



Biohydrogen Production From Biomass Sources: Metabolic Pathways and Economic Analysis

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The commercialization of hydrogen as a fuel faces severe technological, economic, and environmental challenges. As a method to overcome these challenges, microalgal biohydrogen production has become the subject of growing research interest. Microalgal biohydrogen can be produced through different metabolic routes, the economic considerations of which are largely missing from recent reviews. Thus, this review briefly explains the techniques and economics associated with enhancing microalgae-based biohydrogen production. The cost of producing biohydrogen has been estimated to be between \$10 GJ⁻¹ and \$20 GJ⁻¹, which is not competitive with gasoline (\$0.33 GJ⁻¹). Even though direct biophotolysis has a sunlight conversion efficiency of over 80%, its productivity is sensitive to oxygen and sunlight availability. While the electrochemical processes produce the highest biohydrogen (>90%), fermentation and photobiological processes are more environmentally sustainable. Studies have revealed that the cost of producing biohydrogen is quite high, ranging between \$2.13 kg⁻¹ and 7.24 kg⁻¹ via direct biophotolysis, \$1.42 kg⁻¹ through indirect biophotolysis, and between \$7.54 kg⁻¹ and 7.61 kg⁻¹ via fermentation. Therefore, low-cost hydrogen production technologies need to be developed to ensure long-term sustainability which requires the optimization of critical experimental parameters, microalgal metabolic engineering, and genetic modification.

Keywords: microalgae, dark fermentation, photofermentation, biophotolysis, techno-economic analysis, renewable energy

INTRODUCTION

The energy crisis has emerged as the most significant impediment to the advancement of human civilisation (Ahmed S. F. et al., 2021). Rapidly diminishing fossil fuel reserves have been posing a threat to the world's energy security (Mahlia et al., 2020). Therefore, attempts have been made to transition away from an overreliance on fossil fuels and utilize renewable energy sources such as solar

energy, wind energy, geothermal energy, biogas (Mahmudul et al., 2020), ethanol, and biodiesel (Mofijur et al., 2020). With a production of 55 million tonnes every year, hydrogen is an important eco-friendly industrial feedstock due to its high energy density (Wang and Yin, 2018; Bolatkhan et al., 2019; Gielen, 2019). The hydrogen market is experiencing up to 10% growth year on year and is forecast to reach over \$191.80 billion in 2024 (Bolatkhan et al., 2019; Nagarajan et al., 2021). However, the commercialization of hydrogen as a fuel suffers major bottlenecks in terms of the techno-economic and environmental feasibility of conventional methods (Das et al., 2019), the dependence on fossil fuels to drive some processes (Dawood et al., 2020), and challenges related to transportation and storage (Nagarajan et al., 2017; Abdalla et al., 2018). Biological techniques for hydrogen synthesis, such as using organisms like microalgae, can effectively address these limitations. Unlike most biological sources, microalgae have many benefits, such as a high growth rate (Muhammad et al., 2021), low requirements for energy, high-quality land and water; a lower environmental impact (Ahmed SF. et al., 2021); and the absence of a need for costly pretreatment processes due to the lack of lignin (Singh and Das, 2020).

Microalgal biohydrogen production has occupied much interest, especially in recent years after it was revealed that various useful metabolites are also produced as co-products (Siddiki et al., 2020). Nevertheless, difficulties in process engineering, low productivity of microalgae, oxygen sensitivity, and operational costs, and inadequate insights on strain capacity, hinder the commercialization of microalgae-based biohydrogen production. Even though there are several metabolic routes to produce biohydrogen from microalgae with an improved yield, recent reviews (Buitrón et al., 2017; Sharma and Arya, 2017; Shobana et al., 2017; Show et al., 2018; Razu et al., 2019; Show et al., 2019b; El-Dalatony et al., 2020; Jiménez-Llanos et al., 2020; Mona et al., 2020; Salakkam et al., 2021) have not provided the comprehensive economic evaluation of such strategies, which has resulted in a dearth in the understanding of process feasibility. Therefore, in addressing this knowledge gap, this mini-review explicates the metabolic routes that enhance microalgae-based biohydrogen production and provides insights into their economic considerations to guide future work in the scaling-up of these technologies.

METABOLIC PATHWAYS OF BIOHYDROGEN PRODUCTION FROM BIOMASS SOURCES

Biohydrogen production, as an alternative to many conventional energy sources (Foong et al., 2020), is viewed as a strong prospective contender in the energy economy (Wang and Yin, 2018). Microalgae biomass is frequently utilized to generate various types of bioenergy (Ge et al., 2020), with biohydrogen being one of the most promising categories of bioenergy that can be generated from microalgae. Biohydrogen can be generated from microalgae using two alternative processes: i) direct

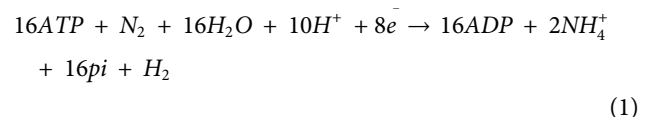
production of biohydrogen by microalgae, and ii) microalgal biomass utilisation as a fermentation substrate by other microbes. The first process can produce biohydrogen ranging from 0.015 to 1.084 mmol/L.h whereas for the second process this may vary from 0.35 to 10.26 mmol/L.h, depending on the feedstocks, culture conditions, and microorganisms used (Oncel, 2015). However, these two processes can be combined to improve the biohydrogen production rate. The metabolic pathways of biohydrogen production from microalgae are illustrated in **Figure 1**. These technologies for producing biohydrogen using microalgae are discussed in the following sub-sections.

Fermentation Process

Biohydrogen production using the fermentation process is found to be effective as it is environmentally friendly, puts less pressure on the consumption of fossil fuel, and controls pollution. Therefore, this method of producing hydrogen has huge potential as an alternative to fossil fuels. The fermentation process can be separated into dark fermentation and photofermentation.

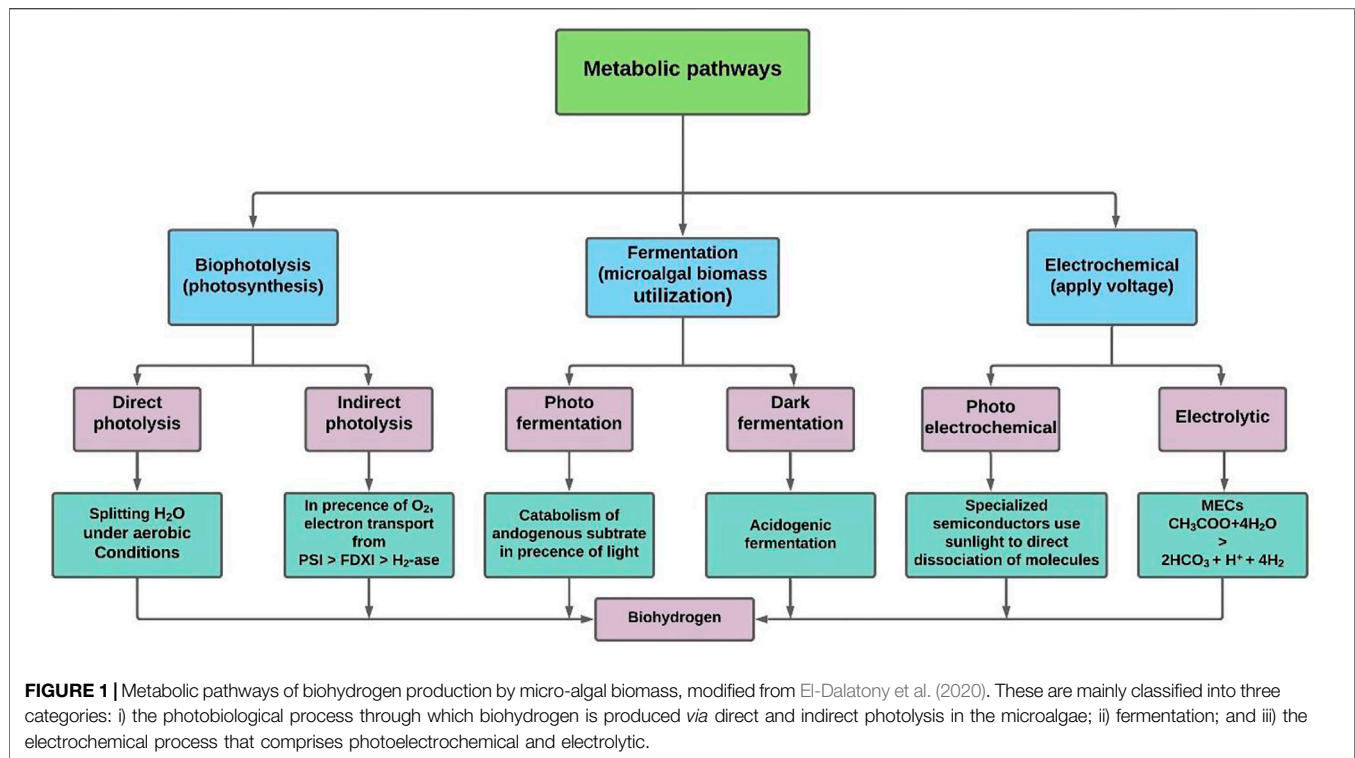
Photofermentation

Photofermentation refers to fermentation processes that employ sunlight as an energy source for photosynthesis. Instead of using sugar, this pathway utilises light as a source of energy. Therefore, photofermentation is advantageous compared to other pathways. Hydrogen production by photofermentation is described by the following equation (Baeyens et al., 2020):



For this pathway, instead of water, the green algae gain electrons from heterotrophic fermentation and the catabolic reaction of the endogenous substrate. Electrons are separated from organic substrate products by photocatabolism. Light energy and oxidative carbon metabolism also play a role in the extraction of electrons. Here e^- is generated from the endogenous substrates using catabolism. These electrons undergo the plastoquinone (PQ) pool which is situated in Photosystem I (PSI) and Photosystem II (PSII). The transfer of electrons to the PQ pool is associated with nicotinamide adenine dinucleotide phosphate (NADPH)- plastoquinone oxidoreductase (PQOR) in the chloroplast of different types of vascular plants (Dalena et al., 2017). In this process, PSI assimilates light energy and generates electrons. The redox potential of this electron can be elevated through the transfer of these electrons to the corresponding level of [Fe]-H₂ase and ferredoxin (FDX) (Mosebach et al., 2017).

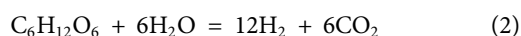
The dark and anoxic environment induce ([Fe]-H₂ase) and help to produce a high yield of biohydrogen. When DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) is present, incubating microalgae culture in these conditions is beneficial for biohydrogen production (El-Dalatony et al., 2020; Mu et al., 2020). Compared to dark fermentation, photofermentation is



considered a better process to produce biohydrogen. Zhang et al. (2020) compared dark-photo fermentation, photo fermentation and dark fermentation in terms of biohydrogen production. Photo fermentation was found to be the most effective as it had a high hydrogen content (maximum 58.90%), and the efficiency in energy conversion (10.12%) was found to be the highest in this method. In terms of hydrogen yield, photo fermentation was also found to be effective compared to the other two pathways. Photo fermentation achieved the maximum hydrogen yield of 141.42 ml(g TS)⁻¹ while dark fermentation generated the minimum hydrogen yield of 36.08 ml(g TS)⁻¹. Therefore, the photo fermentation process can be a good choice in producing biohydrogen. However, compared to biophotolysis and electrochemical processes, it has low efficiency in sunlight conversion and biohydrogen yield.

Dark Fermentation

The dark fermentation pathway is light-independent. It performs heterotrophic fermentation using microbes or microalgae. Green algae rich in carbohydrates are the most common type of microalgae used to produce biohydrogen in this process (Banu et al., 2020). Substrates such as glucose, sucrose, starch, and cellulose are used to enhance the production rate of hydrogen. 1 mol of glucose gives 12 mol hydrogen atoms through complete conversion:



This produces 4 mol of hydrogen through the method of dark fermentation. The by-products of the reaction are organic acids (Mona et al., 2020):



The dark fermentation method involves converting complex matter into simpler products through hydrolysis (Kucharska et al., 2019), for instance, complex carbohydrates into reducing sugars. These simple compounds then go through acidification instigated by enzymes. Fermentative bacteria secrete these types of enzymes. In the next stage, these acid products further transform into biohydrogen and acetate through a process called acetogenesis. It is essential to stop the progress of the methanogenesis and collect biohydrogen produced in the prior steps for the production of biohydrogen in the same pathway (Osman et al., 2020).

In the process of dark fermentation, biohydrogen is produced by two mechanisms. One of them is by a catabolic transformation of formic acid while the other one is through re-oxidation of nicotinamide adenine dinucleotide hydride (NADH), which is catalysed by the H₂ase pathway (El-Dalatony et al., 2020). Pyruvate ferredoxin oxidoreductase (PFR) is used to catalyse pyruvate oxidation. Consequently, there is a need to stabilize the oxidative-reductive reaction. This can be done through the production of compounds like butanol and ethanol from the transformation of NADH (Wang et al., 2020). Biohydrogen produced by the dark fermentation process has a comparatively higher rate and amount than other methods. The issue is with the low hydrogen concentration of about 40–60%. For this, without a purification step, the fermentative effluent gas cannot be used by the fuel cells (Mona et al., 2020).

To enhance the performance of the dark fermentation process, Song et al. (2020) employed enzymolysis and acid pretreatment to discharge reducing sugars from *A. phloxoeroides*. The reducing

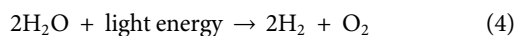
yield of sugar was found to be 0.35 g/g *A. philoxeroides* under the optimal pretreatment condition, and after enzymolysis, it was increased further to 0.56 g/g *A. philoxeroides*. As a result, an increase of 59.9% in biohydrogen yield was achieved for the pretreated *A. philoxeroides*. Optimizing bacteria suspensions, the maximum production rate of biohydrogen was increased to 4.64 from 1.42 ml/gVS/h, which accelerated the biohydrogen yield peak and contributed to a 42.8% increase in the biohydrogen production to 89.8 ml/gVS. However, attention needs to be paid to the by-products of *A. philoxeroides* pretreatment and their influence on biohydrogen generation, which were not addressed in this study.

Biophotolysis Process

In the biophotolysis process, solar energy is used by photosystem II to break water into oxygen, energy, and a reducing agent. This agent is used to produce hydrogen by reducing protons utilizing nitrogenase or hydrogenase enzymes. Electrons gained from breaking water pass through the electron transport chain consisting of PSI and PSII. Then it produces adenosine triphosphate (ATP) and reduces the ferredoxin compound. These take part in various metabolic reactions to produce hydrogen (Razu et al., 2019). The biophotolysis process is divided into two categories: indirect and direct biophotolysis. Endogenous substrates catabolize and produce electrons which are used in indirect biophotolysis whereas direct biophotolysis utilizes the electrons produced from the splitting of water in photosystem II.

Direct Biophotolysis

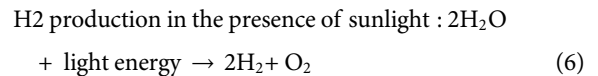
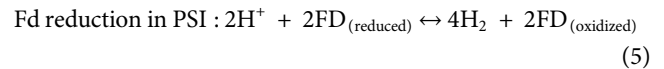
In the process of direct biophotolysis, water molecules are split using sunlight as a source of energy in the PSII. After transferring the residual e^- to photosystem I, the hydrogenase helps to produce hydrogen without generating harmful greenhouse gases. This process also simultaneously releases oxygen into the atmosphere (Jiménez-Llanos et al., 2020). The reaction for this process is as follows (Kumar S et al., 2019):



In the direct biophotolysis pathway, the oxidation of water molecules *via* the PSII produces electrons. The photons gained from sunlight are used by the PSII. To power the electrons these energized electrons then move through the electron transport chain. Ferredoxin (Fd) and PSI assist the transfer of electrons. NADP⁺ is reduced to nicotinamide NADPH by plastoquinol produced through electron transfer. To form O₂ and H⁺, the electrons are substituted by oxidizing water. These electrons gained from water are used for photosynthesis by photosystem II. A proton gradient is needed to generate ATP through ATP synthase. These protons act as the terminal acceptors for the electrons in the chloroplast of the algae. As a result, this process produces hydrogen and oxygen gases simultaneously (Show et al., 2019a).

Green microalgae are noted as the only microorganism that undergoes direct biophotolysis in the absence of oxygen in the production of biohydrogen by splitting water. Green microalgae

and cyanobacteria are referred to as “oxygenic photosynthetic microorganisms” due to their ability to take up sunlight and split the water molecular thus converting it into chemical energy (Yilmaz et al., 2016). Water-PQOR, i.e., PSII, energizes electrons in the presence of sunlight transferring them to PSI and then Fd (Eroglu and Melis, 2016); Fd-oxidoreductase produces reduced Fd, which then donates an electron to [FeFe]-hydrogenase enabling the reduction of the proton (H⁺) to a hydrogen molecule (H₂) and so, produces O₂ at the PSI.



The process of direct biophotolysis is a sustainable pathway. It has a lot of promise as the main reactants such as water, sunlight, and CO₂ are commonly available products. In addition, it converts solar energy into chemical energy to produce hydrogen and oxygen (Jiménez-Llanos et al., 2020). However, the pathway still needs to overcome many challenges to become a good and feasible option for biohydrogen production. One of the major disadvantages of this pathway is the inhabitation of hydrogen production due to the accumulation of oxygen as mentioned in **Table 1**. Other drawbacks include the risk of using oxygen, the requirement for high light intensity, and having low photochemical efficiency (Kumar S et al., 2019). Massive cultivation of algae is required to capture enough sunlight to provide sufficient free energy ($\Delta G = +237 \text{ kJ mol}^{-1}$). Some other barriers include the processing and concentration of cell biomass, respiration, and photosynthetic capacity ratio (Jiménez-Llanos et al., 2020).

Indirect Biophotolysis

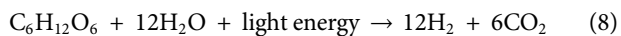
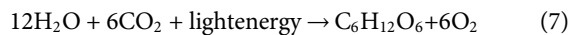
Indirect biophotolysis is performed by microalgae under anoxic conditions. Under these conditions, microalgae can produce biohydrogen through fermentation or respiration. The indirect method is not continuous as a return of the light period makes the growth become photosynthetic and hinders H₂ase (El-Dalatony et al., 2020). Indirect biophotolysis is a two-step process: i) the first stage involves oxygen producing and carbon dioxide fixing into chemical energy carbohydrates, and lipids; and ii) the second stage is functionally the same and a small sealed photo-bioreactor is used with CO₂ synthesis which is divided periodically in the absence of light exposure (Razu et al., 2019).

Electrons released through catabolism of the endogenous substrate are used for a non-photochemical reduction on oxygen depletion which causes the initial electron transport chain to stop during the anaerobic phase. The electron is then transferred to [FeFe]-hydrogenases resulting in biohydrogen production (Lam and Lee, 2013). This hydrogen production method using biological photolysis was first trialed on a non-heterocystous microalga such as *cyanobacteria Plectonemaboryanum* which was subjected to frequent cycles of “aerobic light-driven CO₂ fixation and O₂” (Razu et al.,

TABLE 1 | Advantages and disadvantages of basic biohydrogen production technologies.

Technology	Advantages	Disadvantages
Photo fermentation	<ul style="list-style-type: none"> -Photosynthetic bacteria can exploit varieties of spectral energy -Process a variety of substrates -Can process dark fermentation effluent -Bioremediation 	<ul style="list-style-type: none"> -The efficiency of light conversion is lower -Biohydrogen production through photosynthetic bacteria remains low -Inhomogeneity of light distribution and metabolic shifting from biohydrogen generation to polyhydroxybutyrate synthesis
Dark fermentation	<ul style="list-style-type: none"> -As a by-product of the process, numerous metabolites can be produced and various substrates can be utilized -Process a variety of substrates -Light independent method -No O₂ limitation issues -Bioremediation 	<ul style="list-style-type: none"> -This technique produces a minimal amount of H₂, making it thermodynamically unfavourable -Inhabitation of biohydrogen production due to the accumulation of oxygen -Low biohydrogen production
Direct biophotolysis	<ul style="list-style-type: none"> -An integrated approach to producing H₂ from water and sunlight -Requires simple cultivation -Simple substrate of H₂O -CO₂ consumption 	<ul style="list-style-type: none"> -High light intensity and O₂ work as an inhibitor in the process -Separate high purity O₂ and H₂ streams are not attainable
Indirect biophotolysis	<ul style="list-style-type: none"> -Hydrogen generation from water using blue-green algae -Separate O₂ generation from H₂ production requirement -N₂ fixing capability from air -Metabolite by-product conversion to H₂ 	<ul style="list-style-type: none"> -Hydrogenate uptake is reduced -Uptake hydrogenates can be removed -Hydrogenase enzyme generates carbon dioxide and provides a low yield of hydrogen
Microbial electrolysis cells	<ul style="list-style-type: none"> -A pollution-free technology that produces electricity in a more environmentally friendly manner Bioremediation -High removal of chemical oxygen demand (COD) -Can process dark fermentation effluent -High recovery of H₂ -High degradation of substrate 	<ul style="list-style-type: none"> -Solar systems can give a more efficient system, but they are more expensive -Capital cost is comparatively high -Hydrogen loss and purity -Suffers from scalability, source of power, stability, and mode of operation

2019). The reactions to this process are as follows (Sharma and Arya, 2017):



To produce biohydrogen, the microalgae uses stored glycogen (Acar and Dincer, 2018a). This approach also helps to solve the oxygen sensibility issue by briefly separating oxygen and hydrogen evolution reactions coupled through CO₂ fixation/evolution. In the aerobic phase, the microalgae grow by converting light energy and fixing carbon dioxide into chemical energy such as lipids, carbohydrates, and other biomolecules (Lam et al., 2019). When there is a shortage of oxygen, the electron transport chain stops functioning. If oxygen is absent in the medium, it can create an anaerobic condition. This is why activation of oxygen-sensitive hydrogenase is necessary for producing biohydrogen from electrons produced in this pathway.

Several microalgae (oleaginous) were screened to assess their hydrogen production ability by utilizing crude glycerol as a low-cost source of exogenous carbon to develop a simultaneous hydrogen and lipid production process (Sengmee et al., 2017). All microalgae grew on crude glycerol and accumulated a high lipid content of more than 20%, however only *Chlorella sp.* could produce a significant amount of hydrogen under the anaerobic condition. The optimum condition resulted in a maximum

hydrogen generation of 11.65 ± 0.65 ml/L in the 1L bioreactor and 10.31 ± 0.05 ml/L in the serum bottle, as well as a lipid content of more than 40% in the retrieved microalgal biomass. Exploring biohydrogen generation integrated with the production of lipid by oleaginous microalgae could make a significant contribution to the long-term viability of biofuel production using microalgae.

Electrochemical Process

Electrolysis

The microbial electrolytic cell (MEC) is an alternative way of obtaining sustainable biohydrogen production from various renewable biomass energy sources by lowering the energy requirement. MECs are adaptable bioelectrochemical tools capable of converting CO₂ or organic carbons into useful chemicals. The electrochemically active microalgae produce carbon dioxide and electrons that are transferred through the anode to the cathode to combine with hydrogen atoms in the solution and then releasing H⁺. However, the backdrop of this electrolytic process is that it is not spontaneous and so, an external voltage supply is necessary to produce biohydrogen at the cathode of the microbial cell (Kadier et al., 2018). The use of MEC is a hybrid system that enhances the production of biohydrogen more than fermentation. Therefore, the biohydrogen production efficiency is increased in the presence of photosynthetic and non-photosynthetic microorganisms

TABLE 2 | Microorganisms with their benefits and limitations on biohydrogen production (Razu et al., 2019).

Microorganisms	Strains	Mode of operation	Benefits	Limitations
Green algae	<i>Chlamydomonas reinhardtii</i>	Direct biophotolysis	H ₂ is produced from water and sunlight ten folds more solar conversion energy is produced than to trees, and crops CO ₂ is decreased in the environment	H ₂ production is inhibited by oxygen
	<i>C. moewusii</i>	2H ₂ O + light + Fd _(ox) → 4H + Fd _(red) (4e ⁻) + O ₂		
	<i>Chlorella Vulgaris</i>	4H ⁺ + Fd _(red) → (4e ⁻)Fd _(ox) + 2H ₂ Photo-fermentation 6CO ₂ + 12H ₂ O → 6O ₂ + C ₆ H ₁₂ O ₆ 6H ₂ + C ₆ H ₁₂ O ₆ → 12H ₂ + 6CO ₂		
Cyanobacteria	<i>Anabaenavariabilis</i>	Direct biophotolysis light + 2H ₂ O + Fd _(ox) → 4H + Fd _(red) (4e ⁻) + O ₂	H ₂ is produced from water and sunlight CO ₂ is decreased in the environment	H ₂ is consumed by the hydrogenase enzyme H ₂ production is inhibited by oxygen
	<i>Cyanothece sp.</i>	4H ⁺ + Fd _(red) (4e ⁻) → Fd _(ox) + 2H ₂		
	<i>Synechocystis PCC 6803</i>	Indirect biophotolysis		
	<i>Anabaena sp. PCC7120</i>	N ₂ + 8H + Fd _(red) (8e ⁻) + 16ATP 2NH ₃ + H ₂ + Fd _(ox) + 16ADP + 16Pi 8H ⁺ + 8e ⁻ + 16ATP → 4H ₂ + 16ADP + 16Pi 12H ₂ O + 6CO ₂ + C ₆ H ₁₂ O ₆ + 6O ₂ C ₆ H ₁₂ O ₆ + 6H ₂ + 6CO ₂ + 12H ₂		
Photosynthetic bacteria	<i>R. capsulatus</i>	Photo-fermentation	Different types of waste resources like, whey, distillery, and so on can be utilized A wide spectrum of light can be used	Light is required for the H ₂ production Fermentation causes water pollution
	<i>R. sulidophilus</i>	CH ₃ COOH + 2H ₂ O + light → 4H ₂ + 2CO ₂		
	<i>Thiocapsa roseopersicina</i>	N ₂ + 8H ⁺ + 8e ⁻ + 16ATP → 2NH ₃ + H ₂ + 16ADP + 16Pi		
Fermentative bacteria	<i>Enterobacteraerogenes</i>	Dark fermentation C ₆ H ₁₂ O ₆ + 6H ₂ O → 6CO ₂ + 12H ₂	H ₂ can be produced without the presence of light in anaerobic condition Different carbon sources like starch, cellobiose, sucrose, can be used Valuable metabolites such as butyric acid, lactic acid can be made	Carbon and nitrogen sources are needed for the growth Fermentation causes water pollution Gas contained CO ₂
	<i>Clostridiumbutyricum</i>	Pyruvate + CoA → acetyl - CoA + formate		
	<i>Magashaeraelsdenii</i>	Pyruvate + CoA + 2Fd _(ox) → acetyl - CoA + CO ₂ + 2Fd _(red)		

inoculated in a biomass source, such as wastewater, in an electrolytic cell.

The microorganisms that produce biohydrogen are summarized in **Table 2**, along with their limitations and benefits. Cyanobacteria and a collective species of green algae are proven to be great sources of alternative renewable biofuels for biogas, biodiesel, biohydrogen, and bio-oil (Khetkorn et al., 2017). Nitrogen fixation in cyanobacteria and the reduction of H⁺ electrons in green algae that are generated through photosynthesis, therefore completing a bio photolysis process, is a common biohydrogen production process for these respective organisms (Park et al., 2021). Microorganisms that are either photosynthetic or non-photosynthetic and dark fermentative bacteria can produce biohydrogen.

For the production of biohydrogen, MEC systems have several advantages over traditional fermentation. For instance, fermentation processes yield 2 mol acetate and 4 mol H₂ from 1 mol of glucose, whereas MECs are capable of producing 12 mol H₂/mol glucose due to the fact that they also utilize residual organic materials (Parkash, 2016). To explore hydrogen production in the MEC using sugar industrial effluent as a

substrate versus phosphate buffer catholyte, Jayabalan et al. (2020) used two graphene metal oxide nanocomposites as catalysts. At a 1.0 V voltage, the NiO.rGO nanocomposite demonstrated 4.38 ± 0.11 mmol/L/D as a maximum hydrogen generation rate, 20.8% cathodic hydrogen recovery and 65.6% coulombic efficiency. The results revealed that the MEC system can contribute to a 2.68- and 1.19-times higher efficiency in biohydrogen production than uncoated Ni-Foam and Co₃O₄.rGO, respectively. However, energy recovery *via* industrial effluents, combined with technological improvements and simultaneous treatment, must be further improved for the long-term sustainability of biohydrogen production using this method.

Photoelectrochemical

The microbial photoelectrochemical cell (PEC) is a cutting-edge technology that generates sustainable biohydrogen from organic waste using light-aided synergistic microbial conversion. In the PEC, electrons are synthesized electrochemically from organic compounds on the microbial- or bio-anode by active microorganisms. The PEC

TABLE 3 | Overview of biohydrogen production technologies from microalgae.

Technology	Basic principle	Microorganism	Yield of H ₂	Efficiency	Conditions	References
Photo fermentation	In the presence of light, photosynthetic bacteria convert complex organic microalgal biomass into simpler organic or inorganic components	Photoheterotrophic bacteria (<i>Rhodospseudomona</i> , <i>Rhodobacter</i>)	Maximum 160.40 ± 2.70 ml/gCS	15.93% efficiency in energy conversion	Biohydrogen production, as well as energy conversion efficiency, can be enhanced from corn stalk	Lu et al. (2021)
		(<i>Rhodospirillum</i> and <i>Rhodobium</i>)	36.08–141.42 ml (g TS) ⁻¹	10.12% efficiency in energy conversion	Promising technology for the production of biohydrogen from corn stover	Zhang et al. (2020)
			< 59% depending on the microbes cellulose structure	10% efficiency in energy conversion	-Substrate conversion efficiency -Optimum light penetration - Use of Oxygen absorbers	Ei-Dalatony et al. (2020), Jim and Rami, (2020), and Mona et al. (2020)
Dark fermentation	In the absence of light, complex organic microalgal biomass is converted into simpler organic or inorganic components.	Fermentative bacteria (<i>Escherichia coli</i> , <i>Clostridia</i> , <i>Enterobacter</i>)	89.80 ml/gVS	42.80% improvement	Further optimization can significantly increase the rate of biohydrogen production from 1.42 to 4.64 ml/gVS/h	Song et al. (2020)
		<i>Alcaligenes</i> , <i>Citrobacter</i>	36.08–141.42 ml(g TS) ⁻¹	2.58% efficiency in energy conversion	Effective production of biohydrogen from corn stover	Zhang et al. (2020)
		(<i>Bacillus</i>)	Approximately 19.30%		A very efficient hydrogen isolation technique is required	Ei-Dalatony et al. (2020), Jim and Rami, (2020), Mona et al. (2020), and Lee, (2021)
Direct biophotolysis	In the presence of a direct light source, pigment-containing microorganisms are used in a sequence of processes to generate hydrogen from water molecules	Cyanobacteria Green algae	Greater than 10%	More than 80%	-Availability of ferredoxin -High ATP, NADPH contents -Utilization of O ₂ absorber -Nitrogenase inactivation	Mona et al. (2020), Ei-Dalatony et al. (2020), Jim and Rami, (2020), Kant et al. (2021), Mona et al. (2020), and Razu et al. (2019)
Indirect biophotolysis	In PS I and PS II compartments, sulphur-deficient microorganisms are used in one or more step reactions to generate hydrogen from complex carbohydrates or pyruvate	Cyanobacteria	Approximately 10–15% 6.40 times more	16.30% efficiency in light conversion	-Increased efficiency in light conversion -Hydrogenase inactivation Biomass yield can be improved 7.30 times more by a two-stage biophotolysis system compared to a single-stage system	Ei-Dalatony et al. (2020), Jim and Rami, (2020), Kant et al. (2021), and Mona et al. (2020) Rather and Srivastav, (2021)
Microbial fuel cells	The bioelectrochemical approach produces an electric current by decomposing microorganisms	Electrochemically active bacteria (<i>Aeromonas hydrophila</i> , <i>Shewanella putrefaciens</i>)	Approximately 90%	60–78% conversion efficiency	-Pressure, temperature, and the sorts of electrodes used should all be selected carefully - Integrating with other techniques ensures performance enhancement	Azwar et al. (2014), Kumar et al. (2017), Ei-Dalatony et al. (2020), and Fadakar et al. (2020)

(Continued on following page)

TABLE 3 | (Continued) Overview of biohydrogen production technologies from microalgae.

Technology	Basic principle	Microorganism	Yield of H ₂	Efficiency	Conditions	References
Microbial electrolysis cells	The bioelectrochemical method that decomposes bacteria by using electricity	Exoelectrogenic bacteria such as <i>Geobacteraceae</i>	4.38 ± 0.11 mmol/L/D Approximately 90%	2.68 and 1.19-times higher efficiency in biohydrogen production than uncoated Ni-Foam and Co ₃ O ₄ -rGO, respectively	Energy recovery via industrial effluents, combined with technological improvements and simultaneous treatment, must be emphasized for long-term sustainability -Pressure, temperature, and the sorts of electrodes used should all be selected carefully -Expenditure is contingent upon the cost of external power	Jayabalan et al. (2020) Azwar et al. (2014), Kumar et al. (2017), El-Dalatony et al. (2020), and Fadakar et al. (2020)

consists of semiconductors (electrodes) that are separated by a membrane in an electrolyte. Semiconductors and sunlight are utilized directly in the photoelectrochemical process to separate water into hydrogen and oxygen. During the photoelectrochemical process, water molecules oxidize resulting in oxygen production and biohydrogen reduction as the H₂ production occurs in the opposite electrode (El-Dalatony et al., 2020). This method has a high potential for biohydrogen production with minimal environmental effects.

Few investigations have been conducted on solar microbial photoelectrochemical systems on the basis of photo-bioanodes, in which bio and solar energy are integrated at the same electrode. Utilising a photo-bioanode with two sides, biocarbon material and photocatalyst (α-Fe₂O₃), researchers expedited biofilm formation, improved extracellular electron transport, and enriched exoelectrogens (Feng et al., 2016). The metal-free carbon film based on CeO₂-rGO was recently employed as a photo-bioanode, with the suggested system achieving a hydrogen evolution rate of 5 m³/m³/d while simultaneously treating the wastewater (Pophali et al., 2020). Zhu et al. (2017) demonstrated that output power and photocurrents are increased at visible light using a hematite nanowirearrayed photoanode and designed exoelectrogens. However, this technique necessitates more research to attain a sustainable level of biohydrogen production. **Table 3** provides an overview of commonly available technologies of biohydrogen production from microalgae.

ECONOMIC ANALYSIS OF BIOHYDROGEN PRODUCTION

Even though biohydrogen production has increasingly exhibited promising results at the pilot and laboratory scale, studies focused on optimization of process parameters and the feasibility of scaling up to the industrial level are largely absent (Oncel,

2015). Unlike the chemical synthesis of hydrogen, biohydrogen production has many challenges. Solving the technical and engineering problems may cost approximately \$1.42 million (El-Dalatony et al., 2020). Thus, even though 1 kg of hydrogen is equivalent to 3.79 kg gasoline (Show et al., 2018), for hydrogen to be competitive with an energy price of gasoline (\$2.50 GJ⁻¹), the commercial goal for hydrogen production cost needs to be \$0.30 kg⁻¹ (Goswami et al., 2021).

The microalgae cultivation system and reactor design primarily determine the production cost of biohydrogen. For instance, when biohydrogen is produced from a biophotolysis system, cultivation in a 140 ha open pond and 14 ha photobioreactor can cost \$6 m⁻² and \$100m⁻², respectively, with a \$43 million capital cost and \$10 million annual operational costs, leading to a production cost of \$10 GJ⁻¹ (Mona et al., 2020). The capital investment was almost 90% of the total cost with the capital charge calculated at 25% annually. Operating at 90% capacity, the system aims to produce 1200 TJ of biohydrogen per year (Mona et al., 2020). However, the hydrogen handling and storage costs were not factored in the total calculation, even though these are significant cost factors that can amount to about \$0.58 million (El-Dalatony et al., 2020).

Following the initial failure of continuous biohydrogen production using S-deprived wild type *C. reinhardtii*, the National Renewable Energy Laboratory in collaboration with the researchers at UC-Berkeley developed a two-stage continuous phase system that promoted an aerobic growth phase and a subsequent anaerobic hydrogen evolving phase (Nagarajan et al., 2021). Their design reduced the photobioreactor costs to \$1 m⁻², determined the selling price of hydrogen at \$8.97 kg⁻¹ (or €22.80 kWh⁻¹), and further revealed the capital costs of custom-built photobioreactors can be drastically reduced if open ponds are used. Their first design used a photobioreactor costing \$10 m⁻² and considered utilization of pressure swing adsorption for hydrogen purification and the compression of biohydrogen to 20 MPa for transporting and storing at a facility with the capacity of

300 kg/day. Changes in the flow in accordance with requirements led to production costs of between $\$0.57 \text{ kg}^{-1}$ and 13.53 kg^{-1} which was deemed feasible. However, the production stopped 4 days after sulphur deprivation.

Gholkar et al. (2021) conducted a techno-economic feasibility study of a plant in India that cultivated microalgae in an open pond at the rate of $12,790 \text{ kg h}^{-1}$ and produced 1,239 kg of biohydrogen per hour. The total capital investment was estimated at \$144.6 million, 11% of which was used to purchase the gasifier and its accessories. In addition, 76% of the total operating cost, which was \$7,692 per year was attributed to the cultivation of microalgae, while the remaining was spent on utilities. Having assumed a market price of $\$10 \text{ kg}^{-1}$, they concluded that biohydrogen production was economically feasible, with a payback period of 3.78 years and an internal rate of return of 22%. Because profitability was primarily determined by the biohydrogen composition and the cost of microalgae production, the researchers recommended developing and using highly selective catalysts.

James et al. (2009) attempted to estimate the production cost of biohydrogen using oxygen tolerant microalgae and found a cost of $\$1.38 \text{ kg}^{-1}$, excluding the cost for storage (Jiménez-Llanos et al., 2020). 42% of the cost was derived from photobioreactor operation, 34% from the resources required for the biohydrogen collection, and 13% from recycling and pumping systems. Moreover, 4% of the costs were required for the microalgae feed assembly, with the remaining costs attributable to other control systems and consumables. Storage is an important cost consideration as it serves as a major limitation. To make the production price affordable for direct biophotolysis, it was recommended that the conversion efficiency and biomass production be raised to 13.4 and 9.4%, respectively. Another study (Melis and Happe, 2001), which assumes biohydrogen is produced 100% by photosynthesis, found that with a plant running at half capacity and an estimated production rate of $80 \text{ kg biohydrogen acre}^{-1} \text{ day}^{-1}$, biohydrogen would cost about $\$2.80 \text{ kg}^{-1}$ which can be competitive with gasoline (Jiménez-Llanos et al., 2020).

Some bioreactor designs can also significantly raise the production cost, particularly depending on the manufacturing materials (Sathyaprakasan and Kannan, 2015), because the cost of materials and nutrients can make up more than 80% of major expenses (Show et al., 2018). A near-horizontal tubular reactor system built using single-stage algal biophotolysis cost $\$50 \text{ m}^{-2}$, assuming a 17% capital charge per year and a 10% efficiency of sunlight conversion. This led to an overall biohydrogen production cost of $\$15 \text{ GJ}^{-1}$. An assessment of a simplified design of a pilot modular photoreactor for 6 months' field operation not only provided overall comparative costs of different technology compartments but also established a construction cost of $\$0.75 \text{ m}^{-2}$, which was significantly lower than frequently reported costs of $\$20\text{--}\100 m^{-2} . The study revealed that chemical nutrients and fabrication materials represented 84% of the main expenditure.

A techno-economic evaluation of a system producing biohydrogen through supercritical and thermal gasification

of microalgae biomass suggested that generating 2,000 dry tonnes of biohydrogen per day required a total capital investment of \$215.3 million and \$277.8 million for thermal and supercritical gasification, respectively, to yield biohydrogen with a product value of $\$4.59$ and $\$5.66 \text{ kg}^{-1}$, respectively (Kumar M et al., 2019). The total capital cost comprised individually purchased equipment, installation, and indirect costs, such as construction, engineering, and contingencies. For thermal gasification, the installed capital cost was \$131.48 million whereas it was \$169.6 million for supercritical gasification. The construction of the thermochemical plant was considered to occupy 20, 35, and 45% of the total capital cost for the first, second, and third years, respectively. Maintenance made up 3% of the total project investment, while labour cost was assumed to be 25% of the total operating cost. Plant overheads comprised half of the total labour and maintenance costs. General and administrative expenses were assumed to be 8% of the operating cost. Overall, the production cost of biohydrogen was reported to be fourfold the cost of hydrogen from natural gas.

The efficient microalgal biohydrogen production is hindered by numerous process parameters that affect the progress of metabolic activities. For instance, light intensity or the accessibility to radiation energy alone can significantly affect biohydrogen synthesis (Oey et al., 2016). In particular, in the artificial environment, the different light intensities reduce the efficiency of the metabolic capacity of microalgae, which is often not an issue in natural habitats where algae can adapt based on needs. Consequently, proper mixing of microalgal culture is crucial for effective light capture. Light can be either an inhibiting factor or a limiting factor depending on the metabolic route utilized for biohydrogen production. Furthermore, pH is also an important physical factor that influences biohydrogen production using microalgae (Razu et al., 2019). Even though the appropriate pH depends on the microalgae species in use, minimal changes in the pH can significantly affect the hydrogen-producing enzyme as well as the growth and metabolic activity of microalgae, affecting their biohydrogen production capacity.

Temperature is another physical parameter that affects biomass productivity and the biohydrogen production capacity within a medium. The optimum temperature for the sustainable proliferation of microalgae and the maximum biohydrogen production has been reported to be $15\text{--}85^\circ\text{C}$ in a mixed culture (Razu et al., 2019). The availability of essential micro and macronutrients for enhanced biomass productivity also affects cost. Furthermore, the availability of these nutrients may initiate a stress response within the microalgal cells, which may be exploited to produce different kinds of biofuels. Biohydrogen has been reported to be generated when microalgae are subjected to sulphur (Gonzalez-Ballester et al., 2015), nitrogen (Nagarajan et al., 2017), phosphorous (Batyrova et al., 2015), magnesium (Volgusheva et al., 2015), and potassium deprivation.

The presence of an organic carbon source is also important for cultivation, except for phototrophic strains. The processes involved with microalgal biomass-led biohydrogen production face additional challenges for the smooth and sustainable production of biohydrogen. Oxygen sensitivity is regarded as one of the most challenging obstacles to overcome for many biohydrogen production technologies. The high binding potential of oxygen to the active sites of hydrogenase (El-Dalatony et al., 2020) create irreversible bonds that make it difficult to extract hydrogen and oxygen molecules from the complex compound (Eroglu and Melis, 2016). In addition, issues like altered thylakoid proton gradient, light capture disruption, and low efficiency of photosynthetic activities also contribute to high production costs.

Finally, there are significant differences in the costs of biohydrogen production depending on the routes discussed in the previous section. For instance, utilizing direct biophotolysis for biohydrogen costs an estimated $\$2.80 \text{ kg}^{-1}$ and generated 20 kg per $1,000 \text{ m}^{-2} \text{ day}^{-1}$ (Show et al., 2018). Resnick (2004), undertook comprehensive calculations of the production costs of each of the metabolic pathways. He estimated that direct biophotolysis, with a capital cost of $\$1220 \text{ GJ}^{-1} \text{ year}^{-1}$ calculated at $\$2.47 \text{ m}^{-2}$ and an operating cost of over $\$70,000$ million broken down by power ($\$2.49$ million), water ($\$0.02$ million), labour ($\$50.4$ billion), supplies ($\$7.75$ billion), culture production ($\$5.8$ billion), gas separation and handling ($\$23$ million), and a subtotal operating cost ($>\$63,900$ million) with a contingency of 10%, resulted in a biohydrogen production cost of $\$11,170.33 \text{ GJ}^{-1}$. Biohydrogen produced from indirect biophotolysis with a capital cost of approximately $2.40 \text{ GJ}^{-1} \text{ year}^{-1}$ and an operating cost of about $\$102.1$ million on an annualized basis had a production cost of $\$16.26 \text{ GJ}^{-1}$ (Ghosh et al., 2017). The complete breakdown of the operating cost is as follows: power ($\$2.5$ million), water ($\$0.02$ million), labour ($\$65$ million), supplies ($\$17.3$ million), culture production ($\$8.2$ million), gas separation and handling ($\$0.09$ million), and subtotal operating costs (about $\$93$ million) with a contingency of 10% (Resnick, 2004).

Another study (Hallenbeck and Benemann, 2002) reported that biohydrogen using direct biophotolysis assuming an optimistic capital cost of $\$50 \text{ m}^{-2}$ maintained a production cost of $\$2.13 \text{ kg}^{-1}$ (Nikolaidis and Poullikkas, 2017; Acar and Dincer, 2018a). This implies that a 20% increase in capital cost would raise the production cost by 33%. However, the calculations considered minimal operating costs, at an overall solar conversion efficiency of 10%, and did not account for many significant cost components such as engineering, gas separation and handling. On the other hand, the capital cost using indirect biophotolysis was $\$135 \text{ m}^{-2}$ and the production cost was $\$1.42 \text{ kg}^{-1}$ (Nikolaidis and Poullikkas, 2017). Dincer and Acar (2015) illustrated that direct biophotolysis yielded biohydrogen at a cost of $\$7.27 \text{ kg}^{-1}$, whereas photofermentation and dark fermentation cost $\$7.61 \text{ kg}^{-1}$ and $\$7.52 \text{ kg}^{-1}$, respectively.

Resnick (2004) calculated a production cost of $\$30.7 \text{ GJ}^{-1}$ using photofermentation. An operating cost of over $\$193$ million was assumed, incorporating the costs for power ($\$2.5$ million), water ($\$0.01$ million), labour ($\$23$ million), supplies ($\$3.5$ million), culture production ($\$2.7$ million), gas separation and handling ($\$0.13$ million), and a subtotal operating cost

($>\$175$ million) with a contingency of 10%. The Tredeci-style reactor serves as a significant cost-driver, resulting in a capital cost of $\$1.41 \text{ GJ}^{-1} \text{ year}^{-1}$. Dark fermentation, on the other hand, was found to produce biohydrogen at a cost of $\$155.59 \text{ GJ}^{-1}$. Dark fermentation has potentially the lowest capital cost of about $\$0.64 \text{ GJ}^{-1} \text{ year}^{-1}$ (Resnick, 2004), attributed to the small footprint of the bioreactor. The operating cost of over $\$980$ million can be broken down into power ($\$2.5$ million), water ($\$0.02$ million), labour ($\$17.8$ million), supplies ($\$2.7$ million), culture production ($\$2$ million), gas separation and handling ($\$0.6$ million), and a subtotal operating cost (about $\$891$ million) with a contingency of 10%.

Overall, photofermentation exhibits a higher yield but is relatively expensive (Bolatkhani et al., 2019). Others have demonstrated that photovoltaic processes are more expensive than direct biophotolysis (Goswami et al., 2021), costing up to $\$170 \text{ m}^{-3}$ whereas the latter resulted in a cost of $\$25 \text{ m}^{-3}$. Many of recent studies (Eroglu and Melis, 2016; Khetkorn et al., 2017; Anwar et al., 2019; El-Dalatony et al., 2020; Peng et al., 2020; Singh and Das, 2020; Goswami et al., 2021; Limongi et al., 2021; Wang et al., 2021) primarily extol the advantages of synthetic biology, as advancements in molecular and genetic engineering have allowed these options to be explored for improving the strains of algae to optimize biohydrogen yields. This is no surprise, as synthetic biological methods have a higher yield and output compared to the metabolic routes discussed (Goswami et al., 2021). In general, however, biohydrogen production tends to be more expensive than other fuel alternatives (Show et al., 2018). Nevertheless, the economic analyses of biohydrogen production have been scanty, especially for electrochemical processes.

Most of the economic studies were conducted between 10 and 20 years ago, making economic feasibility analysis of biohydrogen production using state-of-art technology ambiguous. Moreover, many of the existing studies on the economics of biohydrogen are substantially optimistic. It has been calculated that biohydrogen can be produced at a cost between $\$10$ – 20 GJ^{-1} (Show et al., 2019b). Regardless, such production costs are too high for biohydrogen to serve as a competitive alternative to gasoline, which costs $\$0.33 \text{ GJ}^{-1}$. Future work needs to be directed towards comprehensive evaluations with regards to facility feasibility and operation, material and operation costs, light, weather, and land requirements, the mixing of biomass, and stability. Economic feasibility can be improved by recycling residual metabolites and biomass.

CONCLUSION

Different techniques for biohydrogen production offer diverse benefits, even though none are currently feasible for large-scale implementation. The fermentation processes are more eco-friendly, however they have low efficiency in sunlight conversion and biohydrogen yield. Biophotolysis methods also have a low biohydrogen yield ($<15\%$) and face several challenges in terms of oxygen sensitivity and the availability of sunlight. Nevertheless, direct biophotolysis offers an efficiency of more than 80%. The

electrochemical processes are found to be significantly energy-intensive, with high requirements of pressure, temperature, power, and sophisticated equipment, but yield 90% biohydrogen. The current production cost of biohydrogen is quite high, ranging between \$10–\$20 GJ^{-1} , depending on the microalgae cultivation system, reactor design, and the metabolic route used. In order to compete with gasoline, this cost must be less than \$0.33 GJ^{-1} . On the other hand, most economic assessments are fairly optimistic but fail to account for important cost factors such as storage, handling, and transportation. There is also a gap in current economic evaluations as most studies were conducted decades ago. Thus, future research should be directed towards properly assessing the economic feasibility of biohydrogen production processes in order to scale up the technology.

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