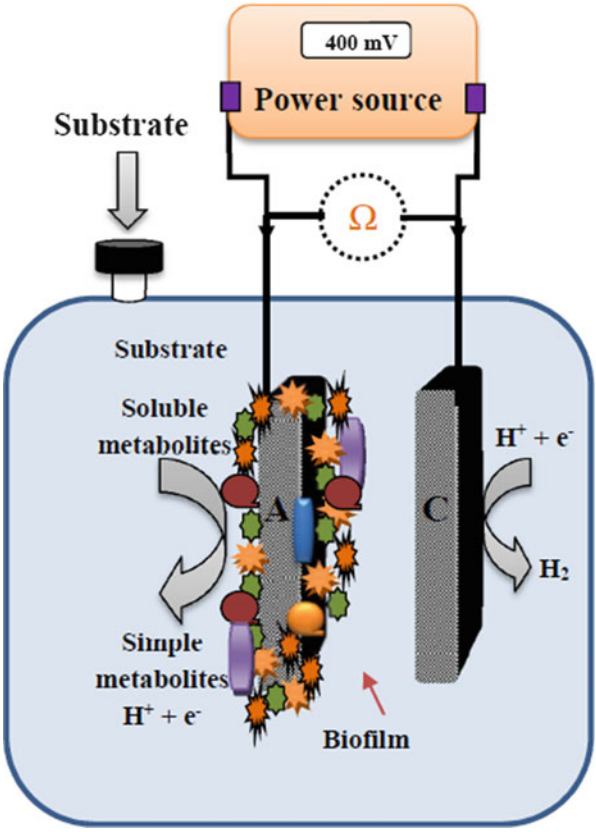

Fig. 7 Schematic illustration of single-chambered microbial electrolysis cell (MEC)

stoichiometric restriction (to produce closely 4 mol H_2/mol glucose; theoretical hydrogen yield) of the dark fermentation, an efficient metabolically engineered microorganism must be examined.

6 Strategies to Improve the Process Efficacy

Low substrate conversion efficacy and accumulation of by-products/acid intermediates were considered as major deterrents to the conventional biohydrogen process (Chandrasekhar and Venkata Mohan 2014a). In particular, the dark fermentative hydrogen production process has a major difficulty for practical applications due to low yield (4 mol of hydrogen yield from each mole of glucose), with a conversion efficiency of 33% (Cheng et al. 2012). In addition, at the end of the dark fermentation process, significant quantities of residual organic acids are still existent in the bioreactor effluents (Kumar et al. 2016a). Consequently, further treatments are necessary prior to disposal. Considering environmental and economic factors, it is

Table 1 Biohydrogen production processes and technical challenges

S. No	Bioprocess type	Technical challenge
1	Direct biophotolysis	• Low hydrogen yield • Inferior light conversion efficiency • Oxygen production(due to the activity of PS II) • Requisite for custom-made bioreactors
2	Indirect biophotolysis	• Necessity of an external light/solar energy source • Lower hydrogen yield caused by hydrogenase • Poor total light conversion efficiency
3	Photo-fermentation	• This photo-fermentation bioprocess is limited by sunlight as the energy source (day and night cycles) • Necessity of an external energy (light energy) source • Low hydrogen yield • Poor light conversion efficacy
4	Dark fermentation	• Low hydrogen yield • Low substrate conversion efficacy • Mixture of hydrogen and carbon dioxide gases as products, which require separation (biogas separation) • Thermodynamic limitations • Absence of terminal electron acceptors • Accumulation of acid-rich intermediate metabolites

wise to reuse the leftover organic fraction of effluents for additional energy/biofuel production together with waste treatment.

6.1 Integration Approaches

In recent years, various process integration approaches have been proposed to address numerous process confines to increase dark fermentative hydrogen production. Utilization of the residual organic substances (such as VFAs) from the bioreactor effluents as potential feedstock for additional energy recovery is practical and an excellent idea, as in the form of integrated bioprocesses (Table 2). Several secondary bioprocesses together with dark fermentation for hydrogen production, photobiological process for hydrogen production, methanogenesis process for methane production (Laurinavichene et al. 2012; Chandrasekhar et al. 2015a), microbial electrolysis system for hydrogen production (Cheng and Logan 2007), anoxygenic nutrient-limiting process for bioplastics production, hetrotrophic algae cultivation process for lipids production, and microbial fuel cell technology for electricity generation were possible secondary bioprocess which can be integrated with dark fermentation process considering as primary hydrogen production process. These integrated approaches enable further utilization of effluents from the primary process as potential substrate in secondary process for additional energy generation; consequently, whole process becomes economically more viable and practically applicable.

Table 2 List of few two-stage integration approaches investigated with dark fermentation

	First stage		Second stage		
Substrate	Process type	Reported yield	Process type	Reported yield	References
Cassava wastewater	H_2 (F_D)	54.22 ml H_2/g	CH_4 (F_D)	164.87 ml CH_4/g	Intanoo et al. (2014)
Food waste	H_2 (F_D)	85 L/kg TS	CH_4 (F_D)	63.3 L/kg TS	Kumar et al. (2014a)
Vegetable waste	H_2 (F_D)	17 L/kg TS	CH_4 (F_D)	61.7 L/kg TS	Kumar et al. (2014a)
Microalgal biomass	H_2 (F_D)	135 ± 3.11 ml H_2/g/VS	CH_4 (F_D)	414 ± 2.45 ml CH_4/g/VS	Wieczorek et al. (2014)
Glucose	H_2 (F_D)	1.20 mmol	H_2 (F_P)	5.22 mmol	Chandra and Venkata Mohan (2011)
Cheese Whey wastewater	H_2 (F_D)	2.04 mol	H_2 (F_P)	2.69 mol	Rai et al. (2012)
Fruit juice industry wastewater	H_2 (F_D)	1.4 mol H_2/mol hexose	Electricity (F_D)	0.55 W/m^2	Gonzalez del Campo et al. (2012)
Corn stover lignocellulose	H_2 (F_D)	1.67 mol H_2/mol-glucose	H_2 (MEC)	1.00 L/L-d	Lalaurette et al. (2009)
Spent wash	H_2 (F_D)	39.8 L	Bioplastic	40% dry cell weight	Amulya et al. (2014)
Pea shells	H_2 (F_D)	5.2 L H_2 from 4 L	Bioplastic	1685 mg PHB/L	Patel et al. (2012b)
Food waste	H_2 (F_D)	69.94 mmol	Lipid	26.4% dry cell weight of algae	Venkata Mohan and Devi (2012)
Food waste	Bioelectricity	85.2 mW/m^2	H_2 (F_D)	0.91 L	Chandrasekhar and Venkata Mohan (2014b)

F_D dark fermentation, *F_P* photo-fermentation, *TS* total solids, *MEC* microbial electrolysis cell

6.1.1 Photobiological Process

Residual organic acids can be readily consumed by photosynthetic bacteria (Rai et al. 2012). While metabolic intermediates from dark fermentation process can be efficiently utilized by few PNS bacteria, the two-stage integration approach of dark fermentation with anoxygenic photo-fermentation process will have double benefit of enhanced hydrogen yield together with concurrent substrate degradation. Green algae can also utilize residual metabolic intermediates from dark fermentation process, especially when acetic acid is used as a potential substrate (Bala Amutha and Murugesan 2011; Chandra and Venkata Mohan 2011). Nonetheless, photo-fermentation of residual organic substances from the hydrogen bioreactor is considered to be more difficult than dark fermentation process with respect to the process effectiveness, due to poor light diffusion, maintaining microenvironment, substrate inhibition, nutritional requirements for microbial growth, and risk of contamination (Özkan et al. 2012).

6.1.2 Biopolymers/Bioplastics

The organic acid-rich bioreactor reactor effluents from first-stage dark fermentation process are a promising feedstock for the production and accumulation of biopolymers [such as polyhydroxyalkanoates (PHA)] in bacterial cells at second-stage integrated bioprocess. The PHAs are a biopolyester which accumulates as cellular reserve storage materials, formed under additional nutrient and carbon-deprived conditions (Amulya et al. 2014; Kumar et al. 2014b, 2015c). These biodegradable polyesters are deposited as cytoplasmic inclusions in microbial cells, while extreme carbon source is offered and while other nutrients are growth-limiting. Production of PHA using pure strains cultures by supplementing synthetic substrates as carbon source (e.g., acetate, butyrate, etc), which is neither economically viable nor cost-effective for its production at large-scale bioreactors. VFA are simple acid-rich organic substrates with a lower number of carbons, which facilitates PHA production by the contribution of a less number of metabolic enzymes when compared to glycolysis and β-oxidation (Amulya et al. 2014). Production of PHB from different fatty acids (such as acetate, propionate, butyrate, etc) and organic effluents from a dark fermentation process were investigated under anoxic conditions using a mixed microbial population (Amulya et al. 2014; Chandrasekhar et al. 2015a; Kumar et al. 2016b). The bioplastic production coupled with H_2 production process and utilization of their effluents for methanogenesis enabled the whole process to be more economically viable (Patel et al. 2012b).

6.1.3 MEC-Driven Biohydrogen Production from Acid-Rich Effluents

Recently, considerable interest is focused on microbial electrolysis cells (MECs) due to the fusibility of integrating with other bioprocesses such as dark fermentation process (Lalaurette et al. 2009; Chandrasekhar et al. 2015a). MEC is an innovative and promising technology for hydrogen production from organic substrates, including carbon-rich wastewater and additional renewable resources. MEC as an alternate (electrically driven) biohydrogen production process can enable the biotransformation of substrate into hydrogen under applied external voltage. Certainly, this electro-fermentation process was viable for the utilization of a wide range of organic substrates to produce hydrogen together with simultaneous wastewater remediation (Wagner et al. 2009). A two-stage bioprocess was investigated to utilize organic effluents of dark fermentation bioreactor as potential feedstock for extra hydrogen production (Fig. 8).

In consideration of superior substrate conversion efficacy (90%) of MEC process, such a two-stage process integration approach, i.e., integration of MEC process with dark fermentative biohydrogen production process, could be a feasible and potential thought to attain higher hydrogen yield and to enhance substrate conversion efficacy (Chandrasekhar et al. 2015a).

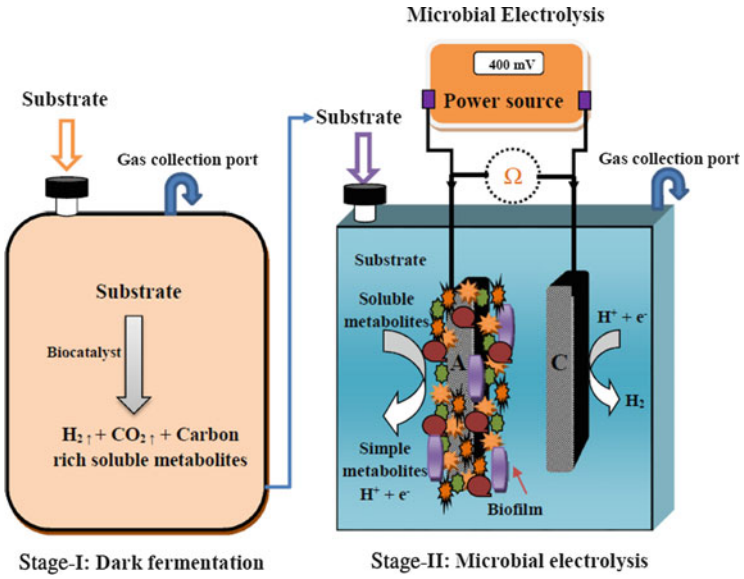

Fig. 8 Schematic representation of a two-stage process integration approach, i.e., integration of MEC process with dark fermentative biohydrogen production process to attain higher hydrogen yield (*A* anode, *C* cathode, *Ω* resistance)

7 Conclusion

In recent years, hydrogen has emerged as a clean, eco-friendly, and promising substitute fuel since it can be derived from renewable energy sources. It appears as the hopeful substitute to fossil fuel-based energy sources. To make hydrogen-based economy sustainable, it is necessary to practice renewable resources as an alternative of fossil fuels to produce hydrogen. Especially, bioconversion of carbon-rich substrate or H_2O into hydrogen is promising and eco-friendly technology. This chapter described the significance of biologically produced hydrogen as a clean energy carrier to build up hydrogen-based economy in the coming years. The importance of useful organisms involved in this technology was discussed toward the awareness of a stable hydrogen-based economy. Environmental impact of hydrogen as carbon-neutral energy carrier is also mentioned. Among the several bioprocesses identified to produce hydrogen, dark fermentation process has the dual advantages of gaseous energy generation and waste treatment. However, production of hydrogen from renewable resource through biological routes, using biocatalyst, is one of the potential areas to develop hydrogen-based economy. Even if numerous innovative approaches are expected in future to overcome few of the existing technical challenges, biohydrogen production process necessitates a multidisciplinary method for the biohydrogen technology to be economically viable and eco-friendly technology.

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