



Microbial biomass conversion for hydrogen production: A review

Muhamad Reda Galih Pangestu^a, Shaikh Abdur Razzak^{a,b}, Shihab Uddin^{c,d,*}

^a Department of Chemical Engineering, King Fahd University of Petroleum and Minerals, Dhahran, 31261, Saudi Arabia

^b Interdisciplinary Research Center for Refining and Advanced Chemicals, King Fahd University of Petroleum & Minerals, Dhahran, 31261, Saudi Arabia

^c Department of Bioengineering, King Fahd University of Petroleum and Minerals, Dhahran, 31261, Saudi Arabia

^d Interdisciplinary Research Center for Bio Systems and Machines, King Fahd University of Petroleum and Minerals, Dhahran, 31261, Saudi Arabia



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ABSTRACT

The escalating demand for clean and sustainable energy sources has propelled hydrogen to the forefront of alternative fuel research. Microbial biomass conversion, a bio-based process utilizing microorganisms to convert organic matter into hydrogen, presents a promising avenue for achieving this goal. This review provides a comprehensive overview of possible microbial biomass conversion methods, including both light-dependent and light-independent methods, and compares their hydrogen production rates (HPRs). Light-dependent methods such as photo-fermentation offer HPRs exceeding $3 \text{ m}^3/\text{dm}^3$, suggesting highly efficient hydrogen generation possibilities. However, most rely on indirect processes or specific light conditions, potentially hindering H_2 production. Dark fermentation (DF) demonstrates significantly higher HPRs, up to $12 \text{ m}^3/\text{d}/\text{m}^3$, with no light requirements, making it a strong contender for large-scale production. Microbial electrolysis cells (MECs) show even greater HPRs of up to $72 \text{ m}^3/\text{d}/\text{m}^3$, competing favorably in hydrogen generation feasibility. Despite promising advancements, challenges remain in scaling up these processes for commercial viability. While current research achieves high HPRs, reactor volumes are typically below 1 L. This review explores opportunities and challenges associated with scaling up, particularly focusing on integrating DF and MECs. Combining these methods holds promise for enhancing stability and achieving efficient energy recovery.

1. Introduction

Hydrogen is emerging as a pivotal solution to reducing carbon dioxide emissions and combating climate change (Ofremu et al., 2024). With its high energy density (120 MJ/kg) and versatility, hydrogen can replace fossil fuels in energy-intensive sectors such as transportation, chemical production, and manufacturing (Osman et al., 2024; Pangestu et al., 2024; Singh et al., 2023). Clean hydrogen production methods, including renewable-powered water electrolysis and microbial biomass conversion, are gaining attention for their potential to enable decarbonization and meet global emission reduction targets, such as those outlined in the Paris Agreement (Howarth and Jacobson, 2021; Osman et al., 2023).

The clean hydrogen production developed nowadays is mostly based on water electrolysis technology, which requires significant electricity energy. To ensure a carbon dioxide emission-free process, the electricity should come from solar, wind, or geothermal energy sources (Gabriel et al., 2022; Osman et al., 2021). In recent years, researchers have also investigated microbial biomass conversion as a viable technology for clean or biohydrogen production (Khalil et al., 2023; Singh et al., 2023;

Wang et al., 2021). These biohydrogen methods leverage the capacity of microorganisms to ingest and break down biomass, ultimately generating hydrogen. Depending on the pathway pursued, this research has the potential to lead to the development of commercial-scale systems within the medium to long-term horizon (Honarmandrad et al., 2022). Given the abundance of biomass as a local resource and the natural adaptation of numerous microorganisms to efficient biomass decomposition, the potential for hydrogen production through this avenue is substantial (Cai et al., 2024; Mahfud et al., 2024).

The purpose of this review paper is to explore the promising field of microbial biomass conversion for hydrogen production (biohydrogen production). Biohydrogen production could be categorized into light-dependent and light-independent methods. The light-dependent methods encompass biophotolysis and photofermentation, while light-independent techniques include dark fermentation (DF) and microbial electrolysis cells (MECs) (Singh et al., 2023). Biophotolysis, a biological process entailing the splitting of water molecules into molecular oxygen and hydrogen gas, relies on light as the primary energy source. This process unfolds through two distinct pathways: direct and indirect

* Corresponding author.

E-mail address: shihab.uddin@kfupm.edu.sa (S. Uddin).

(Ghiasian and Ghiasian, 2019; Javed et al., 2022). Meanwhile, photo-fermentation, facilitated by anaerobic bacteria such as *Rhodobacter* and *Rhodobium*, entails the conversion of organic acids become H_2 and CO_2 through photosynthesis. This hydrogen production occurs concomitantly with the reduction of molecular nitrogen by nitrogenase, a process that also converts protons into hydrogen. Notably, the absence of oxygen release confers an advantage to this process by circumventing the inhibition of nitrogenase activity. The yield of hydrogen production is comparable to that of biophotolysis and is subject to various influencing factors, including the type of microorganism, growth medium, photo-fermenter design, and light intensity (Monir et al., 2022).

DF utilizes organic materials as substrates and hinges on the biological conversion of organic compounds, encompassing waste and biomass, into hydrogen and other biobased compounds. In contrast to light-dependent processes, DF operates independently of light, rendering it particularly appealing due to its continuous operability (24 h/d), simplistic reactor design, and straightforward operational protocols, all of which contribute to significant cost reductions in production (Cao et al., 2022). Lastly, MECs represent a novel technology wherein microorganisms harness the energy and protons generated during organic substance decomposition, supplemented by an external electric current, to produce hydrogen. This nascent technology is undergoing continuous refinement, with ongoing efforts aimed at enhancing various facets of the system, including the quest for cost-effective materials and the identification of optimal microbial strains for utilization (Fudge et al., 2021). Despite the array of microbial biomass conversion pathways available for hydrogen production, this technology still faces challenges in competing with other green hydrogen methods, such as electrolysis water splitting and thermochemical cycles, particularly concerning production volume, stability, and maturity (Megia et al., 2021; Tan et al., 2023; Zhang et al., 2024).

According to the 2023 International Energy Agency (IEA) technology recap, the maturity level of biological water splitting remains at rank 4 (early prototype), while alternative green hydrogen production methods such as alkaline electrolyzers have attained rank 9 (commercial operation in relevant environments) (IEA, 2023). Regardless of its position on the maturity development scale, microbial biomass conversion for clean hydrogen production holds significant potential for integration into various technology platforms. The microbial technology has the potential to be seamlessly incorporated into any pre-existing chemical or hydrogen facility, enabling the conversion of their wastewater, which often contains biomass or organic matter, into additional hydrogen production (Singh et al., 2023). Additionally, microbial biomass conversion aligns with the goals of reducing greenhouse gas emissions and transitioning towards a circular economy. In the subsequent sections, this review will delve into the latest advancements and research in microbial biomass conversion for hydrogen production. It will provide an analysis of various

microbial pathways including the HPR comparison and integration opportunities between each method. By offering a comprehensive overview of this field, this paper aims to highlight the current state, limitations, and promising prospects in microbial hydrogen conversion, setting the stage for a deeper exploration in the subsequent chapters.

2. Biomass for hydrogen production

Throughout the history of hydrogen production, biomass has played a pivotal role, and its significance continues to grow as we transition towards more environmentally sustainable methods. As illustrated by Fig. 1, biomass, derived from organic materials such as plants, agricultural residues, and municipal waste, serves as a promising feedstock for producing carbon-neutral fuel such as biohydrogen or biofuel through thermochemical and biochemical processes (Osman et al., 2023b).

Thermochemical methods, including gasification and pyrolysis, decompose biomass into simpler products such as hydrogen at high temperatures and pressures. These methods are efficient and can produce high hydrogen yields, but they often require significant energy input and precise control of reaction conditions to minimize by-product formation (Reda Galih Pangestu and Zahid, 2024a; Reda Galih Pangestu and Zahid, 2024b). On the other hand, biochemical methods, such as dark fermentation and microbial electrolysis cells, rely on microorganisms to break down biomass under milder conditions, making them more energy-efficient and environmentally friendly (Gurubel Tun et al., 2025; Muhire et al., 2024). However, these methods typically yield lower hydrogen quantities and may require optimization of microbial strains and operating conditions to enhance efficiency. While biomass production and conversion have the potential to achieve carbon neutrality, the choice of hydrogen production method depends on factors such as feedstock availability, process efficiency, and scalability. Table 1 summarizes these methods, highlighting their advantages and limitations.

Several recent studies from India, Saudi Arabia, and Indonesia illustrate the significant potential of biomass residues for biohydrogen production. For example, in India, an assessment of agricultural residues estimates that over 500 million tons of biomass are produced annually, primarily from rice, wheat, and sugarcane, which can be leveraged for bioenergy generation (Deka et al., 2023).

In Saudi Arabia, date palm residues—one of the country's main agricultural byproducts—have been identified as a promising feedstock for bioenergy production. These residues align with the Kingdom's Vision 2030 goal of diversifying its energy resources and promoting sustainable solutions (Abdel Daiem and Said, 2022). The research emphasizes the importance of utilizing locally available biomass for energy production, particularly in arid regions where organic waste is abundant but often underutilized.

Similarly, in Indonesia, research has estimated that the country

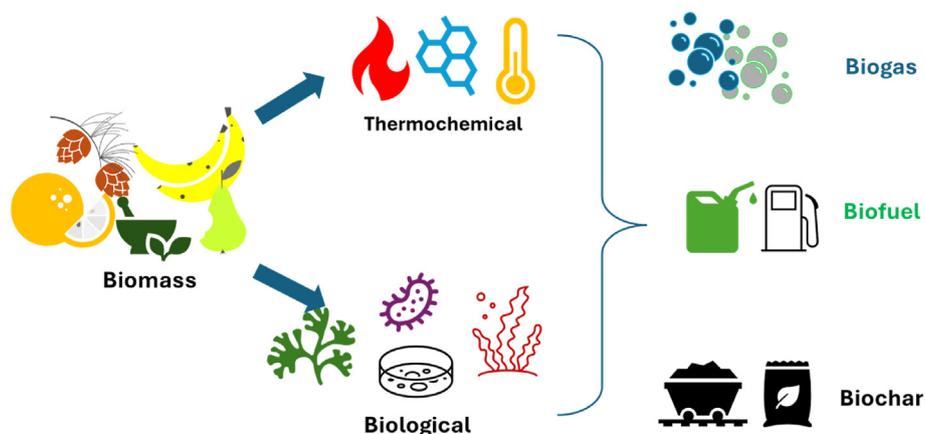


Fig. 1. Biomass could be converted into biogas or biofuel (including biochar) by thermochemical processes or biological pathways.

Table 1
Recent status of several hydrogen production pathways.

Methods	Raw Materials	CO ₂ emission (kg CO ₂ e/kg H ₂)	Temperature Range	H ₂ Cost (\$/kg)	Maturity level	Ref.
Steam Methane Reforming (SMR)	Natural gas, Steam	10.0–12.0	700–1000°C	0.7–2.2	Mature, widely used worldwide	Oni et al. (2022)
SMR with CCS	Natural gas, Steam	1.5–2.0	700–1000°C	1.4–3.8	Commercial stage, but limited application (CCS)	Shahid and Kim (2023)
Electrolysis	Water	0–3.5	25–200°C	3.2–7.7	Early commercial stage, high cost	Peng (2023)
Coal Gasification	Coal, Steam	19.0–23.0	700–1200°C	1.9–2.6	Mature, predominantly in China	Dai et al. (2023)
Gasification with CCS	Coal, Steam	2.0–5.0	700–1200°C	2.0–3.5	Commercial stage, but limited application (CCS)	Liu et al. (2023)
Biomass Gasification	Biomass, Steam	3.0–5.0	700–1200°C	1.9–2.6	Similar to coal gasification, mature	Zhu et al. (2024)
Methane Pyrolysis	Natural gas	0.5–16.0	500–2000°C	0.5–3.5	Early commercial stage, emerging	Pangestu et al. (2024)
Biomass Pyrolysis	Biomass	3.0–5.0	700–1500°C	1.5–2.5	Similar to biomass gasification, emerging	Veksha et al. (2023)
Dark Fermentation	Biomass, Microorganisms	0–3.0	25–70°C	N/A	Experimental stage, ongoing development	Talapko et al. (2023)
MECs	Biomass, Microorganisms	0–2.0	25–40°C	N/A	Pilot scale, demonstration projects	Arun et al. (2024)

generates over 146 million tons of agricultural residues annually, including rice husks, sugarcane bagasse, and palm oil mill effluent (Budhijanto, 2024). This biomass availability offers significant opportunities for integrating biohydrogen production into Indonesia's agricultural and industrial sectors, supporting sustainable energy development. These case studies highlight the importance of region-specific biomass assessments to optimize feedstock supply chains, enhance sustainability, and reduce the costs of biohydrogen production in diverse geographical contexts.

Beyond carbon emissions, the sustainability of biomass-based hydrogen production also hinges on its broader environmental impacts, including water use and land use changes (Alqarzaee et al., 2024; Faruque et al., 2024). Biomass cultivation often requires significant water resources, particularly for feedstocks such as sugarcane or corn. In water-scarce regions, this can exacerbate existing resource pressures and reduce the overall sustainability of biohydrogen systems. Additionally, large-scale biomass sourcing can lead to land use changes, including deforestation, habitat loss, and competition with food crops, which raises ethical and environmental concerns (Irfan et al., 2025; Nawaz et al., 2024).

To mitigate these issues, future research should prioritize the use of non-edible feedstocks, such as agricultural residues and waste streams,

which do not compete with food production. Implementing sustainable agricultural practices, such as agroforestry or intercropping, can also minimize land degradation and optimize resource use. A comprehensive life cycle assessment (LCA) of biohydrogen production methods is essential to quantify and address these environmental impacts effectively (K. Wang and Tester, 2023; Wang, 2023).

3. Microbial biomass conversion methods for hydrogen production

An alternative avenue for biohydrogen production is microbial biomass conversion, offering distinct advantages over traditional biomass thermal gasification. Notably, microbial conversion demonstrates the potential for reduced or neutral carbon dioxide emissions during hydrogen production, owing to its lower energy requirements and operating temperatures, as depicted in Table 1. A visual representation of various biohydrogen production methods is provided in Fig. 2.

Microbial biomass conversion methods could be broadly categorized into two main groups based on their dependence on light, as illustrated in Fig. 2: light-dependent and light-independent methods (Singh et al., 2023). Light-dependent processes encompass two primary pathways: biophotolysis and photo-fermentation. Conversely, light-independent

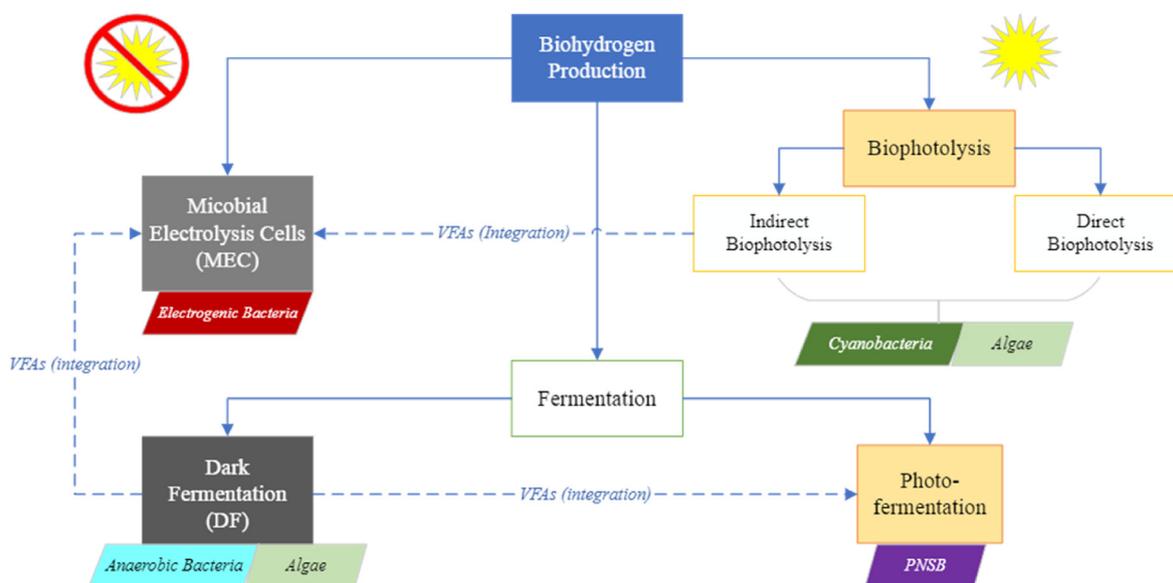


Fig. 2. Biohydrogen production methods and typical integration.

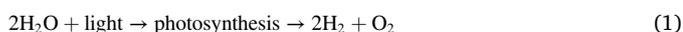
methods include DF and MECs.

3.1. Light dependent methods

Light-dependent techniques for microalgae are those in which their cells use light energy, usually from the sun to power photosynthesis and generate biomass, oxygen, and possibly other useful substances like lipids or carbohydrates. These techniques are essential for a number of applications, such as the production of biofuel, the treatment of wastewater, and dietary additions. Light-dependent techniques for microalgae include photosynthesis, light-condition optimization, photobioreactors, light harvesting, culture of mixotrophic and heterotrophic algae, light stress management, and biorefinery technologies. All things considered, light-dependent techniques are essential for growing microalgae and constitute an important field of study for long-term biotechnological applications. Optimizing these methods can enhance microalgal productivity and contribute to the development of renewable energy sources and environmentally friendly products.

3.1.1. Bio-photolysis

Biophotolysis, as exemplified in Reaction (1), represents a biological process that uses light energy to help split molecules of H₂O to become H₂ and O₂ (Ghiasian and Ghiasian, 2019). Certain microorganisms, notably specific strains of algae and cyanobacteria, harness this process as an integral facet of their photosynthetic metabolism (Javed et al., 2022). Cyanobacterial species such as *Anabaena cylindrica* and *Synechocystis*, along with green microalgae such as *Chlamydomonas reinhardtii*, have demonstrated proficiency in biohydrogen production through biophotolysis when supplemented with biomass under conducive environmental conditions (Ahmed et al., 2021). By harnessing light energy, these microorganisms facilitate the generation of hydrogen gas as a byproduct of the water-splitting reaction under anaerobic conditions (Javed et al., 2022). Biophotolysis encompasses two distinct modes: direct and indirect biophotolysis (Melitos et al., 2021).

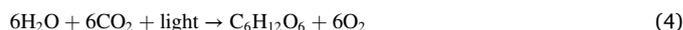


3.1.1.1. Direct biophotolysis. Direct biophotolysis converts H₂O into chemical energy in one or two stages by utilizing sun energy and the photosynthesis step of cyanobacteria or algae. This process involves the absorption of sunlight by photosystems I (PS-I) and II (PS-II), which are instrumental in both biohydrogen generation and energy transmission to ferredoxin, particularly in a one-stage process. Illustrated in Reaction (2), sunlight absorbed by PS-II initiates the oxidation of water into protons, electrons, and oxygen molecules. Subsequently, electrons are shuttled via the electron transport chain from PS-I to ferredoxin and then to the hydrogenase enzyme without the necessity of intermediary CO₂ fixation. As depicted in Reaction (3), hydrogenase catalyzes the combination of protons and electrons to become hydrogen gas (Melitos et al., 2021).



In a two-stage process, the system operates continuously and is enhanced by the removal of oxygen in Reaction (2) through the sulfur deprivation method. The removal of oxygen is crucial for increasing hydrogen production, as the hydrogenase is highly sensitive to O₂ (Ghiasian and Ghiasian, 2019). For instance, Chader et al. (2009) utilized *Chlorella sorokiniana strain Ce* to achieve biohydrogen production via direct biophotolysis, reporting a maximum HPR of 1.35 mL H₂/L/h or 0.0324 m³H₂/d/m³-reactor. Similarly, Tamburic et al. (2011) demonstrated a comparable maximum rate of 1.1 mL H₂/L/h or 0.0264 m³H₂/d/m³-reactor using *Chlamydomonas reinhardtii CC-124* bacteria strains.

3.1.1.2. Indirect biophotolysis. Indirect biophotolysis has 2 steps for producing bio-hydrogen. Firstly, carbohydrate is produced by the photosynthetic system as shown in Reaction (4). Secondly, the carbohydrate becomes H₂ and other chemical through Reaction (5). In this process, oxygen could be separated in the first step so that the hydrogenase enzyme would not be poisoned and could produce more hydrogen. Several types of cyanobacteria in indirect biophotolysis are *Gloeobacter* sp., *Synechococcus* sp., and *Synechocystis* sp. (Melitos et al., 2021; Rahman and Masdar, 2015).



Ohta et al. (1987) proved that indirect biophotolysis could achieve a higher HPR than direct. The study reported that the maximum HPR was 4.25 mLH₂/L/h or 0.102 m³-H₂/d/m³ using *Chlamydomonas MGA 161*. Another study by Tsyganov et al. (Tsygankov et al., 2002) in London used an outdoor photobioreactor (PBR) and *Anabaena variabilis* showed maximum volumetric productivity of 23 mL H₂/L/h and an average rate of 7 mL H₂/L/h or approximately 0.168 m³-H₂/d/m³.

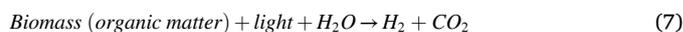
Biophotolysis offers the advantage of a straightforward hydrogen production method without the need for extra nutrients, utilizing water as an abundant source of electrons, while solar energy and carbon dioxide serve as the essential elements for cyanobacteria or algae. Nonetheless, the process demands high light intensity, and the low light conversion efficiency of green algae and cyanobacteria affects its overall performance. The reduced efficiency of photobioreactors in densely cultured environments further hampers hydrogen production due to limited light penetration (Ghiasian and Ghiasian, 2019; Singh et al., 2023).

3.1.2. Photo-fermentation (PF)

PF is a biological method that uses solar or light energy to convert biomass become H₂ and CO₂. This process is under anaerobic conditions performed by purple non-sulfur bacteria (PNSB) (Lu et al., 2021). PNSBs are colored purple, red, brown, and orange due to the pigments called bacteriochlorophylls and carotenoids that coat them. (Cohen-Bazire et al., 1957). In contrast to sulfur-oxidizing bacteria, PNSBs do not use sulfur as an electron donor. Instead, they use organic compounds or hydrogen as electron donors (Dhar et al., 2023). PNSBs can be found in water, soil, or wastewater (Grattieri, 2020). For instance, the conversion of glucose into hydrogen and organic acids by a PNSB, *Rhodospseudomonas palustris*, the reaction could be written as follows:



These reactions are exergonic, meaning they release energy as heat. The energy required for the reactions comes from the light absorbed by the bacteria. The bacteria use special pigments, such as bacteriochlorophylls and carotenoids, to capture light and transfer it to the electron transport chain, where H₂ is produced by hydrogenase (Elgarahy et al., 2022). The acetic acid (CH₃COOH) from Reaction 6 could be further reacted with water and the help of PNSB into the H₂ and CO₂. Therefore, the overall reaction for PF is as follows:



Ma et al. (2018) reported that using *Rhodobacter sphaeroides HY01* bacteria with mixed substrates (acetate, butyrate, and glutamate) through the photofermentation method could achieve a HPR of 156.1 mL H₂/L/h or 3.74 m³ H₂/d/m³. Krujatz et al. study gained a slightly higher HPR up to 195 mL H₂/L/h (4.68 m³ H₂/d/m³) using *Rhodobacter sphaeroides DSM 158* with glutamic acid and lactic acid as substrate (Krujatz et al., 2015). PF has numerous advantages over other methods, such as low energy requirement, higher HPR (compared to biophotolysis), and environmental friendliness (Gupta et al., 2024). However, it also faces some challenges, such as low hydrogen yield and

limited substrate range. To overcome these challenges, various strategies have been proposed, such as pretreatment of lignocellulosic biomass, optimization of reactor design and operation, and genetic engineering of microorganisms (Hitam and Jalil, 2023). PF has great potential for neutral emission of biohydrogen from biomass, especially when combined with other processes, such as MECs.

3.2. Light independent methods

When microalgae use carbon fixation routes or alternate energy sources that don't depend on light, they are said to be using light-independent methods. These methods are essential for microalgal survival in low-light conditions or in environments where light availability is limited. Microalgae production techniques offer significant opportunities to boost yield, broaden the range of products available, and diversify the product range. Comprehending and optimizing these pathways is imperative to propel the domain of microalgal biotechnology forward and actualize its complete capacity for sustainable bioproduction.

3.2.1. Dark fermentation

Dark fermentation (DF) is a biological method that converts substrates such as sugar, starches, or cellulose into biohydrogen, carbon dioxide, and volatile fatty acids (VFAs) (Jain et al., 2022). Anaerobic bacteria or microalgae execute this process devoid of light (El sharkawy et al., 2019). In cases where polymers such as starch or cellulose are employed, a hydrolysis reaction is requisite to enzymatically break down the polymers into simple sugars (Gurubel Tun et al., 2025). For instance, the hydrolysis of starch could be represented by the following reaction:



Sugars then further reacted to produce bio-hydrogen. Hydrogen production through DF has 2 paths: fermentation with acetic acid as the by-product or butyric acid (Talapko et al., 2023). Both reactions are shown below:



Microorganisms used to produce biohydrogen through DF could be strict or facultative anaerobic bacteria (El sharkawy et al., 2019; Talapko et al., 2023). The hydrogen production through DF with those types of bacteria and the yield are shown in Table 2.

Hydrogen produced by DF has some advantages compared to biophotolysis and photo-fermentation processes such as higher production rate, continuous day and night production, simple reactor design (Ajayi-Banji and Rahman, 2024), and low energy consumption (Srivastava et al., 2023a). Kim et al. (Kim and Lee, 2010) reported that the HPR

of DF using anaerobic digester sludge was up to 500 mL H₂/L/h or 12 m³ H₂/d/m³-reactor. However, the H₂ yield in DF is low due to the VFA by-products, which act as hydrogen scavengers (Wang et al., 2011). For instance, a study by Chookaew et al. (2014) using crude glycerol as substrate in batch reactor DF has a hydrogen yield of 43 mL-H₂/g COD. Another DF experiment by Marone et al. (2017) used sugar wastewater as a substrate yielding H₂ 52 mL-H₂/g COD. These yields are relatively low compared with MECs for the same substrate. The factors influencing hydrogen production in the DF process are temperature, pH, nutrition, retention time, and the inoculum sources. Among others, pH has the most significant impact due to its influence on hydrogenase enzyme activity (El sharkawy et al., 2019; Talapko et al., 2023). Fig. 3 illustrates the pH influence in biohydrogen production by DF of palm oil mill by Khongkliang et al. (2019).

Fig. 3 illustrates the significant impact of pH on the DF process. The investigation utilized a palm oil mill in thermophilic conditions, within 50–60°C temperature. The results indicate that at an initial pH of 5.50, the cumulative hydrogen production remains below 1 L H₂/L after 10 days. The hydrogen production increases when the pH is raised to 6.5, but it decreases again with further increases in pH. The optimum cumulative hydrogen production is observed at pH 6.5, reaching approximately 3 L H₂/L after 10 days.

3.2.2. Microbial electrolysis cells (MECs)

MECs are bio-electrochemical tools that use microorganisms to produce H₂ from biomass in the presence of water by applying a small amount of electric current (Srivastava et al., 2023b). Utilizing exoelectrogenic microorganisms, the MECs system transforms organic and biodegradable materials found in the waste stream into protons and electric currents. Exoelectrogenic microbes are microorganisms that can transfer electrons to external electron acceptors. These microbes could

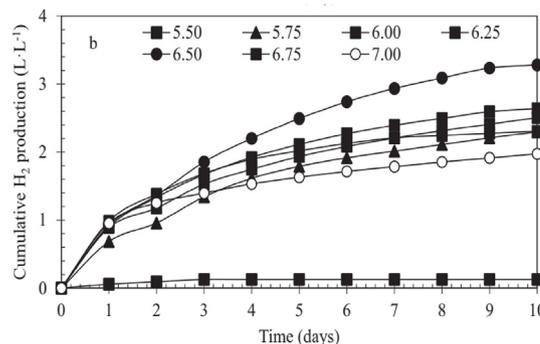


Fig. 3. Effect of pH on biohydrogen production through DF. This figure was adapted with permission from (Khongkliang et al., 2019). Copyright 2019 Elsevier.

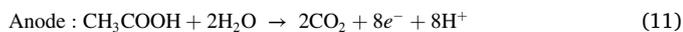
Table 2

Strict and facultative bacteria for DF with hydrogen yields per substrate.

Type of Bacteria	Substrate	Genus	Species	H ₂ -yields (mol/mol)	Ref.
Strict bacteria	Cellulose	<i>Ruminococcus</i>	<i>R. flavefaciens</i>	0.59	Shi et al. (1997)
Strict bacteria	Glucose	<i>Thermoanaerobacterium</i>	<i>T. thermosaccharolyticum</i>	10.86	(Cao et al., 2014)
Strict bacteria	Glucose	<i>Thermotoga</i>	<i>T. maritima</i>	2.20	Boileau et al. (2016)
Strict bacteria	Glucose	<i>Ruminococcus</i>	<i>R. albus</i>	2.01	Ntaikou et al. (2009)
Strict bacteria	Glucose	<i>Clostridium</i>	<i>C. acetobutylicum</i>	2.00	Chin et al. (2003)
Strict bacteria	Lactose	<i>Clostridium</i>	<i>C. thermolacticum</i>	3.00	Collet et al. (2004)
Strict bacteria	Saccharose	<i>Clostridium</i>	<i>C. butyricum</i>	2.78	Chen et al. (2005)
Strict bacteria	Xylose	<i>Clostridium</i>	<i>C. beijerinckii</i>	2.31	An et al. (2014)
Facultative	Glucose	<i>Enterobacter</i>	<i>Enterobacter cloacae</i>	2.20	Rezaei et al. (2009)
Facultative	Glucose	<i>Klebsiellae</i>	<i>K. pneumoniae</i>	2.07	Niu et al. (2010)
Facultative	Glucose	<i>Escherichia</i>	<i>Escherichia coli</i>	2.00	Bisailon et al. (2006)
Facultative	Glucose	<i>Citrobacter</i>	<i>C. amalonaticus</i>	1.24	Oh et al. (2008)
Facultative	Glucose	<i>Citrobacter</i>	<i>C. intermedius</i>	1.10	Brosseau et al. (1980)
Facultative	Glucose	<i>Citrobacter</i>	<i>C. freundii</i>	0.83	Hamilton et al. (2010)

generate electrical currents as part of their metabolic processes (Nazeer and Fernando, 2022). At the anode, microorganisms work with the biodegradable waste to produce protons and power. After that, the electrons are sent to the cathode, where they decrease the protons to produce H_2 (Katuri et al., 2023). Depending on the substrate, different microorganisms are employed in the MECs. Acetate is the suitable substrate for *Thermincola* sp., *Aeromonas hydrophila*, and *Gluconobacter oxydans*; acetate lactate is suitable for *Shewanella putrefaciens* and *Shewanella oneidensis*; and acetate glucose is good for *Klebsiella pneumoniae*, and *Rhodofex ferrireducens* (Kadier et al., 2020).

Since the MECs are oxygen-sensitive, anaerobic conditions are used throughout their execution. The following is a typical reaction scheme that uses MECs to create hydrogen from acetate (Dikshit et al., 2023):



3.2.2.1. Electrode materials. In MECs, the electrode facilitates the electrochemical reactions which convert organic matter into H_2 . The anode is the electrode where the oxidation of organic matter occurs, and the cathode is the electrode for the reduction of protons to hydrogen. The anode in MECs are typically carbon-based materials, such as graphite or carbon cloth, due to their good conductivity, biocompatibility, and low cost. The anode material and design could affect the performance of MECs, and various factors, such as electrode spacing and surface area, have been studied to optimize HPR (Koo and Jung, 2022). Additionally, the application of voltage is a critical factor that influences hydrogen production efficiency and microbial activity. A typical operating voltage ranges between 0.6 and 1.0 V, which is sufficient to drive the hydrogen evolution reaction at the cathode while minimizing energy input (Park et al., 2022). However, excessive voltage or current can lead to adverse effects, such as the formation of reactive oxygen species (ROS), which may inhibit microbial growth or even result in cell death (Gautam et al., 2023; Rossi et al., 2022).

To optimize performance, it is essential to balance the applied voltage with the microbial community's tolerance levels and the system's overall energy efficiency. For example, studies have shown that voltages around 0.7 V produce high hydrogen yields while maintaining stable microbial activity (Khongkliang et al., 2019). Table 3 now reflects these considerations, providing a clearer understanding of the role of voltage and current in MECs operations.

In MECs, the cathode is a crucial part that is required to produce hydrogen gas. The cathode, which is typically made up of metal catalysts and materials that support them, helps to lower the activation energy barrier and improve the reaction kinetics, especially on the cathode surface. Platinum is a hydrogen evolution process (HER) catalyst metal that is frequently utilized to improve the HER on cathodes. One of the biggest obstacles to growing MECs, meanwhile, is the high price of platinum. As a result, several studies have been done on lower-cost material catalysts in water electrolyzers, especially for hydrogen production. However, the applicability of the alternative catalysts for MECs is still being investigated (Gautam et al., 2023; Park et al., 2022).

MECs are classified into two types: single-chamber MECs, which place both electrodes in the same chamber, and two-chamber MECs, which place the anode and cathode separately. In a two-chamber system, the anode and cathode chambers are separated by a membrane, which helps maintain the purity of the H_2 and functions as a separator to prevent short circuits. In two-chamber MECs systems, the introduction of a membrane could lower the number of contaminants in the biogas while raising internal resistance. In single-chamber systems, on the other hand, membrane removal simplifies reactor design but raises the risk of methanogenic bacteria consuming hydrogen (Gautam et al., 2023; Park

Table 3
Microorganisms used in MECs with the anode material and current density.

Chamber Type	Microorganisms	Current density (A/m ²)	Anode material	Ref.
Single	<i>Brevibacillus</i> spp. PTH1	0.009	Graphite rod	Pham et al. (2008)
Single	<i>Thermincola ferriacetica</i> Z-0001	0.400	Solid graphite	(Marshall, 2009)
Single	<i>Escherichia coli</i> K12 HB101	1.000	Graphite-PTFE	(Zhang et al., 2006)
Single	<i>Desulfotobacterium hafniense</i> DCB2	1.100	Graphite block (treated)	Wagner et al. (2009)
Double	<i>Desulfuromonas acetoxidans</i>	0.005	Solid graphite	Bond et al. (2002)
Double	<i>Shewanella oneidensis</i> DSP10	0.013	Graphite felt	Ringeisen et al. (2007)
Double	<i>Pseudomonas aeruginosa</i> KRA3	0.017	Graphite rods	Rabaey et al. (2004)
Double	<i>Shewanella oneidensis</i> MR-1	0.018	Graphite felt	Bretschger et al. (2007)
Double	<i>Geobacter sulfurreducens</i>	0.065	Graphite sticks	Bond and Lovley (2003)
Double	<i>Enterobacter cloacae</i>	0.130	Carbon cloth	Rezaei et al. (2009)
Double	<i>Ochrobactrum anthropi</i> YZ-1	0.710	Carbon cloth	Zuo et al. (2008)
Double	<i>Klebsiella pneumoniae</i> L17	1.200	Carbon felt	(Zhang et al., 2008)

et al., 2022). Fig. 4 illustrates the typical configuration of MEC with two chambers and Table 4 shows several MECs performance with the highest HPR is $72 \text{ m}^3/\text{d}/\text{m}^3$ by Rossi et al. (2022).

In comparison to the fermentation method, MECs technologies have the advantage of using a wider range of biomass resources. Fermentation requires a substrate that has a high amount of carbohydrates, which limits the utilization of biomass that contains organic acid or proteins. On the other hand, bacterial respiration in MECs is possible with a diverse range of substrates, such as glucose, sugars, alcohols, carboxylic acids, proteins, and even a mixture such as domestic wastewater, animal waste, or industrial wastewater (Koo and Jung, 2022). Table 5 summarizes the advantages and limitations of microbial biomass conversion for producing hydrogen.

Recent advancements in the bioconversion of biomass have revealed several key challenges and opportunities for biohydrogen production. One significant constraint is the low efficiency of current bioconversion processes, particularly in the breakdown of lignocellulosic biomass. Recent literature highlights the need for improved pretreatment methods and microbial strain optimization to increase hydrogen yields from these complex feedstocks (Osman et al., 2023). Additionally, bioconversion processes face challenges related to scalability and the cost of feedstock supply. Integrating multiple biohydrogen production methods offers a

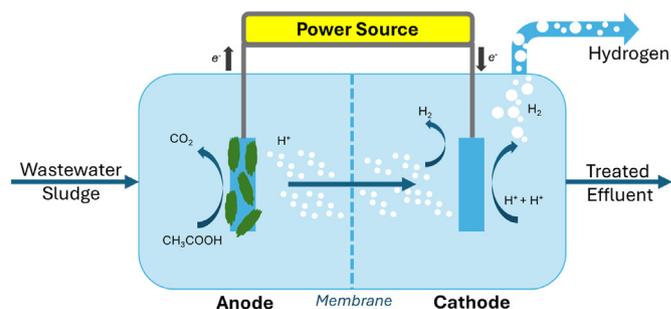


Fig. 4. MECs configuration to produce bio-hydrogen. The membrane is optional.

Table 4
MECs performance with different anode, cathode, and substrate.

Anode	Cathode	Substrate	Chamber	Volume (mL)	HPR ($\text{m}^3/\text{d}/\text{m}^3$)	Ref.
Carbon felt	Titanium with Pt	Acetate	Two	6600.0	0.02	ROZENDAL et al. (2006)
Carbon felt	Ni-foam	Wastewater	Two	250.0	0.04	Jayabalan et al. (2019)
Carbon felt	NiMoO ₄ -Ni foam	Wastewater	Two	250.0	0.12	Jayabalan et al. (2021)
Carbon felt	Carbon cloth with Pt catalyst	Wastewater	Tubular Two	1000.0	0.92	Heidrich et al. (2014)
Treated Carbon fiber brush	Carbon cloth with Pt catalyst	Glucose	Single	28.0	1.87	Selemba et al. (2009)
Treated Carbon fiber brush	Carbon cloth with Pt catalyst	Acetate	Single	28.0	3.12	Call and Logan (2008)
Stainless-steel fiber felt	Titanium with Pt	Acetate	Tubular Two	1000.0	7.06	Guo et al. (2017)
Treated graphite fiber brush	Carbon cloth with Pt	Acetate	Single	10.0	17.80	Cheng and Logan (2011)
Carbon felt	Vapor fed with Pt	Acetate	Two	4.5	72.00	Rossi et al. (2022)

Table 5
Biohydrogen methods comparison with the maximum hydrogen yield reported.

Method	Max HPR ($\text{m}^3/\text{day}/\text{m}^3$) [Ref.]	Advantages	Limitations
Direct biophotolysis	0.032 (Chader et al., 2009)	Straightforward process	Oxygen by-products inhibit hydrogenase enzyme; require high light intensity; low HPR
Indirect biophotolysis	0.168 (Tsygankov et al., 2002)	Uncomplicated process	Require high light intensity, low HPR
Photo-fermentation	4.680 (Krujatz et al., 2015)	Low energy requirement, HPR higher than biophotolysis	Need light, low H ₂ yield, limited substrate range (need of pretreatment)
Dark fermentation	12.000 (Kim and Lee, 2010)	Easy to operate, no need for light, simple reactor design	Low H ₂ purity, low energy efficiency; mixture of H ₂ and CO ₂ need further separation
Microbial electrolysis cells	72.000 (Rossi et al., 2022)	Operate with a variety of organic substrates, high H ₂ yield, high energy efficiency, high HPR	Complex reactor design and operation, high electrode cost for cathode catalyst

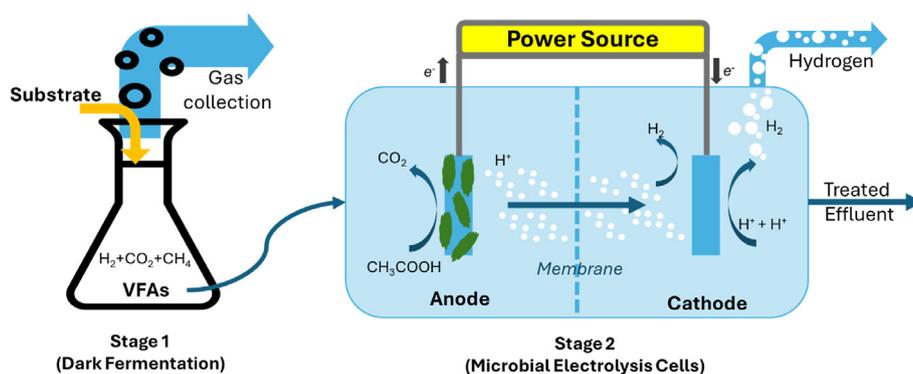


Fig. 5. Schematic illustration of DF and MECs integration.

promising approach to mitigate these challenges, enabling cost-effective and continuous biomass conversion. This integration will be explored further in the following section.

4. Integration of fermentation and microbial electrolysis cells

The limitations in the fermentation method such as low hydrogen yield due to the formation of volatile fatty acid (VFA) and incomplete substrate degradation could be mitigated and turned into advantageous when coupled with microbial electrolysis cells as shown in Fig. 5 (Lee et al., 2022; Srivastava et al., 2023a).

Several studies showed higher hydrogen yield and HPR when fermentation and microbial electrolysis cells were combined or integrated (Khongkliang et al., 2019; Lee et al., 2022; Sittijunda et al., 2022; Srivastava et al., 2024). Lu et al. (Lu et al., 2009) examined the bacterial fermentation of sugars and reported that without coupled with MECs, the HPR from fermentation was only $1.41 \text{ m}^3 \text{ H}_2/\text{m}^3/\text{d}$, while the integrated system of fermentation and MECs achieved HPR up to $2.11 \text{ m}^3 \text{ H}_2/\text{m}^3/\text{d}$ and 96% hydrogen recovery. Wang et al. (Wang et al., 2011) also showed similar results approximately a 41% increase in hydrogen yield from cellulose biomass by the integrated process of DF and MECs.

To maximize biomass conversion into biohydrogen, a two-stage DF and MECs functioning under thermophilic conditions is a highly promising alternative. Fig. 6 illustrates the HPR of DF and MEC independently as studied by Khongkliang et al. (2019). According to the findings, the optimal COD content is 66 g/L and pH of 6.5 for specific palm oil mill effluent substrate. The optimal voltage for MECs to produce hydrogen from DF effluent was 0.7 V. With this configuration, the HPR achieved $7.81 \text{ L H}_2/\text{L}/\text{d}$ or 3 times higher compared with just DF.

5. Conclusion and future prospect

Microbial biomass conversion offers a promising avenue for sustainable hydrogen production, presenting a clean and renewable alternative to fossil fuels. This review provides a comprehensive analysis of both light-dependent and light-independent methods, elucidating their strengths, limitations, and key factors influencing hydrogen production efficiency. Despite the impressive hydrogen production rates (HPRs) demonstrated by vapor-fed microbial electrolysis cells (MECs), achieving scalability for commercial applications remains a significant challenge.

Practical hurdles in scaling up these technologies include limited availability of economically viable feedstocks, high costs of bioreactor

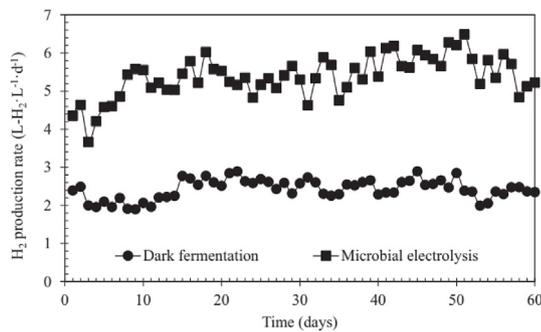


Fig. 6. H₂ Production Rate of DF and MECs. This figure was adapted with permission from (Khongkliang et al., 2019). Copyright 2019 Elsevier.

materials, and maintaining stability under industrial-scale conditions. For instance, microbial electrolysis cells require advanced electrode materials, which significantly increase costs at scale, while dark fermentation processes face difficulties in pretreatment and achieving hydrogen purity suitable for industrial use. Moreover, integrating these technologies into existing hydrogen infrastructure is complex, involving logistical, economic, and regulatory challenges. For example, the combination of dark fermentation with MECs enhances hydrogen yield but demands sophisticated engineering solutions and higher capital investments, posing additional barriers to real-world application.

Future research should prioritize addressing these practical challenges by focusing on pilot-scale demonstrations, optimizing reactor designs, reducing material costs, and identifying cost-effective and sustainable feedstocks. Innovations in synthetic biology, nanotechnology, and advanced materials could also accelerate the transition from lab-scale to commercial-scale systems. Furthermore, comprehensive techno-economic analyses and life cycle assessments are essential to evaluate the feasibility and environmental benefits of these technologies at scale. By addressing these critical areas, researchers can unlock the full potential of microbial biomass conversion for a clean and sustainable hydrogen future, contributing meaningfully to global decarbonization goals.

CRediT authorship contribution statement

Muhamad Reda Galih Pangestu: Writing – original draft, Software, Resources, Data curation, Conceptualization. **Shaikh Abdur Razzak:** Writing – review & editing, Supervision, Investigation, Data curation. **Shihab Uddin:** Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Project administration, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors assert that they don't have any identifiable conflicts.

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