An Overview of Biotechnologies for Hydrogen Production in South Africa

Racquel Sherise Lallie *, Simika Kanniappen, Emmanuel Kweinor Tetteh, and Sudesh Rathilal

Abstract**—** The usage of fossil fuel dominates the global energy landscape, leading to drastic environmental consequences such as climate change. In this context, the shift towards green hydrogen production is becoming a pressing need. This transition from carbon-based fuels to renewable energy is crucial to mitigate environmental challenges. Hydrogen, an energy carrier based on renewable energy resources, offers several technological production routes. Among them is biohydrogen, a biological pathway that produces sustainable energy from natural resources like biomass and wastewater. This study reviews various biological production technologies, their operational and design parameters, enhancement techniques and prospects. Furthermore, the development of nanomaterials and their significance in enhancing microbial growth for biohydrogen production is also emphasized. This study then underscores the prospects of biohydrogen production as a promising and remarkable technology for sustainable future energy carriers such as hydrogen. Therefore, exploring the performance indicators will bridge the gap between laboratory-scale and large-scale applications.

Keywords**—**Biohydrogen, Nanomaterials, Renewable Energy

I. Introduction

The progress that has emanated from humanity, which has led to the development of civilization, socio-economic growth, industrialization and the transition into the era of modern technology has issued a cause of concern. The world is currently confronted with a new affair, energy insecurity. The rise of the global energy demand is owing to the upsurge in the reliance of energy resources because of overpopulation. The current reserves of fossil fuels, which are primary energy resources, face a major decline because these resources are non-renewable. In addition, burning fossil fuels threatens environmental security by contributing towards greenhouse gas (GHG) emissions [1]. The world is approached by a multitude of risk factors from high GHG emissions such as climate change and global warming. Based on studies by Nagarajan [2], the global statistics reveal that fossil fuels make for 81% of the total energy supply of which 66% is used in the power sector. Based on these quantities, the reserves for coal, oil and natural gas are expected to last 200, 40 and 60 years respectively. South Africa's energy sector is driven by coal which causes this country to be one of the largest GHG emitters in the world. Burning coal constitutes for at least 67% of the country's energy supply [3].

In response to the energy and environmental concerns, the United Nations Sustainable Development Goals (UNSDGs) were established in the year 2015 to achieve a better standard of life by the year 2030 [4]. There were 17 acclaimed goals which require nations to practice sustainable usage of resources and avoid major impact to the environment. UNSDG #7 and #13 focus on clean energy production and counteracting climate change. The Paris agreement was also drawn up as a goal to avoid drastic climate change by preventing the temperature from being raised to an additional 2 degrees. In order to reach a carbon neutral and net zero emission goal, cleaner and sustainable energy production methods must be researched in grave detail with much attention given to renewable resources such as wind, hydro, solar, geothermal.

Hydrogen, one of the world's most abundant elements, is an attractive green fuel because of its energy potential, biocompatibility and applications in fuel cells to produce electricity. One of the many favorable characteristics of hydrogen is that during combustion, it only yields water as a by-product which entails no threat to the environment [5]. It has the capacity to lead the world towards the decarbonization goal and become a safer and sustainable substitute for fossil fuels [6]. The current hydrogen production methods involve natural gas via steam methane reforming and emit large quantities of carbon dioxide which threatens environmental well-being. This has caused researchers to draw their attention to a promising concept, biohydrogen production. Hydrogen that is produced via biological routes through microbial pathways of bacteria and microorganisms is known to be biohydrogen. It is an effective solution that is less energy intensive, cost efficient and environmentally sustainable that also mitigates the secondary issue of waste management, aiding with environmental remediation [7].

The development of a stable bio-hydrogen economy is a significant step in the transition to clean and sustainable energy production. The consideration of biohydrogen production is still in the conception phase and requires comprehensive research for the implementation of larger scale applications. Specifically, in the nation of South Africa, waste management is achieved through anaerobic digestors that emit gases (such as hydrogen), there have been little studies on the conversion of waste to biohydrogen [8]. According to Ivaneko [9], biohydrogen yields are low as opposed to the theoretical calculations and optimizing and understanding the process

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characteristics is one of the major concerns of biotechnology. Although there have been many notable papers and works done on this field, there is a prominent lack of information regarding bioreactor design. In addition, there is minimal work done on the use of nanoparticles for the enhancement of biohydrogen production [10].

This review paper explores the various biohydrogen production technologies along with the mechanisms of each pathway, operational parameters, bioreactor design, limitations of each procedure and the prospects of this study. In addition, the process of dark fermentation and use of nanoparticle technology is highlighted as a promising solution for the global energy crisis. Research conducted by others is compared and discussed extensively, all recent work that has been done have been tabulated and diagrams are provided where necessary.

II. Biotechnologies for Hydrogen Production

Among the various types of hydrogen that are classified according to their production route, biohydrogen refers to hydrogen that is produced as a result of biological processes that involve the metabolic activity of microorganisms and bacteria. Ultimately, this would involve the use of plant species and microorganisms that are able to replicate the processes found in nature such as photosynthesis or fermentation. The biological processes may be light dependent or light independent, in which case, solar energy is considered for light dependent processes [11]. As it stands there are four various techniques for biohydrogen production which are bio-photolysis, photo-fermentation, dark-fermentation and bioelectro-hydrogenesis via microbial electrolysis cells, these processes are categorized in Fig 1. Each of the aforementioned methods use principles of biology or electrochemistry to achieve hydrogen production [12].

Biohydrogen is recognized as an energy carrier that has the lowest pollution output and is less energy intensive in production stages because it doesn’t require high temperatures or pressure. Biohydrogen production can be perceived as a more complex form of anaerobic digestion that can also proceed with the presence of oxygen [13]. The feedstock for biohydrogen production can range from municipal or industrial waste, animal manure, biomass, algae, cyanobacteria making it a very versatile process [14].

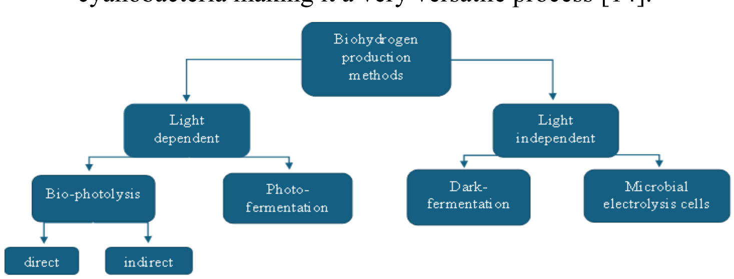

Fig.1 Classification of the various biohydrogen production techniques

A. Light dependent pathways

Biological processes involving the presence of a light source typically rely on the process of photosynthesis to proceed. The mechanisms of bio-photolysis and photo fermentation are diagrammatically presented in Fig 2 and Fig 3 respectively. Organisms such as algae, cyanobacteria and anoxygenic photosynthetic bacteria are commonly used as they can photosynthesize and can lead to the development of cleaner energy by utilizing solar energy to produce hydrogen [15].

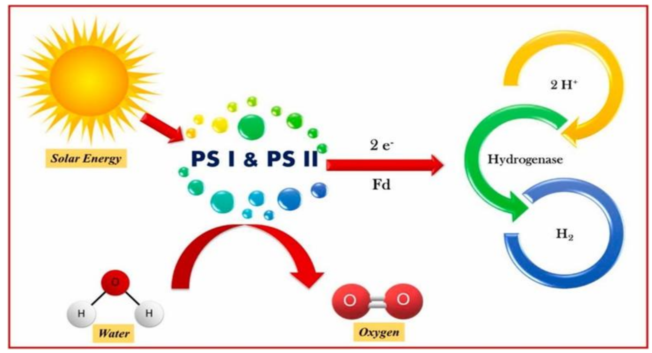

Fig.2 Mechanism of bio-photolysis [16]

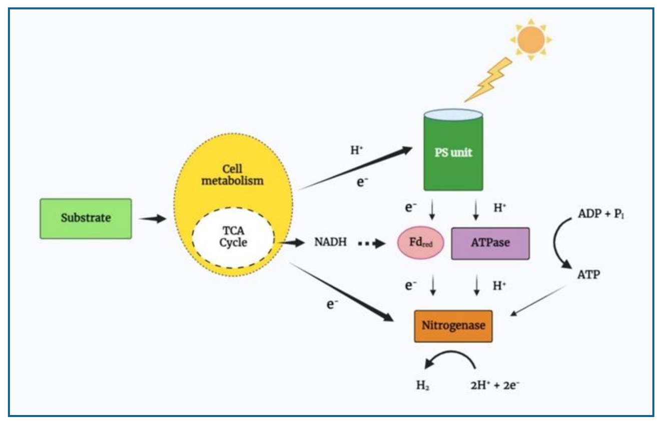

Fig.3 Mechanism of photo fermentation [17]

Microorganisms such as algae and photosynthetic cyanobacteria are used in the process of bio-photolysis. Hydrogen production occurs via the absorption of light and the transfer of electrons to the hydrogenase or nitrogenase enzymes [16]. The reaction proceeds as shown in reaction 1. Under circumstances where there is a surplus of energy, the excess electrons convert the hydrogen ions to hydrogen gas. However, oxygen is also produced and can sometimes suppress the production of hydrogen. There are two categories of bio-photolysis, namely direct bio-photolysis (DbP) and indirect bio-photolysis (iDbP) which are compared in Table 1.

$$2H_2O + light \rightarrow 2H_2 + O_2 \dots\dots\dots\dots (1)$$

TABLE 1: LIMITATIONS AND RESEARCH GAPS OF BIO-PHOTOLYSIS

	Limits	Research areas needed	Ref
Direct photolysis	*Requires large light intensity *Production of oxygen inhibits hydrogen production *Requires anaerobic conditions	*Optimization of reactor configuration for increase in light exposure *Oxygen control to prevent enzyme deactivation	[9, 18,7]
Indirect photolysis	*Oxygen may inhibit nitrogenase enzyme *Low photon conversion *Lower hydrogen yield	*Methods for increasing hydrogen yield	

The production of biohydrogen using photo-fermentation was initially pioneered in 1949 by Gest and Kaman which kickstarted research in this field. In the presence of a light source, photosynthetic microorganisms such as microalgae, can undergo photo-fermentation. Fermentative hydrogen production is known to be a much faster process than bio-photolysis which is also light driven [19]. Commonly used bacteria, known as purple non-sulphur bacteria (PNS), can utilize volatile fatty acids (VFA) for hydrogen production. The hydrogenase and nitrogenase enzymes have been found in these bacterial strains, however, under anoxygenic conditions, nitrogenase is the main enzyme that is responsible for hydrogen production. In the absence of oxygen, PNS utilizes solar energy to reduce ferredoxins and create adenosine triphosphate (ATP). Electron donors, which are usually organic compounds, receive electrons through the water splitting process. The fermentation process can be carried out under ambient conditions and is also known to be environmentally friendly making it a very attractive research area pertaining to sustainable and clean energy production [20]. The general reaction proceeds as shown in reaction 2.

$$C_6H_{12}O_6 \rightarrow 6CO_2 + 12H_2 \; \ldots\ldots (2)$$

PNS bacteria are known for having a higher substrate conversion efficiency and does not result in oxygen evolution [21]. The reaction by use of PNS bacteria is presented in reaction 3. The research gaps and limitations are presented in Table 2.

$$CH_3COOH + 2H_2O + light\ energy \rightarrow 4H_2 + 2CO_2 \; \ldots\ldots (3)$$

TABLE II: LIMITATIONS AND RESEARCH GAPS OF PHOTO-FERMENTATION

	Limits	Research areas needed	Ref
Photo-fermentation	*Poor light conversion efficiency *Oxygen evolution creates an inhibitory effect enzyme *Hydrogen yield is lower	*Kinetic studies needed *Pretreatment of the feedstock and modification to avoid contamination	[20] [9]

B. Light independent pathways

Biohydrogen production techniques not requiring the presence of a light source offer a greater advantage as it can be conducted at any time and will be less energy intensive. These techniques often involve fermentation or bioelectrochemcial procedures and the mechanisms are respectively presented in Fig 4 and Fig 5. There are still limitations and setbacks like creating a specific environment for the process to occur without incurring any losses or additional input.

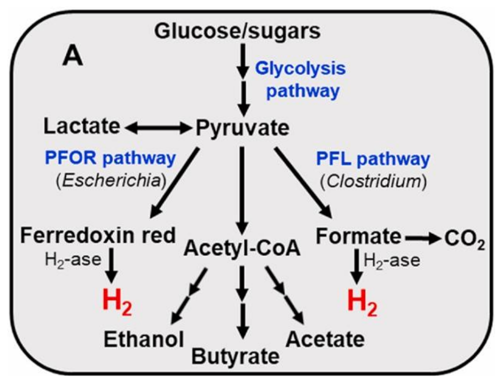

Fig.4 Mechanism of dark fermentation [22]

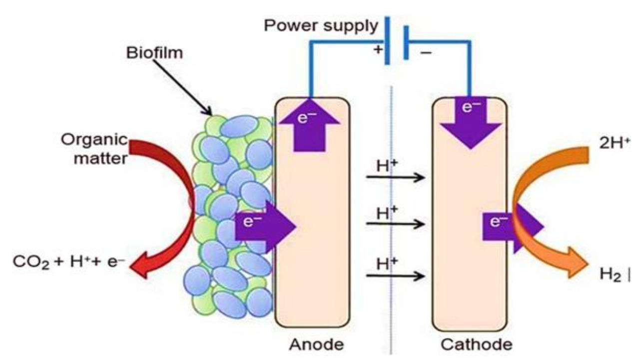

Fig.5 Mechanism of microbial electrolysis cells [23]

Fermentation is a biological process that involves the decomposition of organic material or resources which are converted into energy, usually carried out in the absence of oxygen. Dark fermentation (DF) is known to produce more hydrogen than photo fermentation because of the biological pathways taken such as pyruvate breakdown and glycolysis [23]. The microorganisms used are generally obligate or facultative anaerobic bacteria that show the most potential in anoxygenic conditions. Obligate anaerobes generally produce 4 mol of biohydrogen for every 1 mol of glucose, however, the presence of oxygen will terminate hydrogen production. Facultative anaerobes produce 2 mol of biohydrogen per mol of glucose and can quickly consume oxygen that may inhibit hydrogen production [24]. The metabolic pathways taken by the dark fermentation process depend on the type of microorganism that is used. Pyruvate, which is catabolized in the initial stages of DF, can undergo conversion through facultative bacteria that produces formate and acetyl coenzyme by assistance from the pyruvate lyase enzyme. The formate is then used for the production of hydrogen. If obligate bacteria are involved, then the pyruvate may take a different path whereby coenzymes and carbon dioxide is produced via pyruvate ferredoxin oxidoreductase. The ferredoxin is reduced which then leads to the reduction of the hydrogen ions, producing hydrogen as a by-product [17]. Dark fermentation uses anaerobic bacteria for molecular hydrogen formation and the general reaction pathway is presented in reaction 4.

$$C_{12}H_{22}O_{11} + 9H_2O \rightarrow 4CH_3COO + 8H^+ + 4HCO_3 + 8H_2 \ldots\ldots (4)$$

Amidst the dark fermentation process, volatile fatty acids and a range of solvents such as butyric, propionic, acetic or lactic are produced, depending on the feedstock [25]. DF for hydrogen production offers many advantages but similar to the other biological processes are obstructed by various factors that are mentioned in Table 3.

TABLE III: LIMITATIONS AND RESEARCH GAPS OF DARK FERMENTATION

	Limits	Research areas needed	Ref
Dark fermentation	*Some by products such as ethanol can be toxic to the enzymes producing hydrogen *Slow rate of production	*Optimization of bioreactors *Improving mixed culture maintenance	[7, 24]

A bioelectrochemical system uses principles of electrochemistry and biology that are capable of converting chemical energy into electrical energy within a bioreactor. Microbial electrolysis cells combine the behaviour of microorganisms and their metabolic pathways with electrochemical reactions and undergo bioelectrohydrogenesis to produce hydrogen. These microorganisms aid with the degradation of organic compounds and may also be used for contaminant removal which contributes towards environmental remediation. An MEC consists of electrode material in a reactor, applied voltage usually a direct current (DC) and a gas collection system, sometimes a membrane may be used to separate hydrogen and oxygen reactions. An external voltage is required to overcome thermodynamic barriers and provide a stable circuit for transportation of the electrons [26]. Electrons are transported from the anode material where substances are oxidized via exo electrogenic material, to the cathode material where reduction occurs, and hydrogen is produced.

Electrochemically active microorganisms degrade the organic matter which produces electrons and protons, and the electrons are transferred to the cathode whereby hydrogen is produced. To avoid the mixture of oxygen and hydrogen production, cation and anion membranes are used. Microorganisms that can be used include *E.coli, Pseudomonas, Geobactersulferreducens, Bacteroides* [27]. Under anaerobic conditions, hydrogen gas is produced. The reactions at the anode and cathode are presented in reactions (5) and (6) respectively. MECs can use a variety of feedstock such as biomass, wastewater effluent which can also reduce costs for waste treatment. In comparison to other hydrogen production technologies, MECs only require about half of the energy input as opposed to the requirements to that of water electrolysis for hydrogen production[23]. The limitations and research areas needed to improve the performance of MECs are shown in Table 4.

$$Anode: 8H^+ + 8e^- \rightarrow 4H_2CH_3 \ldots\ldots (5)$$
$$Cathode: COOH + 2H_2O \rightarrow 2CO_2 + 8e^- + 8H^+ \ldots\ldots (6)$$

TABLE IV: LIMITATIONS AND RESEARCH GAPS OF MECS

	Limits	Research areas needed	Ref
MEC	*Configuration of the reactor might be more expensive *Low hydrogen production rate	*Improvements on double chambered MECs	[28, 29]

III. Performance Parameters of Each Technology

The mechanisms of each biohydrogen production technology have been discussed in the previous section. This section provides comprehensive details on how the operational parameters work and affect the overall process. It is imperative to understand the governing factors of each procedure in order to optimize it and identify areas that require more attention.

A. Bio-photolysis

One of the most significant factors that affects hydrogen production during bio photolysis is the activity of the hydrogenase enzyme and the control of oxygen concentration. Microalgae can consist of various hydrogenase enzymes that are classed according to their metal compositions such as FeFe, NiFe, and Fe hydrogenase. The FeFe hydrogenase enzyme is known to be the most efficient which produces the highest yield of hydrogen as opposed to the other enzymes [30]. However, enzyme activity may be inhibited by oxygen

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evolution. Other parameters such as pH, light intensity, temperature and cell density can be controlled to maintain algal cell activity and extend their life cycle as well as increasing the hydrogen production by influencing the hydrogenase enzyme. Understanding these parameters can aid in the enhancement of hydrogen production.

B. Fermentative routes

A reliable carbon source is mandatory for PNS bacterial growth, substrate conversion and hydrogen production with the photo-fermentative route. PNS bacteria already consist of substrates but will vary based on the bacterial strains that are to be used for photo fermentative processes. The capability of PNS for hydrogen production using waste such as industrial, agricultural, food is known to be very high [31]. The utilization of waste as a carbon substrate makes a cost-effective energy source and allows for waste treatment by biological oxygen demand (BOD) and chemical oxygen demand (COD) reduction. The setback with this method is that pretreatment is required before using the waste as a feedstock for biohydrogen production [32]. Nitrogenase enzyme is crucial for the metabolism of PNS bacteria. This enzyme consists of two proteins known as molybdenum and Iron protein. A suitable amount of molybdenum and iron within the medium have a significant impact in enhancing hydrogen production during photo-fermentation [33].

Anaerobic fermentative bacteria can use carbon sources, mostly sugars, like glucose, sucrose, lactose, for hydrogen production. The concentration of substrate also plays a role. Hydrogen production is facilitated by the hydrogenase enzyme which creates a series of electrochemical reactions. To improve the enzyme activity, supplementation of iron is advised which is the addition of Fe^{2+} ions or Ni^{2+}. During fermentation, the pH levels decrease because of the accumulation of products such as volatile fatty acids. Dark fermentation can occur under mesophilic, thermophilic or hyper-thermophilic conditions. Optimal temperature depends on the type of substrate used however higher temperatures are preferred. At relatively high temperatures, the level of entropy increases making the process more thermodynamically positive. Hydraulic retention time (HRT) is the average time that microorganisms need to use the substrate in the reactor. Low HRTs can lower the concentration of metabolites and bacteria but can lower the substrate conversion efficiency. During fermentation, excessive hydrogen gas concentration or hydrogen partial pressure (HPP) can have an inhibitory effect on the process [33].

C. Microbial electrolysis cells

In MECs, the process indicators consist of the type of feedstock and characterization of the feed, the environmental conditions such as temperature and pH, and some physical aspects such as the material of electrodes and the reactor configuration [23]. The pH affects the electrochemical active bacteria within the anode chamber and the hydrogen evolution in the cathode chamber. The temperature directly affects the power density and the energy efficiency of the system. The choice of substrate is crucial for enriching and providing nutrients to the microbial community. Substrates have various degradation rates, proton release rates, hydrogen yield [26]. To aid electron transport, additional energy is required usually in the form of applied voltage. The voltage proportionally influences the hydrogen yield where lower voltages will produce lower hydrogen yields. HRT strongly influences microbial activity. Lower HRT may Both anode and cathode material must be durable, biocompatible, have good cell adherence, and conductivity which will directly affect the efficiency of the reaction. Carbon-based electrodes are the preferred ones because of their affordability and availability [27].

D. Bioreactor design

Operational parameters are important to necessitate biohydrogen production routes, to maintain these parameters, the reactor topology must be considered as it will impact the substrate conversion. The performance of the bioreactor does not just depend on the physical design aspects but also the custom reformation based on specific conditional requirements [34]. The configuration and overall design of the bioreactor that is used for the production of hydrogen has a massive influence on the hydrogen production rate. Bioreactors are meant to create and maintain favorable conditions for the necessary reactions to proceed to produce biohydrogen [33]. For light driven biohydrogen production technologies, the most important focus is the capturing of solar energy, which is contrary to nonlight driven technologies., This requires research attention to designed bioreactors for specific feedstocks and operational requirements[21]. Fig 6 depicts some of the various bioreactors for biohydrogen production.

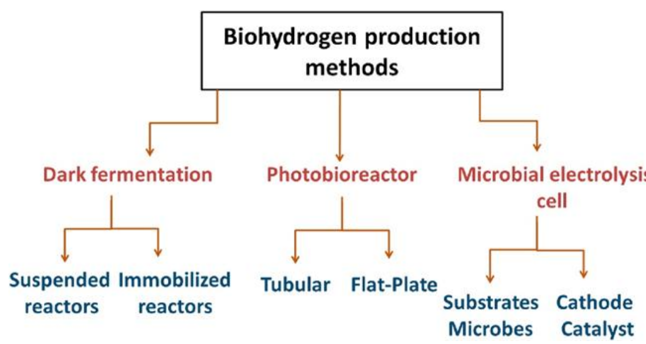

Fig.6 Different bioreactor types [33]

IV. STRATEGIES TO ENHANCE HYDROGEN PRODUCTION

To optimize any process, the parameters affecting the procedure, design parameters and mechanisms of the technology must be understood. Through reviews of biohydrogen production technologies, limitations were found that hindered the hydrogen production yield and production rate. These limitations must be overcome to consider any procedure economically and commercially viable. There are techniques and strategies that are in the works which are promising for biohydrogen production enhancement as shown in Fig 7.

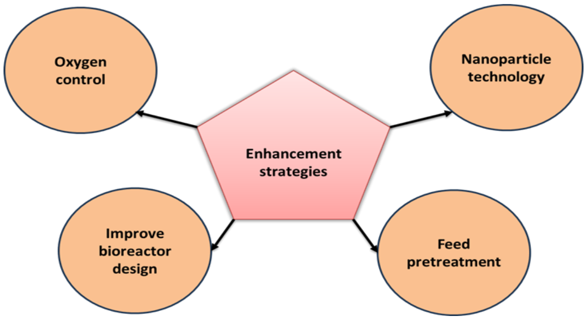

Fig.7 Various enhancement strategies [34]

Hydrogen is recognized as the fuel of the future and has the potential to replace fossil fuels. However, to employ hydrogen as a viable substitute, the production methods must be clean and sustainable [32]. As discussed in previous sections, biohydrogen is a promising concept that has minimal environmental impact and sufficient resources as a feedstock. There are various enhancement strategies to overcome barriers and limitations brought by the current procedures for producing biohydrogen. These techniques require more research and experimental work to confirm practicality but have been suggested by others in an attempt to maximize the efficiency of the current processes [22]. By exploring these strategies, it may lead to the possibility of commercialization of biohydrogen production technologies.

Nanoparticle technology is a potential solution to issues such as low bioconversion, enzyme inactivity and many other bottlenecks of biohydrogen production. Specifically inorganic nanomaterials may be used to enhance hydrogen production owing to their structural and chemical nature which allows them to maintain stable conditions during biohydrogen production [35]. They can act as oxygen scavengers preventing oxygen inhibition.

Other techniques such as oxygen control [28] and feed pretreatment are recommended to prevent enzyme inactivity during the biological routes which impact the formation of hydrogen [17,18]. Bioreactor design is fundamental in ensuring that a favorable environment is maintained to allow for reactions to proceed. Optimizing the designs is significant in light driven processes because of the solar conversion efficiency. Generally, a large surface area is required, which may not be feasible thus more cost-effective bioreactors must be considered [32].

V. Challenges and Future Prospects

Globally there is about 368 trillion cubic meters of hydrogen produced per year and of this overall value, using fossil fuels in processes such as steam reforming, oil reforming and coal gasification make up for 96% of hydrogen production [36]. The usage of fossil fuels has created a problematic situation with regards to climate change and the risk of resource depletion. The contribution towards GHG emissions from combustion of fossil fuels is about 70 % primarily from the release of carbon dioxide [36]. A paradigm shift from the current techniques into renewable energy methods must be asserted. There has been significant progress with hydrogen production techniques, however, despite the research and development being done, the use of renewable energy for hydrogen production is still not economically viable.

The transition into clean energy is one that requires collective action from society, especially from research pertaining to this field. Biohydrogen, one of the most promising energy alternatives, has various methods of production that utilize renewable energy making it clean and sustainable. Biohydrogen production not only can aid in the energy crisis but as a secondary function can aid in waste handling and management as most of these processes can use waste as a feedstock and will result in contaminant removal and treatment [36]. However, there are financial barriers, chemical and thermodynamic limitations, research gaps, that need confrontation in order to upscale biohydrogen production. Overcoming the economic hurdle of energy costs and the input requirements must also be addressed to ensure that the process is feasible. Other notable challenges that are associated with biohydrogen production would be building proper infrastructure to support production without being a direct hinderance on the environment. High capital investments will be required to commercialize the process, and funds will also be required for experimental work for researchers [37].

The production of biohydrogen has great potential for a future in the energy economy. However, there are obstructions that must be considered before considering the upscaling of any biohydrogen production route. Factors such as bioreactor design for large scale applications, operating costs and energy requirements, storage and transport of hydrogen and return of investments are significant for considering biohydrogen production for industrial purposes [38]. The current research being done in this field focuses on enhancement strategies to improve hydrogen yield from biological processes. Nanoparticle technology holds more potential to address challenges such as reaction rate, product quality, environmental control. Using this strategy may incur more costs so it is mandatory that more research is conducted to ensure the feasibility of biohydrogen production.

Hydrogen is the fuel of the future and with zero carbon emissions, offers one of the safest routes towards clean and sustainable energy production. The largest obstruction that stands in the way of commercializing biohydrogen production technologies is the production costs required [39]. Building an economical reactor, selection of an affordable and available substrate and inoculum, maintaining a favorable environment will drastically improve the performance of biological hydrogen production. This review has summarized and highlighted research areas that require further attention which may improve the current knowledge in this research field.

VI. Conclusion

This overview has shown that biological production technologies for biohydrogen production offer numerous benefits. There is versatility in feedstock, low energy requirements, and poses no threat to the environment.

Amongst the various technologies, dark fermentation holds the most promise. It is proposed that further research on this process coupled with nanoparticle technology can enhance biohydrogen production rates.

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