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Investigating Materials for Green Hydrogen Generation with Potential and Challenges in Biological Hydrogen Production

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Abstract

The global pursuit of sustainable and clean energy sources has intensified interest in green hydrogen production, particularly through biological methods that offer eco-friendly alternatives to conventional technologies. This review investigates the potential of various materials and strategies used in biological hydrogen production, including phototrophic and fermentative pathways involving algae, cyanobacteria, and photosynthetic bacteria. Special emphasis is placed on the role of advanced nanomaterials and bio-catalysts that enhance the efficiency and scalability of hydrogen generation processes. Key materials such as metal-based catalysts, semiconductor photocatalysts, and carbon-based nanostructures are explored for their roles in optimising biological pathways and improving electron transfer mechanisms. Additionally, the paper discusses integrated systems that couple biological processes with engineered materials to boost hydrogen yields. Despite promising advancements, several challenges hinder large-scale application, including low production rates, oxygen sensitivity of hydrogenase enzymes, and difficulties in reactor design and process optimisation. Addressing these barriers requires multidisciplinary efforts involving material science, microbiology, and process engineering. This review provides a comprehensive overview of the state-of-the-art materials and methodologies, offering insights into their potential and limitations, and proposes future research directions to overcome existing challenges for viable, sustainable hydrogen production from biological sources.

Keywords: *Bio-catalysts; Biological Hydrogen Production; Green Hydrogen; Photocatalysis; Sustainable Energy; Waste-to-Energy*

Introduction

As the global demand for clean and renewable energy grows, hydrogen has emerged as a key player in the transition to a sustainable energy future (Hassan *et al*., 2024). Hydrogen, the most abundant element in the universe, can be utilised as a clean energy carrier due to its high energy density and versatility (Zhang *et al*., 2024a). When used as a fuel, hydrogen emits only water as a byproduct, making it an attractive alternative to fossil fuels. The potential of hydrogen as a clean energy source extends across multiple sectors, including transportation, power generation, and industrial processes, offering a pathway to significant reductions in carbon emissions and air pollution (Hassan *et al*., 2024, Ofélia de Queiroz *et al*., 2024).

Among various forms of hydrogen, "green hydrogen" is produced using renewable energy sources, such as solar, wind, or hydropower, ensuring minimal environmental impact (Figure 1) (Nnabuife *et al*., 2024). Unlike "gray hydrogen," which is produced from natural gas and results in substantial carbon emissions, and "blue hydrogen," which relies on carbon capture technologies, green hydrogen is considered a truly sustainable solution (Saha *et al*., 2024). It aligns with the global efforts to achieve carbon neutrality and mitigate climate change, particularly as nations set ambitious targets for reducing their reliance on fossil fuels. Green hydrogen has the potential to revolutionise energy systems by enabling long-term energy storage, decarbonizing heavy industries such as steel and cement production, and facilitating the widespread adoption of hydrogen fuel cells in transportation (Zaiter *et al*., 2024). Additionally, hydrogen can play a crucial role in balancing electricity grids by storing excess renewable energy and releasing it during periods of high demand. As the world transitions to a low-carbon economy, green hydrogen is expected to become an integral component of future energy infrastructures, supporting both energy security and environmental sustainability.

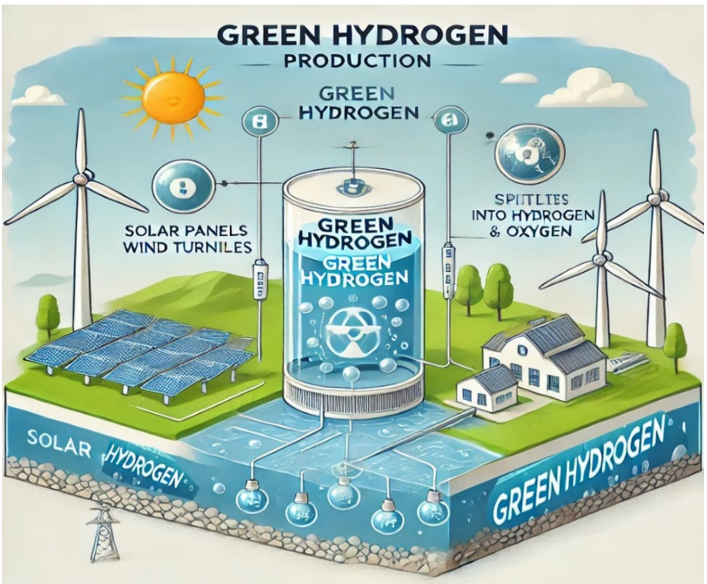

Figure 1: Schematic Diagram of Green Hydrogen Production *(Source: Author)*

The production of green hydrogen can be achieved through various methods, each utilising renewable energy to split water molecules (H_2O) into hydrogen (H_2) and oxygen (O_2) (Worku et al., 2024). Electrolysis process involves the use of electricity generated from renewable sources to split water into hydrogen and oxygen in an electrolyser (Akyüz, Telli & Farsak,2024). There are different types of electrolysis, including alkaline electrolysis, proton exchange membrane (PEM) electrolysis, and solid oxide electrolysis (Sebbahi *et al*., 2024). Among these, PEM electrolysis is widely researched due to its high efficiency and compatibility with intermittent renewable energy sources like solar and wind power (Kumar & Samuel, 2024). Photocatalytic water splitting sunlight leverages to drive the reaction that separates water into hydrogen and oxygen. This method relies on the development of efficient photocatalysts, such as semiconductors, that can absorb sunlight and convert it into chemical energy. Although still in the research and development phase, photocatalysis represents a promising route for large-scale, low-cost hydrogen production in the future (Ahmed *et al*., 2024). Biological hydrogen production is gaining attention as a green and sustainable alternative to conventional methods (Ahmad *et al*., 2024a). This approach harnesses the natural abilities of microorganisms to produce hydrogen through various biological processes. The key biological methods for hydrogen production include biophotolysis, dark fermentation, and microbial electrolysis.

Biological hydrogen production offers several advantages, such as the use of renewable feedstocks, low energy requirements, and minimal environmental impact (Jain *et al*., 2024). Unlike electrolysis, which requires significant amounts of electricity, or photocatalysis, which depends on advanced materials, biological methods utilise naturally occurring biological systems, making them inherently sustainable. Furthermore, these methods align with circular economy principles by utilising waste materials, such as agricultural residues or wastewater, as feedstocks for hydrogen production. Biophotolysis is a process where photosynthetic microorganisms, such as cyanobacteria and green algae, use sunlight to split water molecules, producing hydrogen and oxygen (Kossalbayev *et al*., 2024). This method mimics natural photosynthesis but redirects the energy to produce hydrogen instead of biomass. However, the efficiency of biophotolysis is currently limited by the low hydrogen yield and the oxygen sensitivity of the hydrogen-producing enzymes (hydrogenases) involved in the process (Ram, Rani & Kumar,2024). Dark fermentation involves the anaerobic digestion of organic matter by microorganisms to produce hydrogen (Srivastava *et al*., 2024). This process can occur in the absence of light, making it more flexible than biophotolysis. Various organic substrates, such as food waste, agricultural residues, and industrial effluents, can be used as feedstock. Although dark fermentation has shown potential for large-scale applications (Ahmad *et al*., 2024b), the hydrogen yield is often limited, and additional treatment of byproducts, such as organic acids, is required. Microbial electrolysis cells (MECs) represent a hybrid approach that combines biological activity with electrochemical processes (Arun *et al*., 2024). In MECs, microorganisms oxidise organic matter and release electrons, which are used to reduce protons and produce hydrogen at the cathode. MECs offer a promising route for integrating biological and electrochemical methods, but challenges such as the need for external power inputs and low hydrogen production rates still need to be addressed.

Biological hydrogen production holds great promise for contributing to a sustainable energy future, particularly by enabling the conversion of organic waste into clean energy. The simplicity and potential cost-effectiveness of biological systems make them attractive for both developed and developing regions. Moreover, advances in synthetic biology and metabolic engineering have opened new avenues for optimising microorganisms for hydrogen production, enhancing their efficiency, and overcoming the limitations of traditional biological methods. However, several challenges remain in the widespread adoption of biological hydrogen production (Jiao *et al*., 2024). These include the low efficiency of natural processes, the complexity of scaling up laboratory systems to industrial levels, and the economic feasibility of large-scale implementation. Additionally, maintaining optimal conditions for microbial growth and hydrogen production, such as temperature, pH, and nutrient availability, can be challenging in real-world environments. Hydrogen has the potential to play a transformative role in achieving global sustainability goals, and biological hydrogen production offers a promising and environmentally friendly pathway for green hydrogen generation. However, further research and development are essential to overcome the existing obstacles and unlock the full potential of this technology. This paper will explore the opportunities and challenges in biological hydrogen production, highlighting innovative approaches and future directions for this field.

Literature Review

The growing urgency to transition towards a low-carbon economy has prompted a surge in research focused on sustainable hydrogen production. Green hydrogen, produced through methods that utilise renewable resources and have minimal environmental impact, stands at the forefront of these efforts. Various materials and processes have been developed for green hydrogen production, including advanced photocatalysts, electrochemical materials, enzymes, and biological systems (Alinejad *et al*., 2024). This section provides an in-depth overview of the key materials and methods used in green hydrogen production, focusing on the role of biological hydrogen production processes and recent technological advances.

The materials used in hydrogen production play a critical role in determining the efficiency, scalability, and environmental impact of the process (Chelvam *et al*., 2024). These materials can be broadly categorised based on the hydrogen production method they support—photocatalysis, electrochemical water splitting, or biological processes. Photocatalysts are materials that can absorb sunlight and convert it into chemical energy to drive reactions, such as water splitting, that produce hydrogen. Semiconductor materials, such as titanium dioxide (TiO_2), cadmium sulphide (CdS), and zinc oxide (ZnO), have been extensively studied for their photocatalytic properties (Ghamarpoor, Fallah & Jamshidi, 2024). The efficiency of a photocatalyst depends on its ability to harvest light across a broad spectrum, its stability under irradiation, and its ability to facilitate charge separation and transfer. TiO_2 is one of the most widely studied photocatalysts due to its abundance, chemical stability, and non-toxicity (AlMohamadi *et al*., 2024). However, it can only absorb ultraviolet light, which limits its overall efficiency. Researchers are exploring ways to enhance TiO_2's performance by doping it with metal ions or nonmetals to extend its light absorption into the visible spectrum. Cadmium Sulphide (CdS) can absorb visible light, making it a promising candidate for photocatalytic hydrogen production. However, its application is limited by its toxicity and photocorrosion (Jie *et al*., 2024). Recent research has focused on stabilising

CdS by coupling it with other materials, such as carbon nanotubes or reduced graphene oxide, to improve its durability and efficiency.

Perovskite materials, with their tunable band gaps and excellent light absorption properties, have recently garnered attention for photocatalytic (Figure 2) hydrogen production (Khan *et al*., 2024). The challenge with perovskites lies in their stability, as many perovskites degrade quickly under exposure to moisture and heat. The ongoing research is aimed at developing more stable perovskite structures for hydrogen production. Electrochemical Materials water splitting, also known as electrolysis, involves the use of an electrolyser that splits water molecules into hydrogen and oxygen using electricity. The materials used for the electrodes and electrolytes in electrolysers are crucial for determining the efficiency of this process. Platinum (Pt) and Iridium (Ir) are benchmark materials for hydrogen and oxygen evolution reactions (HER and OER), respectively, due to their high catalytic activity (Guo *et al.*, 2024). However, their high cost and scarcity limit their widespread application in commercial electrolysers. Researchers are actively seeking alternative materials that can match the performance of Pt and Ir while being more cost-effective and abundant. Nickel-Based Catalystsis a more abundant and less expensive alternatives to platinum, making them a popular choice for alkaline electrolysis (Emam *et al.,* 2024). Nickel-based catalysts, such as nickel-iron (NiFe) and nickel-molybdenum (NiMo) alloys, have shown promise for both the HER and OER in alkaline media. These materials are being further optimised to enhance their catalytic efficiency and durability under operating conditions. Solid Oxide Electrolysis Cells (SOECs) use solid oxide materials, such as yttria-stabilised zirconia (YSZ), as the electrolyte, allowing for water splitting at high temperatures (700-1,000°C). These high temperatures improve the efficiency of the electrolysis process, but the development of robust materials that can withstand such conditions remains a challenge.

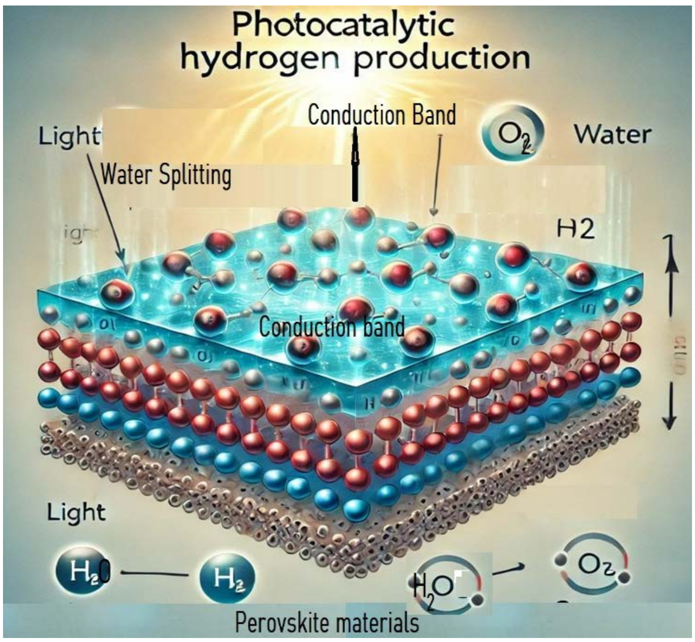

Figure 2: Schematic Image of Photocatalytic Hydrogen Production Using Perovskite Materials *(Source: Author)*

Enzymes play a central role in biological hydrogen production methods. In particular, hydrogenases and nitrogenases are the key enzymes involved in microbial hydrogen production (Cui *et al*., 2024). Hydrogenases are enzymes that catalyse the reversible conversion of protons into hydrogen gas. These enzymes are found in a wide variety of microorganisms, including bacteria and algae. Hydrogenases are classified into three major types based on their metal active sites: [FeFe] hydrogenases, [NiFe] hydrogenases, and [Fe] hydrogenases (Lachmann *et al*., 2024). [FeFe] hydrogenases are generally more efficient at hydrogen production but are sensitive to oxygen, which limits their application in oxygenic photosynthesis. Efforts to engineer oxygen-tolerant hydrogenases are underway to enhance their utility in green hydrogen production. Nitrogenases are enzymes primarily responsible for nitrogen fixation but can also produce hydrogen as a byproduct (Happe & Marx, 2024). These enzymes are less efficient at hydrogen production than hydrogenases but are more tolerant of oxygen. Recent research has focused on manipulating nitrogenase pathways to increase hydrogen yield and make them more viable for industrial-scale hydrogen production.

Biological hydrogen production harnesses the natural metabolic pathways of microorganisms to produce hydrogen. The key processes involved in biological hydrogen production include biophotolysis, dark fermentation, and microbial electrolysis (Rathi *et al.*, 2024). These methods offer unique advantages, such as the use of renewable feedstock (e.g., water, organic waste) and low energy requirements, making them attractive for sustainable hydrogen production. Biophotolysis refers to the production of hydrogen through the action of photosynthetic microorganisms, such as cyanobacteria and green algae. These microorganisms use sunlight to split water into hydrogen and oxygen through the process of photosynthesis. Biophotolysis can be further divided into two types: direct and indirect biophotolysis.

Direct Biophotolysis is when photosynthetic organisms directly split water molecules into hydrogen and oxygen using sunlight. This process occurs in the thylakoid membranes of microorganisms, where the absorbed light energy drives the water-splitting reaction (Akram *et al*., 2024). However, the efficiency of direct biophotolysis is limited by the oxygen sensitivity of hydrogenase enzymes and the competition between oxygen and hydrogen production pathways (Kumar & Fiori, 2024). Indirect biophotolysis separates the stages of photosynthesis and hydrogen production. For example, in some cyanobacteria, photosynthesis is used to produce organic compounds, such as carbohydrates, which are then metabolised under anaerobic conditions to generate hydrogen (Ananthi *et al*., 2024). This separation helps mitigate the inhibitory effects of oxygen on hydrogen production, but it also adds complexity to the process. Dark fermentation is a process in which microorganisms degrade organic matter to produce hydrogen in the absence of light. This process typically involves anaerobic bacteria that convert carbohydrates, proteins, and lipids into hydrogen, carbon dioxide, and organic acids. The simplicity of dark fermentation, along with its ability to utilise a wide range of feedstocks, makes it one of the most promising methods for biological hydrogen production (Rathi *et al*., 2024). Dark fermentation can utilise various substrates, including food waste, agricultural residues, and industrial effluents. The choice of substrates significantly affects the hydrogen yield, with carbohydrates typically producing higher yields than proteins or lipids. The diversity of potential feedstock makes dark fermentation adaptable to different waste management systems supporting the circular economy. Despite its potential, dark fermentation faces several challenges, including low hydrogen yields and the production of organic acid byproducts, which require further treatment (Jain *et al*., 2024). Research is ongoing to optimise microbial communities, metabolic pathways, and process conditions to enhance hydrogen production and minimise byproduct formation.

Microbial electrolysis cells (MECs) represent a hybrid approach that combines biological activity with electrochemical processes (Figure 3) to produce hydrogen (Swaminathan *et al*., 2024). In MECs, microorganisms oxidise organic matter, releasing electrons that are used to reduce protons and produce hydrogen at the cathode. Unlike conventional electrolysis, MECs operate at lower voltages, making them more energy efficient. MECs consist of an anode, where microbial oxidation of organic matter occurs, and a cathode, where hydrogen is produced. The electrons generated at the anode are transferred to the cathode through an external circuit, driving the hydrogen production reaction. MECs can be powered by renewable energy sources, further enhancing their sustainability. MECs offer several advantages, such as the ability to convert organic waste into hydrogen, lower energy requirements compared to traditional electrolysis, and the potential for integrating with renewable energy systems (Arun *et al*., 2024). However, challenges remain, including the need for external power inputs, low hydrogen production rates, and the development of stable and efficient electrode materials.

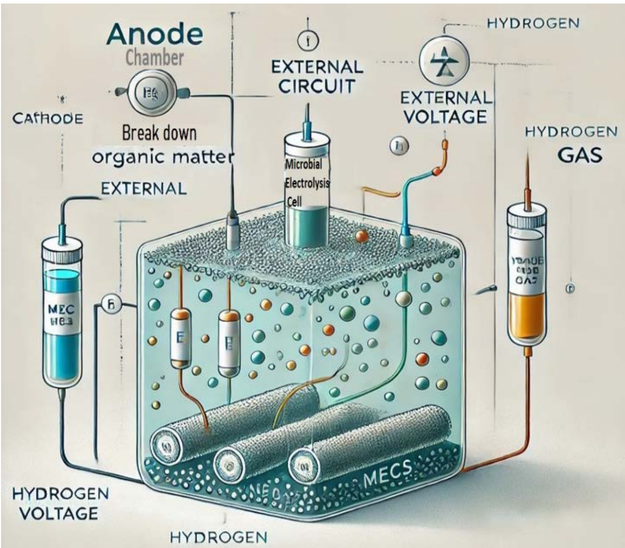

Figure 3: Schematic Drawings of Microbial Electrolysis Cells (MECs) Represent a Hybrid Approach that Combines Biological Activity with Electrochemical Processes to Produce Hydrogen *(Source: Author)*

Recent advances in materials science and technology have significantly enhanced the efficiency and viability of green hydrogen production methods, including biological processes. Innovations in synthetic biology, metabolic engineering, and materials science are helping to overcome the limitations of traditional biological hydrogen production systems. Synthetic biology and metabolic engineering have opened new avenues for optimising microorganisms for hydrogen production (Zhang *et al*., 2024b). Researchers are engineering microorganisms to enhance their hydrogen production pathways, improve enzyme stability, and increase tolerance to environmental conditions. For example, the introduction of oxygen-tolerant hydrogenases into photosynthetic organisms has shown promise in improving the efficiency of biophotolysis (Rady, Ali & El-Sheekh, 2024). Genetic engineering techniques are being used to create genetically modified strains of bacteria and algae with enhanced hydrogen production capabilities. By manipulating the genes responsible for hydrogenase expression, researchers can increase hydrogen yield and reduce the inhibition.

Opportunities in Biological Hydrogen Production

Biological hydrogen production offers a range of opportunities in the pursuit of sustainable energy, with the potential for large-scale deployment and unique advantages over conventional methods (Bhandari & Adhikari., 2024). The adaptability of biological processes, the ability to use renewable feedstocks, and the alignment with circular economy principles make biological hydrogen production an appealing avenue for green hydrogen generation. This section explores the opportunities for large-scale production, the advantages of biological methods, and case studies demonstrating successful implementation.

Potential for Large-Scale Production

The scalability of biological hydrogen production methods is a crucial factor in determining their viability as a major contributor to global hydrogen supply (Teke *et al*., 2024). Biological hydrogen production can utilise a wide range of renewable feedstocks, including water, organic waste, agricultural residues, and wastewater. This flexibility in feedstock selection is a significant advantage, as it allows for the integration of hydrogen production into existing waste management systems. For example, agricultural waste and food waste, which are often disposed of in landfills, can be diverted to bioreactors for hydrogen production, reducing both waste and emissions. The use of renewable feedstocks not only contributes to environmental sustainability but also provides opportunities for decentralised hydrogen production (Chelvam *et al*., 2024). In rural and agricultural regions, local waste streams can be converted into hydrogen, reducing the need for transportation and distribution of energy resources. This decentralised approach can help reduce energy poverty and increase energy security in remote or underdeveloped areas. Biological hydrogen production processes, such as dark fermentation and microbial electrolysis cells (MECs), can be integrated with existing industrial and agricultural systems to create synergies between waste management, energy production, and resource recovery. For example, wastewater treatment plants can implement dark fermentation processes to convert organic waste into hydrogen while simultaneously treating wastewater (Martínez-Fraile *et al.,* 2024). This approach not only produces clean energy but also enhances the sustainability of wastewater treatment systems. Similarly, agricultural operations that generate large quantities of organic waste can incorporate biological hydrogen production into their processes, creating a closed-loop system where waste is converted into energy (Kazmi *et al.,* 2024). This type of integration reduces the environmental footprint of agriculture and enhances the overall efficiency of resource use.

Scaling up biological hydrogen production requires the development of efficient bioreactor systems that can accommodate large volumes of feedstock and optimise microbial activity. Recent advances in bioreactor design have focused on improving mass transfer, maintaining optimal conditions for microbial growth, and enhancing hydrogen production rates (Zarei *et al*., 2024). Continuous-flow reactors, which allow for the constant input of feedstock and output of hydrogen, have shown promise for large-scale applications. These reactors can operate for extended periods without the need for frequent shutdowns or cleaning, increasing their overall efficiency. The design of continuous-flow systems also allows for better control of process conditions, such as temperature and pH, which are critical for maximising hydrogen production. Membrane bioreactors (MBRs) have been explored as a means of enhancing the separation of hydrogen gas from the liquid phase, reducing the loss of hydrogen and improving overall efficiency (Tran *et al*., 2024). In MBRs, a semi-permeable membrane separates the microbial culture from the hydrogen gas, allowing for more efficient gas collection. This technology is particularly useful in processes like microbial electrolysis, where hydrogen must be efficiently harvested from the bioreactor. Hybrid systems that combine biological and electrochemical methods, such as microbial electrolysis cells, offer potential for scaling up. These systems can utilise both biological activity and electrical inputs to produce hydrogen more efficiently than either method alone. Advances in electrode materials and cell design are helping to make these systems more viable for large-scale deployment. The flexibility of biological hydrogen production systems allows for the potential development of large-scale production facilities. These facilities could be located near sources of renewable feedstock, such as agricultural regions, food processing plants, or wastewater treatment facilities. The ability to co-locate hydrogen production with feedstock sources reduces transportation costs and emissions, making the overall process more sustainable. Large-scale biological hydrogen production facilities could also benefit from economies of scale, reducing the cost of hydrogen production and making it more competitive with conventional methods. As technology continues to advance and the cost of bioreactor systems decreases, the economic feasibility of large-scale biological hydrogen production will improve.

Advantages of Conventional Methods

Biological hydrogen production offers several distinct advantages over conventional hydrogen production methods, such as steam methane reforming (SMR) and traditional electrolysis (Nemitallah *et al*., 2024). These advantages position biological methods as a key component of future green hydrogen production systems. One of the most significant advantages of biological hydrogen production is its alignment with sustainability and environmental goals (Martins *et al*., 2024). Unlike conventional methods, which often rely on fossil fuels and generate carbon emissions, biological processes utilise renewable resources and produce minimal environmental impact. For example, dark fermentation uses organic waste as a feedstock, transforming waste products into clean energy rather than contributing to landfills or pollution. Biological hydrogen production also has the potential to contribute to carbon sequestration efforts. Some microorganisms involved in hydrogen production, such as algae, can absorb carbon dioxide during photosynthesis, further reducing the carbon footprint of the process. Additionally, integrating biological hydrogen production with carbon capture and utilisation (CCU) technologies could create a closed-loop system where carbon emissions are captured and reused in the production process (Zhang *et al*., 2024c). Biological methods generally have lower energy requirements than conventional electrolysis or thermochemical processes. For example, dark fermentation and microbial electrolysis cells operate at ambient temperatures and do not require the high temperatures or pressures needed for processes like steam methane reforming. This reduces overall energy consumption and makes biological methods more energy efficient. In addition, the ability to use sunlight as a direct energy source in biophotolysis offers a unique advantage over conventional methods, which often rely on electricity generated from renewable sources. By directly harnessing solar energy, biophotolysis bypasses the need for intermediate energy conversion steps, potentially increasing the overall efficiency of hydrogen production.

Biological hydrogen production methods are highly adaptable to a wide range of feedstocks, from water and organic waste to agricultural residues and industrial effluents (Garg *et al*., 2024). This adaptability provides flexibility in choosing the most appropriate feedstock for a given region or application, allowing for the optimisation of hydrogen production based on local resources. The use of diverse feedstocks also enables biological hydrogen production to contribute to waste management efforts. By converting waste materials into hydrogen, biological processes can help reduce the environmental impact of waste disposal and promote the circular economy. This is particularly important in regions with large agricultural or industrial sectors, where waste management is a significant challenge. The ability to implement biological hydrogen production systems on a smaller scale compared to conventional methods offers the potential for decentralised production. Decentralised systems can be deployed in remote or rural areas where access to centralised energy infrastructure is limited. This decentralisation can increase energy access, reduce reliance on fossil fuels, and promote energy independence in these regions. Decentralised biological hydrogen production systems can also be integrated with local renewable energy sources, such as solar or wind power, further enhancing their sustainability. For example, a rural community with access to both agricultural waste and solar energy could implement a combined biological and solar hydrogen production system, providing clean energy for local use without relying on external energy supplies.

Case Studies of Successful Implementation

Several successful case studies have demonstrated the potential of biological hydrogen production on both small and large scales. These case studies highlight the versatility of biological methods and their ability to contribute to green hydrogen production in various contexts.

In 2015, researchers at the University of California, Berkeley, developed a strain of cyanobacteria that could produce hydrogen through biophotolysis using sunlight and water as inputs (Pereira *et al*., 2024, Barry *et al*., 2016). By introducing mutations that enhanced the efficiency of the hydrogenase enzyme, the researchers were able to increase hydrogen production rates while reducing the inhibitory effects of oxygen. This work represents a significant step forward in the development of biophotolysis for large-scale hydrogen production. The success of this project highlights the potential of genetic engineering and synthetic biology in optimising biological systems for hydrogen production. The engineered cyanobacteria demonstrated the feasibility of using biophotolysis as a sustainable hydrogen production method, particularly in regions with abundant sunlight. Further development of these systems could lead to commercial scale biophotolysis facilities that produce hydrogen using only sunlight and water. In 2019, a wastewater treatment plant in Seoul, South Korea, implemented a dark fermentation system to convert organic waste from wastewater into hydrogen (Gebreslassie *et al*., 2021). The system used a continuous-flow reactor that allowed for the constant input of organic waste and output of hydrogen, reducing the need for frequent maintenance. The plant successfully produced hydrogen while simultaneously treating wastewater, demonstrating the potential for integrating hydrogen production with waste management.

This case study highlights the scalability and practicality of dark fermentation for hydrogen production. By using waste materials as feedstock, the plant reduced its overall environmental impact and created a new revenue stream through the sale of hydrogen. Similar systems could be implemented in other wastewater treatment facilities around the world, contributing to the circular economy and reducing the environmental footprint of waste treatment. In 2020, a pilot project in the Netherlands tested the use of microbial electrolysis cells (MECs) to produce hydrogen from agricultural waste (Kadier *et al*., 2020). The project involved a dairy farm that generated large quantities of manure, which was fed into the MEC system to produce hydrogen. The hydrogen was then used to power farm equipment and vehicles, reducing the farm's reliance on fossil fuels.

Obstacles in Biological Hydrogen Production

Despite the numerous opportunities and advantages, biological hydrogen production faces significant challenges that must be addressed before it can be adopted on a large scale (Emetere *et al*., 2024). These challenges include technical limitations, economic barriers, and environmental and sustainability issues. Each of these obstacles presents unique difficulties in scaling up and optimising biological hydrogen production, but ongoing research and technological advancements continue to seek solutions. In this section, the key obstacles that hinder the widespread implementation of biological hydrogen production will be discussed.

Technical Challenges

Efficiency Limitations: One of the primary technical challenges in biological hydrogen production is the low efficiency of the processes. Compared to conventional methods like steam methane reforming (SMR) and electrolysis, biological methods generally produce lower yields of hydrogen (Nemitallah *et al.*, 2024). Several factors contribute to these efficiency limitations, including the intrinsic metabolic constraints of microorganisms, competition between metabolic pathways, and the sensitivity of enzymes involved in hydrogen production.

Microorganisms, such as bacteria, cyanobacteria, and algae, are not naturally optimised for large-scale hydrogen production. Their metabolic pathways often prioritise other cellular functions, such as growth, reproduction, and the synthesis of organic molecules (Zhang *et al*., 2024b). As a result, the proportion of energy dedicated to hydrogen production is limited. Genetic engineering and metabolic optimisation can help address these constraints, but these approaches are still in the developmental stage. In photosynthetic microorganisms, hydrogen production often competes with other metabolic pathways, such as oxygenic photosynthesis. For example, in biophotolysis, the oxygen produced during water splitting can inhibit the activity of hydrogenases, reducing hydrogen yield. Overcoming this competition requires innovative approaches to either separate or optimise these pathways (Goveas *et al.,* 2024). Enzymes like hydrogenases and nitrogenases play a critical role in biological hydrogen production. However, these enzymes are often sensitive to environmental conditions, particularly oxygen levels (Chen *et al*., 2024). [FeFe] hydrogenases, for instance, are highly efficient at producing hydrogen but are also highly sensitive to oxygen, which can inactivate them. This sensitivity poses a significant challenge in developing robust and reliable biological hydrogen production systems, particularly in aerobic environments.

While biological hydrogen production shows promise at the laboratory scale, scaling up these processes to industrial levels presents several challenges. Biological systems are inherently complex, and scaling up often introduces new variables that can affect performance. Effective scaling requires the development of bioreactor systems that can maintain optimal conditions for microbial growth and hydrogen production over large volumes. Achieving uniform mixing, mass transfer, and temperature control in large bioreactors is challenging. In addition, maintaining the viability and activity of microbial cultures over extended periods can be difficult, especially in continuous-flow systems where microbes are exposed to changing conditions (Sarkar *et al.*, 2024). Biological systems are often sensitive to fluctuations in environmental conditions, such as pH, temperature, and substrate concentration. These fluctuations can affect microbial activity and reduce hydrogen production efficiency. Maintaining stable process conditions at scale requires advanced monitoring and control systems, which can add complexity and cost to the production process.

Efficient separation and collection of hydrogen gas from the bioreactor is another scalability challenge. In many biological processes, hydrogen is produced in a gaseous form that must be separated from the liquid phase and other gases, such as carbon dioxide. This separation process can be energy-intensive and reduce the overall efficiency of hydrogen production. Biological hydrogen production often relies on organic waste or biomass as feedstock, which can vary widely in composition. The variability in feedstock can affect the efficiency and consistency of hydrogen production, as different substrates can produce different yields of hydrogen (Perat *et al.,* 2024). The composition of organic waste can vary depending on its source, seasonal changes, and processing methods. For example, food waste from different regions or industries may have different ratios of carbohydrates, proteins, and fats, which can influence microbial metabolism and hydrogen yield. This variability makes it difficult to standardise biological hydrogen production processes (Economou *et al*., 2024). Organic waste streams can contain contaminants, such as heavy metals, antibiotics, or toxic compounds, which can inhibit microbial activity and reduce hydrogen production efficiency. Managing these contaminants requires pre-treatment steps, which can add complexity and cost to the process.

Economic Challenges

High Cost of Materials and Production: The economic viability of biological hydrogen production is currently limited by the high cost of materials and production methods (Singla *et al*., 2024). Biological processes generally require specialised materials, such as enzymes, catalysts, and bioreactors, which can be expensive to produce and maintain.

The production of enzymes, such as hydrogenases and nitrogenases, can be costly, particularly when these enzymes are used in large quantities (Happe & Marx,2024). Enzyme production often requires complex fermentation processes, purification steps, and stabilisation techniques, all of which add to the overall cost of hydrogen production. Additionally, the sensitivity of enzymes to environmental conditions means that they may need to be replaced frequently, further increasing costs. In microbial electrolysis cells (MECs), the electrodes play a crucial role in facilitating hydrogen production. However, the materials commonly used for electrodes, such as platinum and other precious metals, are expensive and limited in supply (Swaminathan *et al.,* 2024).

Developing cost-effective and durable electrode materials is essential for reducing the overall cost of MEC-based hydrogen production. The construction and operation of large-scale bioreactors represent a significant capital investment. Bioreactors must be designed to maintain optimal conditions for microbial growth and hydrogen production, which often requires advanced control systems, monitoring equipment, and specialised materials (Jiao *et al*., 2024). Operating costs can also be high, particularly if energy inputs, such as heating or mixing, are required to maintain process stability. Biological hydrogen production is currently less economically competitive than conventional methods, such as steam methane reforming (SMR) and electrolysis (Nemitallah *et al*., 2024). The cost of producing hydrogen through biological methods is generally higher due to the lower efficiency of the processes and the higher cost of materials.

Cost of Hydrogen Production: The cost of producing hydrogen through biological methods is often measured in terms of dollars per kilogram of hydrogen produced (Ghasemi, Nikafshan & Akrami, 2024). Biological processes typically produce lower yields of hydrogen compared to SMR or electrolysis, which means that more feedstock and larger production facilities are needed to achieve the same output. This increases the overall cost of production, making biological hydrogen less competitive in the market.

The market for green hydrogen is still in its early stages, and demand for hydrogen produced through biological methods is relatively low. Most current hydrogen demand is met by conventional methods, which are cheaper and more widely available (Singla *et al*., 2024).

As a result, biological hydrogen producers may struggle to compete in a market dominated by lower-cost alternatives. The development and scaling of biological hydrogen production systems require significant investment in research, development, and infrastructure. Securing funding for these projects can be challenging, particularly in a market where conventional hydrogen production methods are more established and cost-effective. Developing new biological hydrogen production technologies, such as genetically engineered microorganisms or advanced bioreactors, requires substantial investment in research and development (R&D) (Zhang *et al.,* 2024a). These R&D costs can be high, and the return on investment may not be immediate, making it difficult to attract private investment. Scaling up biological hydrogen production to commercial levels requires significant infrastructure investment, including the construction of production facilities, bioreactors, and distribution networks. Securing funding for these large-scale projects can be challenging, particularly in regions where hydrogen infrastructure is still underdeveloped.

Environmental and Sustainability Issues

While biological hydrogen production is generally more sustainable than conventional methods, it is not without its environmental impacts. The use of resources, such as water, land, and energy, must be carefully managed to ensure that biological hydrogen production remains environmentally sustainable (Jeje *et al*., 2024). Some biological hydrogen production processes, such as biophotolysis, require large amounts of water as a feedstock. In regions where water resources are scarce, the high-water consumption of these processes could pose a sustainability challenge. Developing water-efficient processes and recycling water within the production system could help mitigate this issue. Large-scale biological hydrogen production facilities, particularly those that rely on biomass or algae, may require significant amounts of land. This could compete with other land uses, such as agriculture or conservation, particularly in densely populated regions. Ensuring that land use for biological hydrogen production does not contribute to deforestation, habitat loss, or food insecurity is essential for maintaining sustainability. Biological hydrogen production processes often generate waste products, such as organic acids, carbon dioxide, and other metabolites. Managing these byproducts in an environmentally responsible manner is essential for ensuring the sustainability of the process.

Organic Waste: Dark fermentation, for example, produces organic acids and other byproducts that must be treated or disposed of properly. Failing to manage these byproducts could result in environmental pollution or contribute to greenhouse gas emissions (Tiwari & Nakamura,2024). Developing integrated systems that convert byproducts into useful products, such as biofuels or fertilisers, could enhance the sustainability of biological hydrogen production. While biological hydrogen production generally produces fewer carbon emissions than conventional methods, some processes, such as dark fermentation, still generate carbon dioxide as a byproduct (E Silva *et al*., 2024). Capturing and utilising this carbon dioxide, either through carbon capture and utilisation (CCU) technologies or by integrating the process with carbon-sequestering microorganisms, could help reduce the overall carbon footprint of biological hydrogen production. The large-scale deployment of biological hydrogen production systems could have unintended impacts on biodiversity and ecosystems. For example, the cultivation of algae for hydrogen production could disrupt aquatic life.

Discussion

A Comparative Look at Biological Hydrogen Production

Biological hydrogen production methods, particularly biophotolysis (Figure 4), dark fermentation, and microbial electrolysis cells (MECs), offer promising alternatives to conventional techniques like steam methane reforming and water electrolysis (Swaminathan *et al*., 2024). However, each method comes with its own set of advantages and challenges. Biophotolysis harnesses the natural photosynthetic abilities of organisms such as algae and cyanobacteria to split water into hydrogen and oxygen using sunlight. While it promises high energy efficiency by directly tapping solar energy, the method is hampered by the sensitivity of hydrogenases to oxygen and the competition between photosynthetic and hydrogen-producing pathways. Scalability is another challenge due to the requirement for extensive areas for cultivation, and the high costs of constructing and maintaining photobioreactors can further complicate large-scale deployment (Kumar, Mishra & Singh, 2024). Nonetheless, biophotolysis stands out as a relatively low-cost method in terms of energy input and has the potential to minimise environmental impact by relying on water as a feedstock, provided water consumption and land use are managed effectively.

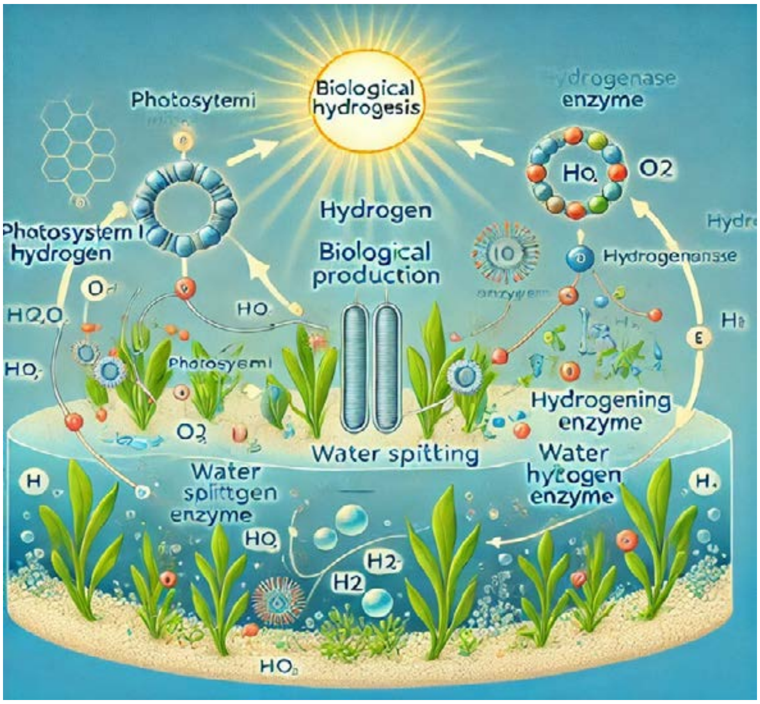

Figure 4: Schematic Illustration of Biological Hydrogen Production Methods, Focusing on Biophotolysis *(Source: Author)*

The diagram includes sunlight interacting with photosynthetic microorganisms, highlighting key components like Photosystem I and II, the water-splitting enzyme, and hydrogenase, leading to hydrogen gas production.

Dark fermentation, on the other hand, operates in anaerobic conditions, breaking down organic substrates such as agricultural waste and biomass to produce hydrogen. This method is less dependent on light and can be integrated into existing waste management infrastructures, making it more scalable than biophotolysis. However, dark fermentation struggles with lower efficiency as only a fraction of the substrate's energy is converted into hydrogen, with the rest dissipating as byproducts like organic acids (Srinadh & Neelancherry ,2024). The process’s economic viability largely depends on feedstock availability and cost, with waste materials offering a promising route to cost reduction. However, scalability is limited by challenges like process stability and feedstock variability, and the management of byproducts is essential to prevent environmental contamination. Despite these hurdles, dark fermentation holds significant potential in waste-to-energy applications, particularly with optimised feedstock processing and microbial consortia.

Microbial electrolysis cells (MECs) combine biological and electrochemical processes to convert organic substrates into hydrogen, utilising an external voltage to drive the electrochemical reaction (Srivastava *et al*., 2024). This hybrid approach can achieve higher hydrogen yields than dark fermentation, but its efficiency is closely tied to the materials used for electrodes and the energy required for the reaction. While MECs are scalable and can be integrated into waste treatment systems, they are hindered by the high cost of electrode materials, such as platinum, and the need for external energy input. Advances in non-precious metal catalysts and renewable energy integration could improve both cost-effectiveness and sustainability. MECs offer significant environmental benefits by converting waste into hydrogen, though their full potential can only be realised if the external energy used is derived from renewable sources. As a result, MECs present a compelling case for further research and development in the pursuit of scalable, cost-effective, and environmentally sustainable hydrogen production.

Conclusion

Biological hydrogen production presents a promising alternative to conventional methods of hydrogen generation, offering a sustainable and environmentally friendly solution. This review has explored various biological methods, including biophotolysis, dark fermentation, and microbial electrolysis cells (MECs), highlighting their respective advantages and challenges. While biological methods provide opportunities for utilising renewable resources and waste materials, they currently face technical challenges related to efficiency, scalability, and economic viability. Key findings suggest that each method has unique strengths: biophotolysis leverages solar energy but struggles with low efficiency due to enzyme sensitivity; dark fermentation is well-suited for waste-to-energy applications but requires better process optimisation; and MECs offer high hydrogen yields by combining biological and electrochemical processes, though they are hindered by the high cost of electrode materials. Addressing these challenges requires a multi-faceted approach. Advances in genetic engineering and synthetic biology can optimise microbial metabolic pathways, while innovations in bioreactor design and hybrid systems can improve scalability and stability. Furthermore, reducing the cost of key materials, such as enzymes and catalysts, is crucial for enhancing the economic viability of these processes. Looking ahead, future research must focus on enhancing microbial efficiency, developing cost-effective and sustainable production systems, and scaling up processes for industrial applications. Exploring hybrid systems that integrate biological and electrochemical methods, as well as valorising waste byproducts, will be pivotal in overcoming current limitations. The importance of further research in biological hydrogen production cannot be overstated. As the global demand for green hydrogen grows, advancing biological methods will play a critical role in meeting this demand sustainably. By addressing technical, economic, and environmental challenges, biological hydrogen production can contribute significantly to the transition toward a low-carbon, renewable energy future. Continued investment in research, innovation, and infrastructure is essential for realising the full potential of biological hydrogen production and achieving large-scale implementation.

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