Biohydrogen Production: An Outlook of Fermentative Processes and Integration Strategies

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Abstract

The emanations upon combustion of petroleum fuels cause grave pessimistic impact on surrounding ambiance and global climate change. Therefore, the contemporary stance of scientific fraternity is to generate energy and mercantile products through biological methods with waste as a resource. Biohydrogen production from wastewater seems to be a promising green option for sustainable renewable energy. The process is feasible from practical point of view and can be operated under ambient conditions. It has been attracting attention due to its applicability to different types of wastewaters, and the production costs of biohydrogen can compete economically with other traditional methods. However, the crucial challenges like enhancing rate and yield for sustainable biohydrogen production still persist. During fermentation process, the undissociated volatile fatty acids (VFAs) and alcohols accumulate in the system leading to inhibition and redundancy in substrate degradation. Employing integration strategies with other bioprocesses like photo-fermentation or bio-electrochemical systems is the sanguine option to make the process frugally possible.

Keywords

Biohydrogen • Fermentation • Renewable • Energy • Photosynthetic

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12.1 Present Scenario

Paucity of worldwide petroleum reserves and apprehensions concerning environmental pollution prompt for the advancement of green energy substitutes (Arimi et al. 2015). Bioenergy has the sustainable potential that is appealing great curiosity amongst researchers across the world (Rama Mohan 2015). Recently, biohydrogen is cited to be one of the prospective areas of interest (Venkata Mohan 2009). In 2010, the market demand of international hydrogen production was 53 million metric tons and is anticipated to rise with a growth rate of 5.6% per annum. Worldwide commercial H_2 production presently is produced majorly from natural gas, oil and coal and to certain extent by water electrolysis (Markets and Markets 2011; Bhaskar et al. 2013; Rama Mohan 2015). Alternatively, H_2 from waste biomass/wastewater using biological methods as renewable resource is gaining much interest. By this way negative-valued organic waste is transformed to green energy abating the pollution (Ghimire et al. 2015). The governing prerequisite for wastewater treatment is an ideal prospect to produce biohydrogen. By this way, it diminishes the overall expenditure towards effluent treatment, and additional revenue could be incurred from biohydrogen as a fuel (Nissilä et al. 2014; Thi et al. 2016).

12.2 Biohydrogen Production

There are several routes for H_2 production, viz. physical, chemical, biological and thermochemical (Fig. 12.1). BioH_2 production is majorly contributed by anaerobic fermentation which is categorised as light-independent and light-dependent (Venkata Mohan et al. 2007a; Hallenbeck 2013). Light-independent fermentation process is usually referred to as dark fermentation that hires both strict anaerobes and facultative bacteria. This process results a higher rate of H_2 and formation of

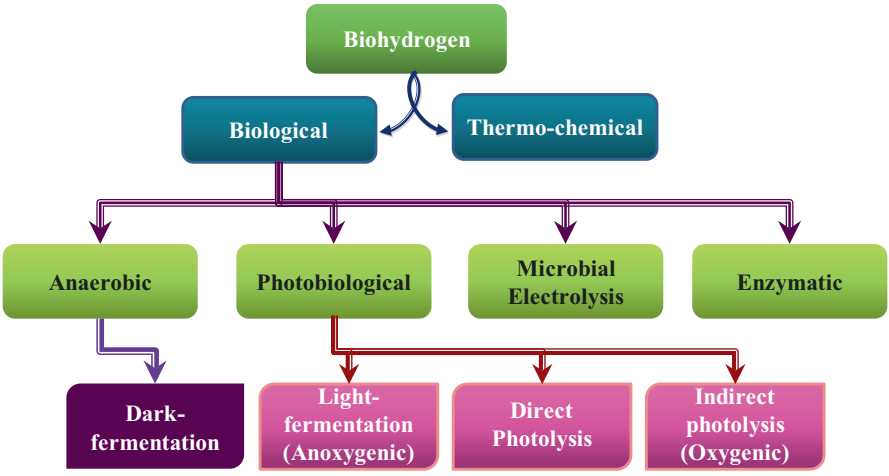

Fig. 12.1 Flowchart representing various routes for biohydrogen production

soluble metabolic products (SMPs), viz. volatile fatty acids, solvents, etc. Acidogenic anaerobes are incompetent of consuming these undissociated organic acids resulting in the accumulation of these acids leading to a drop in pH value. Subsequently, H_2 production is inhibited and, finally, results in low H_2 yields (Guo et al. 2010; Venkata Mohan et al. 2017). Light-dependent processes used by cyanobacteria and green algae are direct and indirect biophotolysis or via photo-fermentation mediated by photosynthetic bacteria. H_2 production by photosynthetic bacteria occurs by photo-fermentation of inorganic/organic acids (Lam and Lee 2013). The biochemistry varies considerably based on the biocatalyst, operational conditions, microenvironment and substrate (Lin et al. 2012).

12.2.1 Photo-Fermentation

Photosynthetic microorganisms, viz. cyanobacteria, photosynthetic bacteria and algae, have varied photosynthetic machinery (anoxygenic or oxygenic) that functions for H_2 production (Allakhverdiev et al. 2010). The process involves generation of a proton gradient by energy from sunlight and electron generation either by direct photolysis of water (Fig. 12.2a) or by indirect photolysis involving a parallel photosystem II (PSII)-independent process (Fig. 12.2b). In direct photolysis, the sunlight functions as a powerhouse for PSII ensuing production of oxidising equivalents that are used for the water oxidation into protons, electrons and O_2 (Krassen et al. 2009). Then, ferredoxin in reduced state is the electron donor for [FeFe]-hydrogenases in both types of photolysis. Thereafter, these reducing equivalents are transported to the chlorophyll α-dimer (P700) in photosystem I (PSI). The P700 is energised upon absorption of sunlight and then discharges electrons to the acceptor site of PSI containing iron–sulphur clusters via the electron transport chain (Hallenbeck 2013). Later, PSII is driven by water splitting where in the electrons are transported to reducing equivalents which then finally reduced by the [FeFe]-hydrogenase (*Hyd*A) to H_2. Hydrogenase enzyme performs as a discharge valve in presence of protons and electrons from the reduced ferredoxin to produce H_2. The activity of hydrogenase enzyme relies on the number of reducing equivalents from either of two photosynthetic processes (Constant and Hallenbeck 2013).

Microalgae are outstanding for their ability of high translation of solar energy to molecular H_2 (12–14%) by the oxidation of water molecules (Sambusiti et al. 2015). In direct biophotolysis, O_2 is generated as by-product of PSII that is a major suppressor of the hydrogenase enzyme; therefore, it can be operated for short periods of time (Blankenship et al. 1995). In indirect photolysis, the generated electrons and protons are generated and stored as starch during photosynthesis. Under certain stress conditions, the electrons and protons are fed into the plastoquinone pool and then onto *Hyd*A via PSI resulting in H_2 production. In case of microalgae and cyanobacteria, compounds for reserve accumulate during the Calvin cycle. At night, the compounds in reserve act as source energy for the cell activities. The change from aerobic to anaerobic condition is complemented by termination of a photosynthetic light reaction and generation of a surplus reductant, which is finally converted to H_2 by hydrogenase (Allakhverdiev et al. 2010). Under anaerobic conditions,

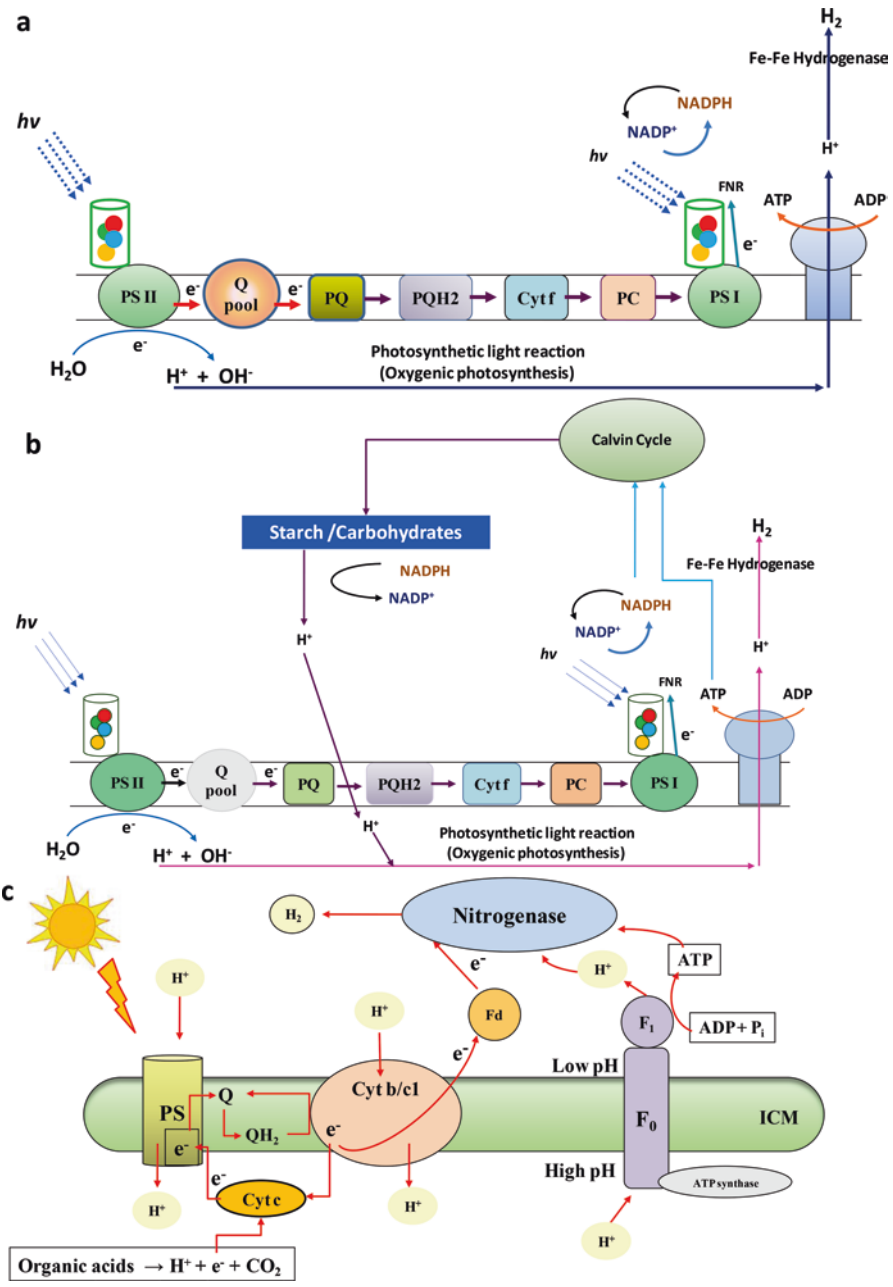

Fig. 12.2 Schematic illustration of the photosynthetic mechanisms for biohydrogen production – (**a**) direct photolysis involved in oxygenic photosynthesis; (**b**) indirect photolysis involved in oxygenic photosynthesis; (**c**) anoxygenic mechanism involved with photosynthetic bacteria

[FeFe]-hydrogenases (*Hyd*A1 and *Hyd*A2) catalyse the molecular H_2 by proton reduction. In cyanobacteria, H_2 is produced during nitrogen fixation by action of nitrogenase enzyme (Greenbaum 1988a, b; Melis et al. 2000).

Photosynthetic bacteria utilise the visible and/or near-infrared spectrum to produce H_2 by catabolising organic molecules by anoxygenic photosynthesis (Fig. 12.2c). In these bacteria, the [FeFe]-hydrogenase can easily evade the distress of oxygen, since they do not use water as source for electron generation (Melis and Happe 2001; Krassen et al. 2009). In this process, the bacteriochlorophyll (*BChl*) elicits for the formation of bacteriopheophytin (*BPh*) upon light absorption. The electrons from *BPh* are transferred to quinine pool (QA) and then to the cytochrome subunit of the reaction centre that generates a proton gradient for ATP synthesis and finally to H_2 (Hallenbeck and Benemann 2002). The efficacy of light energy translation to H_2 is significantly higher in photosynthetic bacteria than cyanobacteria since the quantum of light energy requirement is less than the water photolysis. Thus, the photosynthetic bacteria make photo-fermentation process more feasible, viable and its adaptability in consuming a variety of substrates (Chandra and Venkata Mohan 2011, 2014).

12.2.2 Dark Fermentation

Dark fermentation involves a multitude of biochemical metabolic reactions, viz. hydrolysis, acidogenesis, acetogenesis and methanogenesis (Lin et al. 2012). The composite organic matter is catabolised to simple molecules during hydrolysis and consequently fermented to organic acids during acidogenesis (Dahiya et al. 2015). As a special case, H_2 production is feasible from acetic acid by microbial consortia of acetogens and homoacetogens. Further, the acetoclastic methanogens translate the volatile fatty acids to methane during methanogenesis (Thi et al. 2016). Most often, acetogens nurture in syntrophic alliance with the hydrogenotrophic methanogens, thereby retaining low partial pressure to allow acidogenesis to become thermodynamically favourable. Therefore, the methanogenic activity is suppressed to increase the yields of bioH_2 as a sole metabolic by-product (Venkata Mohan et al. 2008a, b; Goud and Venkata Mohan 2012a; Sarkar et al. 2016).

The organic sugars are first metabolised during the glycolysis to pyruvate, a key precursor for subsequent microbial fermentation (Venkata Mohan et al. 2007b). Consequently, pyruvate can result in a variety of short-chain organic fatty acids, and H_2 is also produced during the metabolism (Fig. 12.3a). Anaerobes that are facultative metabolise pyruvate to acetyl-CoA and then to formate catalysed by pyruvate formate lyase and subsequently to hydrogen by formate hydrogen lyase (Nikhil et al. 2014b). Anaerobes that are obligate oxidise pyruvate to acetyl-CoA through pyruvate ferredoxin oxidoreductase. Metabolites formed during anaerobic substrate metabolism raise the accessibility of reducing equivalents within the bacteria (Wong et al. 2014). Protons from the redox mediators separate in presence NADH dehydrogenase enzyme and subsequently reduced to H_2 with electrons offered by the oxidised ferredoxin upon action of hydrogenase enzyme. The membrane-bound NADH dehydrogenase, cytochrome complex and other carrier proteins channel the

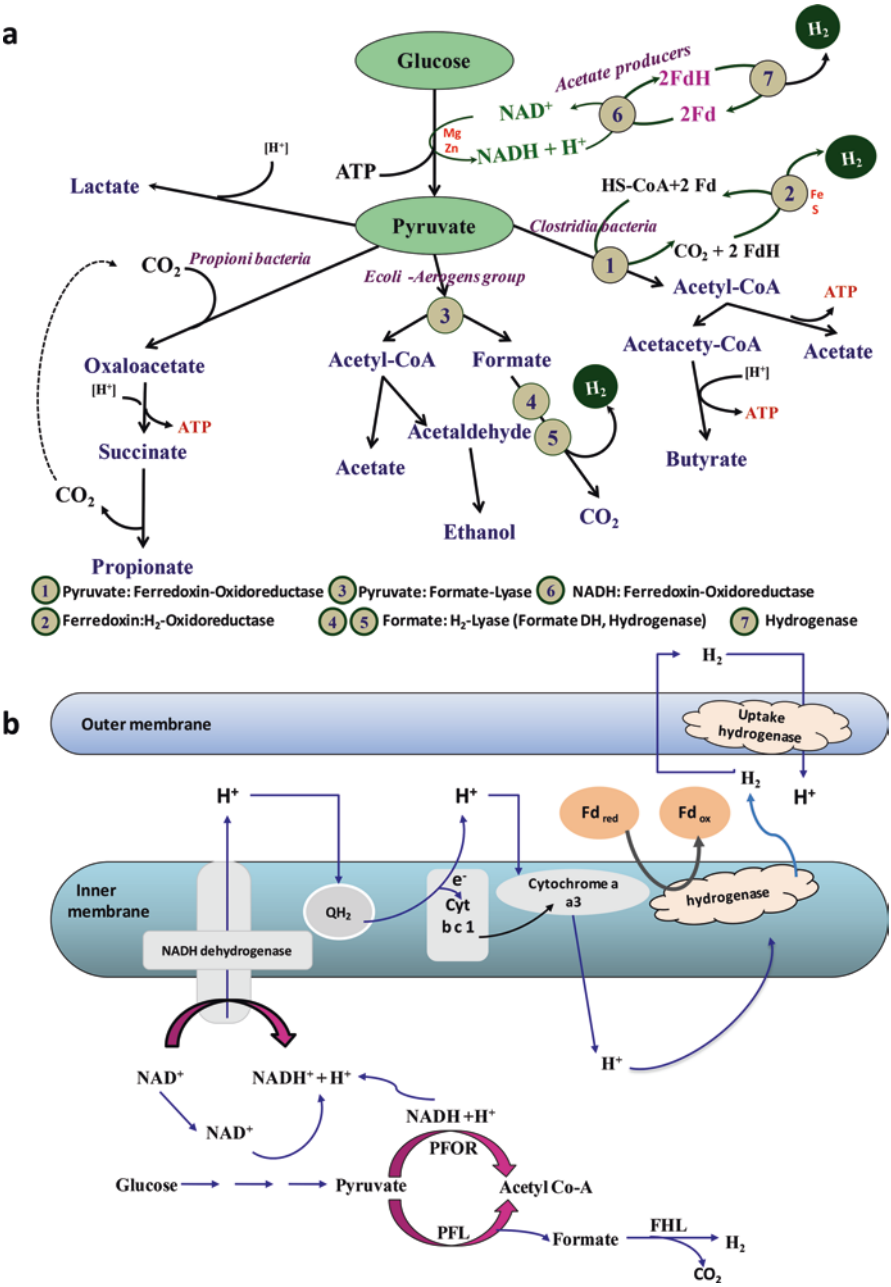

Fig. 12.3 (**a**) Different biochemical steps involved in the dark fermentation of glucose (organic matter); (**b**) schematic illustration of substrate conversion and H_2 production mechanism during dark fermentation

electrons via the quinone (Q) pool (Fig. 12.3b). Uninterrupted interconversions of quinone and protons assist in the transport of electrons to cytochrome b–c_1 complex and then to cytochrome aa_3. Ultimately, the electrons are transferred from cytochrome aa_3 to iron-containing protein ferredoxin. The ferredoxin in reduced state transfers electrons to the catalytic site of hydrogenase where protons are combined to form H_2 (Saratale et al. 2013).

Dark fermentation has gained significant credit as a practically feasible scheme amongst the other biological ways for H_2 production, especially when wastewater is considered as a substrate and assorted bacterial consortium as a biocatalyst (Agler et al. 2011; Nikhil et al. 2014a). The prominent features of this process are: process simplicity, less energy intensive, less carbon footprints, use of broad spectrum of organics and operation at ambient temperatures. Besides, the process is economically steady and robust for large scale of H_2 production (Venkata Mohan 2009). If the fermentation favours for the acetic acid synthesis, then the stoichiometric yield of H_2 is 4 mol per mol of glucose, whereas if the fermentation pathway is butyric acid, the yield of H_2 is 2 mol per mol of glucose. Yet, the experimental H_2 yield is lower than the stoichiometric yield, since a fraction of the substrate is consumed for bacterial cell mass and in few cases by-products might reduce the H_2 yield. Strategies are developed to attain superior H_2 yields at better rates, viz. novel reactor designs, variable mode of operation, inoculum type and pretreatment, nature and pretreatment of substrate and many more (Venkata Mohan et al. 2008a; Monlau et al. 2015; Sarkar et al. 2016).

The potential sources for anaerobic cultures are found almost in all types of natural environments. Anaerobic bacteria under the class of *Firmicutes, which* are apt biocatalysts for H_2 production (Venkata Mohan et al. 2011). A mixture of bacterial flora is largely preferred for continuous H_2 production; however, the microbial culture can show a divergence in H_2 production competence because of the co-occurrence of H_2-consuming bacteria in overall microbial diversity. In such conditions, conditioning of inoculum is performed to augment H_2 producers due to their compliance in hostile environments (Sarkar et al. 2013; Goud et al. 2017). Moreover, proton fluxes in/out bacteria affects the enzyme activity, biochemical pathways and substrate decomposition. pH is also acute to sustain ample cellular ATP levels, since surplus H^+ ions are pumped out using ATP to ensure cell neutrality (Srikanth and Venkata Mohan 2012). The initial pH can impact the duration of lag phase involved in spore germination and enzyme synthesis. The operational organic load of bioreactor can affect several functional issues which include accumulation of undissociated VFA and pH variations. This subsequently changes the diversity of microbial flora with subsequent amendments of the allied metabolic pathways (Van Ginkel and Logan 2005a; Goud and Venkata Mohan 2012b). Commercial biohydrogen production still poses certain process impede like the inhibitory effect caused by the undissociated VFA (Van Ginkel and Logan 2005b; Goud et al. 2014; Srikanth and Venkata Mohan 2014; Sarkar et al. 2017). Improving H_2 production rate and yield are the grave challenges to sustain economic production. In this regard, various strategies were reported in the literature. A few of them are selection and pretreatment of microbial consortia, immobilisation of consortia, statistical techniques for process optimisation, sequencing of bioreactors, bio-electrochemical treatment,

multiple process integration and bio-augmentation (Pasupuleti and Venkata Mohan 2015a, b). Commercial operation still poses certain process impedes like inhibitory effect of undissociated VFA (Van Ginkel and Logan 2005b; Goud et al. 2014; Srikanth and Venkata Mohan 2014; Sarkar et al. 2017).

12.2.3 Integration Strategies

12.2.3.1 Biohythane

A proportionate mixture of hydrogen and methane is commercially available as Hythane™, HCNG or methagen (Eden 2010). The recommended composition of H_2 with CH_4 ranges between 10% (v/v) and 25% (v/v) (Liu et al. 2013). Conventional methods could be unsustainable due to their reliance on exhausting petroleum reserves. Yet, the contemporary trends in bioenergy exploit the different biological routes for the production of both methane and hydrogen from waste organic matter. Literature reports are available on two-step fermentation process to produce a mixture of H_2 and CH_4 from wastewater (Venkata Mohan et al. 2008b; Mohanakrishna and Venkata Mohan 2013). This biological design assures the composition of biogas mixture ($CH_4 + H_2$) by regulating the environments of microbial fermentation. The usual operation is a two-step process that has a few advantages, but operation of two bioreactors is frugally not that sustainable (Cavinato et al. 2012; Willquist et al. 2012). A recent investigation reported biohythane from single-stage bioreactor using spent-wash wastewater (Pasupuleti and Venkata Mohan 2015b). In another study, a unique strategy was reported that augments acidogenesis for the production of biohythane (Sarkar and Venkata Mohan 2016, 2017).

12.2.3.2 Hybrid Dark–Photo Fermentation

A blend of both dark and photo-fermentation is a credible option to accomplish a yield of 12 mol H_2/mol glucose. Integrative fermentation can be considered as an operational and competent option to harness maximum H_2 yield with simultaneous wastewater treatment (Srikanth et al. 2009; Laurinavichene et al. 2012). A variety of photosynthetic bacteria are capable of H_2 production which can uptake the volatile fatty acids as carbon source and light as energy sources. Studies have been carried out with two-stage integration of heterotrophic dark with photo-fermentation for bioH_2 production (Chandra et al. 2015). It was noticed that photo-fermentation of acid-rich effluents is considered to be complicated due to light infiltration complications, multifaceted nutritional requirements, obligatory operational conditions, substrate (VFA and ammonia) inhibitions and vulnerability for impurity (Wang et al. 2009; Chandra and Venkata Mohan 2014).

12.2.3.3 Microbial Electrolysis

Microbial electrolysis is an electrically driven H_2 production process wherein the exchange of electron equivalents in carbon-based composites to H_2 gas involves bio-electrochemical reactions (Call and Logan 2008). A microbial electrochemical cell (MEC) has a prerequisite of additional potential to assist the metabolism of

undissociated organic acids into H_2 which is obligatory to cross the endothermic barrier (Cheng and Logan 2011). Therefore, the protons and electrons are driven with the supplementation of an external voltage from anode to cathode which is reduced to H_2. The typical redox potential for H_2 reduction is −0.414 V, and a voltage >0.11 V facilitates H_2 formation at the cathode. These applied potentials are moderately low compared to the applied external potential of 1.23 V for electrolysis of water. For example, H_2 (−0.414 V) from acetate (−0.279 V) at the cathode occurs upon appliance of voltage (−0.135 V). In reality, a relatively higher voltage is required than the prerequisite; owing to system overpotentials aroused by physicochemical and microbial influences.

It is reported that the applied potentials selectively enrich the growth of electroactive bacteria that efficiently reduce electrons (Arunasri et al. 2016). Operating an MEC can recover more than 90% of H_2 as against 33% by the dark fermentation. Thus, the application of MEC can be seen with the usage of acidogenic effluents rich in short-chain fatty acids for recovery of additional H_2/CH_4 production (Wagner et al. 2009; Modestra et al. 2015). The prospects of MEC over conventional water electrolysis are low-energy consumption, appreciable H_2 yields and wastewater treatment. Nevertheless, biocatalyst diversity, electrode materials, membrane, applied potential, substrate loading rate and reactor configuration critically affect the functioning of MEC (Nam et al. 2011). To begin with, studies were carried out in double chamber that allows separate capture of H_2 at cathode and prevents fouling by anodic bacteria (Pisciotta et al. 2012). But, separation leads to acidification at the anode chamber; so, removing the partition creates a single-chamber MEC that reduces the applied potential attenuating the pH and energy losses. Electrodes coated with platinum are commonly used as chemical catalysts for H_2 evolution as it significantly diminishes the cathode overpotential. Conventional methods use platinum which is costly, consumable and vulnerable to contamination by constituents in the effluents. Recently, research on biocathodes is of prime attention as they are eco-friendly and renewable (Jeremiasse et al. 2010; Nikhil et al. 2015b).

During the operation of MEC, the hydrogen evolution reaction (HER) is linked to the pH. The buffer capacity of wastewater is typically low resulting in accumulation of protons resulting in acidification at anode (Hamelers et al. 2010). The use of chemical buffers could be a possible option that alleviates the pH gradient. The efficacy of a buffer relies on its dissociation constant (pKa) and diffusivity. Buffers with pKa slightly above the operating pH (near neutral) aid to sustain the internal pH close to the external pH (Zhu et al. 2009; Liang et al. 2014). Inappropriate use of buffer (above 300 mM) affects the system and unsuitable for the effluent discharge; besides addition of salts would escalate the operating economics (Ambler and Logan 2011). It was reported that the external voltage exhibited a dual consequence over process performance by regulating the in situ buffering capacity using a biocathode (Lenin Babu et al. 2013b; Nikhil et al. 2015a). Integrating MEC with other processes to harvest biohydrogen generation is the state-of-the-art research amongst the scientific fraternity (Escapa et al. 2014). Small additional voltage with a high current density will be a crucial challenge for scaling-up of MEC to mercantile applications (Lenin Babu et al. 2013a; Venkata Mohan et al. 2014).

12.2.3.4 Bio-based Products

The dark-fermentative effluents are rich in VFA, and these can be used as possible substrate for production of polyhydroxyalkanoates (PHAs) commonly referred to as 'bioplastics' (Venkata Mohan et al. 2010; Sarkar et al. 2016). PHAs are biopolyesters of hydroxyalkanoates that amass as cellular storage materials produced under surplus carbon and nutrient-deprived environments (Patel et al. 2012; Fradinho et al. 2013). Commercial scale of PHA is produced using plant-derived resources and pure cultures that rise the production costs. Compared to other substrates (carbohydrates or proteins), the use of VFA facilitates PHA synthesis depriving the participation of glycolysis and β-oxidation pathways. Reports are available on PHA production from synthetic VFA and biohydrogen effluents (VFA-rich) under an anoxic microenvironment using a mixed bacterial culture (Venkateswar Reddy and Venkata Mohan 2012). If acetate and butyrate compositions are relatively higher amounts, then polyhydroxybutyrate (PHB) is the product type, and if propionate and valerate are relatively higher amounts, then polyhydroxyvalerate (PHV) is the product type. The combined production of H_2 and PHA followed by methanogenesis is possible option to make the whole process economical and sustainable (Patel et al. 2012; Venkateswar Reddy et al. 2014).

12.3 Waste Treatment vs Biohydrogen Production

Ideally, a unit kilogram of chemical oxygen demand (COD) equals to 5.2 mol of glucose, and upon dark fermentation each mole of glucose results in 4 mol and 2 mol of H_2 depending upon acetic acid and butyric acid pathways, respectively (Venkata Mohan et al. 2007c). On the other hand, photo-fermentation yields 12 mol of H_2 per mol of glucose. H_2 produced by acetate pathway of dark fermentation yields 89.6 l of H_2 per mol of glucose which is equivalent to 466.6 l of H_2 per kg COD. Assuming with only 40% of COD removal, then H_2 yield in dark and photo-fermentation is 125 g and 16.6 g, respectively. For example, if an industry discharging ~3×10^{11} l of effluent per annum containing a typical COD of 20 g/l, then it amounts to 6000×10^6 g of COD for that year. The yields of H_2 with dark and photo-fermentation are about 5×10^6 kg and 300×10^6 kg per year that accounts for many million dollars/year (at a rate of $ 4/kg H_2) (Markets 2011).

Rapid industrialisation is resulting in immense amounts of waste. Conventional treatment of wastewater is an energy-exhaustive process. Probing means to produce or harness valuable products from wastewater remediation are significantly pursued in recent times (Angenent et al. 2004; Venkata Mohan et al. 2010). The prerequisite requirement for their management markedly makes wastewater a choice for biohydrogen production, besides abating the overall treatment cost (Guo et al. 2010; Elsharnouby et al. 2013). Till date, considerable efforts were put on the application of various wastewaters for the production of biohydrogen through fermentation processes (both light-dependent and light-independent) (Hallenbeck 2013). Both wastewater treatment and H_2 production are equally important, and a balance should exist between technical expertise and substrate uptake with operating circumstances (Show and Lee 2013). Assessing these conditions is particularly important in

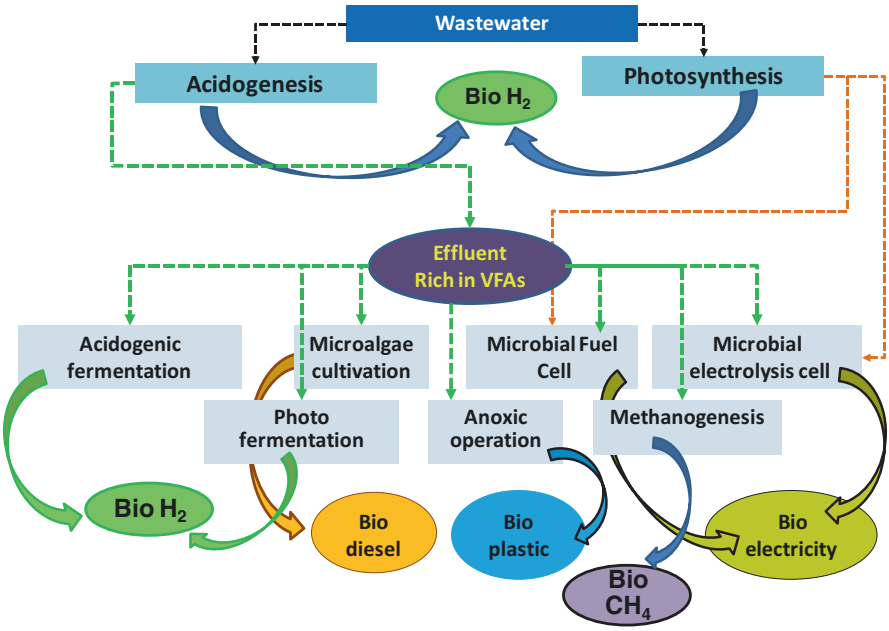

Fig. 12.4 Schematic details of various strategic routes possible for effective H_2 production and other value-added products

sustaining the financial possibility and environmental competence of the process (Arimi et al. 2015). An illustrative plan of strategies to recover energy and products for value additions along with wastewater treatment is represented in Fig. 12.4.

12.4 Future Perspectives

Hydrogen is an imperative substitute for energy domain and can be produced from a variety of production technologies (Dunn 2002; Nouni 2012). Presently, investigations are being dedicated on viable and eco-friendly biohydrogen from biomass to substitute fossil fuels (Hallenbeck and Ghosh 2009; Rama Mohan 2015). Waste biomass can be deliberated as the paramount choice and has the foremost prospective that encounters energy supplies and could assure fuel stock in the upcoming future. Utilisation of remaining organic portion after acidogenesis is also the most significant aspect to be paid momentous consideration (Mohanakrishna et al. 2010; Ghimire et al. 2015). Photobiological processes with acidogenic wastewater as substrate are comparatively less studied and need further exploitation. Multi-process combination is effectively evaluated for economic viability and commercialisation (Mohanakrishna and Venkata Mohan 2013; Wang and Ren 2013). Overall, a biorefinery concept with closed-loop approach visualises negative-valued waste as a potential renewable feedstock that will pave new opportunities for the growth of bio-based society with circular economy (Venkata Mohan et al. 2016).

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