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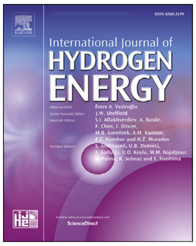

Bioreactors and biophoton-driven biohydrogen production strategies

Sadia Anjum [a], Shakira Aslam [a], Nazim Hussain [a], Muhammad Bilal [b], Grzegorz Boczkaj [c,d], Wojciech Smułek [b], Teofil Jesionowski [b,**], Hafiz M.N. Iqbal [e,f,*]

[a] *Center for Applied Molecular Biology, 87-West Canal, Bank Road, University of the Punjab, Lahore-53700, Pakistan*
[b] *Institute of Chemical Technology and Engineering, Faculty of Chemical Technology, Poznan University of Technology, Berdychowo 4, PL-60695 Poznan, Poland*
[c] *Department of Sanitary Engineering, Faculty of Civil and Environmental Engineering, Gdańsk University of Technology, 11/12 Narutowicza Str., Gdańsk 80-233, Poland*
[d] *EkoTech Center, Gdansk University of Technology, G. Narutowicza St. 11/12, Gdansk 80-233, Poland*
[e] *Tecnologico de Monterrey, School of Engineering and Sciences, Monterrey, 64849, Mexico*
[f] *Institute of Advanced Materials for Sustainable Manufacturing, Tecnologico de Monterrey, Monterrey 64849, Mexico*

HIGHLIGHTS

- Biophoton-driven biohydrogen production strategies are reviewed.
- Pathways for biohydrogen production are discussed with key examples.
- Role of various bioreactors and impact of nanoparticles on biohydrogen production.

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ABSTRACT

Given the current issues with global warming and rising greenhouse gas emissions, biohydrogen is a viable alternative fuel option. Technologies to produce biohydrogen include photo fermentation, dark fermentation, direct and indirect bio-photolysis, and two-stage fermentation. Biological hydrogen generation is a green and promising technique with mild reaction conditions and low energy consumption compared to thermochemical and electrochemical hydrogen generation. To optimize hydrogen gas output using this method, the activity of hydrogen-consuming bacteria should be restricted during the production stages of hydrogen and acetate to prevent or limit hydrogen consumption. Raw material costs, poor hydrogen evolution rates, and large-scale output are the main limitations in biological hydrogen generation systems. Organic wastes would be the most preferred target feedstock for hydrogen fermentation, aside from biodegradable wastes, due to their high amount and simultaneous waste treatment advantage. This study examined the three primary methods for converting waste into bio-hydrogen: microbial electrolysis cell, thermochemical gasification, and biological fermentation, from both a technological and environmental standpoint. The effectiveness and applicability of these bioprocesses in terms of aspects influencing processes and their constraints are discussed. Alternative options for improving process efficiency, like microbial electrolysis, bio-augmentation, and

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* *Corresponding author.*
** *Corresponding author.*

E-mail addresses: teofil.jesionowski@put.poznan.pl (T. Jesionowski), hafiz.iqbal@tec.mx (H.M.N. Iqbal).

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multiple process integration, are also considered for industrial-level applications. Biohydrogen generation might be further enhanced by optimization of operating conditions and adding vital nutrients and nanoparticles. Cost reduction and durability enhancement are the most significant hindrances to fuel-cell commercialization. This review summarizes the biohydrogen production pathways, the impact of used organic waste sources, and bacteria. The work also addresses the essential factors, benefits, and challenges.

Introduction

One of the most significant future issues will be the depletion of energy resources and increased pollution due to overusing fossil fuels. In the near future, renewable energy sources such as wind, sun, and biomass energy (biomethane, bioethanol, biohydrogen, etc.) are predicted to replace traditional energy sources such as fossil fuels. Furthermore, hydrogen has a larger energy mass-based content than other fuels and may be generated from renewable sources [1]. Biohydrogen is based on the green chemistry idea, in which food, vegetable, and manure wastes are processed and utilized to create hydrogen gas rather than being discharged into the environment. Chemical absorption, such as amine scrubbing and water washing, as well as membrane separation and physical adsorption, such as pressure swing adsorption (PSA) and temperature swing adsorption (TSA), allows for enrichment and separate CO_2 generated by fermentation [2]. Agricultural, food processing, forestry waste, sludges, effluents, an organic household, and yard trash are the most common organic waste feedstocks. Proteins, carbohydrates, fats, vitamins, fibers, and bioactive agents (antioxidants, enzymes, and antibacterial agents) are all significant components of such diverse materials. Pigments, flavors, medicines, biofuels, organic acids, biopolymers, and soil improvers could be obtained or created using a mixture of treatments followed by adequate purification and separation techniques [3].

Researchers have been particularly interested in dark fermentation since it can be performed in the absence of light, with little energy consumption, at surrounding temperature and pressure. It may yield valuable products such as H_2, CH_4, and other compounds from waste substrates. However, dark fermentation has a significant disadvantage in hydrogen generation since only about 33% of electrons in the substrate can be converted to H_2. The remaining 66% produces soluble liquid metabolites like alcohol, volatile fatty acids (VFAs), etc. To increase total energy recovery and minimize organic content, hydrogen-fermented discharge should be employed in photofermentation, methane synthesis, and microbial fuel cells (MFCs) [4]. Dark fermentative hydrogen generation via anaerobic hydrogen-producing bacteria is an environmentally friendly, long-term, and emission-free method of manufacturing hydrogen [5]. Nitrogen is a necessary ingredient for hydrogen generation via dark anaerobic fermentation. In the presence of 0.1% polypeptone, starch produced the greatest quantity of hydrogen (2.4 mol/mol glucose). Lin discovered that the C/N ratio had a more significant impact on hydrogen productivity than the particular hydrogen production rate [6].

Hydrogen is a frequent reactant in the petrochemical sector and has been identified as a possible fuel within the next 20 years. During the next five years, HIS (information handling service) Chemical predicts a nearly 5% yearly rise in global demand for hydrogen. Due to the continual increase of its economies, Asia continues to lead the way in growing demand. The use of hydrogen in transportation fuel desulfurization and the expansion of the transportation industry has both affected the increased demand for hydrogen. At the same time, the quality of crudes is deteriorating, resulting in a reduction in hydrogen production from crude processing. This has prompted refineries to reconsider their supply of hydrogen. Many studies have been done on the best way to produce hydrogen [7]. In metabolic processes involving molecular hydrogen creation, carbohydrate content as a carbon source has a beneficial influence on hydrogen production. As a result, carbohydrate-rich food and beverage industry effluent might be darkly fermented to convert carbohydrate content to organic acids and, ultimately, hydrogen gas. Furthermore, cumulative hydrogen generation from wastes surged in early studies before gradually decreasing until the batch reactor ran out of biogas. The development of granules or biofilms significantly improved biomass preservation. However, rapid hydrogen-producing culture growth and higher outgoing long-wave radiation conditions may limit the use of biofilm anaerobic biohydrogen routes [8]. Table 1 illustrates waste sources to produce hydrogen and its reported yield. A viable option for the large-scale, environmentally responsible production of hydrogen required to power a future hydrogen economy is biological hydrogen production. High potential exists for creating useful H_2 generation bioprocesses using currently available technologies. It is imperative to do additional research and development geared at raising H_2 synthesis rates and final yields. The future holds many promising possibilities for biohydrogen systems, including bioprocess integration, bioreactor design optimization, quick hydrogen removal and purification, directed hydrogenase evolution, metabolic engineering of the hydrogen-evolving microbe, and some unique approaches. The quick development of biological and engineering sciences will make it much easier to overcome current barriers and upcoming difficulties and open fresh possibilities for cost-effective hydrogen generation soon.

Techniques for biohydrogen production

Techniques, such as physicochemical, thermal, and biological ones, can be used to synthesize hydrogen. Chemical methods

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Table 1 – Waste sources for production of hydrogen and the yield.

Waste Source	Substrates	Pre-treatment	pH	Inhibitors	Hydrogen Yield	References
Industrial	Paper solid waste	Crushed to <0.5 mm	2.5% H_2SO_4	–	61.1 mmol/h/g	[74]
	Waste Peach Pulp	Boiled for 45 min	–	–	123.27 mL/g TOC	[75]
	Wastewater from citrus processing	Suspended	–	–	85.4 mmol/L	[76]
	Waste activated sludge	Gamma irradiation	12.0	Low pH	1.07 mL/L sludge	[77]
	Sewage sludge	Heat treatment (150 °c for 30 min) with alkaline conditions	–	Ammonia	39.0–220.3 mL/L sludge	[78]
Agricultural	Dairy cow solid waste	Dried and crushed; hydrolyzed the dilute acid	–	VFAs and alcohols	0.3 mol H_2/mol	[79]
	Sugarcane bagasse	Raw baggase heated at 120 °c for 30 min	Sulfuric acid H_2SO_4 (1%, g/v)	Phenolics	62.1 mL/g non-detxified sugarcane bagasse	[80]
	Wheat straw	Enzyme treatment	–	–	19.4 mL/g-VS	[81]
	Corn starch	Heated at 100°c for 30 min	–	Low pH	1.94 mol/mol glucose 1.19 mol/mol glucose	[82]
Municipal	Waste pastry	Crushed <0.5 mm	–	–	241.4 mL/g glucose	[83]
	Fruit and vegetable wastes	Crushed 2 mm	–	Acetate and Lactate	3.46 mol/mol	[84]
	Wastepaper	Crushed <0.15 mm and heat (100 °c for 90 min)	2.2	Furan	140 mL/g sugar	[85]
	Food waste	Crushed <0.5 mm	–	VFAs	57mL/g-VS (150°c)	[85]
Forestry	Poplar leaves	2% vicrozyme	–	Furfural	44.92 mL/g dry poplar leaves	[86]
	Waste sorghum leaves	Heat at 150 °c for 176 min	5.95% HCl	–	47.3 mL/g sugar	[87]

involve fuel reforming, partial oxidation, coal gasification, and steam reforming. Because biological technologies, such as photosynthetic and dark fermentation processes, utilize minimal energy and work at low temperatures and pressures, they are efficient and cost-effective. In the biological method, many bacteria can digest diverse chemical compounds and produce hydrogen as a metabolite. The primary selection factors for substrates are availability, affordability, carbohydrate content, and biodegradability. Glucose, sucrose, starch, and cellulose have been intensively studied to be used as carbon sources for biohydrogen generation. Because of their easy biodegradability and presence in many carbohydrate-rich wastewaters and agricultural wastes, they have been employed as model substrates for research reasons. Protein and fat-rich wastes are also suited for biohydrogen generation. Despite being less readily accessible than carbohydrate-rich wastes, they signify potential feedstock for biologically conversing organic wastes into hydrogen. Fig. 1 portrays various routes for biohydrogen production [9]. As demonstrated in Table 2, there are differences in environmental friendliness, substrate type, substrate efficiency, and hydrogen generation efficiency. Pure bacteria and cell immobilization techniques, like the choice of hydrogen-producing strains and embedding agents, have received most of the studies attention. However, using innovative biotechnology and mixed-culture technologies will increase the possibility of developing technology for biohydrogen production [10].

Pathways for biohydrogen production

Microbial electrolysis

Microbial electrolysis cell is a promising method for converting organic matter into an increased hydrogen yield. Most organic wastes are used as feedstock in microbial electrolysis, a light-independent biological process for hydrogen synthesis. For the conversion of organic molecules into biohydrogen, it combines bio-electrochemical and microbiological processes. In principle, the MEC uses minimal extra voltage (<1.23 V) and electrogenic microorganisms to transform organic molecules into useful hydrogen energy [11]. Electrochemically active microorganisms are utilized in this process, which can generate electricity from organic waste oxidation and biohydrogen generation at the cathode. Microorganisms that are thermophiles or extremophiles, as well as the proper electrode material, play a key role in hydrogen production in MEC. Carbonaceous materials, including carbon brushes, paper, cloth, and graphite, are frequently used as probable electrodes in MEC. Endothermic nature substrates (such as wastewater from acid, pharmaceutical, textile industries, and so on) may also be employed for biohydrogen generation via the microbial electrolysis process.

Electro-hydrogenises is a process in which anaerobic bacteria consume organic matter and produce hydrogen gas, known as MEC [5]. Anode-respiring bacteria (ARB) are

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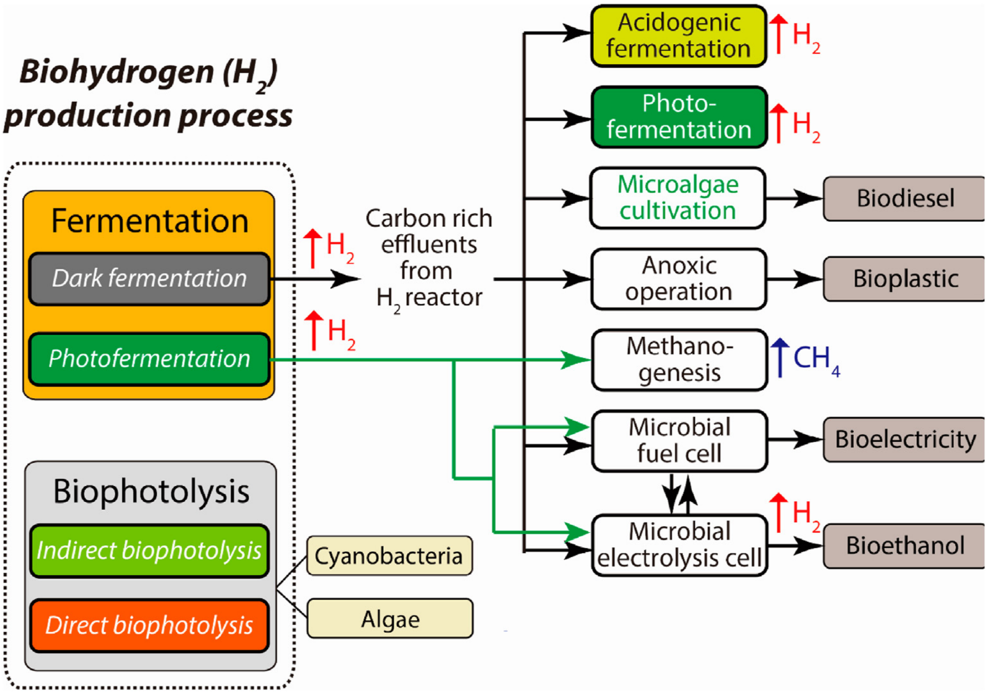

Fig. 1 – Various routes for biohydrogen process. Reprinted from Ref. [9] with permission under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/4.0/).

anaerobic bacteria capable of transferring electrons from a biodegradable substrate to a solid electrode [12]. To surpass the endothermic barrier and generate H_2 at the cathode, the applied voltage must be ≥ 0.11 V [13]. However, the kind of microbe, electrode materials, type of membrane employed, substrate concentration and composition, applied voltage range, and design of MEC all influence MEC performance [14]. Furthermore, this method has many substrates, requires low amounts of energy, and is more environmentally friendly than other biohydrogen generation methods.

Dark fermentation

Fermentation is the process of converting natural resources into energy by consuming microbes with the help of nitrogenases, hydrogenases, and enzymes without oxygen [15]. Dark fermentation is a less energy-intensive and straightforward method of producing biohydrogen. Although it's a conventional method, it's still promising for reducing sludge, ensuring wholesomeness, and recovering nutrients such as fatty acids (short chain) and hydrogen or energy on time [16]. With 1.9 as the net energy ratio (the ratio of hydrogen yield to non-renewable energy intake), this method was determined the most advantageous method of producing biohydrogen via biomass conversion, generating low-yield hydrogen [17]. Dark fermentation produces more hydrogen than photosynthetic fermentation [18], which happens through biological events, including glycolysis, pyruvate breakdown, and hydrogen creation [19]. Under anaerobic conditions, strongly anaerobic or facultative anaerobic bacteria produce biohydrogen through dark fermentation [20]. Anaerobic absorption of substances like glucose has been shown to convert quickly into hydrogen by forming hydrogen lyase. As a result, the pathway (formate) is important for hydrogen production in facultative anaerobes [21]. Although various organic materials, like carbohydrates, lipids, sugar, and protein, could be utilized as substrates, the glucose bioconversion process to acetate is frequently suggested for speculative hydrogen generation calculation. According to Zhang et al. [22], adding NADH increased hydrogen synthesis through the formate pathway while decreasing it through the NADH pathway, resulting in a net drop in manufacture. The NADH oxidation on the cell membrane causes a flow of electrons through the membrane, causing alteration in the cell's metabolic pattern and oxidation state. Because many countries prohibit the direct disposal of organic wastes containing energy, this strategy positively influences waste removal [23]. Fig. 2 represents biohydrogen production via indirect and direct biophotolytic routes [9].

Photo-fermentation

Photosynthetic bacteria that use sunlight and biomass can be used to generate biohydrogen. Gest and Kaman reported biohydrogen production through photo-fermentation utilizing photosynthetic bacteria in 1949. Since then, this method has demonstrated a productive synthesis of premium hydrogen without oxygen formation [24]. Biohydrogen is created via photo-fermentation by anaerobic or photosynthetic bacterial strains, such as *Rhodobacter, Rhodopseudomonas, Rhodospirillum, and Rhodobium,* via a nitrogenase-catalyzed process during the degradation of organic compounds in the presence of light energy [25]. Photo-fermentation production of hydrogen has become a worldwide key study topic in recent years caused of its main advantages of extensive raw material resources and

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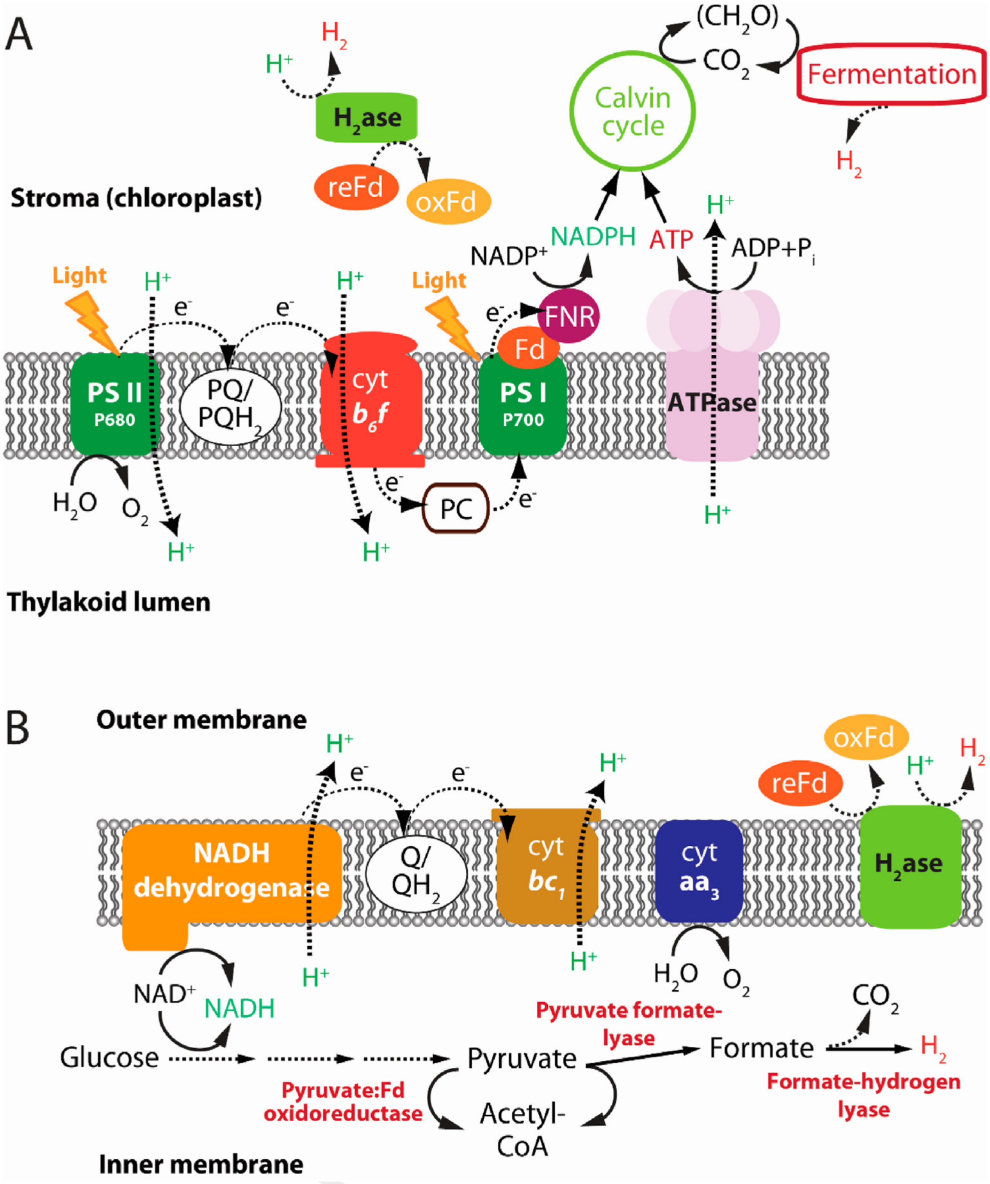

Fig. 2 — Schematic illustration of H_2 evolution through (A) direct/indirect biophotolysis and (B) dark fermentation: (A) PS II, photosystem II; PQ, plastoquinone; PQH_2, plastoquinol; cyt *b_6f*, cytochrome *b_6f* complex; PC, plastocyanin; PS I, photosystem I; Fd, ferredoxin; and FNR, ferredoxin-NADP $^+$ reductase. Approximately half of the evolved H_2 is from water splitting, and the rest of the H_2 is produced with e^- made from the fixed carbon by the activity of the PS I; (B) Q, quinone; QH_2, quinol; cyt *bc_1*, cytochrome *bc_1* complex; and cyt *aa_3*, the cytochrome *aa_3* oxidase. Reprinted from Ref. [9] with permission under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/4.0/).

thorough substrate usage [26]. Furthermore, it is efficient, environmentally benign, and capable of producing enormous amounts of hydrogen at room temperature and pressure. According to a previous study, the temperature was usually kept at 30 (°C) [24]. The hydrogen production was carried out for several days with constant stirring and a sampling interim of 12 h [27]. A unique bag was employed to capture hydrogen, and hydrogen concentration was then measured using the gas chromatography technique [27].

Several challenges have been recognized in photo-fermentation biohydrogen generation. Photosynthetic bacteria, for example, have limits in collecting sunlight's energy, which could result in a low light transformation efficiency for biohydrogen generation [28]. Bacteria require sterile environmental conditions for growth and hydrogen production [29]. Furthermore, nitrogenase enzymes need considerable energy to accomplish the photo-fermentation process due to increased activation energy. Furthermore, the cell shadowing effect reduces light penetration inside the photo-reactor, resulting in lower light intensity and worse biohydrogen generation performance. In order to create an efficient anaerobic photobioreactor on a big scale, a significant land covering area is required [27]. Organic substrates for hydrogen synthesis are currently an appealing concept for upcoming

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sustainable and renewable technologies. Even though lignocellulosic biomass was once thought to be a worthless raw material that should be discarded, various research groups are now attempting to turn it into new value-added products [18]. Agricultural residues, energy crops, industrial, forestry, home waste, algae, and animal manure can all be classified as lignocellulosic biomass [27].

Biophotolysis

Bio-photolysis is a photonic-driven biohydrogen generation method widely used in cyanobacteria and blue-green algae. It works in a similar way to plant photosynthesis [27]. Some microbes may use light energy to break water molecules and generate H_2. Biophotolysis is the name for the light-driven process, which may be classed as direct or indirect biophotolysis. Microalgae such as green algae (*Chlamydomonas reinhardtii*) and cyanobacteria (*Synechocystis*) use direct biophotolysis to convert water (substratum) into hydrogen and oxygen in the light presence and carbon dioxide in photosynthesis [30]. Photosystem I and photosystem II might absorb photons to form potent oxidants for water oxidation into O_2, protons, and electrons at photosynthetic reaction sites in microalgae chloroplasts. When an electron reduces a proton on condition that reduced ferredoxin of hydrogenase enzyme present in cells, hydrogen is produced [31]. It's worth noting that the hydrogenase enzyme, which is very O_2-sensitive and has been identified as the main bottleneck of algae photolysis H_2 synthesis [32], is responsible for most H_2 generation in blue-green algae. As a result, upon illustration, H_2 evolution occurs for a brief duration before the hydrogenase is inhibited by the accumulated O_2 [33].

The first step of indirect biophotolysis involves cyanobacteria photosynthesis, in which CO_2 and H_2O are transformed to organic compounds and O_2. In a light-independent mechanism, the cyanobacteria further break down the organic molecules from the first step into H_2, CO_2, and other soluble metabolites [34]. A single-celled, non-heterocystous cyanobacterium Cyanothece generated sustained H_2 synthesis when grown in media augmented with glycerol for respiratory conservation or when photosynthetically produced O_2 was replaced with Argon (Ar) gas [9]. By impermanently dividing H_2 and O_2 evolution into two different phases via CO_2 evolution or fixation, cyanobacteria and microalgae may manufacture H_2 from stored glycogen, and this strategy has solved the O_2 sensitivity problem [33].

Enzymatic in vitro *hydrogen biosynthesis*

Biohydrogen production using microorganisms has several significant limitations, among which are losses due to competing metabolic pathways, problems with maintaining sterile conditions and anaerobic conditions or relatively low volumetric productivity. Intensively developed enzymatic processes using pure biocatalysts may become the answer to these problems [35]. Cell-free synthetic enzymatic pathway biotransformations (SyPaB) allow to produce biohydrogen from carbohydrate substrates using enzymatic cascades driven by recombinant enzymes. In the process described by Ye et al. [36] H_2 is produced from cellulosic materials in a one-pot process, which was catalyzed by up to 14 enzymes and one coenzyme under modest reaction conditions (32 °C and atmospheric pressure). Another example is the work of Zhang et al. [37] who developed a synthetic enzymatic pathway involving 13 enzymes for producing biohydrogen from starch and water. As simple as it may be in theory, this solution requires overcoming several challenges and optimizing process conditions to make the most efficient use of all participating enzymes. Moreover, the costs of pure enzymes are definitely higher than costs of pure microbial cultures. Hence, this approach is still a long way from being implemented on an industrial or even pilot scale.

Bioreactors for biohydrogen production

Operational circumstances, process parameters, and reactor topologies all influence substrate alteration effectiveness and biohydrogen generation capability of microbial biocatalysts during dark fermentation. Bioreactor performance is governed not just by reactor design but also by custom reformation for individual conditions.

Continuous stirred tank reactor (CSTR)

The bacteria-producing hydrogen is mixed thoroughly in the CSTR, and the liquor biomass is retained. Since it has an identical constitution to the effluents due to mixing, it cannot preserve a massive portion of fermentative flora. These bioreactors are widely utilized to generate biohydrogen continually. Hydraulic retention time, temperature, pH are all extremely sensitive operating factors in these reactors. However, due to washout and a short HRP, its performance has been hampered in recent years. This constraint is primarily due to the biomass's poor settling characteristics. This constraint can be solved by physically retaining the microbial biocatalyst. It has recently been suggested that appropriate self-granulation [38], hydrogen producers flocculation, or bacterial immobilization on supporting materials [39] could aid in microbial retention, increasing biohydrogen output. Pong-chol et al. explored the effect of HRT on hydrogen production in both vertical and horizontal CSTRs. Shorter HRT limits hydrogen-consuming bacteria while also increasing treatment capacity [40].

Anaerobic fluidized bed reactor (AFBR)

The biocatalysts in these reactors generate biofilms and get adhere to the surface. The biomass suspension is held in place by an upward flow of wastewater. As a result, biomass serves as a catalyst, boosting biohydrogen synthesis. In contrast to CSTR, these reactors have excellent mixing and minimal shearing. As a result, mass transfer proficiency is high in these reactors. Despite the efficient mass transfer, these reactors are susceptible to biocatalyst washout, like CSTR. As a result, the output of biohydrogen falls [41]. These reactors can handle a higher substrate load and a shorter HRT [41]. The total soluble metabolites produced in the AFBR directly connect to the hydrogen yields. The only restriction of AFBR is the high energy required for fluidization [22].

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Up-flow anaerobic sludge blanket reactor (UASBR)

The UASBR system has obtained favor for biohydrogen production via anaerobic digestion [42]. The decrease in HRT has been connected to an elevation in HPR and H_2 content in the biogas generated; this may be due to the higher substrate feeding rate providing additional carbon sources for the microorganisms, which boosted microbial activity to make H2 more abundantly [43]. Because of their active substrate consumption, the bacterial mixed culture could adapt to increased substrate availability, resulting in early and high H_2 generation [43]. Over the 70 days of operation, the biogas generated by the UASB bioreactor contained only H_2 and CO_2, with no CH_4, showing that inceptive heat treatment of sludge and slightly acidic fermentation medium (pH 5.5–5.8) had successfully restricted the activity of CH_4-producing bacteria [44].

Membrane bioreactor (MBR)

In contrast to CSTR membranes, MBR membranes can separate solids from liquids and maintain biomass in the system, allowing HRT and SRT to be decoupled [45]. Modifications in SRT might boost microbial diversity in the system, allowing both hydrogen-producing bacteria and competing for hydrogen-consuming microorganisms to flourish more quickly, resulting in a shift in biohydrogen production efficiency [46]. Membrane fouling and high utility costs are the main drawbacks of membrane bioreactors. Buitron et al. looked into the biohydrogen generation potential of a granulated bio-solids membrane bioreactor fed brewery effluent as a substrate [40]. Membrane fouling, produced by depositing foulant materials on the membrane surface and within the pore matrix, has long been a problem with an MBRs because it reduces permeability and necessitates frequent chemical cleaning, shortening membrane life [47]. Fouling is an unavoidable occurrence that can be managed if the method and substances responsible are identified [48]. Fig. 3 represents different types of bioreactors for bio-hydrogen production.

Nanoparticles for enhancing biohydrogen production

Rapid advancements in nanotechnology have broadened its applications in various industries, including food, agriculture, pharmaceuticals, and energy. Nanomaterials have also been shown to improve a variety of biological functions. As a result of their influence on microorganism expansion, intracellular electron transport, and activity of metalloenzymes implicated in hydrogen creation, their use in improving biohydrogen production is favorable. The use of nanoparticles (NPs) as additives to boost biohydrogen generation has recently gained high interest, and a few studies confirmed its potential in this field. The influence of nanosized TiO_2 particles on hydrogen generation was investigated utilizing the photosynthetic bacterium *R. sphaeroides*. The adding up of titanium NPs augmented biohydrogen generation from organic wastewater as a feedstock considerably, demonstrating the capacity to create biohydrogen on a profitable scale from renewable organic wastewater. Iron NPs have been shown to enhance biohydrogen generation by enhancing the action of major biohydrogen-producing

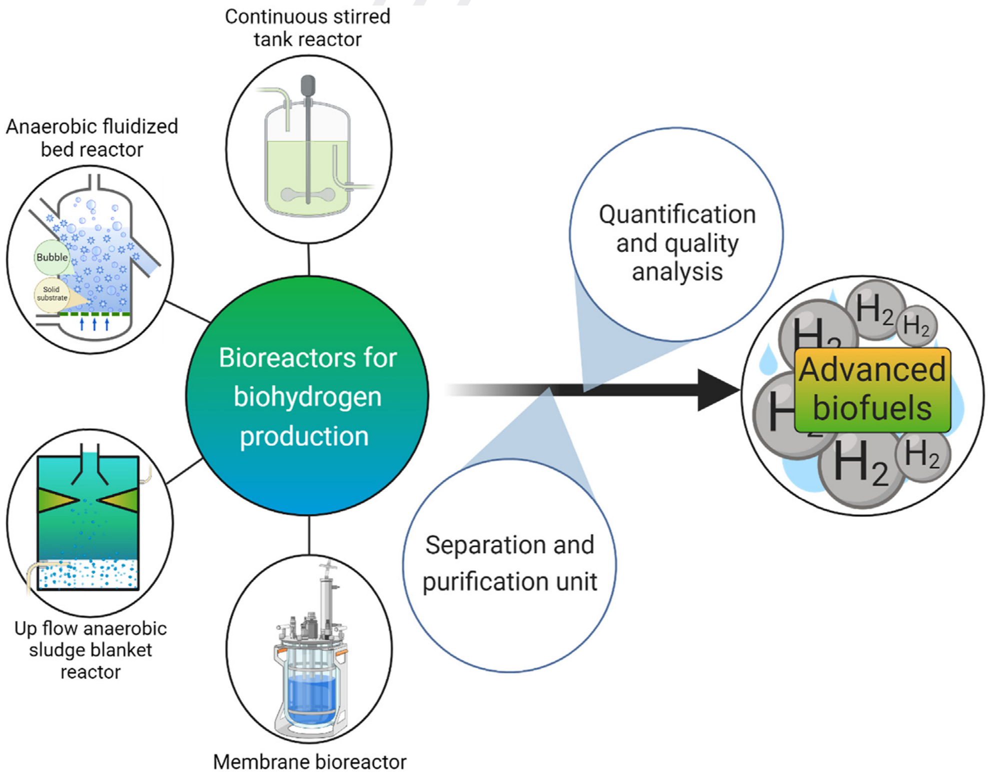

Fig. 3 – Types of bioreactors used for biohydrogen production. Created with BioRender.com.

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enzymes such as hydrogenases. This large improvement may be linked to the presence of Fe $^{2+}$ ions [49].

Raw material for biohydrogen production

Hydrogen may be produced from a variety of carbohydrate sources. However, most studies in reviewed literature have focused on hydrogen production by dark fermentation using pure sugars as substrates. Biohydrogen should be made from renewable, unrefined resources to benefit the environment and humanity. Renewable raw resources for biohydrogen generation include livestock wastes, agriculture, aquatic plants, leftovers from nutrition processing, lignocellulosic commodities, and biomass. If recycled properly, these feedstocks contain the potential to be converted into the chief future energy source. The main raw materials are raw materials: Sucroses, starches, arabinose, xyloses, and glucose. Dry matter biomass resources originating from agricultural waste, like potato peels, sugarcane bagasse, and sweet sorghums, might be another source of raw materials. Algae and microalgae are other raw materials that might be used as biohydrogen feedstocks [50]. Two enzymes are involved in the hydrogen generation pathway: ferredoxin-NAD reductase (FNR) and [Fe]-hydrogenase (FR). Upregulation of the hydA gene, which encodes the FR, has been utilized to boost hydrogen generation. In recombinant *Clostridium paraputrificum* overexpressing the hydA gene, hydrogen output rose 1.7-fold compared to a wild-type bacterium. Nonetheless, findings on hydrogen production are not yet accessible. FNR upregulation may boost hydrogen generation [9].

Pure carbohydrates

Carbohydrates are regarded as the most important biohydrogen sources. As a result, residues and biomass that have been enhanced with sugars and complex carbohydrates appear to be suitable for biohydrogen generation. Monosaccharides, disaccharides, and polysaccharides are the three forms of carbohydrates. Biohydrogen can be produced from a variety of carbohydrates, according to an earlier study. However, most studies have focused on polysaccharide hemicelluloses [51], cellulose [50], starches [29], disaccharides, and pure monosaccharides. Other simple carbohydrate macromolecules that have been used as hydrogen-generating source materials include cellobioses [52], maltoses [53], and lactoses [54].

Pure polysaccharides

Polysaccharides, also known as polycarbohydrates, are the diet's most common type of carbohydrate. They are called high molecular weight polymers because they include at least 10 monosaccharides evenly joined by glycosidic linkages. Natural polysaccharides, particularly seaweed-derived polysaccharides, such as agars alginate, laminarin, carrageenans, and fucoidans, have been discovered to have a wide range of therapeutic healing capabilities and health-promoting effects. Polysaccharides used for energy storage will provide easy access to the monosaccharide while maintaining a solid structure. Polysaccharides, such as chitin, are glucose monosaccharides that have been enhanced by adding a cluster of oxygen, nitrogen, and carbon [50].

Bacterial cellulose degradation is one of the most appealing methods for cellulose degradation. The biggest disadvantage of producing bacterial cellulolytic is that cell development consumes a lot of hydrolysis products. Nonetheless, this approach is thought to be cost-effective and simple to use. *Clostridium cellulolyticums* and cellulose are in close contact during the hydrogen generation stage. The bacteria's cells are shifted to the end of their growth cycle, indicating that the available celluloses have been depleted. These bacteria grow in soils, creating endospores and cellulose abridgment via cellulosomes, which are exocellular enzyme complexes. Chitins are present in the environment in the exoskeletons of flies and crustaceans, as well as the cell walls of most fungi and certain algae. Chitins are Nacetyl-D-glucosamine (GlcNAc) lined β-1,4 linked homopolymers with high biocompatibility and biodegradability that are nontoxic to the environment. After cellulose, chitin is the next most abundant polysaccharide, found mostly in the exoskeleton of arthropods and the fungal cell wall [50]. Zhang et al. [55] used chitin nanofibrous support to construct ultra-small nanosized particles catalyst for hydrogen generation. Gorrasi et al. [56] investigated the viability of employing unprocessed chitin as a substrate for bio-hydrogen production. Their breakthrough paves the path for exploiting this large biomass as a source of electricity. To speed and improve biohydrogen yield, it is advised that future process improvements and optimized culture medium be developed. Starch manufacturing firms release organic wastes and leftovers containing starch.

Pure disaccharides and monosaccharides

During disaccharide synthesis, the hydrogen atom of a monosaccharide reacts with the hydroxyl group of a separate monosaccharide, forming a covalent bond known as glycosidic linkage. Beta and alpha glycosidic connections are the two types of glycosidic connections. The most common disaccharides are sucrose, maltose, and lactose. Lactoses are disaccharides naturally occurring in milk and contain galactose and glucose monomers. Monosaccharides are generated from the hydrolysis of macromolecules abundant in many industrial wastewaters. The most common fermentation substrates are monosaccharides like pentose and hexose. Monosaccharides are the hydrolysate of a wide range of macromolecules that are used in batch culture experiments to maintain a diverse fermentation community. As a result, it's important to look into the utilization of a variety of monosaccharides in anaerobic activated sludge microbial communities, which could be a wonderful way to generate industrial biological hydrogen. Following the lead of past investigations, the new studies focused on agricultural, food, and manufacturing-related elements as potential biohydrogen source materials. Some wastewaters, particularly biodiesel leftovers containing glycerol and oil manufacturing wastewater, have spurred the development of biological hydrogen generation techniques. Microphyte biomass, created by carbon dioxide fixation during photosynthesis, has also been discovered to be a well-founded raw material [57].

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Biohydrogen production using organic waste

Hydrogen is used in a variety of industries, including the chemical industry, as a building block/feedstock for the production of heterogeneity of valuable chemicals and as an environmentally benign energy source in the transportation zone. Nonetheless, H_2 gas is currently mostly produced from non-renewable fossil fuels. Fermentation (including DF and PF processes), gasification, and the MEC system are currently widely developing and discussed technologies for producing biohydrogen from organic waste. Biohydrogen synthesis from biological sources mostly depends on various bacteria's metabolic action to break down complex waste products while simultaneously producing H_2 [58]. Cyanobacteria, anaerobic bacteria, and fermentative bacteria are three kinds of microorganisms that produce hydrogen. Photosynthesis allows cyanobacteria to directly break down water into hydrogen and oxygen in the presence of light energy. Organic substrates are used by photosynthetic bacteria. Anaerobic bacteria transform organic molecules into hydrogen as their only source of electrons and energy. Temperature, pH management, reactor hydraulic retention time (HRT), and other treatment system parameters may all be used to make biohydrogen utilizing bacteria like Clostridia [59].

Pre-treatment of wastes for biohydrogen production

Before biohydrogen production, various pre-treatment techniques of lignocellulosic biomass were used. These phases are critical for increasing sugar synthesis, avoiding carbohydrate loss, and developing inhibitory compounds for the subsequent conversion processes of fermentation and hydrolysis. It should be highlighted that the biohydrogen economy's long-term viability relies heavily on the cost-effective production of hydrogen and accessible availability to substrates. As a result, combining hydrogen generation with the treatment of profuse biomass waste and wastewater substrate is one of the most promising techniques to achieve this goal [60]. A favorable pre-treatment step should have low operating costs, cheap capital, and efficiency on much lignocellulosic biomass while recovering most of the lignocellulosic components [61]. Chemical, physical, biological, and physical-chemical approaches are the most common pre-treatment methods for biohydrogen generation. However, this research will focus on many pre-treatment approaches used in the photo-fermentation process of biohydrogen production. Physical treatment modalities can be classified into two categories: mechanical and irradiation. Milling, grinding, cutting, shearing, chipping, and other mechanical treatments are used.

Challenges in biohydrogen production

Significant technical hurdles include lower feed transformation effectiveness and acid metabolites in the reactor. When the reactor was used to process composite organic waste, the low substrate conversion efficiency was one of the most prevalent issues. Generally, this problem arises owing to the substrate's complexity or a lack of a specialized microbial community capable of hydrolyzing these complex substrates. Furthermore, one of the fundamental limits in practically all bio- H_2 processes is the formation of intermediate acid metabolites. The H_2-generating microbial population uses a simple substrate and produces volatile fatty acids as a by-product during the first step of bio-H_2 development [58]. Pre-treatment is required to break down the complex polymers when using lignocellulose waste materials as a dark fermentation substrate. When processing lignocellulose with the MEC method, the same pre-treatment is used. Furthermore, the MEC system prefers to collect hydrogen from various wastewater rather than complicated wastes like food waste or municipal solid waste [62]. These obstacles can be solved by designing efficient H_2-generating bioreactors, modifying processes, selecting acceptable feedstocks, and selecting suitable and efficient microbial strains.

Factors affecting biohydrogen production

One of the most significant factors influencing hydrogen output is temperature of the process. Many facultative anaerobes may generate hydrogen by breaking down glucose to pyruvate during glycolysis. The hydrogen output is influenced by metabolites produced during pyruvate breakdown. Carbon sources affect nitrogenase activity, which disrupts cyanobacteria's hydrogen production. The beginning load of glucose in the substrate was discovered to improve hydrogen yield during photosynthesis/fermentation. For harvesting biohydrogen, many temperature ranges have been reported: mesophilic (25–40 °C), thermophilic (40–65 °C), severe thermophilic (65–80 °C), or hyperthermophilic (>80 °C) [63]. Hydraulic Retention Time (HRT) allows germs to endure the mechanical dilution generated by uninterrupted volumetric circulation. When the fermentation duration is exceeded, the metabolic change from acidogenesis to methanogenesis occurs, which is not beneficial for hydrogen generation. HRT is influenced by several parameters, including the microorganisms utilized, and the kind and content of the substrate used [64]. High-rate bio- H_2 generation may be obtained by continually converting organic matter while keeping the hydraulic retention time (HRT) short. As a result, a different design technique than a generic microbial reactor is necessary [65]. A life cycle assessment is a well-established scientific approach for quantifying possible environmental consequences by taking into account all inputs (energy, materials, water, and so on) and outputs (products, emissions, energy, and so on) [62]. As a result, various factors must be considered when evaluating a waste biorefinery's environmental sustainability, including: variability, composition, availability, and properties of feedstock, transport and storage issues [3]. In the case of immobilization of free cells, an additional challenge is to provide a suitable carrier, that is, one that has adequate biochemical resistance, durability under process conditions, and at the same time will not adsorb hydrogen or retain it in the pores.

Discussion

Hydrogen is being considered a future energy market contender. Hydrogen is a viable fossil-fuel substitute. It

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creates water instead of greenhouse gases when combusted, making it a clean and ecologically beneficial fuel. Synthesis of hydrogen from organic waste sources via thermochemical and biological processes is essential to bioenergy production. Cyanobacteria, anaerobic bacteria, and fermentative bacteria are the three kinds of microorganisms that produce hydrogen [59]. It is critical to utilize many microorganisms capable of high hydrogen output and improved fermentation techniques of co-culturing to enhance dark fermentation performance. *Anabaena variabilis*, *Aphanocapsa montana*, *Nostoc linckia*, and *Synechococcus* are a few algae reviewed by Levin et al. that can produce biohydrogen. Green algae can either release or utilize hydrogen as an electron donor in the CO_2-fixation process when anaerobic circumstances are present [66]. Theoretically, thermophiles can produce 60–80% more hydrogen via dark fermentation than mesophilic bacteria [67]. Acetic acid can generate roughly 4 mol of H_2 per mol of glucose, while butyric acid can theoretically yield 2 mol of H_2 per mol of glucose [68]. High biohydrogen producers include *Clostridium pasteurianum, Clostridium butyricum,* and *Clostridium beijerinckii* [69]. However, *Clostridium propionicum* is a strain that produces biohydrogen ineffectively. There have been reports of hydrogen production by *Rhodopseudomonas capsulata*, *Rhodobacter sphaeroides,* and *Rhodospirillum rubrum* at rates of up to 50, 100, and 180 mL of H_2/L of culture/h, respectively [66]. Due to its significantly higher substrate-to-hydrogen yields (80%) and capacity to scavenge light energy under various light spectrum, photo-fermentation is the most preferred method of biohydrogen generation [70]. Most of the research focuses on boosting production yield, volume, and pace by altering Dark Fermentation conditions, and most researched reactors were designed on a laboratory scale without considering the viability of scaled-up production. Furthermore, proposed strategies for improving energy recovery at the laboratory level, such as substrate preparation, pH modification, temperature control, and so on, appear costly and difficult to implement on a broad scale [71]. In many experiments, the combined fermentation procedures have also yielded encouraging outcomes. However, scaling these techniques to large-scale manufacturing is still challenging [72].

Conclusion

Biological H_2 generation offers several advantages over traditional and fossil-fuel-based methods, including using carbon-rich industrial wastewater without emitting greenhouse gases. The individual approaches have inherent limitations, such as a poor H_2 yield and the buildup of volatile fatty acids, which render the procedure economically unviable. One viable replacement for fossil fuel is biohydrogen, an environmentally pleasant energy carrier like high-energy produces. Organic-rich substrates, such as organic waste/wastewater, are ideal for improving hydrogen production via dark fermentation. Hydrogen fuel is a viable cause of future energy in a quickly growing world, especially given the accelerated reduction of fossil fuel resources and exponential rise in energy consumption in different industries such as autos, electricity generation, etc. Biological techniques of hydrogen generation are more cost-effective and environmentally friendly than chemical procedures. Biological technologies can produce hydrogen gas from organic waste materials, algae species, non-food items, and other sources. Biohydrogen, which is produced through biological processes, is a clean fuel that may be used to reduce greenhouse gas emissions [73]. The fermentative biohydrogen generation was also influenced by wastewater properties, starting pH, and temperature conditions. It was discovered that sterilizing was the optimal pre-treatment method for restaurant food and raw starch wastes and could increase the maximum hydrogen output from restaurant food waste. The poor rates and yields of hydrogen creation are the key issues in bio-hydrogen synthesis from the garbage. Due to the poor hydrogen production rates, large reactor volumes are necessary for bio-hydrogen synthesis. As a result, more investigation into the impact of environmental factors on biohydrogen production is necessary. Extensive research and development studies are needed to improve the “state of the art” in biohydrogen generation.

Future perspective and recommendations

An essential step forward in the future of bio-hydrogen would be expanding the use and value of the residual waste streams produced by fermentation [7]. In the production of hydrogen, the design of the bioreactor is equally critical. A high-quality blueprint must boost manufacturing competence while keeping prices down. If CO_2 is generated alongside H_2 in dark fermentation and MEC, a suitable bioreactor design might include a gas separation technique that may confine CO_{2-} though, boosting the purity of H_2 formed [34]. The formation of dark fermentative H_2 denotes a fertile ground for renewable energy technology growth. On a research lab scale, several studies have been undertaken to explore strategies to boost the total yield of H_2. Hydrogen is regarded as “energy for the future” as its a sparkling energy resource with elevated energy content when compared to fossil fuels. Hydrogen, contrasting fossil fuels such as petroleum, natural gas, and biomass, is not commonly accessible in nature [6]. Due to its high hydrogen production rate, dark fermentation is undoubtedly the most suited to handle biomass waste. It is possible to make additional efforts to raise the hydrogen yield from lignocellulosic material so that it will eventually approach the Thauer limit. The top strains on the market continue to perform better, thanks largely to metabolic engineering. MEC is a viable second-stage treatment approach for effluent from dark fermentation, provided that the device scale-up and current density issue can be resolved. Renewable energy sources, such as solar systems, can be used to provide electricity for MEC. Before biophotolysis is regarded as feasible, numerous problems must be resolved. Low light conversion efficiency and hydrogenase's oxygen sensitivity are two problems. Although photofermentation has a lower light conversion efficiency than MEC, it can be an excellent alternative for treating dark fermentation effluent in the second stage. The ability of cell-free enzymatic systems to outperform in vivo production methods in terms of production rate and yield has been demonstrated. Many of the problems call for further basic research.

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1. Increasing the hydrogen production rates by enhancing the activity of the hydrogen-producing enzymes and the metabolic pathways required for the processes.
2. Creating strains that can utilize sunlight and other inputs effectively to boost hydrogen production.
3. Creating strains and reactor setups that can eventually be employed on a big scale to produce commercial hydrogen.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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