

Biohydrogen 2.0: Emerging Innovations from Waste Valorisation to Intelligent Bioprocess Design

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Abstract

The growing requirement for sustainable energy, coupled with greenhouse gas emissions reduction, have resulted in increased research efforts towards the production of hydrogen via biohydrogen as an alternative to fossil fuels. Biohydrogen production methods, though environmentally friendly, have limitations in terms of hydrogen yield and other operational issues. The objective of this literature review is to introduce Biohydrogen 2.0 and discuss recent advancements which make biohydrogen production technologically feasible for its incorporation into future energy generation. Special focus will be paid to biohydrogen production using WV approaches through utilization of crop residues, food wastes, industrial effluents, sewage sludge, algae biomass, and new waste sources as sustainable feedstock. Additionally, novel approaches like ME, synthetic biology, microbial consortium engineering, nanobiotechnology-enabled bioprocessing, and DF-PF and microbial electrolysis cell hybrids are also explored. Recent developments in hybrid and MEC-assisted systems have demonstrated higher hydrogen yields than conventional DF, which typically produces approximately 2–4 mol H₂ per mol glucose, with some integrated systems achieving up to 5.2 mol H₂ per mol hexose-equivalent substrate under controlled conditions. In addition, the combination of AI, machine learning, digital twins, and advanced reactor engineering is making huge changes to process optimization with predictive accuracy rates exceeding 95%, coupled with operational savings of 40%. Overall, the combination of biotechnology, digitization, and the circular bioeconomy approach can pave a way towards effective biohydrogen production.

Keywords:

waste valorisation; microbial engineering; dark fermentation; intelligent bioprocess design; microbial electrolysis cells

1. Introduction

There is an ongoing fundamental change within the global energy industry due to increasing energy demand, limited availability of fossil fuel resources, and the need for reducing GHG emissions. At present, fossil fuels represent over 80% of the world's total energy consumption and continue being the main supplier of hydrogen produced through steam methane reforming and coal gasification, responsible for emitting 830 million tons of CO₂ annually [1,2]. Considering climate goals at the global level and the development of net-zero economies, renewable hydrogen has become a critical energy vector to facilitate decarbonization in such fields as transport, manufacturing, and electricity generation [3].

Hydrogen has one of the most elevated gravimetric energy densities amongst other types of fuels, up to 142 MJ/kg compared to gasoline with ~44 MJ/kg, resulting in solely water upon combustion [4]. Yet, the environmental sustainability of hydrogen fuel highly relies on the process of its synthesis. As such, at present, over 95% of all hydrogen is extracted from non-renewable raw materials, emphasizing the necessity for sustainable hydrogen generation methods [3]. In turn, there are several ways to produce hydrogen; nevertheless,

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the use of biohydrogen fuel has been extensively explored due to the simultaneous environmental benefit of waste utilization and renewable energy production under mild conditions [5]. Biologically generated hydrogen fuel can be derived from several natural resources using bioprocessing techniques followed by further usage in the operation of fuel cells and power generation (Figure 1).

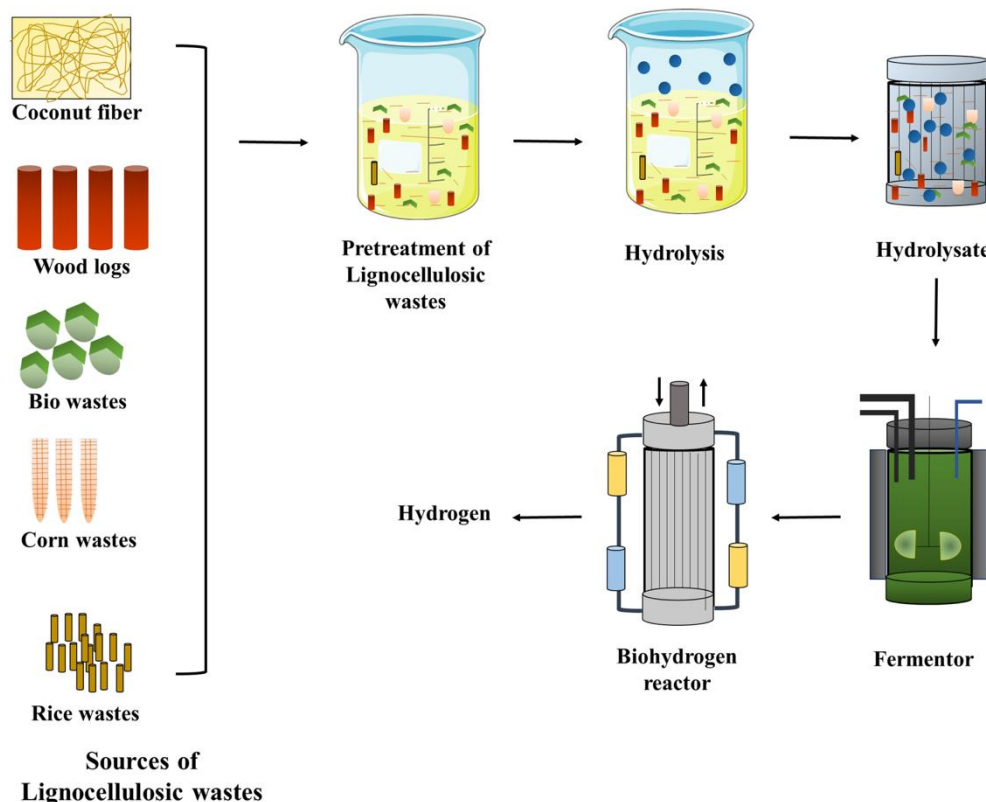


Figure 1. Schematic representation of different sources, production processes, storage techniques, and applications of biohydrogen in fuel cells. Reproduced from Ref. [1] under the CC BY 4.0 license.

Biohydrogen is defined as hydrogen production via biological or mixed biological methods that employ renewable biomass and organic waste streams as raw materials. Agricultural waste products, food waste, lignocellulosic biomass, municipal solid waste, sewage sludge, industrial wastewaters, algae, and agro-industrial residues are examples of plentiful and cheap sources of feedstock for biohydrogen production [6]. Annually, about 1.6 billion metric tons of food waste and billions of metric tons of agricultural wastes are produced globally, thus providing a rich feedstock supply for biohydrogen technologies [7]. The use of such waste streams for hydrogen production not only meets the circular economy criteria but also helps to minimize environmental pollution and landfills [8].

Traditional biological methods for hydrogen generation include dark fermentation (DF), photofermentation (PF), direct and indirect biophotolysis, and microbial electrolysis cells (MEC) [9]. PF and biophotolysis are both light-driven, but they are mechanistically distinct and should not be used interchangeably: PF is carried out by purple non-sulfur photosynthetic bacteria that use light energy and the nitrogenase enzyme to convert low-molecular-weight organic acids, typically the volatile fatty acids left over from DF, into H_2 and CO_2 , whereas biophotolysis is carried out by microalgae and cyanobacteria that use photosynthesis to split water itself: directly, via the concerted action of photosystem II, photosystem I, and a hydrogenase (direct biophotolysis), or indirectly, by first fixing CO_2 into stored carbohydrate and then fermenting that carbohydrate to H_2 in a separate, typically anaerobic, stage using nitrogenase (indirect biophotolysis). In this review, photofermentation (PF) refers strictly to the organic-acid-based photosynthetic-bacterial process, while photobiological hydrogen production and biophotolysis refer to the water-splitting algal and cyanobacterial process; the two terms are not synonyms and are used accordingly throughout. These methods involve DF since it is the most studied one due to its capability of working with numerous carbohydrate substrates in the absence of light. While traditional DF generates $2\text{--}4 \text{ mol } H_2 \text{ mol}^{-1} \text{ glucose}$, the idealized stoichiometric maximum of $12 \text{ mol } H_2 \text{ mol}^{-1} \text{ glucose}$ is approachable only through integrated DF and PF systems, rather than DF alone [10–12]. The disparity between potential and actual

yields indicates significant problems related to metabolism constraints, hydrogen consumption organisms, and reactor inefficiency.

New technological advancements have steered the biohydrogen production studies towards hybrid biohydrogen production systems [13]. The combination of dual-stage DF and PF processes has yielded hydrogen recovery values greater increasing the efficiency of substrate conversion [14]. Micro-organisms that have been genetically modified include *Clostridium*, *Enterobacter* and *photo-synthetic bacteria* which have shown much greater hydrogen production efficiency than their wild types through metabolic engineering. Approaches that have been used to improve hydrogen production efficiency include over-expression of hydrogenase genes, knock-out of competing metabolic pathways and improved substrate utilization [15].

The use of nanotechnology is another innovative technology that can be used to increase the production of biohydrogen. Nanoparticles of metals, carbon nanomaterials, magnetic nanocomposites, and enzyme immobilization nanoparticles have been used for increasing electronic reactions, increasing microbial activity, and improving the reactor's performance. Some studies have indicated an improvement in hydrogen production efficiency between 20% and 50% using nanoparticles in biohydrogen production [16]. Moreover, some advanced membrane technologies and nanostructured catalysts can be used for improving the purity and storage of hydrogen produced [15].

Another characteristic of the next generation of biohydrogen technologies-called "Biohydrogen 2.0" here, is the incorporation of AI, ML, and digital engineering concepts in biohydrogen production. Recent research works have revealed that AI-based prediction models can attain accuracy of more than 95% for hydrogen yield optimization along with cost savings by about 40% using intelligent process control methods. ANNs, SVMs, digital twins, and soft sensors have been increasingly used for characterization of feedstocks, monitoring of reactors, process optimization, and predictive maintenance [4,17]. Though there have been significant developments, various hurdles still exist in the commercial application of hydrogen production using microbes. Some of the barriers that are hindering its commercial success include feedstock inconsistency, high pretreatment cost, low efficiency in the conversion of substrates to hydrogen, reactor scaling problems, hydrogen purification needs, and its economic competitiveness compared with hydrogen produced from fossil fuels [5]. However, only a handful of existing literature discuss all these elements simultaneously.

Consequently, in this review, we present the notion of Biohydrogen 2.0, which symbolizes the confluence of circular WV methods, cutting-edge microbial manipulation techniques, hybrid biohydrogen pathways, enhanced process development through nanotechnology, and intelligent bioprocess design via AI tools. This review will explore new and innovative aspects of potential feedstocks, new reactor technologies, novel combinations of biological and thermochemical conversion processes, ML approaches, sustainability studies, and even potential applications at an industrial scale.

1.1. Review Methodology and Literature Search Strategy

To find recent developments in the production of biohydrogen, WV, novel conversion technologies, microbial and nanotechnology intervention strategies, AI, and sustainability considerations in bioprocess design, a structured literature search has been performed. Literature search was carried out through Scopus, Web of Science, ScienceDirect, Springer Link, and Google Scholar databases, from publications between January 2010 and 2026. The search has been carried out using combinations of keywords such as "biohydrogen," "biomass-to-hydrogen," "WV," "DF," "PF," "microbial electrolysis," "nanotechnology," "ME," "AI," "AI optimization," "TEA," and "LCA." Relevant literature was screened through title, abstract, and full-text screening processes for inclusion into this review. Research articles published in peer-reviewed journals along with relevant reviews on biohydrogen production, waste and biomass valorisation, novel technologies, optimization approaches, AI-driven bioprocess designs, and environmental and TEAs were selected for this review. Literature irrelevant to the scope of this review was not included in this review process. The selected literature has been critically analysed to present a comprehensive review on "Biohydrogen 2.0: Emerging Innovations from WV to Intelligent Bioprocess Design."

2. WV and Emerging Feedstocks for Sustainable Biohydrogen Production

Biohydrogen production is highly dependent on the use of low-cost and renewable carbon-based feedstocks [18]. WV can be considered as an alternative way of converting organic wastes into hydrogen in a sustainable manner while tackling environmental issues related to waste disposal. Agricultural waste, food waste, industrial waste, municipal solid waste, sewage sludge, and algal biomass have been recognized as sustainable feedstocks due to their availability, cheapness, and suitability for biohydrogen production processes [1,2,5,15]. The use of

such organic wastes promotes the concept of circular bioeconomy, which aims to convert waste materials to valuable energy resources and minimize emissions of GHG [2,6].

The most abundant sources of feedstock are lignocellulosic biomass from agricultural wastes [19]. Rice straw, wheat straw, corn stover, sugarcane bagasse, and forestry waste consist of 35–50% cellulose and 20–35% hemicellulose, which upon hydrolysis, can give rise to fermentable sugar for hydrogen producing bacteria [5,20]. However, the lignin component is a barrier to microbial action and usually requires pretreatment like dilute acid hydrolysis, alkali treatment, or steam explosion. DF typically produces hydrogen yields of approximately 2–4 mol H_2 per mol hexose, depending on the substrate characteristics, pretreatment conditions, and microbial metabolism [5,21]. Agricultural residues such as rice straw, wheat straw, corn stover, and sugarcane bagasse are produced in huge amounts across the globe, hence being easily available and inexpensive raw materials for producing sustainable biohydrogen.

Food waste has gained much importance due to its high biodegradability and moisture content along with easily fermentable carbohydrate sources [9,22]. Global food waste generation exceeds 1.6 billion metric tons annually and contributes significantly to greenhouse gas emissions if improperly managed [7]. Unlike lignocellulosic biomass, food waste generally requires minimal pretreatment and can directly support microbial fermentation. Hydrogen yields between 80 and 220 mL H_2 g^{-1} volatile solids have been reported depending on substrate composition and reactor operation [7,23]. Furthermore, integrated DF-photo fermentation systems enable further utilization of volatile fatty acids produced during primary fermentation, thereby improving overall hydrogen recovery and substrate conversion efficiency [2]. Integrated WV strategies are increasingly being developed to maximize resource recovery from food waste through combined biological and thermochemical conversion pathways, enabling the simultaneous production of biohydrogen, biofuels, biochar, and other value-added products (Figure 2)

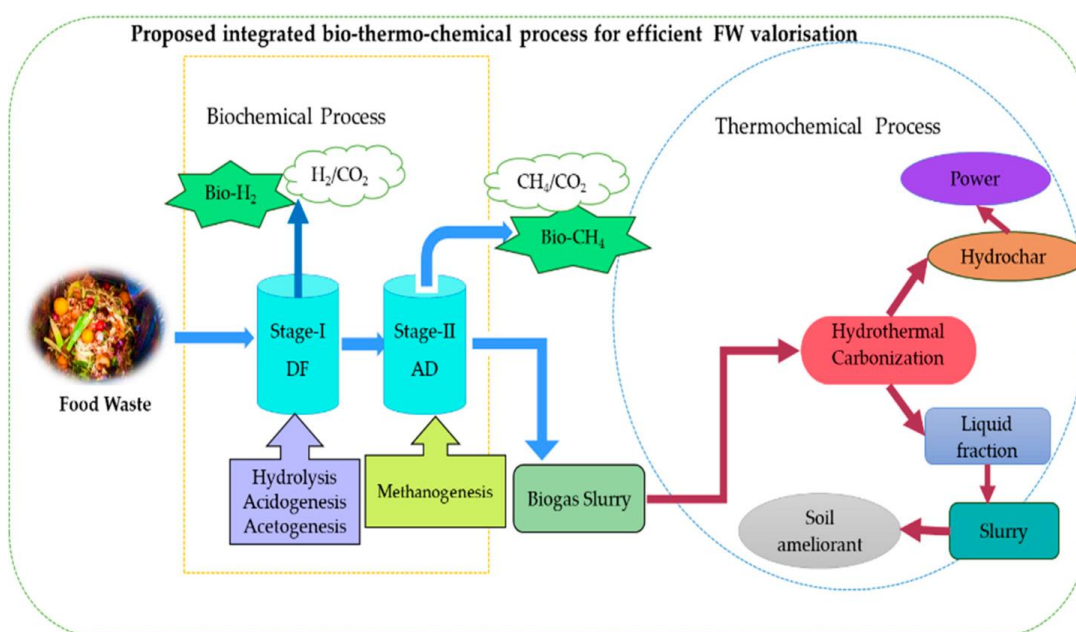


Figure 2. Integrated bio-thermo-chemical pathways for food WV and recovery of value-added products, including biohydrogen, biofuels, biochar, and platform chemicals. Reproduced from Ref. [7] under the CC BY 4.0 license.

Industrial wastewater and sewage sludge constitute another important category of feedstocks due to their high organic content and continuous generation. The wastewaters discharged by the brewery, dairy processing industry, food processing plant, distillery, and sugar mill usually contain high amounts of chemical oxygen demand in the range of 5–100 g L^{-1} , offering adequate substrate for the production of hydrogen by the microbes. Hydrogen and wastewater treatment have been demonstrated to be performed simultaneously with COD removal efficiency greater than 70% [24]. Likewise, sewage sludges obtained from the wastewater treatment plants consist of carbohydrates, proteins, and microbial mass that can be utilized for the production of hydrogen.

Current studies have gone beyond traditional sources of biomass to investigate new ones. Algal biomass offers numerous advantages due to its fast growth, high photosynthetic activity, and atmospheric CO_2 sequestration capability. Algal strains like *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, and *Scenedesmus obliquus* have shown potential in the production of hydrogen via photobiological and fermentative mechanisms. Hydrogen production rates for *Chlamydomonas reinhardtii* of around 150–200 mL H_2 L^{-1} day^{-1} under sulfur

deprivation conditions have been achieved [12]. Moreover, the lack of lignin and easy degradation of carbohydrates makes its pretreatment unnecessary in contrast to terrestrial biomass. Invasive aquatic plants and agro-industrial residues represent novel potential sources of biomass. Among invasive species, water hyacinth (*Eichhornia crassipes*) that ranks among the world's most notorious aquatic weeds offers excellent prospects for biohydrogen production due to its high cellulose content and fast growth rate [25]. The highest yield of 81.7 mL H₂ g⁻¹ glucose was obtained from uncontaminated water hyacinth biomass by DF [26]. In the same way, dairy whey, molasses, brewing wastes, coffee processing waste, cassava effluents, and fruit processing wastes possess considerable amounts of fermentable sugars for efficient hydrogen production along with waste treatment problems [4,6]. As summarized in Table 1, a wide range of emerging waste feedstocks have been explored for biohydrogen production. Among these, macroalgae (seaweeds) offer a promising lignocellulose-free alternative to terrestrial biomass, with their comparatively low lignin content enabling easier hydrolysis and reported yields of 2.0–4.5 mol H₂ mol⁻¹ sugar; their cultivation in coastal and marine environments also avoids competition with arable land and freshwater resources used by terrestrial feedstocks. Similarly, mixed municipal organic waste represents a high-volume, continuously generated urban feedstock (20–50 L H₂ kg⁻¹ VS, Table 1), whose heterogeneous composition poses pretreatment and process-control challenges but offers substantial valorisation potential given its availability in nearly every urban centre, aligning biohydrogen production with municipal waste-reduction and circular-economy objectives.

The future development of Biohydrogen 2.0 will be associated with increased feedstock diversity and effective waste management approaches [27,28]. It has been proven that co-fermentation of different waste sources increases the efficiency of nutrients availability and microbial activity, providing hydrogen yields increase up to 20–40% compared to single feedstocks [2,16]. Additionally, the use of AI and ML techniques for feedstock characterization and process optimization will lead to improved resource utilization and process reliability. Such innovations will allow creating decentralized biohydrogen biorefineries utilizing various wastes and producing hydrogen [8,16].

Table 1. Emerging Waste Feedstocks for Biohydrogen Production: Characteristics, Hydrogen Yield, and Valorisation Potential.

Feedstock Category	Major Components/Characteristics	Reported Hydrogen Yield	Key Advantages	Valorisation Potential	Ref.
Agricultural residues (rice straw, wheat straw, corn stover, sugarcane bagasse)	35–50% cellulose, 20–35% hemicellulose, 10–30% lignin	0.44–1.86 mol H ₂ mol ⁻¹ hexose equivalent	Abundant, inexpensive, non-food biomass	Biohydrogen, bioethanol, biochar, biochemicals	[2,5,6,41,42]
Food waste	Rich in carbohydrates, proteins, lipids; moisture > 70%	63–360 mL H ₂ g ⁻¹ VS	Minimal pretreatment requirement, high biodegradability	Biohydrogen, biomethane, organic acids, biofertilizers	[7,23]
Industrial wastewater (brewery, dairy, distillery, food-processing effluents)	High COD (5–100 g L ⁻¹), soluble organic matter	1.37–2.03 mol H ₂ mol ⁻¹ hexose equivalent	Simultaneous wastewater treatment and energy recovery	Biohydrogen, treated wastewater, nutrient recovery	[2,24]
Sewage sludge	Proteins, carbohydrates, microbial biomass	9.1–16.6 mL H ₂ g ⁻¹ DS	Continuous availability from WWTPs	Biohydrogen, biomethane, biosolids	[24]
Microalgae (<i>Chlorella</i> , <i>Scenedesmus</i> , <i>Chlamydomonas</i>)	High carbohydrate content, lignin-free biomass	39–205 mL H ₂ L ⁻¹ day ⁻¹ (photobiological production rate)	CO ₂ sequestration, rapid growth, non-arable land utilization	Biohydrogen, biodiesel, pigments, nutraceuticals	[1,3]
Macroalgae (seaweeds)	Carbohydrates 30–60 wt%, low lignin content	2.0–4.5 mol H ₂ mol ⁻¹ hexose equivalent	Easy hydrolysis and high productivity	Biohydrogen, bioethanol, bioplastics	[2,3]
Water hyacinth (<i>Eichhornia crassipes</i>)	High cellulose content, invasive aquatic weed	81.7 mL H ₂ g ⁻¹ glucose	Environmental remediation and biomass utilization	Biohydrogen, compost, biochar	[26]
Dairy whey	Lactose-rich industrial by-product	152.2 ± 43.9 mL H ₂ g ⁻¹ VS	Continuous generation and high biodegradability	Biohydrogen, lactic acid, SCP production	[6,20]
Molasses	Rich in sucrose and reducing sugars	700 mL H ₂ L ⁻¹ day ⁻¹ (volumetric production rate)	Low-cost substrate from sugar industries	Biohydrogen, bioethanol, organic acids	[6,21]
Cassava wastewater (Manipueira)	High starch and carbohydrate content	16.90 mL H ₂ g ⁻¹ COD	Reduces environmental pollution from agro-industries	Biohydrogen, volatile fatty acids	[6,20]
Brewery spent grains and brewery wastewater	Cellulose, proteins, soluble sugars	-	Large-scale industrial availability	Biohydrogen, biomethane, animal feed	[6,20]

Mixed municipal organic waste	Heterogeneous organic fractions	20–50 mL H ₂ g ⁻¹ VS	High availability in urban areas	Biohydrogen, waste reduction, circular economy	[16,24]
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Note: Values follow the units and normalization basis used in the cited sources and are not always directly interconvertible. (i) Molar yields (agricultural residues, industrial wastewater, macroalgae) are expressed per mole of fermentable sugar, reported here as mol H₂ mol⁻¹ hexose equivalent. (ii) Specific yields normalized to substrate mass (food waste, sewage sludge, dairy whey, cassava wastewater, water hyacinth) are expressed per gram of volatile solids (VS), dry solids (DS), chemical oxygen demand (COD), or substrate glucose content, as mL H₂ g⁻¹ substrate; mL H₂ g⁻¹ VS and L H₂ kg⁻¹ VS are numerically identical and have been standardized to mL H₂ g⁻¹ VS throughout this table. (iii) Values for microalgae and molasses are volumetric hydrogen production rates, a time-normalized reactor performance metric rather than a per-substrate yield, expressed as mL H₂ L⁻¹ day⁻¹. Because VS, DS, COD, and substrate-specific mass represent different fractions of the feedstock, and production rates additionally depend on reactor hydraulic retention time and organic loading rate, values across these three bases remain reported as published and should not be compared directly without the underlying substrate composition data.

3. Next-Generation Biohydrogen Technologies: Innovations in ME, Synthetic Biology, and Hybrid Systems

Future developments in biohydrogen technologies aim at addressing the challenges associated with traditional biological processes such as low hydrogen production, inefficient use of substrates, metabolic losses, and non-scalability. Despite the fact that traditional DF can yield a mere 2–4 mol H₂ mol⁻¹ glucose when compared to the maximum theoretical yield of 12 mol H₂ mol⁻¹ glucose, technological advancements in ME, synthetic biology, and integrated processes have improved hydrogen yields immensely [1,21,29]. These technological advancements will become the pillar upon which Biohydrogen 2.0 will be based.

ME has been found to be a promising approach in increasing the rate of hydrogen generation by manipulation of metabolism and making microbes harder. Hydrogen-producing microorganisms like *Clostridium*, *Enterobacter*, *Thermotoga*, and photosynthetic bacteria have gained much attention due to their potential to produce hydrogen from various organic compounds [2]. Genetic engineering of hydrogenase and nitrogenase enzymes has led to better electron transport capability and prevention of production of metabolites which compete in producing hydrogen like lactate, ethanol, and acetate. It has been observed recently that hydrogen production rates higher than 41.47 mmol H₂ g⁻¹ dry cell weight h⁻¹ have been achieved through microbial fermentation pathways with genetic manipulations. Furthermore, mixed microbial consortia have demonstrated superior substrate utilization, metabolic flexibility, and process stability compared with monocultures, resulting in improved biohydrogen production efficiency [24].

In addition, advancements in synthetic biology contribute significantly to the development of biohydrogen production, providing an opportunity for the exact regulation of metabolism processes. In particular, modern technologies of genome editing like CRISPR-Cas systems make it possible to introduce genetic modifications into genes involved in hydrogen production, substrate utilization, and oxygen resistance [30]. Engineered strains of *Clostridium butyricum* and *Rhodobacter sphaeroides* provide an increase in hydrogen recovery due to optimized carbon flux redistribution and hydrogenase activity enhancement [31]. Advancements in metabolic engineering using CRISPR, microbiome engineering, digital twins, and smart biorefineries have contributed to higher hydrogen production efficiency and process stability. These new technologies are expected to drive faster advancements in intelligent and commercial biohydrogen production systems [2,16,32]. Furthermore, the application of synthetic biology can contribute to the creation of artificial microbial pathways that utilize more different types of biomass-derived substrates for hydrogen production [15].

Hybrid biohydrogen production technologies have attracted substantial attention due to the benefits provided by combining the best aspects of several biological and thermochemical technologies [32]. DF has been the most investigated technology due to its technical simplicity and capability of processing a variety of substrates; however, the recovery rate of hydrogen from this process is constrained by partial substrate conversion. In order to resolve this issue, dual DF, PF technologies have been developed, which allow utilizing volatile fatty acids produced at the primary fermentation stage. The hydrogen yield of these hybrid technologies is 5.2–8.0 mol H₂ mol⁻¹ substrate, which is significantly higher compared to the yield for the isolated fermentation process [5,21]. The same applies to another hybrid technology, DF-microbial electrolysis cell (DF-MEC), where the recovery of hydrogen exceeds 80% [24]. DF converts organic substrates to hydrogen anaerobically via microbial action (Figure 3).

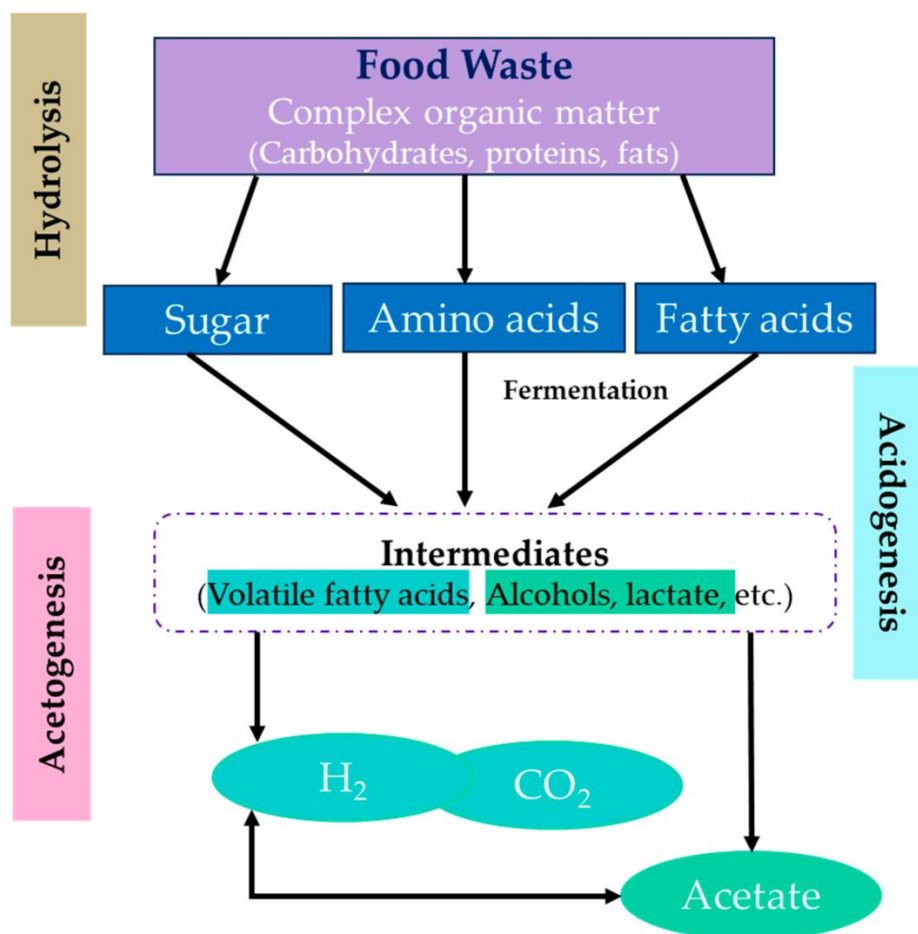


Figure 3. Biohydrogen production via DF. Reproduced from Ref. [7] under the CC BY 4.0 license.

MECs provide yet another advanced next generation system that uses electrochemistry coupled with microbial metabolic activity. The MECs constitute bio electrochemical reactors that combine electrochemical processes and metabolic activities of the microorganisms for production of hydrogen. In MECs, the electro-active microorganisms oxidize the organic substrates, while the external voltage is applied for production of hydrogen through cathodic reactions. Despite some limitations, MECs allow efficient production of hydrogen. In the MEC approach, electroactive microbes oxidize organic compounds, and the resulting electrons are transferred to the anode, whereas a small amount of voltage is applied to evolve hydrogen at the cathode. In comparison to the traditional method of fermentation, MEC can produce more hydrogen with better efficiencies. It has been found that MEC systems could produce 20–35% more hydrogen than DF alone [4,24].

Moreover, nanoparticle-based nanotechnology is increasingly incorporated into state-of-the-art biohydrogen systems due to their positive influence on biological activity and electron transport [33]. Iron oxide nanoparticles, nickel nanoparticles, carbon nanotubes, graphene-based nanomaterials, and magnetic nanocomposites increase enzyme activity, allow interspecies electron transfer, and improve reactor performance. The use of nanomaterials is accompanied by an increase in hydrogen production yield by 20–50% under optimal working conditions [15,29]. Moreover, nanoparticle-based catalysts and membrane materials are under investigation for hydrogen purification and storage to address critical issues related to post-production hydrogen treatment and storage.

The combination of ME, synthetic biology, nanotechnology, and hybrid systems becomes more and more common in the field of biohydrogen production. Future systems are going to be a combination of engineered microorganisms, advanced reactors, electrochemical techniques, and process control based on digital technology to achieve maximum productivity and economic feasibility. Such technological solutions are crucial to the development of new biohydrogen production systems from the lab scale to commercial scale [3,16,21].

4. Intelligent Bioprocess Design: AI, Digitalization, and Advanced Reactor Engineering

The progression of biohydrogen production from lab-scale operations to industrial applications does not only entail technological improvements in biological conversion but also intelligent process control, sustainability considerations, and economical optimization. Emerging techniques in AI, ML, digitization, and reactor design are revolutionizing existing biohydrogen processes into smarter biorefineries that have capabilities for real-time monitoring, prediction, and optimization [34]. Through these technologies, feedstock utilization is optimized while process robustness and hydrogen output are maximized, all while solving economic and environmental concerns associated with scaling up production [8,15,16].

AI has become a very promising tool that can be used for optimization of the biohydrogen production process [35]. ML, ANN, SVM, and deep learning algorithms have become frequently utilized for predicting hydrogen yield, optimizing process parameters, and revealing complex correlations between substrate properties and process outcomes. AI-based models have reached the accuracy level of over 95% for predicting such key process parameters as pH, temperature, hydraulic retention time, substrate concentration, and organic loading rate [16]. Additionally, predictive maintenance and fault detection have become an effective means of preventing process disturbances and have been proved to result in saving of up to 40% of the total process cost in biohydrogen production plants [4,16].

Parallel advances in the field of reactor design have resulted in enhanced production of biohydrogen along with increased efficiency of the process [36]. Continuous stirred tank reactors, anaerobic sequencing batch reactors, membrane bioreactors, packed-bed reactors, and MEC have shown better results when compared with traditional batch reactors. Recent reports indicated that hydrogen production rate using optimized continuous reactors is in the range of 29–40 L H₂ L⁻¹ day⁻¹, whereas hydrogen recovery by the DF-microbial electrolysis system is higher than 80% [21,24]. Usage of nanomaterials, conductive biofilm, and sophisticated electrode structures have resulted in improved electron transfer and enhanced hydrogen production rate [29].

Sustainability has been identified as an important parameter for analyzing new technologies for biohydrogen. Life cycle assessment (LCA) is a tool used to quantify the total environmental impact from biohydrogen production, covering all stages from biomass collection, pre-treatment, conversion, purification, storage, and end uses [37]. Recent LCA studies have shown that biohydrogen generated using wastes may be much less of a source of greenhouse gas emissions than conventional hydrogen produced from fossil fuels, especially when agricultural residues, food waste, and wastewater are used as the sources [2]. Integrating WV with DF and MEC has shown the potential to achieve carbon-neutral or even carbon-negative hydrogen production under optimized conditions, particularly when renewable electricity and carbon sequestration strategies are employed [37–39]. Moreover, the systems combining waste management and biohydrogen production have achieved considerable savings on landfills, wastewater treatment, and carbon emissions for example, organic wastes such as food waste, agricultural residue, industrial wastewater and sludge have been used successfully as substrates for producing biohydrogen.

The techno-economic assessment (TEA) has also become a crucial tool to establish the economic viability of biohydrogen systems [40]. According to current studies, feedstock prices, pretreatment steps, reactor configuration, hydrogen purification, and energy use continue to be major factors contributing to the cost of production. Although being economically competitive with steam methane reforming is quite difficult, substantial savings are possible via process integration, the use of waste materials as feedstock, and enhanced hydrogen yields. The hybrid systems which involve DF, PF, microbial electrolysis, and thermochemical pretreatment have been found to perform better from the perspective of energy utilization and economics than individual processes [5,21]. Furthermore, the optimization of processes and automation with AI assistance can lower the costs [16].

However, despite many breakthrough achievements, several issues still exist which prevent wide-scale commercialization of biohydrogen technologies. Feedstock variability, relatively low hydrogen productivity per volume of bioreactor, difficulty in scaling up, hydrogen purification demands, as well as regulatory uncertainties remain significant challenges. The majority of current biohydrogen approaches belong to TRL 3–5 and require additional pilot plant demonstrations prior to the commercial launch [21]. Thus, the development of standardized sustainability measures, adequate policies, carbon credits and renewable hydrogen incentives will play an important role in future implementation. The Figure 4 gives an insight on the different components of Biohydrogen 2.0 which includes intelligent process engineering, reactor design, sustainability assessment, and techno economic analysis to facilitate the evolution towards smart biorefinery and biohydrogen production.

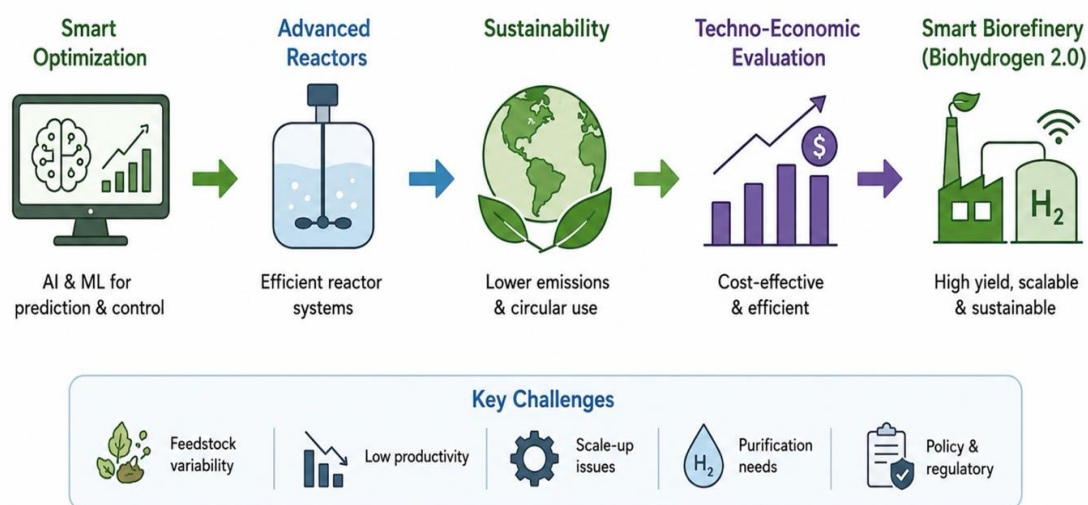


Figure 4. Intelligent bioprocess engineering for sustainable biohydrogen production: Key technologies and challenges.

Taking into account all above-mentioned aspects, it may be stated that the combination of digital transformation, innovative reactor design, sustainability analysis, and principles of circular economy will determine the future of Biohydrogen 2.0. Smart biorefinery facilities based on AI-based optimization technologies, integrated WV tools, as well as real-time monitoring systems could revolutionize biohydrogen industry and make it a valuable part of hydrogen economy of the future. Besides increased energy security, such systems would help address climate change problems and contribute to sustainable development of industries [3,4,15]. The technologies associated with synthetic biology platforms are an advancement of the genetic engineering technologies (Table 2) through the development of CRISPR pathway editing capabilities and creation of synthetic microbial consortia based on division of labor metabolic functions. This technology gives an advantage in comparison with single-strain engineering in terms of carbon flux and product specificity but still faces obstacles during the process of implementation due to large-scale production challenges and regulatory requirements. Nanoparticle-assisted biohydrogen production technologies involve using nanoparticles to increase microbial electron transfer and enzyme action. This has shown 20–50% improvement in yield in laboratory research (Table 2), although the high cost of nanoparticles and possible toxicity to the microbes prevent its use at an industrial scale. The concept of integrated circular biorefinery is the most developed one among the emerging technologies described in Table 2 (TRL 5-6).

The readiness of biohydrogen technology for commercialization is determined by the TRL of the technology. DF technology has achieved the TRL of 5-6 due to successful pilot plant demonstration, while PF and MEC technology have been at the TRL level of 4-5 owing to difficulties in the design of reactor, productivity of hydrogen, and scaling up. Novel technologies based on synthetic biology, AI in bioprocessing, and hybrid integrated system technology have been at the TRL level of 2-4 and need to be validated under industrial settings [2,4,5].

Table 2. Biohydrogen 2.0 Innovations: Emerging Technologies and Their Readiness for Industrial Deployment.

Technology/Innovation	Principle/Approach	Key Advantages	Current Performance Indicators	TRL (TRL)*	Major Challenges	Industrial Deployment Potential	Ref.
Advanced DF	Anaerobic conversion of organic substrates into H ₂ by fermentative bacteria	Simple operation, broad feedstock flexibility	2–4 mol H ₂ mol ⁻¹ glucose; 29–40 L H ₂ L ⁻¹ day ⁻¹ under optimized continuous operation, with peak rates up to 208.3 L H ₂ L ⁻¹ day ⁻¹ reported	TRL 5-6	Low theoretical yield, by-product formation	High	[1–4]
PF	Conversion of organic acids into H ₂ using photosynthetic bacteria under light	Higher substrate conversion efficiency	4.5–5.0 mol H ₂ mol ⁻¹ substrate	TRL 4-5	Light dependency, reactor cost	Moderate	[2,3]

Direct and Indirect Biophotolysis	Photobiological water splitting by microalgae and cyanobacteria	Direct solar-to-hydrogen conversion	Reported H ₂ productivity varies with organism and operating conditions	TRL 2-3	Oxygen sensitivity, low productivity	Low–Moderate	[1,29]
Synthetic Biology Platforms	CRISPR-based pathway engineering and designer microbial consortia	Enhanced metabolic control and product selectivity	Higher carbon conversion efficiency and H ₂ productivity	TRL 2-4	Scale-up and regulatory issues	Moderate	[15]
MECs	Electrochemically assisted microbial hydrogen production	High hydrogen recovery and wastewater treatment	>80% hydrogen recovery efficiency	TRL 4-5	Electrode cost and energy input	High	[2,24]
DF-MEC Hybrid Systems	Fermentation integrated with electrochemical enhancement	Improved energy recovery and substrate utilization	30–40% higher H ₂ production than standalone systems	TRL 3-4	System complexity	High	[5,24]
DF-PF Hybrid Systems	Sequential conversion of sugars and volatile fatty acids	H ₂ yields up to 5.2 mol H ₂ mol ⁻¹ substrate	Enhanced overall hydrogen recovery	TRL 3-4	Multi-stage process control	High	[3,21]
Nanomaterial-Assisted Biohydrogen Production	Use of nanoparticles to improve microbial activity and electron transfer	20–50% increase in H ₂ yield reported	Improved reactor performance and stability	TRL 3-4	Nanomaterial cost and toxicity concerns	Moderate	[4,21]
AI-Assisted Process Optimization	ML, ANN, predictive analytics	>95% prediction accuracy; up to 40% reduction in operational costs	Real-time optimization and control	TRL 2-4	Data availability and model validation	Very High	[8,16]
Digital Twin and Smart Bioreactors	Virtual process modelling with real-time monitoring	Predictive maintenance and adaptive control	Enhanced process stability and scalability	TRL 2-4	Integration complexity	Very High	[8,16]
Integrated Circular Biorefineries	Simultaneous production of hydrogen, biomethane, fertilizers, and chemicals	Maximum resource recovery and WV	Improved techno-economic feasibility	TRL 5-6	Infrastructure requirements	Very High	[2,6,15]

* TRL (1 = Concept validation; 9 = Commercial deployment).

5. Future Perspectives and Conclusions

Biohydrogen 2.0 represents the evolution of conventional biohydrogen production through the integration of WV, ME, synthetic biology, hybrid conversion systems, and intelligent bioprocess optimization. Recent advancements in hybrid biological systems have demonstrated improved H₂ production compared with conventional DF, particularly through the integration of complementary fermentation and electrochemical approaches [2,32]. Moreover, the accuracy rate reached over 95% and decreased costs up to 40% due to optimization models based on AI, indicating the great promise for digital solutions in future biohydrogen technology [16]. Biohydrogen 2.0 is a revolutionary model that combines waste utilization, state-of-the-art ME, AI, and bioprocessing for effective production of green hydrogen. While there are issues associated with feedstock variability, scaling-up of reactors and commercialization of the technology, the combination of digital intelligence, circular economy of biomass and optimization of processes paves the way for a technically feasible and promising biohydrogen model with potential for future industrial application.

Though, there are many difficulties connected to feedstock variation, reactor scaling, hydrogen purifying, and economy that do not allow commercializing these solutions yet. Future research in biohydrogen should take into consideration the implementation of modern techniques of ME, synthetic biology, AI, and digital bioprocessing to enhance the productivity, stability, and scalability of the process. Modern trends in biotechnology including AI-driven strain engineering, digital twin, autonomous bioreactor, and multi-omics-based metabolic engineering possess a considerable potential to bring intelligent bioprocesses to life. At the same time, combining WV with hybrid biorefinery concepts, negative carbon footprint production, and renewable energy sources may significantly speed up the development of a circular bioeconomy. The future research in biohydrogen should also be concentrated on reactor scale-up, hydrogen separation, TEA, LCA, and supportive policies. Taking into consideration the aspect of commercialization, piloting studies, efficient integration of processes, and collaboration between industry and academia will be essential for the transition of Biohydrogen 2.0 from laboratories to industries.

List of Abbreviations

AI	Artificial Intelligence
ANNs	Artificial Neural Networks
COD	Chemical Oxygen Demand
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
DF	Dark Fermentation
DS	Dry Solids
LCA	Life-cycle Assessment
ME	Microbial Engineering
MEC	Microbial Electrolysis Cell
ML	Machine Learning
PF	Photo Fermentation
SCP	Single Cell Protein
SVMs	Support Vector Machines
TEA	Techno-economic Analysis
TRL	Technology Readiness Level
VS	Volatile Solids
WV	Waste Valorisation
WWTPs	Wastewater Treatment Plants

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Availability of Data and Materials

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Conflicts of Interest

On behalf of all authors, the corresponding author declares no conflict of interest regarding the publication of this review article.

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AI Declaration

ChatGPT (OpenAI) was used only to enhance the language, grammar, and clarity of the manuscript. The authors are fully responsible for the content, accuracy, interpretation, and conclusions presented in the manuscript.

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