

Waste-to-hydrogen pathways: A cross-sectoral review of feedstock potentials, process integration, and sustainability metrics

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ABSTRACT

Biohydrogen production from waste offers a dual solution to mounting waste management pressures and fossil fuel dependence. This review addresses the gap through a cross-sectoral analysis of waste categories: agricultural residues, municipal solid waste, and wastewater, synthesised across classification tables and a review on a comparative framework encompassing hydrogen yield, energy output, environmental impact, economic feasibility, technology readiness, grid integration potential, scalability, and regional relevance. Dark fermentation of sugar-rich agro-industrial wastes achieves the highest near-term viability, with pineapple peel juice yielding up to 2.3 mol H₂/mol sugar and POME reaching 1.88 mol H₂/mol sugar under thermophilic conditions, both requiring minimal pretreatment. However, Technology Readiness Level mapping reveals that no reviewed pathway has surpassed TRL 7, and the levelized cost of biohydrogen spans \$2.50–48.96/kg H₂, with pathway selection, not feedstock alone, as the primary cost driver. Life cycle assessment integration remains critically absent from most studies, motivating the standardised cradle-to-grave LCA–TEA framework proposed herein. The review aligns these findings with Malaysia's National Energy Transition Roadmap (NETR), Sarawak Hydrogen Economy Roadmap (SHER), and Post-COVID Development Strategy (PCDS) 2030, identifying waste-to-biohydrogen as a priority deployment pathway for the circular bioeconomy.

1. Introduction

The growing global concern about the escalating generation of waste and the depletion of fossil fuel resources underscores the need for alternative energy sources aligned with sustainable development principles. Biohydrogen production from waste offers a dual advantage, addressing waste management challenges while also contributing to clean energy generation. Waste materials, such as agricultural residues (e.g., wheat straw, corn stover, sugarcane bagasse), food waste, and industrial effluents, serve as valuable feedstocks rich in organic compounds that can be converted into hydrogen through biological processes [1–3]. This process not only diverts waste from landfills but also

mitigates associated environmental problems, such as greenhouse gas emissions and leachate formation, aligning seamlessly with the circular economy paradigm, where waste is repurposed as a resource [4]. Unlike traditional linear models that generate waste at every stage of production and consumption, biohydrogen pathways utilize these wastes as feedstocks, contributing to a closed-loop system that conserves resources and reduces pollution.

Interest in hydrogen generation and utilization is gaining momentum worldwide as a potential fuel of the future. Hydrogen has a calorific value (higher heating value) of 141 MJ/kg, which is the highest of all known commercial fuels. Considering the lower heating value (120 MJ/kg), 1 kg of hydrogen is equivalent to approximately 2.75 kg of gasoline

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[5]. The integration of biohydrogen production into the circular economy is further strengthened by its capacity to recover resources and valorize energy. Processes such as enzymatic saccharification combined with dark fermentation or steam explosion, followed by microbial electrolysis, enable high conversion efficiencies from complex biomass into biohydrogen [6,7].

Life Cycle Assessment (LCA) studies indicate that biohydrogen pathways offer substantial reductions in greenhouse gas emissions compared to conventional fossil-based hydrogen production [8,9]. In addition to environmental benefits, biohydrogen production from waste supports economic sustainability and technological innovation within the circular economy.

A collection of review articles has been gathered and analyzed through cross-analysis to uncover distinctive insights that differentiate this study from previous reviews on biohydrogen synthesis from waste materials, including agricultural waste, municipal solid waste, wastewater, and various types of waste. Through this cross-analysis, key parameters, including synthesis mechanisms (dark fermentation, microbial electrolysis cells, and hybrid systems), hydrogen yield efficiencies, operating conditions, and environmental indicators, were examined in parallel to reveal technological complementarities and performance differences. This broader, multi-waste, and multi-method lens not only highlights critical synergies between different technologies, such as dark fermentation or hybrid conversion to improve hydrogen recovery, but also identifies underutilized feedstocks with untapped potential and the gaps that need to be addressed [10,11].

This review article makes a significant contribution to the field by emphasizing biological conversion technologies, including dark fermentation, microbial electrolysis cells (MECs), photofermentation, and microbial fuel cells (MFCs), and by addressing the limitations of standalone processes. It goes beyond conventional reviews by incorporating Technology Readiness Level (TRL) assessments, offering a realistic view of the deployment potential of various hydrogen production pathways. The inclusion of comprehensive environmental performance metrics such as greenhouse gas (GHG) emissions, energy return on investment (EROI), and life cycle assessment (LCA) provides a holistic evaluation that connects technological feasibility with sustainability goals [12].

Despite the wealth of literature on biohydrogen, existing reviews often remain fragmented, focusing either on isolated feedstock types or specific biochemical pathways without a unified sustainability metric. This manuscript addresses this gap by providing a cross-sectoral analysis that integrates agricultural, municipal, and industrial waste streams into a single evaluative framework. By combining Life Cycle Assessment (LCA) with Techno-Economic Analysis (TEA) and Technology Readiness Level (TRL) benchmarks, this study moves beyond theoretical potential to identify actionable, policy-aligned pathways specifically for the regional hydrogen economies of Malaysia and Sarawak. To underscore the unique contributions of this work, Table 1 provides a comparative summary of how the scope and methodological integration of this manuscript advance beyond existing review paradigms.

Therefore, this article review aims to contribute impactful novelty to the body of knowledge by synthesizing fragmented research into a unified, systems-level framework that evaluates not only technological efficiency but also environmental, economic, and policy dimensions. Its focus is on identifying a critical research gap and the boundaries of current understanding in biohydrogen production. By aligning scientific findings with circular economy principles and local implementation capacity, the review opens new avenues for interdisciplinary research and practical adoption, marking a shift from theoretical exploration toward sustainable, policy-driven innovation in biohydrogen production.

This review extends beyond traditional academic evaluations by aligning technological assessments with the strategic goals outlined in Malaysia's and Sarawak's energy transition frameworks. Specifically, it examines how waste-to-biohydrogen pathways can contribute to the

Table 1
Comparative analysis of the present study against typical review literature, emphasizing the integration of LCA-TEA, TRL, and regional policy alignment.

Comparison Factor	Literature Review	This Study (Waste-to-Hydrogen Pathways)	Advance/Novelty
Scope of Waste	Often limited to a single sector (e.g., only agricultural or only municipal waste).	Cross-sectoral: Integrates agricultural, industrial (POME), and municipal organic waste.	Enables direct comparison of conversion efficiencies across different waste sectors.
Methodological Depth	Primarily focus on biochemical mechanisms or laboratory-scale yields.	LCA-TEA Integration: Simultaneously evaluates environmental burdens and economic viability.	Identifies the economic "cost gap" between lab results and industrial targets.
Readiness Assessment	General discussion on future potential.	TRL Benchmarking: Explicitly identifies the commercial readiness of each pathway.	Differentiates between theoretical research and scalable technologies.
Contextual Application	Often, global or generalized perspectives.	Regional Policy Alignment: Directly linked to Malaysia's NETR and HETR frameworks.	Transforms scientific findings into a tool for national energy planning.

Sarawak Hydrogen Economy Roadmap (SHER), which envisions Sarawak as a global leader in the production of green and blue hydrogen. Leveraging abundant renewable resources such as hydropower and biomass, the roadmap emphasizes waste valorization to enable a circular hydrogen economy [13]. This review identifies locally available waste streams: agricultural residues, municipal organic waste, and agro-industrial effluents. They serve as viable feedstocks for hydrogen production. In doing so, it not only addresses waste management challenges but also enhances regional energy security and sustainability.

At the national level, the review supports the objectives of the National Energy Transition Roadmap (NETR), which identifies hydrogen as one of six key levers for Malaysia's low-carbon future [14]. Through a comparative analysis of cross-waste indicators, including life cycle assessment (LCA), economic feasibility, and technology readiness levels (TRLs), the study contributes to Malaysia's Responsible Transition Pathway (RT Pathway, 2050). It provides a foundation for prioritizing investments in scalable hydrogen technologies. Additionally, the review aligns with the Hydrogen Economy and Technology Roadmap (HETR), which emphasizes the commercialization and innovation potential of waste-derived hydrogen, projected to generate RM400 billion by 2050 [15]. Ultimately, by evaluating biohydrogen synthesis within circular economy and decarbonization frameworks, this review offers a policy-relevant tool for government, industry, and academia to leverage waste resources for clean energy, while advancing Malaysia's Sustainable Development Goals, which are SDG 7 (Affordable and Clean Energy), SDG 12 (Responsible Consumption and Production), and SDG 13 (Climate Action).

2. Review methodology

The research review framework outlines a systematic approach for identifying, selecting, and analyzing publications relevant to the literature on biohydrogen production from waste sources. By leveraging reputable databases, this framework ensures a comprehensive collection of both review and research articles. Through a structured, keyword-based search and defined inclusion and exclusion criteria, the process enables the extraction of high-quality studies aligned with the objectives of circular economy integration, life cycle assessment, and sustainable

energy production. This framework ensures transparency and academic rigor in synthesizing current knowledge and identifying research gaps, as illustrated in Fig. 1.

2.1. Database selection

The literature review began by identifying reliable research article sources to ensure comprehensive, high-quality data collection. Four major scholarly databases were selected: Web of Science, ScienceDirect, Scopus, and Google Scholar. These platforms were chosen for their extensive coverage of peer-reviewed scientific publications on biohydrogen production, waste management, sustainability, and life-cycle assessment. This step ensured an inclusive foundation for article collection, integrating both global and region-specific studies.

2.2. Total number of web-based articles collected

From the four selected databases, 302 articles were initially collected. The distribution was as follows: Web of Science (T = 24), ScienceDirect and Scopus (T = 200), and Google Scholar (T = 78), as shown in Fig. 2. This diverse article pool enabled a multidisciplinary, multisource perspective on biohydrogen production and sustainability.

2.3. Type of articles collected

As shown in Fig. 3, the collected articles were categorized into two types: Review Articles and Research Articles. Specifically, Web of Science contributed 10 review articles and 14 research articles;

ScienceDirect contributed 48 review articles and 52 research articles; and Scopus contributed 38 review articles and 62 research articles. In comparison, Google Scholar added 45 review articles and 32 research articles. In total, this comprised 141 review articles and 161 research articles, offering a balanced mix of synthesis and empirical studies.

2.4. Evaluation of related articles

A structured screening process was applied to assess the relevance of each article. The screening was based on keyword matching and thematic relevance. Key terms used in the search methodology included: “biohydrogen”, “waste-to-hydrogen”, “dark fermentation”, and “life cycle assessment”. Boolean operators were used to refine results, and articles were screened for titles, abstracts, and full texts. The goal was to retain studies that directly address biohydrogen production from waste, providing details on the conversion process and sustainability considerations.

2.5. Not Irrelevant articles and rejection criteria

Following screening, 144 articles were categorized as Not Irrelevant. These articles were excluded based on several criteria: (i) the topics were beyond the scope, focusing on other forms of energy (such as biodiesel or fossil-based hydrogen); (ii) they provided insufficient experimental or technical data, or (iii) they lacked connection to biohydrogen or waste valorization. Hence, it does not support comparison or cross-analysis. These papers did not align with the review's central themes.

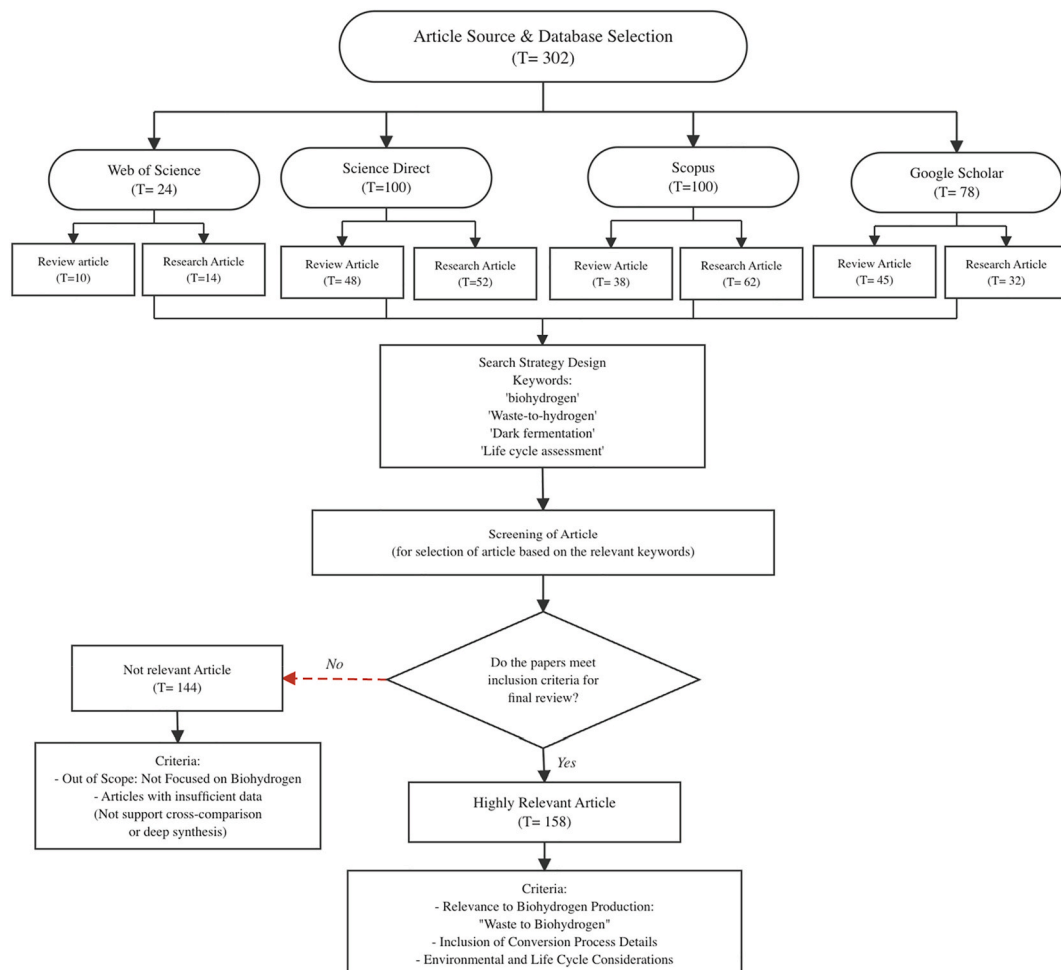


Fig. 1. Flow chart of research methodology framework.

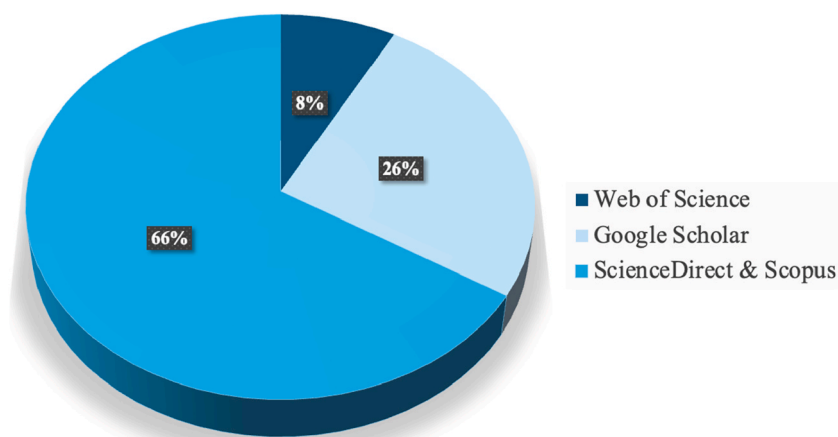


Fig. 2. Pie chart of research database collected from 2015 to 2024.

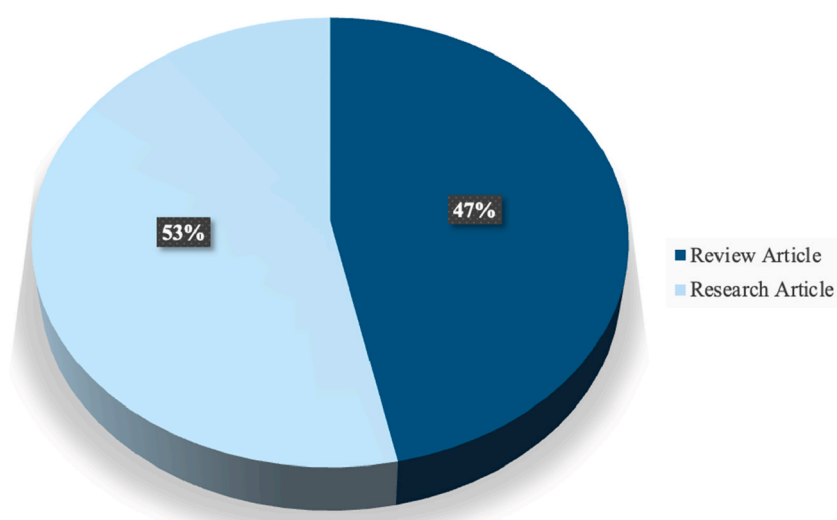


Fig. 3. Pie chart of the type of research articles collected from 2015 to 2024.

2.6. Highly relevant articles

A total of 158 articles were retained as Highly Relevant and included in the final analysis. Articles were considered highly relevant if they met three main criteria: (i) directly related to biohydrogen production using waste feedstock, (ii) inclusion of conversion process details, such as dark fermentation or hybrid processes, and (iii) incorporation of environmental or life cycle analysis, such as GHG emissions, energy efficiency, or circular economy linkages. These studies formed the core dataset for the review and were analyzed in-depth in the cross-analysis table.

3. Classification of waste types and their conversion potentials

3.1. Agriculture waste

Agricultural waste refers to the by-products of processing farming products that are not intended for food consumption. This type of waste can be categorized into crop residues (husks, leaves, straws, and bagasse generated from the harvesting of rice, wheat, sugarcane, and corn), waste generated from the distribution of fruits and vegetables, processing of agricultural products, or managing livestock (fertilizer cans, packaging materials) [16–18] as illustrated in Fig. 4.

3.1.1. Wheat straw

Wheat straw is among the most plentiful agricultural residues

worldwide and has great promise as a biohydrogen feedstock. Its appeal lies in its abundance and its composition. Wheat straw is composed mainly of cellulose, hemicellulose, and lignin, interwoven materials that trap a significant amount of sugars [19]. This complexity is what makes wheat straw a challenging feedstock to work with, yet it is highly attractive for bioenergy applications. To synthesize the findings from the research on wheat straw for biohydrogen production, a comparative summary table has been constructed. This table categorizes each study by wheat straw classification, including hydrolysate type, pretreatment method, and conversion potential (e.g., hydrogen yield and synthesis type). It identifies key research gaps that still need to be addressed, as tabulated in Table 2.

Recent studies on wheat straw as a feedstock for biohydrogen production have revealed a growing shift in how researchers classify and utilize this abundant agricultural residue. Traditionally regarded as lignocellulosic waste, wheat straw is now being classified more precisely based on its hydrolysate composition and behavior during pretreatment, which has proven critical in determining its potential for biohydrogen conversion. For instance, Kongjan et al. [20] explored hemicellulose-rich hydrolysate derived from hydrothermal pretreatment of wheat straw and employed dark fermentation under extreme thermophilic conditions (70 °C) using mixed microbial cultures. They reported a high hydrogen yield of 318.4 mL of H₂ per gram of sugar in batch reactors, and 178 mL H₂/g sugar in continuous stirred tank reactors (CSTR). However, they noted that increasing the hydrolysate

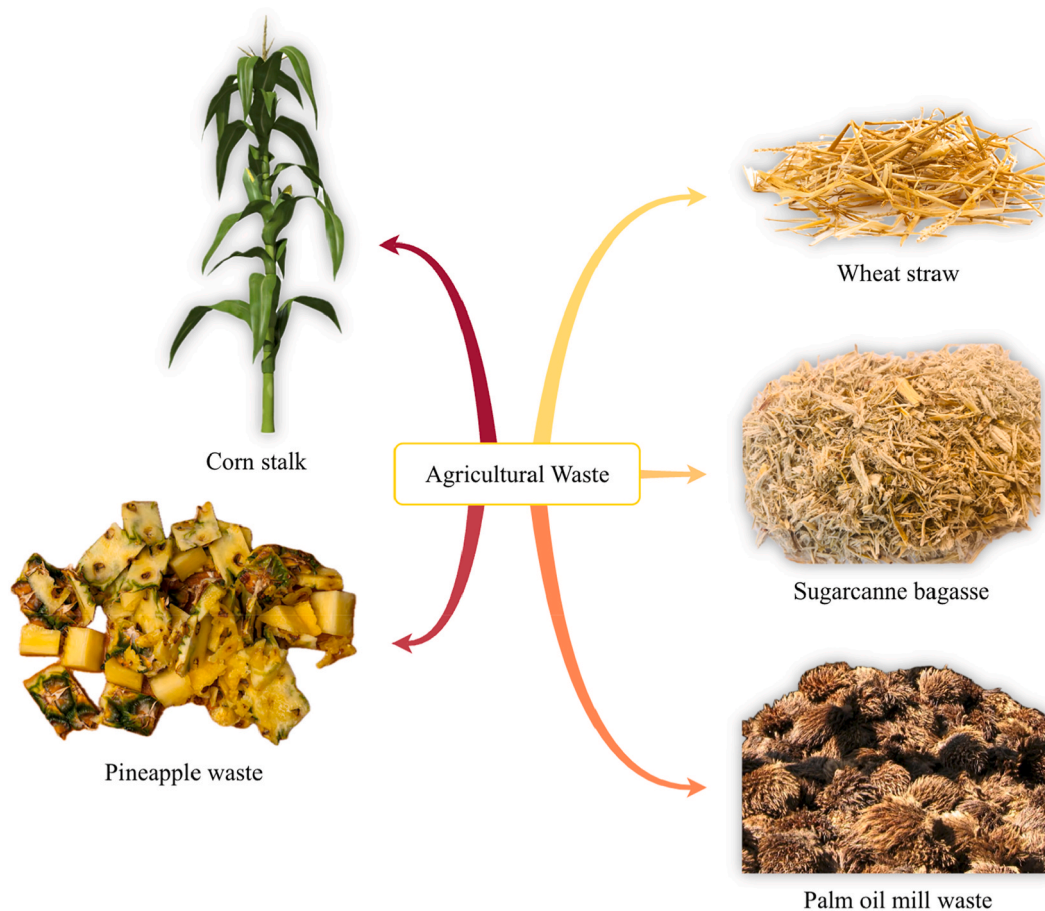


Fig. 4. Types of agricultural waste.

concentration beyond 25% v/v reduced hydrogen production, suggesting that higher substrate loads may introduce inhibitory compounds, such as furfural and HMF, thereby affecting microbial efficiency.

In another study, Zhu et al. [6] focused on enhancing the accessibility of wheat straw for microbial digestion by combining ultrasound and dilute alkali pretreatment, followed by enzymatic saccharification and dark fermentation. This integrated process significantly enhanced hydrogen production, reaching 133.6 mL H_2 per gram of total solids (TS), approximately 5.6 times higher than yields from raw untreated wheat straw. The feedstock amount was not specified in exact kilograms, but the comparative increase indicates a substantial impact of pretreatment in unlocking fermentable sugars. This study also emphasized that optimizing pretreatment conditions is crucial for balancing conversion efficiency and cost, particularly when dealing with large quantities of lignified straw biomass.

A study by Jin et al. [1] adopted a more systems-level perspective by integrating dark and photofermentation in a cascade configuration within a life-cycle analysis (LCA) framework. Their study simulated the treatment of 1000 kg/h of wheat straw, which, after acid hydrolysis with 2% sulfuric acid and sodium acetate supplementation, yielded 340,000 L of hydrogen per hour and generated 348.6 kW of electricity, translating to a hydrogen conversion yield of 340.48 mL H_2 /g biomass. This large-scale model demonstrated the potential of cascade utilization to maximize both energy output and carbon recovery; however, controlling such multi-step systems under industrial conditions remains a significant challenge.

In a semi-continuous digestion system, Chandolias et al. [21] investigated wheat straw hydrolysate derived from phosphoric acid pretreatment, varying organic loading rates (OLRs), and introducing membrane-encased cells to improve productivity. At a low OLR of 4.42 g

COD/L day, the maximum biohydrogen and acetic acid production was recorded. While exact conversion rates were not reported in mL/g terms, the results demonstrated that membrane bioreactors could enhance acid production by up to 121% in a second-stage system, suggesting promise for stable, long-term operation. However, challenges remain in balancing hydrogen and acid production, and more studies are needed on the scalability and cost-efficiency of this approach.

A study also presented an integrated biorefinery concept that utilized wheat straw in a multi-stream process, converting cellulose to ethanol and hemicellulose to hydrogen via dark fermentation, followed by methane recovery from the residues. Their hydrogen yield was 178 mL H_2 /g of sugar from the hydrolysate, while the ethanol yield was 0.41 g ethanol/g of glucose. Although the initial straw mass per batch was not specified, the study focused on maximizing overall bioenergy recovery through synergistic process design. A key limitation noted was the inefficiency of pentose sugar fermentation and the lack of robust co-culture microbial systems capable of fully utilizing the diverse sugar streams present in wheat straw [19].

Taken together, these studies demonstrate not only the versatility of wheat straw as a biohydrogen substrate but also the wide range of conversion mechanisms, from dark fermentation and cascade fermentation to membrane-assisted and enzymatic systems, that are being developed to improve yields. However, across all approaches, shared technical gaps persist, including the need for more efficient pentose-utilizing microbes, cost reductions in pretreatment, inhibitor-mitigation strategies, and scalable reactor designs that can handle variable feedstock loads in real-world biorefinery settings.

3.1.2. Sugarcane bagasse (SCB)

Sugarcane bagasse (SCB) is categorized as agricultural waste,

Table 2

Classification of wheat straw.

Classification of Waste	Conversion Potential of Biohydrogen	Identified Research Gap	References
Hemicellulose-rich hydrolysate from hydrothermal pretreatment	Dark fermentation using extreme thermophiles; up to 318.4 mL H ₂ /g-sugar (batch); 178 mL H ₂ /g-sugar in CSTR	Inhibition occurs at high substrate concentrations, and scalability issues arise in continuous systems.	[20]
Pretreatment-enhanced wheat straw (Ultrasound + Alkali)	Enzymatic saccharification + dark fermentation; up to 133.6 mL H ₂ /g-TS, 5.6× higher than untreated	Energy demand for pretreatment; limited real-world applications and the need to optimize microbial strains.	[6]
Hydrolysate from dilute acid treatment for cascade use	Dark + photofermentation cascade; 340.48 mL H ₂ /g biomass, 348.6 kW electricity from 1000 kg/h feed	There is a lack of real-world-scale integration and a need to optimize heat and energy recycling.	[1]
Phosphoric acid hydrolysate under varied OLRs	Thermophilic digestion with membrane-encased cells; max biohydrogen at OLR 4.42 g COD/L/day; acetic acid up by 121% (stage 2).	Few studies on membrane-cell reactors have addressed the imbalance between hydrogen and acid production, and the process's scalability remains uncertain.	[21]
Biorefinery: Cellulose for ethanol, hemicellulose for H ₂	Dark fermentation of hydrolysate: 178 mL H ₂ /g sugar; ethanol: 0.41 g/g glucose; methane potential from residue effluents.	Integration complexity, inefficient pentose sugar fermentation, and a lack of robust co-culture systems are key challenges.	[19]

Note: Yield units are as reported in the original studies; normalization bases (VS, TS, sugar, COD) vary by study design. See the table for inter-conversion factors. STP = 0 °C, 1 atm.

specifically a lignocellulosic crop residue produced as a byproduct of sugarcane juice extraction. This residue is rich in lignocellulosic material, primarily composed of cellulose (38.1%), hemicellulose (21.2%), and lignin (8.3%), making it an appealing candidate for bioconversion technologies.

One notable study by Sangyoka et al. [22] classified SCB based on its hydrolysate, derived from dilute sulfuric acid pretreatment, which enables the release of fermentable sugars from the hemicellulose-rich fraction. They applied dark fermentation using mixed microbial cultures and optimized the process using Response Surface Methodology (RSM). Under optimal conditions, including a substrate sugar concentration of 22.77 g/L, an inoculum-to-substrate ratio of (3.5:1), and appropriate buffering, the system achieved a remarkable hydrogen yield of 6980 mL H₂ per liter of fermentation broth. However, they also noted that acid hydrolysis could introduce inhibitors, such as furfural, and emphasized the need to optimize inoculum ratios and scale up the process for practical applications.

In contrast, Srivastava et al. [23] approached SCB classification from an enzymatic perspective, utilizing crude enzyme hydrolysis enhanced with magnetite nanoparticles (Fe₃O₄) to enhance saccharification prior to sequential fermentation. This method, which combines enzymatic treatment with dark fermentation, yields 80.45 mL of H₂ per gram of reducing sugar. Although the exact weight of SCB used was not directly specified, the study emphasized that the enhanced hydrolysis significantly increased sugar availability from pretreated SCB, thereby boosting hydrogen production efficiency. The novelty of their work lies in integrating nanomaterials for enzyme reuse and stability. However, the authors acknowledge challenges such as high enzyme costs, process complexity, and the need for improved validation at pilot or industrial

scale.

Meanwhile, Guo et al. [24] explored a more generalized and comparative classification of SCB by evaluating it both as a single substrate and in co-digestion with other agricultural wastes. In batch dark fermentation, SCB used alone yielded 58.2 mL H₂ per gram of volatile solids (VS). While this figure is modest compared to enzymatic or optimized systems, it still demonstrates SCB's potential as a reliable substrate for hydrogen production. Their setup involved variable initial SCB quantities in combination with pH control strategies; however, the study noted issues with inconsistent pretreatment quality, suboptimal pH ranges, and the formation of inhibitory byproducts that could impact microbial performance during fermentation.

Across these three studies, the type of synthesis ranged from traditional dark fermentation to sequential enzymatic-hydrolytic systems, and conversion rates varied widely, from 58.2 mL H₂/g-VS to 6980 mL H₂/L, depending on microbial efficiency and pretreatment type. The initial substrate concentration played a crucial role, particularly in a study conducted by Sangyoka et al. [22], where a precisely tuned sugar load enabled the maximum yield. However, common research gaps persist, including the need to minimize fermentation inhibitors, enhance enzyme recyclability, and scale the processes economically while maintaining high hydrogen output, as summarized in Table 3. Despite these challenges, sugarcane bagasse remains a highly promising feedstock, and these studies collectively demonstrate that with the right combination of pretreatment, fermentation strategy, and process optimization, it can play a significant role in sustainable biohydrogen production.

3.1.3. Rice waste

Recent studies on rice waste and its derivatives have shown promising potential for sustainable biohydrogen production; however, each approach adopts different strategies, mechanisms, and conditions to maximize yield. One of the most comprehensive analyses was conducted by Yakesh et al. [25], who explored the impact of combinative strategic pretreatment. This study specifically evaluated the effects of diluted acid, alkaline, and hybrid treatments on raw rice straw before dark fermentation. Among the various treatment combinations, the DTCD method (dilute alkali + cellulase) yielded the highest hydrogen

Table 3

Classification of sugarcane bagasse.

Classification of Waste	Conversion Potential of Biohydrogen	Identified Research Gap	References
SCB hydrolysate (acid-pretreated)	Dark fermentation using mixed culture; yield: 6980 mL H ₂ /L at optimized conditions (22.77 g sugar/L substrate)	Optimizing the inoculum-to-substrate ratio is necessary, as is addressing acid hydrolysis inhibition and scaling up the RSM optimization.	[22]
Enzyme-treated SCB (crude enzyme with Fe ₃ O ₄)	Sequential fermentation (enzymatic hydrolysis + dark fermentation); yield: 80.45 mL H ₂ /g reducing sugar	Key challenges include enzyme cost, process complexity, the need for high-efficiency magnetite reuse, and limited pilot validation.	[23]
SCB mixed with other agricultural waste	Dark fermentation in batch setup; yield from SCB alone: 58.2 mL H ₂ /g-VS	Several factors, including a lack of standard pretreatment, mixed waste interactions, variable pH tolerance, and metabolic byproduct inhibition, hinder the process.	[24]

Note: Yield units are as reported in the original studies. See the table for inter-conversion factors. STP = 0 °C, 1 atm.

production of 129 mL H₂ per gram COD, compared to 81 mL/g (DTD) and 58 mL/g (DD). The initial substrate concentration was based on a solid loading of 10–15 g/L, and they noted that a higher enzyme-to-substrate ratio significantly enhanced sugar release and fermentation efficiency. However, their study also indicated that chemical-intensive pretreatments may raise environmental and economic concerns due to energy inputs and chemical costs.

In a systems-level evaluation of energy and sustainability, one study compared three biohydrogen production pathways: dark fermentation, gasification, and electrolysis. They are using rice husk as the primary feedstock. While the study did not focus on lab-scale yield values in mL/g, it presented net energy ratios (NERs) and life-cycle performance data. Among the three, dark fermentation was found to be the most energy-efficient and had the lowest greenhouse gas (GHG) emissions, achieving a net energy ratio of up to 1:9. Although large-scale waste input figures were modeled hypothetically, the study emphasized the need for detailed inventory data and validation of integrated systems in real-world Southeast Asian contexts [26].

On the biochemical front, Kim et al. [12] examined acid and alkali hydrolysis of rice straw to prepare hydrolysates for dark fermentation, comparing their effectiveness in terms of sugar recovery and hydrogen yield. Using an initial sugar concentration derived from 15 g/L rice straw, acid hydrolysates yielded 3.35 mol H₂/mol glucose, while alkali hydrolysates produced 2.73 mol H₂/mol glucose. This study highlighted the inhibitory effects of furfural and hydroxymethylfurfural (HMF) in acid-treated hydrolysates, which limited microbial fermentation despite their high sugar content. Therefore, even though acid hydrolysis achieved slightly higher yields, its toxic byproducts remain a significant technical hurdle.

In contrast, Sattar et al. [27] approached rice crop waste holistically, using components such as stems, leaves, husks, and bran in a batch anaerobic fermentation setup without any chemical pretreatment. Their experiments at different temperatures showed that thermophilic conditions (55 °C) yielded the best results, achieving approximately 120 mL of H₂ per gram of dry substrate. The simplicity of their process, which utilizes raw biomass, suggests a cost-effective path forward; however, it also introduces limitations, such as uncharacterized microbial activity, limited sugar accessibility, and sensitivity to temperature changes.

Focusing specifically on rice bran, Azman et al. [28] investigated de-oiled rice bran for fermentative hydrogen production under mesophilic conditions. At 37 °C and pH 6, the process achieved 1.43 mol H₂/mol reducing sugar, using substrate loads of approximately 10 g/L. Their study revealed that while removing oil from the bran enhances microbial accessibility, residual protein and lipid content can still affect microbial pathways, and further work is needed to optimize reactor operation under continuous flow conditions.

Lastly, Morya et al. [29] explored the use of raw rice waste, particularly mixtures of rice leaves and husks, for mixed-acid fermentation. Although specific yield values varied depending on composition, the reported range was approximately 60–100 mL of H₂ per gram of volatile solids (VS). This process emphasized acidogenesis, and the study highlighted that the microbial consortia involved were not fully identified, making it challenging to fine-tune fermentation conditions or predict performance in scaled-up systems. The process is intentionally halted at the acidogenic stage to maximize hydrogen yield. If allowed to progress to the methanogenic stage, the produced hydrogen would be consumed by methanogens to form methane, reducing the purity and yield of the target hydrogen product. Additionally, synergy with enzymatic or thermochemical treatments was not explored, leaving room for further integration and optimization.

Taken together, these studies highlight a rich diversity in both pretreatment classification and synthesis mechanisms that ranging from direct anaerobic fermentation of raw biomass to chemically pretreated hydrolysates and underscore the key factors influencing hydrogen yield: pretreatment efficiency, substrate loading, inhibitor management, and microbial dynamics and what are still lacking based on the research of

using rice straw for conversion of biohydrogen, which have been summarized in Table 4.

3.1.4. Corn stalk

Corn stalk, a commonly discarded agricultural residue, has attracted increasing interest as a lignocellulosic feedstock for biohydrogen production. In one study, Lu et al. [30] investigated the impact of phosphate buffering systems (Na₂HPO₄: NaH₂PO₄) on hydrogen production during dark fermentation. Using untreated corn stalk as the substrate, they observed that the buffering system played a crucial role in stabilizing the pH and enhancing fermentation efficiency. Operating at 37 °C and pH 7 for 48 h, the system achieved a yield of 2.21 mol H₂/mol hexose, equivalent to approximately 52.1 mL of hydrogen per gram of dry corn stalk. Although promising, the study noted that the long-term effects of phosphate salts on microbial communities and potential nutrient imbalances remain underexplored.

In a related study, Lu et al. [31] also investigated the effect of iron (II) (Fe²⁺) supplementation on photofermentation using *Rhodospseudomonas palustris*, a photosynthetic bacterium that produces hydrogen. Corn stalk hydrolysates were fermented under operating conditions (4000 lux, 32 °C, pH 6.8) to examine the influence of various Fe²⁺ concentrations. The study revealed that an optimal concentration of 100 mg/L Fe²⁺ significantly boosted hydrogen production to 3.04 mol H₂/mol hexose, outperforming both lower and higher iron levels due to improved electron transfer and enzymatic activity. Beyond iron supplementation, sulfur availability represents another critical environmental factor influencing photosynthetic hydrogen production efficiency. Sulfur deprivation has been shown to reversibly inactivate PSII (Photosystem

Table 4
Classification of rice straw.

Classification of Waste	Conversion Potential of Biohydrogen	Identified Research Gap	References
Raw rice straw	Dark fermentation; 129 mL H ₂ /g-COD (DTCD), 81 mL/g (DTD), 58 mL/g (DD), 8 mL/g (Control); 742 kJ/kg RS energy input for DTCD	Lacks large-scale validation; high chemical load for alkali use.	[25]
Rice husk (energy LCA of pathways)	Dark fermentation, gasification, electrolysis; DF is the most energy-efficient, with the lowest GHG; net energy ratio up to 1.9	There is a need for real-world energy integration, as life-cycle inventory data remain limited in Southeast Asia.	[26]
Rice straw hydrolysate (acid/alkali-treated)	Dark fermentation; acid: 3.35 mol-H ₂ /mol-glucose; alkali: 2.73 mol-H ₂ /mol-glucose	Variability in hydrolysate purity; acid inhibitors (HMF/furfural) remain a concern.	[12]
Rice crop waste (stem, leaf, husk, bran)	Anaerobic fermentation in batch; max 120 mL H ₂ /g dry substrate at 55 °C (thermophilic)	No pre-treatment integration; temperature sensitivity; gas purity not discussed	[27]
Rice bran (de-oiled)	Fermentative H ₂ production; 1.43 mol H ₂ /mol reducing sugar; optimal pH 6, 37 °C	Not scaled beyond lab-batch setup; lacks continuous flow studies; minor protein/lipid residue inhibition.	[28]
Raw rice waste (leaf + husk mix)	Mixed acid fermentation; variable yield (range: 60–100 mL H ₂ /g VS); acidogenesis pathway dominant	Microbial consortium not characterized; synergy with enzymatic treatment unexplored.	[29]

Note: Yield units are as reported in the original studies. See the table for inter-conversion factors. STP = 0 °C, 1 atm.

II) activity in photosynthetic bacteria, with a temporal window of 24–72 h representing the critical Stage 2 during which this inactivation process becomes pronounced, after which activity may recover upon sulfur repletion [32]. This reversible inactivation mechanism suggests that maintaining adequate sulfur concentration in photofermentation reactors is essential for sustaining continuous hydrogen generation. The recovery of PSII activity upon sulfur restoration indicates that sulfur limitation does not cause irreversible photosynthetic damage, presenting an opportunity for process optimization through precise nutrient management. Understanding these temporal dynamics allows reactor operators to monitor and maintain critical nutrient thresholds, preventing extended periods of reduced photosynthetic efficiency. This finding is particularly relevant for agricultural residue-based photofermentation systems, where varying feedstock compositions may necessitate dynamic sulfur supplementation strategies [33].

This study also explored the use of Response Surface Methodology (RSM) to optimize process parameters for the dark fermentation of corn stalk using *Clostridium butyricum* [34]. This study provided more granular details on substrate load, using a solid concentration of 50.87 g/L, an inoculum size of 10.25%, and a pH of 6.6 under mesophilic conditions at 35.6 °C. Under these conditions, they achieved a hydrogen yield of 40.62 mL/g dry corn stalk, reflecting the synergistic effect of precisely tuned operating parameters. Differing from his previous studies, Lu et al. [30] focused heavily on experimental design and mathematical optimization, while also acknowledging the need for further work to reduce enzyme costs and ensure the genetic stability of bacterial strains during extended fermentation cycles.

The research study on using corn stalk for biohydrogen conversion provides a comparative lens on the various synthesis mechanisms, ranging from chemically buffered and photo-enhanced systems to statistically optimized microbial fermentations, as shown in Table 5. The hydrogen yields varied depending on the conversion pathway and the type of pretreatment. Despite these advancements, shared research gaps persist across all studies, including the need to scale processes, ensure cofactor stability, characterize microbial communities, and integrate pretreatment methods with downstream recovery systems.

Table 5
Classification of corn stalk.

Classification of Waste	Conversion Potential of Biohydrogen	Identified Research Gap	References
Untreated corn stalk (CS) with Na ₂ HPO ₄ , NaH ₂ PO ₄ buffering	Type: Dark fermentation. Conditions: 37 °C, pH 7, 48 h. Yield: 2.21 mol H ₂ /mol hexose (≈52.1 mL/g).	The optimal phosphate buffering concentrations and long-term stability remain uncertain. Need for microbial community profiling.	[30]
Corn stalk + Fe ²⁺ ions (0–200 mg/L)	Type: Photo-fermentation. Conditions: 32 °C, light 4000 lx, pH 6.8. Yield: Max 3.04 mol H ₂ /mol hexose at 100 mg/L Fe ²⁺ .	Photoinhibition at high iron levels; unclear synergy between metal ions and lignocellulosic hydrolysates	[31]
Cornstalk + <i>Clostridium butyricum</i>	Type: Dark fermentation. Conditions: 35.61 °C, pH 6.6, 50.87 g/L substrate, 10.25% inoculum. Yield: 40.62 mL/g dry substrate.	Requires scale-up validation, strain genetic stability, and enzyme cost reduction	[34]

Note: Yield units are as reported in the original studies. See the table for inter-conversion factors. STP = 0 °C, 1 atm.

3.1.5. Pineapple waste

Pineapple waste is a highly abundant by-product generated from both cultivation and processing industries. This waste can be categorized into several types: peel, core, stem, crown, leaves, pomace, and liquid waste (juice residues). Each type has a unique composition and physical characteristics that influence its suitability for biohydrogen production. These compositions or characteristics of pineapple waste require pretreatment for efficient biohydrogen conversion [35,36].

Several studies have explored various conversion strategies utilizing different parts of the pineapple plant, revealing both opportunities and limitations in terms of yield efficiency and system design, and in one study by Ahmad et al. [37], specifically targeted liquid pineapple waste (LPW) for dark fermentation using a mesophilic setup (35 °C, pH 6.5). They reported a hydrogen yield of approximately 1.58 mol H₂ per mol of glucose equivalent, indicating moderate performance for a sugar-rich substrate. The study used diluted LPW as a substrate. However, it did not specify the exact volume-to-substrate ratio, making it difficult to evaluate the influence of initial loading on yield fully. Moreover, variability in substrate composition and the lack of inhibitor profiling were identified as technical gaps requiring further exploration.

In a more complex system, Chu et al. [38] proposed a two-stage continuous fermentation process to produce biohythane, a combination of hydrogen and methane, from pineapple peel juice (PPJ). In the first thermophilic stage (55 °C, HRT 12 h), they achieved a yield of around 2.3 mol H₂/mol sugar, which is among the highest reported for pineapple waste. The initial sugar concentration from PPJ was tightly controlled, and a 3-liter reactor setup ensured consistent flow and microbial retention. However, the authors acknowledged that the energy demand for thermophilic operation and the absence of full-scale validation limit its current practical deployment.

Meanwhile, Kamilah et al. [39] employed a co-culture approach, using immobilized *Clostridium sporogenes* and *Enterobacter aerogenes* to ferment mixed pineapple residues (including peel, crown, and core) in a packed-bed bioreactor. At 37 °C and pH 6.8, the system produced approximately 1.8 mol H₂/mol of substrate, demonstrating the synergistic effect of the microbial consortia. The initial substrate load was consistently maintained across batches; however, the cost and sustainability of the immobilization materials were identified as challenges for industrial-scale applications. The study also lacked detailed microbial profiling to explain the observed fermentation synergy.

Across these studies, it is evident that pineapple waste exhibits diverse hydrogen conversion potential depending on the type of synthesis, ranging from dark fermentation and co-culture systems to multi-stage bioreactor configurations. Yields varied, with higher performance often linked to controlled pretreatment and optimized microbial conditions. However, gaps in substrate variability, inhibitor effects, microbial synergy characterization, and process scalability remain significant hurdles that must be addressed to unlock the full energy value of pineapple waste in a hydrogen economy. These areas require further study, as indicated in Table 6.

3.1.6. Palm oil mill effluent (POME)

Palm Oil Mill Effluent (POME) is classified as an industrial agrowaste, a high-strength liquid waste generated during the palm oil milling process. It is rich in organic materials, including carbohydrates, lipids, proteins, and minerals, which make it an excellent substrate for biohydrogen production. Unlike many other industrial wastes, POME contains a substantial amount of biodegradable material. Still, it requires proper management to prevent severe environmental pollution, given its high Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD) levels [40]. In its fresh form, POME contains significant amounts of cellulose (38.4%), hemicellulose (23.2%), and lignin (26.7%) [41]. These lignocellulosic materials are structurally complex and resist biodegradation unless subjected to appropriate pretreatment. The classification of POME has been studied for its potential in biohydrogen production, as tabulated in Table 7.

Table 6
Classification of pineapple waste.

Classification of Waste	Conversion Potential of Biohydrogen	Identified Research Gaps	References
Liquid Pineapple Waste (LPW)	Type: Dark Fermentation. Conditions: 35 °C, pH 6.5, batch fermentation. Yield: 1.58 mol H ₂ /mol glucose.	Substrate variability not addressed; inhibitory compounds not characterized.	[37]
Pineapple Peel Juice (PPJ)	Type: Two-Stage: Dark Fermentation → Methanogenesis (Biohythane focus). Conditions: Thermophilic (55 °C), 3 L reactor, HRT 12h. Yield: 2.3 mol H ₂ /mol sugar (first stage).	High energy demand in thermophilic systems; scale-up not validated	[38]
Mixed Residue (Peel, Core, Crown)	Type: Dark Fermentation. Conditions: pH 6.8, 37 °C, packed-bed reactor. Yield: 1.8 mol H ₂ /mol substrate.	Microbial synergy not fully characterized; immobilization cost not addressed	[39]

Note: Yield units are as reported in the original studies. See the table for inter-conversion factors. STP = 0 °C, 1 atm.

Table 7
Classification of POME.

Classification of Waste	Conversion Potential of Biohydrogen	Identified Research Gaps	References
Palm Oil Mill Effluent (POME)	Type: Thermophilic Dark Fermentation using mixed cultures (optimized enzymatic pretreatment). Conditions: 55 °C, pH 6.0, 24 h, enzymatic hydrolysis with cellulase/xylanase (via Box-Behnken design). Yield: 61.34 mL H ₂ /g VS.	Requires pilot-scale validation; economic feasibility of enzymes is not discussed; enzyme stability and reusability are not optimized.	[41]
Palm Oil Mill Effluent (POME)	Type: Thermophilic Dark Fermentation with Fe ²⁺ and carbon/nitrogen ratio optimization. Conditions: 55 °C, pH 6.0, C/N = 140, Fe ²⁺ = 100 mg/L. Yield: 1.88 mol H ₂ /mol sugar.	Lab-scale only; lacks techno-economic assessment; real-world variability of POME may affect reproducibility.	[42]
Palm Oil Mill Effluent (POME)	Type: Thermophilic Dark Fermentation with acid pretreatment (H ₃ PO ₄ , HNO ₃). Conditions: 60 °C, pH 5.8, 72 h, 0.8% phosphoric acid or 1% nitric acid. Yield: 1.24 mol H ₂ /mol glucose (97% higher than raw POME).	Pretreatment was tested using a one-factor-at-a-time approach; acid inhibition and treatment cost require further study.	[43]

Note: Yield units are as reported in the original studies. See the table for inter-conversion factors. STP = 0 °C, 1 atm.

In a study by Izzi et al. [41], a novel enzymatic pretreatment approach was developed using cellulase and xylanase, optimized via the Box-Behnken design. Under thermophilic conditions (55 °C, pH 6.0),

this method produced approximately 61.34 mL of hydrogen per gram of volatile solids (VS), indicating a significant improvement over untreated POME. Although this enzymatic hydrolysis enhanced substrate accessibility, the study was limited to laboratory conditions, and questions remain regarding enzyme cost, stability, and large-scale reusability.

In comparison, Abdullah et al. [42] investigated the effect of the carbon-to-nitrogen (C/N) ratio and ferric ion (Fe²⁺) supplementation under similar thermophilic fermentation conditions (55 °C, pH 6.0). By optimizing the C/N ratio to 140 and Fe²⁺ to 100 mg/L, they achieved a higher hydrogen yield of 1.88 mol H₂ per mol of sugar equivalent. This study suggests that nutrient composition and micronutrient balance are crucial for stimulating hydrogenase activity in microbial consortia. However, the study did not quantify the exact volume of POME used, and the results were obtained in controlled batch systems, highlighting the need for real-time testing with variable POME compositions.

In contrast, Mahmud et al. [43] employed acid pretreatment with 0.8% phosphoric acid and 1% nitric acid to break down the complex structure of POME. The study reported a biohydrogen yield of 1.24 mol H₂ per mol glucose, which marked a 97% improvement over untreated samples. Conducted at 60 °C and pH 5.8 over 72 h, this method proved effective for solubilizing fermentable sugars. Nonetheless, the researchers adopted a one-factor-at-a-time optimization approach. They did not address issues such as furfural formation (a known fermentation inhibitor) or the long-term economic feasibility of chemical pretreatment.

Across these studies, the synthesis type was consistently thermophilic dark fermentation, yet conversion rates varied due to differences in pretreatment methods, nutrient additives, and microbial activity enhancement. Although not all studies explicitly reported the volume of POME processed, those that optimized enzyme or chemical loading demonstrated that substrate concentration, expressed as volatile solids or sugar equivalents, strongly influenced hydrogen yield. The key gaps identified across all papers include scale-up feasibility, variability in POME characteristics, and the cost-effectiveness of pretreatment or nutrient supplementation, which remain critical to commercializing biohydrogen from this waste stream.

3.1.7. Comparative assessment of agricultural waste

The cross-analysis of agricultural waste feedstocks across Tables 2–7 reveals a performance hierarchy shaped by the interplay between substrate composition, pretreatment intensity, and fermentation strategy. Among the lignocellulosic residues, Wheat straw achieves the highest sugar-normalized yield (318.4 mL H₂/g-sugar) through hydrothermal-enzymatic dark fermentation [20], though multi-step processing generates furfural and HMF inhibitors. Sugarcane bagasse produces the highest volumetric output (6980 mL H₂/L) via dilute acid hydrolysis [22], with similar inhibitor constraints; enzymatic–nanomaterial approaches improve recyclability at a lower yield of 80.45 mL H₂/g-reducing sugar. Rice waste is limited by silica-rich structures that resist hydrolysis, with the best yield (129 mL H₂/g-COD) requiring combinative DTCD pretreatment [25]. Corn stalk introduces a distinct light-dependent pathway: Fe²⁺-enhanced photofermentation with *Rhodospseudomonas palustris* achieves the highest molar yield in this category (3.04 mol H₂/mol hexose) [31], although photoinhibition at elevated iron concentrations remains unresolved.

Pineapple waste and POME (Tables 6 and 7) occupy a strategically distinct position owing to high simple-sugar content, low lignin, and minimal pretreatment requirements. Thermophilic two-stage fermentation of pineapple peel juice reaches 2.3 mol H₂/mol sugar [44], the highest among sugar-rich pathways, while single-stage mesophilic dark fermentation yields a more moderate 1.58 mol H₂/mol glucose [37]. POME, a high-strength liquid effluent (COD 50,000–80,000 mg/L), can be fed directly into anaerobic systems at existing palm oil mills; C/N ratio optimization with Fe²⁺ supplementation achieves 1.88 mol H₂/mol sugar [42]. Both feedstocks bypass the costly pretreatment step that constrains lignocellulosic routes.

Four cross-cutting gaps persist across all six feedstocks: (i) high enzymatic pretreatment cost, (ii) incomplete pentose utilization, with C5 sugars from hemicellulose largely unconverted in most dark fermentation consortia, (iii) no long-term continuous-operation data beyond batch or short-cycle trials, and (iv) near-total absence of standardized life cycle assessment. Among these feedstocks, pineapple waste and POME emerge as the most deployment-ready candidates for tropical regions, given their natural fermentability, year-round availability in Southeast Asian agro-industrial settings, and minimal upstream processing costs.

3.2. Municipal solid waste

Municipal solid waste (MSW) is the waste generated from everyday activities in households, businesses, and construction sites, as illustrated in Fig. 5. Its management presents a significant global challenge due to rapid urbanization, population growth, and rising living standards. Over 2 billion tonnes of MSW are produced each year, projected to rise to 3.5 billion tonnes by 2050, posing serious risks to public health and the environment. Effective MSW management requires integrated strategies that focus on waste reduction, reuse, and recycling, alongside the use of advanced technologies and sustainable practices. Addressing these issues is crucial for achieving environmental sustainability and enhancing urban living conditions worldwide. Biohydrogen production from MSW has attracted considerable attention due to its dual role in managing organic refuse and generating clean energy.

3.2.1. Kitchen waste

Kitchen waste, often referred to as food or domestic organic waste, primarily consists of biodegradable materials, including carbohydrates, proteins, lipids, fibers, and minerals. According to Srivastava et al. [45], kitchen waste includes food scraps, vegetable peels, fruit residues, and leftover cooked food, all of which are rich in organic compounds suitable for microbial fermentation. The high abundance of starch, cellulose, and simple sugars in kitchen waste makes it particularly favorable for microbial digestion and biohydrogen production.

For instance, Marone et al. [46] focused on vegetable and kitchen waste, using native microflora via dark fermentation at mesophilic conditions (35–37 °C, pH 5.5–6.5). The study reported a hydrogen yield of up to 120 mL per gram of volatile solids (VS), highlighting the potential of untreated organic residues as both substrates and microbial sources. However, the fermentation was not scaled up and lacked further optimization of microbial diversity, limiting its reproducibility at a broader industrial scale.

In a similar approach, Kazmi et al. [47] evaluated the domestic food waste, including rice, noodles, meat, and vegetables, under thermophilic dark fermentation. By adjusting substrate ratios and maintaining

optimal conditions around 55 °C and pH 5.5–6.0, the hydrogen yield ranged from 108 to 160 mL per gram of volatile solids (VS), depending on the composition of the food waste. However, the inconsistency in raw waste input and the tendency of pH instability due to acid accumulation during fermentation presented technical limitations for continuous operation.

Expanding on single-stage fermentation methods, Dinesh et al. [48] explored a sequential fermentation strategy that couples dark fermentation with photofermentation to enhance overall hydrogen recovery. Using hydrolysates from pre-treated food waste, they achieved a cumulative yield of up to 4.5 mol H₂ per mol of glucose, one of the highest reported. This approach leverages the metabolic potential of phototrophic bacteria in the second stage but comes at the cost of higher energy demands. The study also notes the lack of robust continuous-flow models for system integration. When implementing sequential dark fermentation–photofermentation strategies, it is critical to distinguish between different phototrophic organisms and their conversion mechanisms. Bacterial photofermentation, particularly using purple non-sulfur bacteria such as *Rhodospseudomonas* species, directly converts volatile fatty acids (VFAs) from acidogenic dark fermentation into hydrogen under anaerobic light conditions [49]. These bacteria perform anoxygenic photosynthesis, capturing light energy without producing oxygen. In contrast, microalgae-based photofermentation systems employ oxygenic photosynthesis and biophotolysis pathways, where microalgal species accumulate biomass during the light phase and subsequently undergo dark fermentation for hydrogen production [32]. While both approaches leverage light-dependent mechanisms for hydrogen generation, the fundamental photosynthetic pathways, oxygen production profiles, and substrate conversion kinetics differ substantially. Understanding these distinctions is essential when designing sequential fermentation systems, as the choice between bacterial and microalgal photofermentation stages determines reactor configuration, oxygen management strategies, and overall system efficiency [33].

Meanwhile, Hassan et al. [50] utilized anaerobic single-stage fermentation of unsorted cooked food scraps, particularly high in fats and carbohydrates, under mesophilic conditions (37 °C, pH 6.8). The hydrogen yield ranged from 1.2 to 1.7 mol H₂ per mol of hexose equivalent, which is moderate compared to structured systems. However, the study highlighted the inhibitory effects of fatty acid accumulation, which suppresses hydrogenase activity, and emphasized the need for improved pH-buffering strategies.

Lastly, Srivastava et al. [45] tested an innovative nanomaterial-based enhancement method using Fe³⁺ and carbon dots to improve microbial performance during dark fermentation of kitchen waste. Operating at 35 °C and pH 6.0, the use of nanomaterials resulted in a 20–30% increase in hydrogen yield compared to non-treated controls. However, while effective in boosting output, this method raises concerns about



Fig. 5. Type of municipal solid waste (MSW).

environmental toxicity and the lifecycle implications of introducing nanoparticles into biological systems.

Across all studies, the conversion of biohydrogen from food waste warrants further investigation to identify the potential characteristics of the waste that could help close the research gap. Moreover, previous studies have identified factors, such as the amount of volatile solids or sugar equivalents present in the starting waste, as critical determinants of output efficiency. Despite the promising results, common research gaps remain from previous studies, particularly in process stability, inhibitor management, energy consumption, and scalability, which must be addressed to move food-waste-based biohydrogen production closer to commercial viability, as shown in Table 8.

3.2.2. Organic fraction of municipal solid waste (OFMSW)

The Organic Fraction of Municipal Solid Waste (OFMSW) constitutes a significant portion of municipal waste streams worldwide, typically comprising food waste, kitchen waste, garden waste, paper, and other biodegradable materials. It is primarily classified by its physical and chemical properties, with major components including carbohydrates (starch, cellulose, hemicellulose), proteins, lipids, lignin, metals, and inorganic contaminants [51]. This complex mixture presents both

Table 8
Classification of food waste.

Classification of Waste	Conversion Potential of Biohydrogen	Identified Research Gap	References
Vegetable and kitchen waste (mixed peels, residues, pulp)	Type: Dark fermentation using native microflora and microbial enrichment. Operating conditions: 35–37 °C, pH 5.5–6.5. Hydrogen yield: up to 120 mL per gram of volatile solids (VS).	Limited optimization of indigenous microbial consortia. No scale-up validation.	[46]
Domestic food waste (rice, noodles, meat, vegetables)	Type: Thermophilic dark fermentation. Operating conditions: pH 5.5–6.0 with a temperature of 55 °C. Hydrogen yield: 108–160 mL per gram of volatile solids (VS), depending on substrate ratio.	Variability of food waste composition pH instability during fermentation.	[47]
Pre-treated food waste hydrolysate	Type: Sequential fermentation (dark → photofermentation). Operating condition (DF): 35–37 °C, (PF): 28–30 °C with illumination. Hydrogen yield: up to 4.5 mol H ₂ /mol glucose.	High energy cost for the sequential system. Lack of robust continuous process models.	[48]
Unsorted cooked food scraps (high carb/fat)	Type: Anaerobic fermentation (single stage) Operated at 37 °C, pH 6.8. Hydrogen yield: 1.2–1.7 mol H ₂ /mol hexose.	Fatty acid accumulation inhibits hydrogenase. Needs improved buffering systems.	[50]
Kitchen waste (peels, grains, residual biomass)	Type: Dark fermentation + nanomaterial enhancement. Operated at 35 °C, pH 6.0; nanomaterials like Fe ³⁺ and carbon dots were added. Yield increase: up to 20–30% over control.	Environmental toxicity of nanomaterials Need lifecycle impact evaluation.	[45]

Note: Yield units are as reported in the original studies. See the table for inter-conversion factors. STP = 0 °C, 1 atm.

challenges and opportunities for bioconversion, where proper pretreatment is often essential to maximize hydrogen yields.

For instance, Campoy et al. [52] demonstrated a thermophilic dry acidogenic co-digestion process where OFMSW was mixed with food waste (FW) in various ratios (80:20, 70:30, and 50:50). Their findings revealed that the optimal ratio of 70:30 (OFMSW: FW) delivered the highest hydrogen yield of 42.9 L of H₂/kg VS added, with 53.9% hydrogen content in the produced biogas. This improvement was attributed to the synergistic substrate composition, which enhanced hydrolysis; however, the study also highlighted the risk of long-chain fatty acid (LCFA) inhibition and microbial imbalance when excessive food waste was added.

In another approach, Shah et al. [53] employed a batch dark fermentation method using *Bacillus* sp. strains as the inoculum. Operating under mesophilic conditions (35–37 °C) and at pH 5.5–6.0, their process yielded 60.5 mL of H₂/g VS from untreated OFMSW. While promising, the process still faced challenges related to microbial optimization and yield consistency due to the heterogeneous nature of municipal waste.

Taking a more integrated approach, Ebrahimian et al. [54] implemented a cocultivated biorefinery to co-produce hydrogen, butanol, and ethanol. Their system achieved a hydrogen yield of 103.5 mL per gram of volatile solids (VS) during the first stage, outperforming single-stage systems by valorizing multiple fermentation pathways. However, energy consumption and the complexity of separating multiple end-products remained unresolved issues.

In a more practical-scale study, Elsamadony et al. [51] tested a pilot-scale dry anaerobic digester operating under semi-continuous flow and with a total solids content of 25–30%. The system produced a maximum of 55 L of H₂/kg VS, proving that dry digestion is feasible on a larger scale. However, their research emphasized the need to better manage feedstock variability and contaminant interference in real-world municipal waste.

Finally, Rózsenszki et al. [55] explored anaerobic dark fermentation enhanced by hydrolysates derived from pre-composted OFMSW. Under mesophilic conditions (37 °C) and across different pretreatment strategies, the study reported a yield of 65 L H₂/kg VS, a notable increase attributed to enhanced enzymatic activity in the hydrolysate. Despite these improvements, the study acknowledged the necessity for lifecycle comparisons and economic assessments to validate this strategy over conventional chemical pretreatments.

Overall, the comparison across these studies reveals that while dark fermentation remains the central mechanism, innovations such as biological pretreatment, co-digestion, and multi-stage biorefinery systems significantly impact hydrogen yield, as shown in Table 9. The amount and condition of the initial waste, primarily the volatile solids (VS) content, and the proportion of food waste. They play a pivotal role in determining the final output. Nonetheless, future work must focus on enhancing process stability, managing microbial dynamics, and ensuring the scalability and economic feasibility of these systems for real-world deployment.

3.2.3. Textile waste

Research on textile waste for biohydrogen production has revealed a range of innovative approaches, primarily centered on dark fermentation and anaerobic digestion, with variations in pretreatment and operational strategies. Słowski et al. [57] investigated the utilization of cotton textile waste through dark fermentation under both anaerobic and microaerobic conditions. Interestingly, they found that the microaerobic setup, which used only 2% oxygen, significantly enhanced hydrogen yield, achieving 63.5 mL H₂/g volatile solids (VS) compared to 38.4 mL H₂/g VS under strict anaerobic conditions. This study highlights how introducing a minimal oxygen concentration can promote the metabolic activity of hydrogen-producing bacteria without entirely shifting to aerobic pathways.

In contrast, Binczarski et al. [58] studied a mixed textile waste

Table 9
Classification of organic municipal solid food waste (OMFSW).

Classification of Waste	Conversion Potential of Biohydrogen	Identified Research Gap	References
OFMSW mixed with food waste (FW)	Synthesis: Dark fermentation (DF) under thermophilic dry acidogenic co-digestion2. Operating conditions: 55 °C, 20% total solids, various FW/OFMSW ratios (80:20, 70:30, 50:50) 3. H ₂ yield: 42.9 L of H ₂ /kg VS added at 70:30 ratio, H ₂ content in biogas: 53.9%	• Long-chain fatty acid (LCFA) inhibition from FW • Balance between hydrolysis rate and substrate degradation • Microbial inhibition and process instability • Scaling up and kinetics of pre-composting impact	[52]
OFMSW with Bacillus strains	Synthesis: Batch fermentative DF using Bacillus sp.2. Conditions: OFMSW pH 5.5–6.0, mesophilic range (35 °C–37 °C), 3-day retention. Yield: 60.5 mL H ₂ /g VS.	• Microbial community optimization • Controlling inhibitors and metabolic pathway shifts • Consistent hydrogen yield across waste heterogeneity	[53]
OFMSW in co-cultivation biorefinery	Synthesis: Two-stage biorefinery for H ₂ , butanol, and ethanol2. H ₂ yield: 103.5 mL per gram of volatile solids (VS) in stage 1	• Streamlining multiple pathways • Energy balance optimization • Co-product separation efficiency	[56]
OFMSW through pilot dry anaerobic digestion	Synthesis: Pilot-scale dry DF reactor. Solids content: 25–30%, semi-continuous mode. Max yield: 55 L of H ₂ /kg VS	• Optimization of semi-continuous flow • Effect of feedstock variability • Real-world contaminant effects	[51]
OFMSW + anaerobic pre-composting (enhanced hydrolysis)	Synthesis: Pre-composted DF at mesophilic temp (37 °C). OFMSW mixed with the hydrolysate of pre-compost. Yield: 65 L of H ₂ /kg VS.	• Comparative analysis of pre-compost vs thermal/chemical pretreatment • Stability of microbial inoculum • Economic scaling	[55]

Note: Yield units are as reported in the original studies. See the table for inter-conversion factors. STP = 0 °C, 1 atm.

composed of cotton and polyester, subjecting it to sulfuric acid hydrolysis before anaerobic digestion. While this study primarily focused on biogas (methane) production, the process also generated volatile fatty acids (VFAs), which are known precursors for biohydrogen under the right conditions. However, they did not directly quantify hydrogen output, leaving a gap in evaluating the actual conversion potential. Moreover, the heterogeneity of textile blends and the presence of dyes introduce inhibitors that need further study for scalable hydrogen recovery.

Zhuang et al. [59] advanced the acid-thermal pretreatment of cotton textile residues to enhance their fermentability. They achieved a respectable hydrogen yield of approximately 92.6 mL/g chemical oxygen demand (COD) added during the dark fermentation process. The effectiveness of this method suggests that severe pretreatment enhances cellulose accessibility, although it may also generate fermentation inhibitors, such as furfural, necessitating downstream detoxification strategies to maintain conversion efficiency.

Across all studies, one clear trend emerges: pretreatment plays a pivotal role in determining conversion rates, and the type and amount of waste, particularly its cellulose content and inhibitory compounds, significantly influence hydrogen yields as tabulated in Table 10. While enzymatic and acid pretreatments enhance fermentability, their costs, scalability, and by-product management remain key technical gaps that require further research.

3.2.4. Comparative assessment of municipal solid waste

Tables 8–10 reveal a fundamental trade-off between substrate homogeneity and feedstock volume across the organic fractions of MSW. Kitchen and food waste, the most compositionally uniform fraction, achieves the highest yields. Sequential dark–photofermentation reaches 4.5 mol H₂/mol glucose [48], substantially exceeding single-stage yields of 108–160 mL H₂/g-VS under thermophilic conditions [47] and 120 mL H₂/g-VS under mesophilic conditions [46]. Two-stage configurations can thus more than double hydrogen recovery from high-sugar substrates, though the additional photobioreactor infrastructure limits near-term scalability.

OFMSW presents the inverse profile: enormous feedstock volume (50–70% of total MSW in developing countries) paired with compositional heterogeneity. Cocultivated biorefineries achieve the highest per-gram yield (103.5 mL H₂/g-VS), co-producing hydrogen, butanol, and ethanol [56]. However, pilot-scale dry anaerobic digestion (42.9–60.5 L H₂/kg-VS) [51,52] demonstrates greater throughput at more moderate yields, highlighting the yield–scalability trade-off central to MSW-based deployment. Long-chain fatty acid (LCFA) inhibition at high food waste ratios [52]. Moreover, batch-to-batch variability from mixed organic

Table 10
Classification of textile waste.

Classification of Waste	Conversion Potential of Biohydrogen	Identified Research Gap	References
Cotton textile waste (cotton fabric strips, cellulose-rich)	Synthesis: Dark fermentation under anaerobic and microaerobic conditions. Operating conditions: Anaerobic: 37 °C, pH 6.0; Micro-aerobic: 2% O ₂ . Yield: Anaerobic (38.4 mL per gram of volatile solids (VS)); Micro-aerobic (63.5 mL per gram of volatile solids (VS)).	Optimizing oxygen levels in micro-aerobic systems is necessary to increase H ₂ yield, and this can be achieved through integration with energy recovery systems.	[57]
Mixed textile waste (cotton and polyester) hydrolyzed with sulfuric acid	Synthesis: Anaerobic digestion (acid hydrolysis pretreatment). Operating conditions: 5% H ₂ SO ₄ , 121 °C, 1h. Yield: Not directly H ₂ (focus on methane, but volatile acids as precursors for H ₂ observed).	Lacks direct biohydrogen yield validation; insufficient data on scalability and fermentation inhibition from textile dye residues.	[58]
Cotton textile residues subjected to acid-thermal pretreatment	Synthesis: Combined dark fermentation and acid hydrolysis. Operating conditions: 121 °C, 0.1 MPa, pH 5.5–6.0. Yield: 92.6 mL H ₂ /g, COD added.	Pretreatment efficiency must be enhanced to reduce the formation of inhibitory by-products; scaling up challenges remain.	[59]

Note: Yield units are as reported in the original studies. See the table for inter-conversion factors. STP = 0 °C, 1 atm.

fractions remains a persistent challenge.

Three cross-cutting gaps emerge: (i) inhibitor management, whether from volatile fatty acid accumulation in food waste dark fermentation or LCFA formation in OFMSW co-digestion; (ii) the absence of long-term continuous-operation data, as all reviewed studies used batch or semi-continuous modes; and (iii) the lack of integrated techno-economic assessment accounting for collection logistics, sorting costs, and seasonal variation. These gaps are significant because MSW-based biohydrogen must compete on both production cost and waste diversion value, a dual-benefit proposition quantified in the techno-economic analysis.

3.3. Wastewater

Wastewater used for biohydrogen production can be broadly classified into several types based on its origin and physical-chemical characteristics, as illustrated in Fig. 6. These include: (a) domestic wastewater, (b) industrial wastewater (paper, biodiesel, agro-industrial, dairy, sugar, fruit processing, etc.), (c) agro-industrial wastewaters, (d) animal wastewater (e.g., swine wastewater), and (e) municipal sewage sludge. Each of these waste streams contains high concentrations of biodegradable organic matter (carbohydrates, proteins, fats), nutrients (nitrogen, phosphorus), and, in some cases, toxic or recalcitrant compounds (dyes, lignin, heavy metals, antibiotics) that influence their suitability for biohydrogen production.

3.3.1. Industrial wastewater

Industrial wastewater, a by-product of various industries such as textiles, distilleries, breweries, refineries, and pharmaceuticals, has emerged as a promising feedstock for biohydrogen production due to its high organic content and abundance. Across the reviewed literature, the classification of industrial wastewater for biohydrogen production has been explored, and the limitations or gaps in the research studies are identified in Table 11.

In the study by Gunasekaran et al. [60], textile dyeing effluent and sugar industry wastewater were subjected to dark fermentation using mixed microbial cultures. Operating at a pH range of 5.5–6.5 and mesophilic-to-thermophilic temperatures (35–55 °C), the hydrogen yield ranged between 0.8 and 1.5 mol H₂/mol glucose. The quantity of wastewater used was not explicitly scaled industrially. Still, it reflected lab-scale batch optimization, with conversion efficiency influenced by dye toxicity, which inhibited microbial activity at higher effluent volumes.

Prabakar et al. [61] expanded on this by examining wastewater from various industrial sources, including breweries, paper mills, and dairy plants, using dark fermentation and integrated anaerobic digestion.

Their study reported yields of up to 2.5 mol H₂/mol substrate under thermophilic conditions (45–60 °C) and varied retention times depending on the feedstock. The use of larger volumes of mixed industrial wastewater emphasized the importance of buffering agents and microbial adaptability, especially when scaling up from batch to semi-continuous systems.

Meanwhile, Banu et al. [62] explored photofermentation using sugar industry wastewater, operating at approximately 4000 lux and 30 °C. Their work yielded around 2.1 mol H₂/mol hexose, though it highlighted the challenge of maintaining stable light conditions and phototrophic bacterial growth when treating large volumes of diluted wastewater. The hydrogen production efficiency decreased when the substrate concentration was too low or when turbidity impeded light penetration.

In contrast, Moreno et al. [63] used anaerobic dark fermentation to produce biohydrogen from distillery wastewater. With acidic pretreatment and a stable mesophilic temperature of 37 °C, they achieved a relatively high yield of 3.2 mol H₂/mol glucose equivalent. However, this yield was sensitive to the wastewater's buffering capacity and nutrient balance; higher volumes often led to acid accumulation and microbial inhibition without supplementation.

A more advanced setup was employed by Qyyum et al. [64], who integrated dark and photofermentation using composite effluents from the refinery and pharmaceutical industries. Their staged bioreactor system operated at pH 6–7 and achieved yields of 1.7–2.4 mol H₂/mol COD. The system treated larger volumes of wastewater with greater efficiency but faced challenges such as membrane fouling and costly maintenance, especially when scaling from lab to pilot scale.

Lastly, Preethi et al. [65] provided a comprehensive review of various industrial wastewater types and synthesis strategies, including dark fermentation, photofermentation, and microbial electrolysis cells (MECs). They reported that hydrogen yields typically range from 0.5 to 3.5 mol H₂/mol substrate, depending on the process type, pH, and temperature. However, their analysis emphasized that many of these findings lack validation at the pilot or industrial scale, and a consistent techno-economic and lifecycle assessment remains necessary across studies.

3.3.2. Domestic wastewater

Domestic wastewater, primarily from residential sources, contains a mixture of biodegradable organic substances, including carbohydrates, proteins, lipids, and various micronutrients, making it a potential feedstock for biohydrogen production.

Prakash et al. [66] investigated the co-digestion of domestic wastewater with biodiesel industry effluent, using anaerobic fermentation with a mixed inoculum. The process was carried out under mesophilic

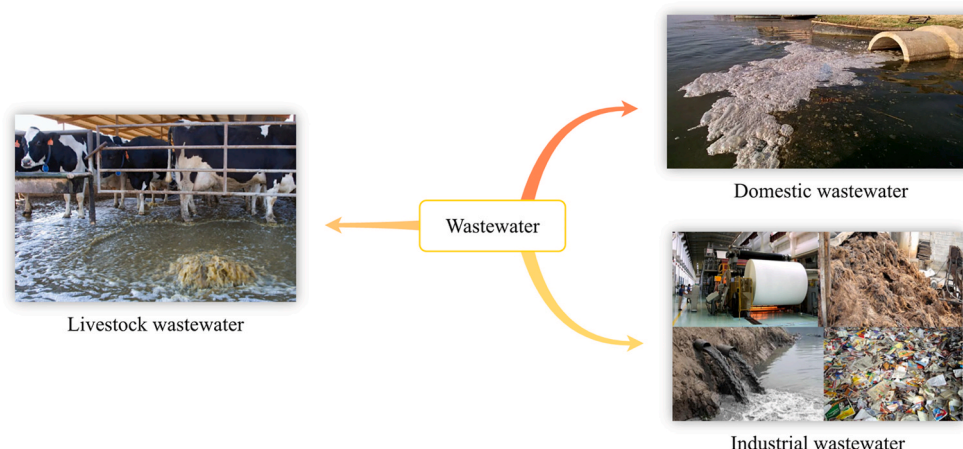


Fig. 6. Type of wastewater.

Table 11
Classification of industrial wastewater.

Classification of Waste	Conversion Potential of Biohydrogen	Identified Research Gap	References
Textile dyeing effluent and sugar industry wastewater	Type: Dark fermentation using mixed cultures. Operating Conditions: pH 5.5–6.5, 35–55 °C. Yield: 0.8–1.5 mol H ₂ /mol glucose (after pretreatment).	There is a need to optimize pH and mitigate the dye's toxicity.	[60]
Multiple industrial sources: brewery, paper mill, textile, dairy	Type: Dark fermentation, sometimes integrated with anaerobic digestion. Operating Conditions: Thermophilic (45–60 °C), variable retention time. Yield: 0.75–2.5 mol H ₂ /mol substrate.	The system lacks integration with energy recovery systems and is not scalable.	[61]
Sugar industry wastewater	Type: Photofermentation. Operating Conditions: Light intensity 4000 lux, pH 6.5, 30 °C. Yield: 2.1 mol H ₂ /mol hexose.	There is a need for stable light-driven reactor designs and addressing scale-up issues.	[62]
Distillery wastewater	Type: Anaerobic dark fermentation. Operating Conditions: Acidic pretreatment, 37 °C, pH 6.0. Yield: Up to 3.2 mol H ₂ /mol glucose equivalent.	Low wastewater buffering capacity and nutrient imbalance.	[63]
Composite effluents (refineries, pharmaceuticals, etc.)	Type: Combined dark-photofermentation with membrane filtration. Operating Conditions: Sequential staged process, pH 6–7. Yield: 1.7–2.4 mol H ₂ /mol COD.	High operating cost and membrane fouling risks.	[64]
General review of various industrial wastewater types	Type: Overview of dark fermentation, photofermentation, and microbial electrolysis cell (MEC) systems. Operating Conditions: pH 5–7, temperature range 30–60 °C, depending on method. Yield: Varies (range of hydrogen yield mostly shown 0.5–3.5 mol H ₂ /mol substrate).	Limited pilot-scale validation, insufficient techno-economic assessment.	[65]

Note: Yield units are as reported in the original studies. See the table for inter-conversion factors. STP = 0 °C, 1 atm.

conditions (36–37 °C) with a total solids (TS) concentration of 5%, pH 7.5, and agitation at 100 rpm. This combination resulted in a hydrogen yield of 20.14 mL H₂ per gram of volatile solids (VS), highlighting the role of co-digestion in improving the substrate's biodegradability. However, the hydrogen production remained moderate, suggesting that further optimization of the inoculum-to-substrate (I/S) ratio and retention time could significantly improve conversion efficiency.

In contrast, Cai et al. [67] explored thermophilic dark fermentation using raw sewage sludge and wastewater. Their synthesis approach involved thermal pretreatment of the sludge and operation at 55 °C with a neutral pH of 7.0. The hydrogen yield achieved was notably higher (52.1 mL per gram of volatile solids (VS)), demonstrating the effectiveness of thermophilic conditions in improving metabolic activity and substrate conversion. Importantly, this study indicates that elevated

temperatures and appropriate sludge pretreatment significantly enhance the breakdown of complex organics, thereby increasing hydrogen productivity, even with a relatively small initial mass of waste sludge.

Šabić Runjavec et al. [68] adopted a hybrid strategy combining dark fermentation and microbial electrolysis cell (MEC)-assisted fermentation using anaerobically digested sludge from a municipal wastewater treatment plant (WWTP). Operating under mesophilic conditions (35 °C) and slightly acidic pH (5.5–6.5), this approach yielded 30.4 mL of hydrogen per gram of chemical oxygen demand (COD). Although the yield was lower than that of the thermophilic process, integrating MEC could potentially recover more energy per unit of waste, which could be beneficial for treating large volumes of digested sludge. This approach also highlighted the importance of microbial adaptation and electrochemical enhancement in maximizing hydrogen recovery from stabilized substrates.

Across all studies, it is evident that the type of synthesis is either thermophilic dark fermentation, mesophilic anaerobic digestion, or MEC-assisted co-fermentation. It plays a critical role in determining hydrogen output. The amount and quality of initial waste used can be a factor affecting conversion potential, whether through pretreatment or nutrient balancing, and can be key drivers of higher yields. Nonetheless, gaps remain in optimizing reactor design, managing microbial communities, and scaling these findings to continuous or pilot-scale operations, as summarized in Table 12.

3.3.3. Livestock wastewater

Livestock farming, including swine, cattle, and poultry operations, generates a highly concentrated organic waste stream rich in biodegradable compounds that are suitable for biohydrogen production. This

Table 12
Classification of domestic wastewater.

Classification of Waste	Conversion Potential of Biohydrogen	Identified Research Gap	References
Domestic wastewater and biodiesel industry effluent (co-digestion)	Type of Synthesis: Anaerobic fermentation using mixed inoculum. Operating Conditions: 5% total solids (TS), 36–37 °C, initial pH 7.5, 100 rpm. Hydrogen Yield: 20.14 mL H ₂ /g VS.	Optimization of retention time and inoculum-to-substrate (I/S) ratio is still needed; the impact of nutrient ratios under co-digestion is underexplored.	[66]
Raw sewage sludge and wastewater	Type of Synthesis: Dark fermentation (thermophilic batch process). Operating Conditions: 55 °C, pH 7.0, HRT 24–72 h, pre-treated sludge. Hydrogen Yield: 52.1 mL per gram of volatile solids (VS).	Pretreatment optimization and long-term stability under continuous operation require further study.	[67]
Anaerobically digested sludge from WWTP	Type of Synthesis: Dark fermentation and MEC-assisted fermentation. Operating Conditions: pH 5.5–6.5, mesophilic 35 °C, HRT 24 h, nutrient supplementation. Hydrogen Yield: 30.4 mL/g COD.	Requires a pilot-scale demonstration; the impact of microbial community adaptation remains unclear.	[68]

Note: Yield units are as reported in the original studies. See the table for inter-conversion factors. STP = 0 °C, 1 atm.

type of wastewater typically contains significant amounts of proteins, carbohydrates, lipids, and ammonia and exhibits high organic loading, with Chemical Oxygen Demand (COD) values often exceeding 20,000 mg/L [69].

The reviewed studies on livestock wastewater report varied strategies and outcomes in efforts to optimize biohydrogen production, as tabulated in Table 13. Feng et al. [69] focused on synthetic swine wastewater and enhanced biohydrogen production by integrating fruit peel crude enzymes (FPCE) into a dark fermentation system. The mechanism enhanced the hydrolysis and acidogenesis phases, yielding a hydrogen production of 1.92 mol H₂ per mol of glucose under mesophilic conditions (35 °C, pH 6.5) with a hydraulic retention time (HRT) of 12 h. While the synthetic nature of the wastewater and the controlled enzyme concentration yielded promising results, the study did not explore how these outcomes would translate in a real-world wastewater setting, where waste composition fluctuates. The long-term impacts of enzymatic pretreatment remain uncertain.

In contrast, Xing et al. [70] investigated dairy manure under dark fermentation conditions. Using total solids (TS) concentrations ranging between 2% and 6%, with a retention time of 72 h at 37 °C and pH 6.5, the conversion process produced a lower hydrogen yield of 30 mL H₂ per gram of volatile solids (VS). This modest performance was attributed to the high solid content of dairy manure, which likely hindered microbial activity and mass transfer efficiency. The study emphasized the need for more effective pretreatment techniques and microbial adaptation strategies to enhance substrate availability and overall hydrogen productivity from manure-based systems.

Meanwhile, another study by Yokoyama et al. [71] also explored co-digestion to increase hydrogen production by mixing cow manure with food waste at a 1:1 ratio. This approach aimed to balance nutrient availability and improve fermentability. Conducted under conditions of pH 6.0 and 37 °C, the co-digestion strategy significantly increased hydrogen yield to 51.2 mL H₂/g VS, nearly doubling the output compared to the mono-digestion of manure. The improved yield highlights the synergistic effect of combining protein-rich manure with easily degradable food residues. However, further research is required to fine-tune the co-substrate ratios and develop microbial consortia better

Table 13
Classification of livestock wastewater.

Classification of Waste	Conversion Potential of Biohydrogen	Identified Research Gap	References
Swine wastewater (synthetic) with fruit peel crude enzyme (FPCE)	Type: Dark fermentation. Operating conditions: pH 6.5, 35 °C, HRT 12 h. Yield: 1.92 mol H ₂ /mol glucose.	There is a lack of clarity regarding the long-term effects of enzyme dosage and the variability of real wastewater.	[69]
Dairy manure	Type: Dark fermentation. Operating conditions: pH 6.5, 37 °C, TS 2%–6%, HRT 72 h. Yield: 30 mL H ₂ /g VS.	Low hydrogen yield due to high solid content; requires improved pretreatment and microbial acclimation.	[70]
Cow manure co-digested with food waste	Type: Dark fermentation. Operating conditions: pH 6.0, 37 °C, cow manure: food waste (Ratio 1:1). Yield: 51.2 mL H ₂ /g VS.	A synergistic effect was observed; however, more optimized feedstock ratios and microbial community engineering are still required.	[71]

Note: Yield units are as reported in the original studies. See the table for inter-conversion factors. STP = 0 °C, 1 atm.

adapted to heterogeneous feedstock combinations.

In summary, while dark fermentation remains the predominant synthesis pathway across all studies, the hydrogen yield varied substantially depending on both the type and treatment of livestock waste. Higher yields were generally associated with lower solid concentrations or co-digestion strategies, pointing to the importance of waste composition and pretreatment in optimizing conversion efficiency. However, further research is necessary to optimize operational parameters, pretreatment methods, and microbial consortia to maximize hydrogen yields and ensure the economic viability of full-scale livestock wastewater-to-biohydrogen systems.

3.3.4. Comparative assessments of wastewater

Tables 11–13 demonstrate that wastewater-based biohydrogen spans the widest performance range among the three waste categories, driven by extreme diversity in effluent composition and inhibitory compounds. Industrial wastewater (Table 11) offers the richest organic substrate base: sugar and distillery effluents achieve the highest yields through conventional dark fermentation, owing to naturally high biodegradable sugar content [62,63] while paper industry wastewater achieves competitive yields through MEC-assisted configurations [61]. Notably, none of the industrial wastewater studies reports EROI, preventing meaningful economic comparison with the agricultural and MSW feedstocks assessed above.

Domestic wastewater (Table 12) has lower organic loading than industrial streams, making standalone dark fermentation less productive. MEC-assisted co-fermentation compensates by achieving the highest hydrogen recovery per unit COD removed [68], though the external voltage requirement (0.2–0.8 V) elevates operating costs above \$20/kg H₂ in the LCOH analysis (Table 14). Co-digestion with higher-strength industrial effluents, such as biodiesel wastewater [66], offers a strategy to boost organic loading without additional electrode infrastructure.

Livestock wastewater (Table 13) occupies a middle ground: FPCE-enhanced dark fermentation of swine wastewater yields 1.92 mol H₂/mol glucose, the highest in the wastewater category [69], though the cost of sourcing fruit peel crude enzymes at scale remains unresolved. In textile wastewater studies, microaerobic dark fermentation enhances yield to 63.5 mL H₂/g-VS versus 38.4 mL H₂/g-VS under strict anaerobic conditions [59], while acid-thermal pretreatment of cotton residues achieves the category's highest yield at 92.6 mL H₂/g-COD [58]. Dye-derived inhibitors and synthetic fiber blends introduce toxicity absent from organic food-based streams.

Four cross-cutting gaps span all wastewater subcategories: (i) absent EROI quantification, which is available for agricultural feedstocks but unreported for any wastewater study; (ii) no continuous-flow operation data, with all studies limited to batch or semi-continuous modes; (iii) uncharacterized inhibitor profiles, from dye-derived furfural in textile waste to antibiotic residues in livestock effluents; and (iv) the absence of techno-economic assessment crediting wastewater treatment cost offsets, a critical omission, since these systems can offset costs through avoided disposal fees (Section 5.3). Among wastewater feedstocks, industrial effluents with high sugar content offer the most immediate deployment potential. In contrast, MEC-assisted domestic wastewater treatment represents the highest long-term efficiency ceiling pending electrode cost reduction.

4. Synthesis pathways and mechanisms

Biohydrogen production employs various biological and electrochemical pathways, each leveraging specific microbial advantages and processes as illustrated in Fig. 7. In dark fermentation, hydrogen is produced anaerobically from carbohydrates by acidogenic and acetogenic bacteria. The hydrogen yield is influenced by the predominance of acetate- and butyrate-utilizing pathways, as well as by microbial competition [73]. In microbial electrolysis cells (MECs), it enhances this process by employing electrogenic bacteria to oxidize organic materials.

Table 14
Estimated levelized cost of hydrogen (LCOH) across biohydrogen pathways.

Pathway	Representative Feedstock	Pretreatment Cost (\$/ton feed)	Reactor CAPEX (\$/kg H ₂ capacity)	OPEX (\$/kg H ₂)	Estimated LCOH (\$/kg H ₂)	Key Cost Driver	References
Dark fermentation (lignocellulosic)	Wheat straw, SCB, rice straw	15–40	8–15	3.5–8.0	4.80–12.50	Pretreatment chemicals + enzyme cost; high biomass logistics cost	[6,7,22,72]
Dark fermentation (sugar-rich waste)	Pineapple waste, kitchen waste, food waste	0–5 (minimal)	6–12	2.0–5.0	2.50–6.80	Reactor + inoculum; near-zero feedstock cost; no chemical pretreatment needed	[37,45,46,48]
Two-stage DF–PF (sequential)	Food waste, agri-residue, corn stalk	10–25	15–30	5.0–12.0	8.20–22.40	Photobioreactor + light energy input; complex process control	[3,31,73,74]
MEC (electrochemical)	Industrial WW, rice mill WW, domestic WW	5–15	20–50	8.0–25.0	12.00–48.96	Electrode + membrane replacement (30–50% of OPEX); scaling losses	[75–78]
MFC (bioelectrochemical)	Fruit waste, POME, oily kitchen waste	0–5	10–25	4.0–15.0	6.20–35.00	Electrode material + scaling; low power density; high internal resistance	[79–82]
DF + MEC hybrid (integrated)	POME, food waste, agri- residues	5–15	15–35	6.0–18.0	8.50–30.00	MEC capital cost dominates; electrode replacement + applied voltage	[76,83,84]
DF + ABE co- production (biorefinery)	OFMSW, municipal waste	5–15	12–25	4.0–10.0	5.60–18.00	Multi-product revenue offsets cost; ABE downstream separation complexity	[54,85,86]

Notes: LCOH estimates compiled from published TEA studies and analogous system analyses cited in this review. Ranges reflect differences in scale (lab vs pilot), geography, and process optimization. The upper range of \$48.96/kg for MEC reflects early-stage, lab-scale systems [77]. This figure decreases substantially at higher current densities and optimized electrode configurations [75].

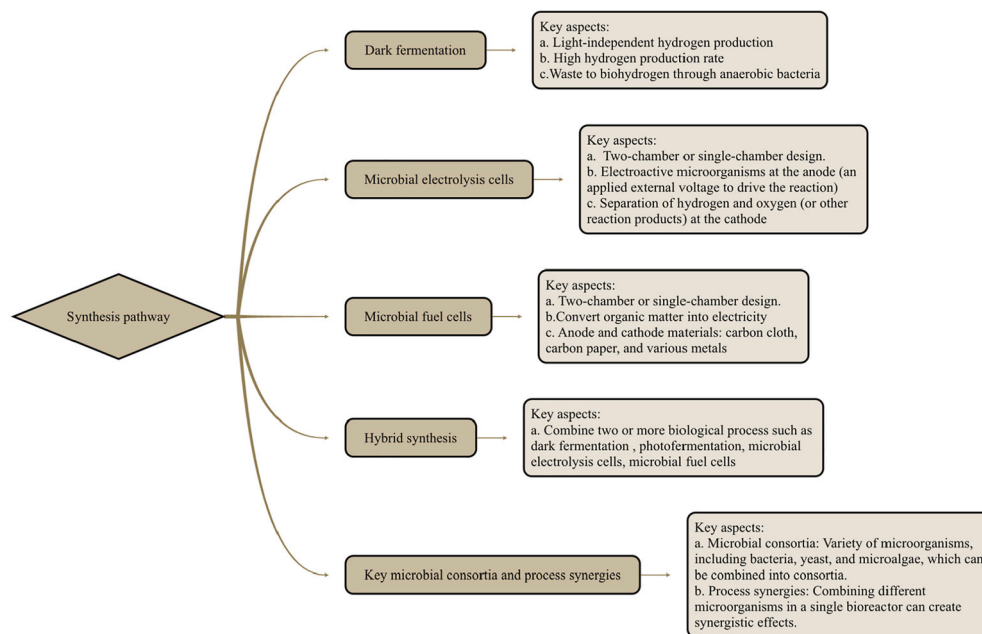


Fig. 7. Synthesis pathway and mechanism in biohydrogen production.

By applying a small external voltage, these bacteria can reduce protons to hydrogen at the cathode, effectively recovering energy from volatile fatty acids, such as acetate [75]. While microbial fuel cells (MFCs) are mainly designed for electricity generation, they can also produce hydrogen under specific anodic and cathodic conditions. This dual functionality supports simultaneous waste treatment and energy recovery [87]. Emerging hybrid systems and co-fermentation strategies combine dark fermentation with MECs or photofermentation and may involve multiple substrates. These approaches aim to enhance hydrogen yield through metabolic synergy and improved nutrient balance [76, 88]. Central to these processes are microbial consortia, which operate through cooperative interactions, such as cross-feeding and syntrophic metabolism. These interactions contribute to the stability, resilience,

and productivity of the systems, particularly when supported by appropriate pretreatments and environmental controls [89,90].

4.1. Dark fermentation

Unlike light-dependent processes, dark fermentation operates under anaerobic and light-independent conditions, using bacteria such as *Clostridium* and *Enterobacter* to convert carbohydrate-rich biomass into hydrogen. The core of the process lies in the microbial breakdown of glucose through glycolysis to produce pyruvate, which is then further metabolized into hydrogen gas and valuable byproducts, such as acetic and butyric acids. This transformation is driven by key enzymatic activities, most notably hydrogenase and pyruvate: ferredoxin

oxidoreductase (PFOR), which facilitate the release of electrons and protons to form molecular hydrogen [85]. Although various organic substances, including carbohydrates, sugars, proteins, and lipids, can, in principle, serve as substrates for bioH₂ production, the metabolic pathway is commonly demonstrated using glucose as a model substrate (Fig. 8) [91].

The main metabolic pathway involves the glycolytic breakdown of glucose to pyruvate, followed by its conversion into hydrogen, acetic acid, and butyric acid, as shown in **Reaction 1**:

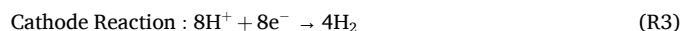
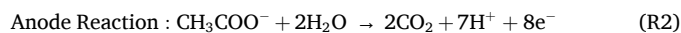


Based on a comprehensive analysis of studies on biohydrogen production using dark fermentation, the key lies in integrating metabolic engineering, system optimization, and co-fermentation strategies to improve hydrogen yields using diverse biowaste substrates, such as lignocellulosic residues, agricultural wastes, and food waste [92].

4.2. Microbial electrolysis cells (MEC)

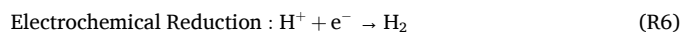
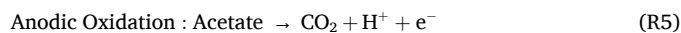
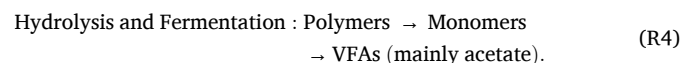
Unlike conventional electrolysis, MECs require only a small external voltage of approximately 0.2–0.8 V, since part of the energy needed is generated by microbial metabolism, as illustrated in Fig. 9. This system makes MECs more energy-efficient than traditional water electrolysis, which typically requires more than 1.2 [93]. MECs usually consist of an anode, a cathode, and, in some cases, a membrane separator. The microorganisms present on the anode serve as biocatalysts, oxidizing organic waste and transferring electrons either directly or via mediators, such as cytochromes and conductive pili. The selection of microbes, substrates, electrode materials, and the reactor configuration significantly influences the performance of MECs [94].

Recent studies have shown that wastewater from the sugar industry is rich in readily biodegradable sugars and volatile fatty acids, making it an ideal candidate for microbial electrochemical cells (MECs). In the study by Florio et al. [79], the organic matter in sugar wastewater was oxidized at the anode by electrogenic bacteria, such as *Geobacter* spp. This process produced electrons and protons. The electrons traveled through an external circuit to the cathode. At the same time, the protons moved through the electrolyte with a small applied voltage of approximately 0.6 V. The protons and electrons then recombined at the cathode to form hydrogen. The overall anode and cathode reactions can be expressed as shown in **Reactions 2 and 3**:



The study enhanced this process using a TiO₂/graphene nanocomposite catalyst, which improved electron transfer efficiency and boosted hydrogen yield by over 45% compared to conventional electrodes.

In a recent study, Florio et al. [79] examined microbial electrolysis cells (MECs) using wastewater from paper mills. This wastewater contains byproducts from lignocellulose degradation, including xylose, glucose, and organic acids. Microbial consortia fermented these compounds to produce acetate and hydrogen, precursors. The study found that nickel-based nanocatalysts, such as Ni-Co-P, applied to the cathode surface significantly reduced the overpotential needed for hydrogen evolution. The reaction mechanism followed a consistent pattern, as shown in **Reactions 4 to 6**:



This study demonstrated that the Ni-Co-P catalyst enhanced catalytic activity, achieving a hydrogen yield of 3.4 m³ H₂/m³/day under optimal conditions.

4.3. Microbial fuel cells (MFC)

Microbial fuel cells (MFCs), as illustrated in Fig. 10, are bio-electrochemical systems that generate electricity by harnessing the metabolic activity of microorganisms to break down organic materials, often derived from waste. These cells convert chemical energy from substances such as wastewater, agricultural residues, or glucose into electrical energy via microbial metabolism. Unlike traditional fuel cells, MFCs use exoelectrogens, living bacteria that thrive in anaerobic conditions. As these microbes consume organic matter, they release electrons and protons. Electrons travel to the anode surface, generating an electric current as they move through an external circuit to the cathode. Protons pass through a proton exchange membrane, where they combine with oxygen and electrons to form water. This process not only

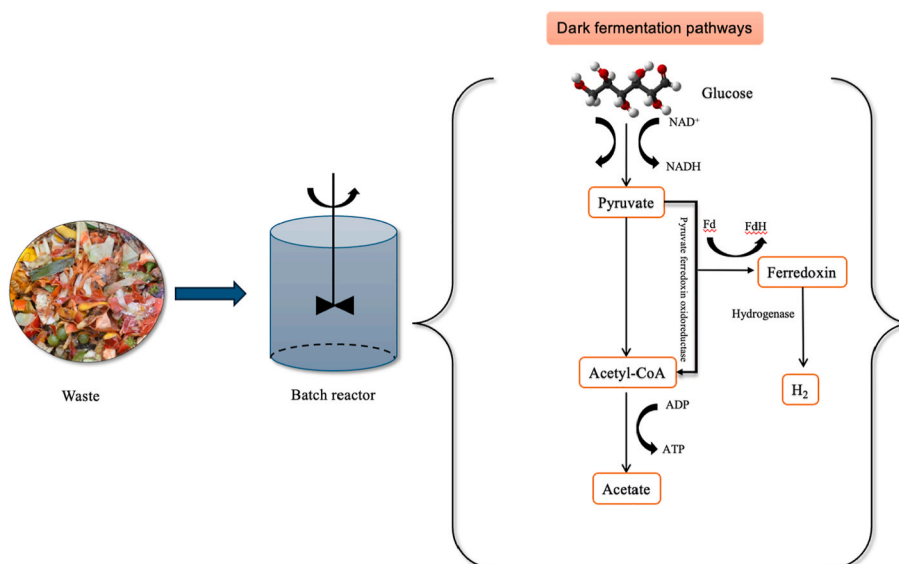


Fig. 8. Diagram of dark fermentation metabolic pathways.

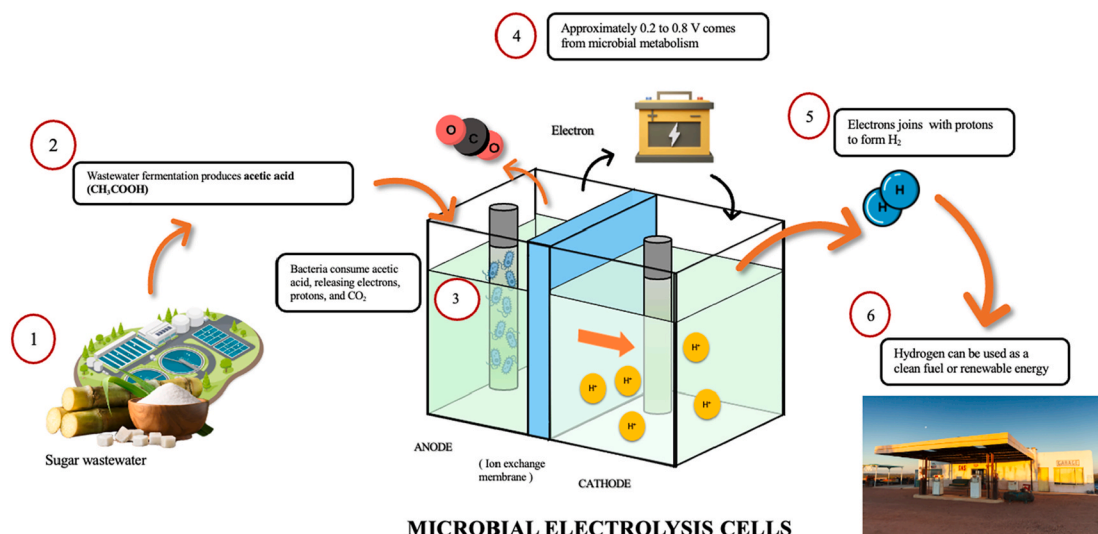
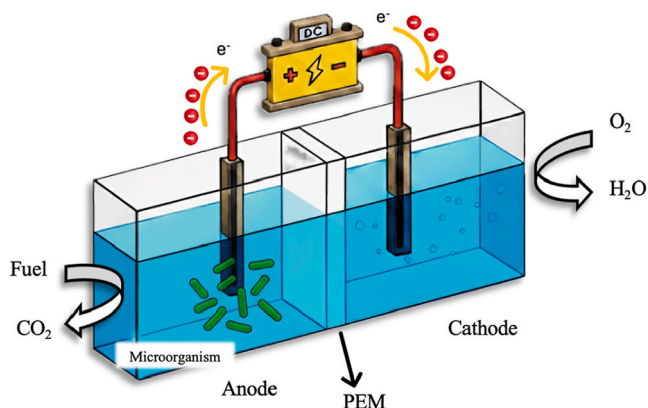


Fig. 9. Microbial electrolysis cell pathways.



produces sustainable energy but also treats wastewater. Operating efficiently under mild conditions (ambient temperature, neutral pH, and without expensive catalysts). The microbes' ability to form electroactive biofilms on electrodes enables the anode to function as a living power generator, bridging biology and electrochemistry to create cleaner energy technologies and promote enhanced environmental sustainability.

In the study by Florio et al. [79], municipal solid waste was used in a single-chamber air-cathode microbial fuel cell (MFC). Microorganisms within the system decompose the organic matter, releasing electrons and protons in the process. The electrons then travel through an external circuit to the cathode, where they react with oxygen to produce water. The remaining substrate, which is rich in microbial metabolites, was subsequently used for anaerobic digestion. This study resulted in a significant enhancement in biohydrogen production, increasing it by 276% compared to untreated waste. This study demonstrates a sequential mechanism: first, electricity is generated in the MFC, followed by hydrogen production from the MFC's spent substrate.

Kafaei et al. [80] demonstrated the use of oily food waste in a high-solid-content microbial fuel cell (MFC). In this process, organic matter is hydrolyzed and fermented anaerobically by microbes such as *Clostridia*, producing volatile fatty acids (VFAs) and hydrogen. Electrons generated during fermentation are transferred by electroactive bacteria, including those from the *Geobacteraceae* and *Clostridiaceae* families, to the anode, where they generate electricity. Notably, a 6% oil

concentration enhanced hydrogen production; however, higher oil concentrations suppressed electrical output, likely due to microbial inhibition or the accumulation of VFAs.

Kalagbor et al. [81] investigated the utilization of fruit waste, including banana, tomato, and pineapple, in microbial fuel cells (MFCs). These fruits, which are rich in sugars and starches, serve as excellent substrates for fermentative bacteria. The metabolic process begins with the hydrolysis of polysaccharides, which is followed by fermentation into acids and alcohols. These byproducts are then oxidized by electrogenic bacteria, producing electrons and hydrogen ions (protons). Electricity is generated when the electrons reach the cathode and react with oxygen, while the protons migrate through the medium. This study demonstrates a direct link between fermentation and electrogenesis in fruit-based MFCs.

Segundo et al. [95] investigated the use of pickled chili as an acidic substrate in a single-chamber microbial fuel cell (MFC). The microbial community adapted to the low pH conditions, effectively degrading organic acids and sugars. During this breakdown process, electrons were released and captured by the anode, then transferred to the cathode. Although acidic waste typically inhibits microbial activity, the adapted microbes still generated electricity, demonstrating a unique mechanism that enables them to thrive in harsh chemical environments.

According to Mohyudin et al. [96], microbial fuel cells (MFCs) used for treating textile wastewater operate via redox-based degradation. In this system, bacteria oxidize dye compounds, transferring electrons to the anode. These electrons are then used to reduce electron acceptors at the cathode, which contributes to both electricity production and pollutant removal. Specifically, azo dyes undergo cleavage through microbial reduction, a process that can be enhanced by improving the electrode material and promoting microbial adhesion.

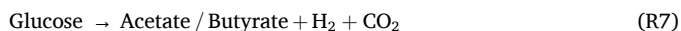
4.4. Hybrid and Co-fermentation systems

Hybrid fermentation systems are integrated bioprocesses that combine two or more biological stages, such as dark fermentation (DF), photofermentation (PF), and microbial electrolysis cells (MECs). The aim is to maximize hydrogen yield and energy recovery from organic waste. The key principle behind these systems is synergy; each step addresses the limitations of the previous one. For instance, DF breaks down complex waste into volatile fatty acids (VFAs) and produces some hydrogen. PF or MECs can then use these VFAs to generate additional hydrogen, while anaerobic digestion (AD) can convert the remaining

solids into methane, thereby improving overall energy recovery [74,76,83].

In contrast, co-fermentation systems involve the simultaneous fermentation of two or more substrates, typically with complementary characteristics. The goal here is to enhance substrate biodegradability, balance the carbon-to-nitrogen ratio, and promote microbial diversity and activity. This approach results in increased hydrogen production compared to using a single substrate alone [88].

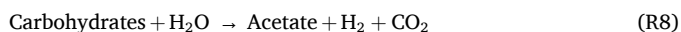
In a hybrid system that utilizes agro-industrial wastewater, such as that from cheese, fruit juice, and paper industries, dark fermentation (DF) is employed to break down organic matter into hydrogen and volatile fatty acids (VFAs). This DF process primarily follows the acetate-butyrate fermentation pathway, resulting in the production of hydrogen through microbial metabolism, as shown in **Reaction 7**:



The remaining VFAs are then introduced into a Microbial Electrolysis Cell (MEC). In the MEC, exoelectrogenic bacteria oxidize the VFAs at the anode, generating electrons that are transferred to the cathode. This process reduces water into hydrogen by applying a small voltage. As a result, this combined approach can increase hydrogen yields by up to 13 times compared to DF alone [84].

This method focuses on complex and unsterilized agro-waste, where direct fermentation (DF) serves as a pre-treatment to produce short-chain fatty acids (SCFAs). The primary microbial process involves the anaerobic oxidation of glucose and other polysaccharides, yielding acetate and butyrate. In the second stage, microbial electrolysis cells (MECs) utilize these SCFAs. Microorganisms such as *Geobacter* sp. and *Shewanella* sp. transfer electrons to an electrode via respiratory mechanisms, facilitating secondary hydrogen evolution at the cathode [97].

In this co-fermentation setup, buffalo slurry (BS) provides a stable pH environment and essential micronutrients, while cheese whey (CW) and crude glycerol (CG) supply readily available sugars and carbon. The combined metabolic interactions accelerate fermentation initiation, resulting in a shorter lag phase and higher hydrogen yields. The typical reaction can be represented as shown in **Reaction 8**:



This mixture effectively balances nutrient levels and pH, thereby improving hydrogen production efficiency. The peak yield recorded was 111.6 mL H₂/g of volatile solids added [98].

Šabić Runjavec et al. [68] investigated the potential of combining sewage sludge with industrial effluents to enhance hydrogen production, capitalizing on the complementary biodegradability of these substrates. The process involves fermentative bacteria that break down proteins, carbohydrates, and fats via acidogenesis, producing volatile fatty acids (VFAs). These VFAs are then converted into hydrogen and carbon dioxide (CO₂) by hydrogenase enzymes. Co-fermentation in this context improves chemical oxygen demand (COD) removal and facilitates nutrient recovery.

4.5. Key microbial consortia and process synergies

Key microbial consortia are groups of interacting microorganisms that collaborate to perform complex biochemical processes more efficiently than single-species cultures. These consortia capitalize on positive interactions, such as mutualism and commensalism, where different species mutually support each other's growth and metabolic activities. This collaboration leads to improved stability and productivity in bioprocesses, including biofuel production, waste treatment, and the synthesis of valuable chemicals [89,99,100].

Process synergies emerge from the complementary metabolic capabilities of different microbes, enabling the division of labor, cross-feeding of metabolites, and enhanced tolerance to environmental stresses [90,101]. Strategies such as substrate separation, byproduct elimination, and the use of quorum-sensing circuits help regulate

population dynamics, preventing the dominance or collapse of specific strains. Interkingdom consortia involving bacteria, fungi, and microalgae further enhance robustness and productivity by leveraging unique metabolic pathways and stress responses [3,90]. Interkingdom consortia can be divided into distinct biological systems with different mechanisms. Bacterial consortia, particularly photosynthetic purple non-sulfur bacteria such as *Rhodospseudomonas* species, perform photo-fermentation directly under light conditions, converting volatile fatty acids into hydrogen through anoxygenic photosynthesis [102]. In contrast, microalgae-based systems leverage oxygenic photosynthesis and biophotolysis pathways, where microalgal species accumulate biomass and subsequently undergo dark fermentation or photo-fermentation for hydrogen production [103]. While both approaches utilize light-dependent mechanisms, the photosynthetic pathways, oxygen production, and substrate conversion routes differ fundamentally.

Recent research into the synthesis pathways and mechanisms for biohydrogen production highlights the essential role of specific microbial consortia and their interactions. These combinations are tailored to the type of waste being processed: agricultural biomass, municipal waste, or industrial wastewater. It determines the efficiency of hydrogen extraction through various biological processes. For example, a multi-stage bioprocess using cassava, corn, and domestic wastewater. This integrated system combined dark fermentation, photofermentation, and microbial electrolysis cells (MECs), using a mix of fermentative and electrogenic bacteria [104]. In this collaboration, *Clostridium* species performed dark fermentation to produce VFAs and hydrogen, which were then utilized in photofermentation by *Rhodospseudomonas* under light conditions. Meanwhile, MECs recovered additional hydrogen through electron flow facilitated by *Geobacter*-like exoelectrogens. This coordination of microbial functions maximized hydrogen recovery from mixed waste streams.

Similarly, recent studies on food waste, such as fruit and vegetable scraps, employ a two-stage process. The first stage involved thermophilic dark fermentation at 45 °C, using microbial consortia rich in *Thermoanaerobacterium* to generate hydrogen and organic acids [50]. In the second stage, the remaining substrates underwent methanogenesis under anaerobic conditions. This sequential treatment not only boosted total hydrogen yield but also significantly reduced waste volume, demonstrating how temperature-optimized microbial pairing can enhance system performance. In the context of industrial wastewater, Ramu et al. [105] reported biohydrogen production from food and sugar industry effluents using dark fermentation with isolated strains of *Bacillus cereus* and *Clostridium perfringens*. These bacteria effectively converted simple sugars and organic acids under anaerobic digestion, illustrating how indigenous microbes can adapt to specific wastewater profiles and drive localized hydrogen production.

Another example involves paper industry wastewater (PIW) applied using microbial electrolysis cells with nickel and nickel-cobalt electrodes. The MECs were inoculated with mixed microbial communities enriched in *Pseudomonas* and *Acinetobacter* strains. These electrogenic microbes transferred electrons extracellularly, enabling hydrogen production at the cathode with minimal voltage input. The system leveraged electrochemical synergies and microbial biofilm stability to enhance performance even under high organic loading rates [77].

5. Comparative cross-analysis of key parameters

Fig. 11 shows the eight-dimensional comparison developed in this section across six panels, each mapping directly to the subsections that follow. Panel (a) ranks hydrogen yield by feedstock category and conversion pathway, revealing that sugar-rich wastes (pineapple peel, food waste) consistently achieve higher molar yields than lignocellulosic residues, a pattern corroborated by the classification data in Tables 2–7. Panel (b) overlays energy output (kWh/kg H₂) against GHG emission intensity, identifying MEC-assisted systems as occupying the high-efficiency, low-emission quadrant. Panel (c) maps economic feasibility

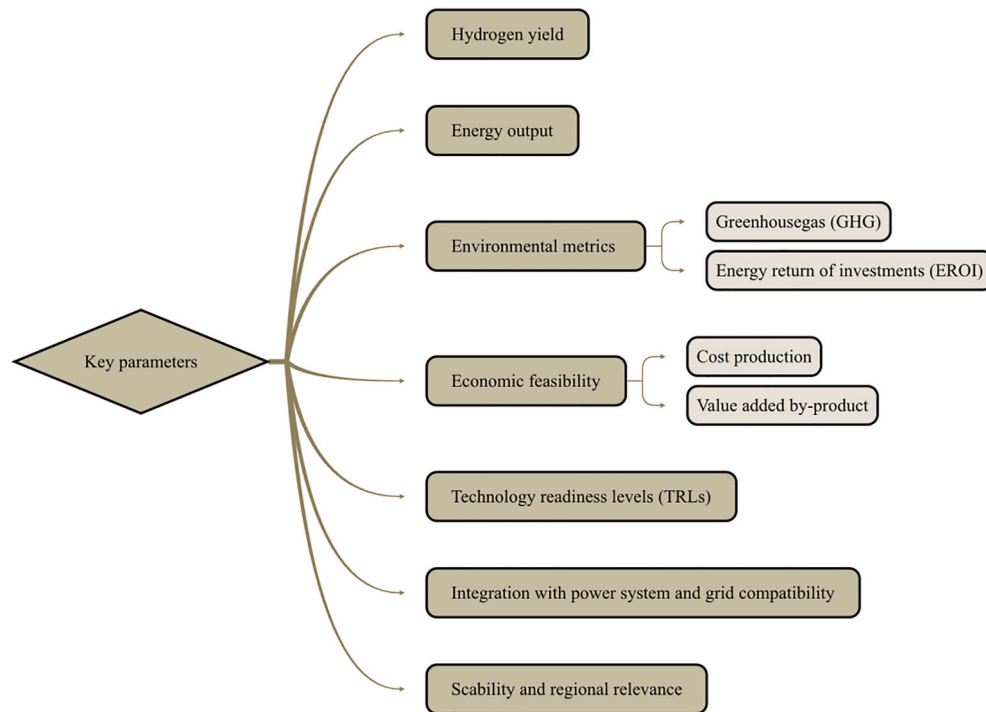


Fig. 11. Comparative cross-analysis of key parameters.

against technology readiness level (TRL), showing that dark fermentation of food and pineapple waste clusters at the lowest LCOH (\$2.50–5.80/kg H₂) and highest TRL (4–6), while MEC systems remain at TRL 3–4 with LCOH exceeding \$20/kg H₂. Panel (d) presents the TRL distribution across all 12 feedstock categories, confirming that no waste-to-biohydrogen pathway has surpassed TRL 7, a finding that anchors the research roadmap. Panel (e) evaluates grid integration readiness using the five-criterion traffic-light matrix developed (as in Section 5.5), and panel (f) maps scalability potential against regional feedstock availability in Southeast Asia, connecting directly to the regional cost analysis in Table 15. The subsections below unpack each dimension in detail.

5.1. Hydrogen yield and energy output (kWh/kg H₂)

The principle of hydrogen yield concerns the efficiency with which organic substrates, such as agricultural residues, food waste, or wastewater, are converted into hydrogen gas through biological processes. This concept is crucial to biohydrogen production, in which microorganisms break down complex organic matter in anaerobic environments. Those that lack oxygen release hydrogen as a byproduct. One of the most common and widely studied processes is dark fermentation, in which bacteria metabolize simple sugars, such as glucose, to generate hydrogen, along with organic acids and gases.

The actual amount of hydrogen produced is determined by the

Table 15
Regional cost comparison for biohydrogen.

Cost Factor	Malaysia	Indonesia	Thailand	Brazil	India
Industrial electricity tariff (\$/kWh)	0.085–0.097	0.07–0.11	0.09–0.13	0.06–0.10	0.05–0.08
Pineapple waste availability (\$/ton, gate fee)	0–5 (Sarawak, Johor; abundant)	0–3 (Lampung, E. Java)	0–5 (Prachuap Khiri Khan)	0–8 (Minas Gerais; transport cost)	2–10 (Kerala; scattered)
POME availability (\$/ton, gate fee)	–5 to 0 (waste disposal credit; >100 M ton/yr)	–3 to 0 (2nd largest palm oil producer)	N/A (limited palm oil sector)	N/A	N/A
SCB availability (\$/ton, ex-mill)	8–15 (limited sugar industry)	5–12 (E. Java; moderate)	8–15 (co-located with mills)	3–8 (São Paulo; largest global producer)	5–10 (UP, Maharashtra; abundant)
Rice straw (\$/ton, collected)	10–20 (Kedah, Perak; seasonal)	8–15 (Java, Sumatra; abundant)	10–18 (Central Plains; seasonal)	12–20 (transport from fields)	5–12 (Punjab, W. Bengal; field burning alternative)
H ₂ policy framework	HETR, NETR, SHER, PCDS 2030	National H ₂ Strategy (draft)	BCG Economy Model	National H ₂ Programme (PNH2, 2022)	National Green H ₂ Mission (NGHM, 2023)
H ₂ selling price target (\$/kg)	2.00–4.00	2.50–5.00	3.00–5.00	2.00–4.00	1.50–3.00
Comparative advantage for bioH ₂	POME (free/neg. cost); low electricity; dedicated H ₂ policy	Low labor; abundant POME & agri-waste; large domestic market	Rice + SCB co-location near mills; BCG policy support	Lowest SCB cost globally; strong carbon credit value (RenovaBio)	Lowest labor + electricity; abundant rice straw; NGHM target \$2/kg

Notes: Electricity tariffs reflect industrial/commercial rates (2024–2025). Gate fees represent the cost to acquire feedstock at the processing facility; negative values for POME indicate waste disposal credits where the facility is paid to accept waste. H₂ selling price targets drawn from national hydrogen strategies/roadmaps. Malaysia POME row highlighted in green. All values in 2024 USD unless otherwise stated.

metabolic pathway followed by the microbes. For instance, when microbes convert glucose via the acetate pathway, the theoretical maximum yield is 4 mol of hydrogen per mole of glucose. This pathway is considered more efficient because it extracts more hydrogen from the same amount of feedstock. However, suppose the microbes follow the butyrate pathway. In that case, the yield is reduced to 2 mol of hydrogen per mole of glucose, as more of the energy is locked in the end products, such as butyric acid, instead of being released as hydrogen [106].

Several factors influence which pathway is dominant and, consequently, the overall hydrogen yield. These include the type of substrate, pH, temperature, presence of specific bacterial strains, and reactor conditions. Carbohydrate-rich wastes, such as food residues or molasses, tend to yield more hydrogen due to their high biodegradability [11]. Additionally, maintaining ideal environmental conditions, such as a neutral pH, mesophilic temperatures, and low hydrogen partial pressure, can favor the more hydrogen-efficient acetate pathway.

In essence, hydrogen yield reflects the system's ability to convert organic waste into a valuable, clean fuel. Understanding and optimizing this principle is crucial for designing biohydrogen systems that are not only efficient and scalable but also aligned with sustainability goals. As part of a circular bioeconomy, high-yield biohydrogen production can transform problematic waste streams into renewable energy sources, offering a win-win solution for energy generation and environmental protection [107].

5.2. Environmental metrics (GHG emissions, EROI)

Based on cross-analysis of environmental metrics, it is clear that the potential to reduce greenhouse gas (GHG) emissions and improve energy return on investment (EROI) varies significantly across different waste types and synthesis methods. Dark fermentation, commonly applied to agricultural waste such as wheat straw and lignocellulosic residues, often shows indirect GHG benefits through the valorization of crop residues and prevention of open burning. Jin et al. [1] found that a combined dark-photofermentation process resulted in CO₂ emissions of approximately 27.1 kg per ton of wheat straw. Theoretical improvements in EROI have been proposed through the integration of waste-to-energy systems. However, most studies on dark fermentation tend to stop short of conducting complete life-cycle assessments, focusing instead on theoretical emissions reduction and introductory energy balances [72].

In contrast, hybrid systems, such as combining dark fermentation with photofermentation or microbial electrolysis, demonstrate a more comprehensive environmental performance, particularly when applied to municipal solid waste (MSW) and industrial wastewater. For example, Srivastava et al. [45] indicated that using kitchen waste in hybrid dark-photo systems can significantly divert organic loads from landfills, thereby indirectly reducing methane emissions. Meanwhile, a study by Batista et al. [108] emphasized that integrating urban wastewater treatment with microalgae-based hydrogen production can achieve nutrient removal efficiencies of up to 99.5%, significantly reducing eutrophication and improving the system's EROI through biomass recycling.

Microbial fuel cells (MFCs), while promising for processing pineapple and oily food wastes, generally lack comprehensive GHG quantification. Rojas-Flores et al. [82] and Kafaei et al. [80] suggest that these systems could reduce emissions by avoiding reliance on fossil-fueled electricity; however, EROI metrics are often underdeveloped, with a greater focus on proof-of-concept rather than scalable energy return calculations. Similarly, co-fermentation systems, especially those utilizing multiple feedstocks such as glycerol, cheese whey, and manure, highlight the environmental advantages of substrate synergy. Marone et al. [98] also reported reductions in synthetic media usage, which lower costs and emissions; however, explicit GHG or EROI metrics were not provided.

The most robust EROI figures are seen in microbial electrolysis cells

(MECs), particularly when applied to industrial wastewaters. Marone et al. [84] also highlighted that net energy inputs as low as 11–13 kJ per gram of COD removed, resulting in high EROI values, especially when combining dark fermentation with MECs in a biorefinery context. Chaurasia et al. [109] also documented significant energy recovery in acetate-fed MECs with nickel-based electrodes, highlighting the low energy threshold needed for hydrogen evolution when external voltage is applied. Although few full-scale life-cycle assessments exist, available data support MECs as the most environmentally optimized approach in terms of energy efficiency and carbon impact.

Overall, while all systems show promise, MECs and hybrid systems present the most compelling evidence for GHG mitigation and energy efficiency, especially when integrated with wastewater or MSW treatment. Dark fermentation and co-fermentation remain effective, particularly when using low-cost agricultural and agro-industrial residues, but further life-cycle assessments are needed to validate these environmental claims. MFCs, although innovative, require more in-depth environmental and EROI evaluations to transition from pilot projects to commercial applicability.

5.3. Economic feasibility (cost reduction, value-added byproducts)

The economic viability of biohydrogen production is significantly influenced by the type of waste used and the conversion methods employed. A notable example of dark fermentation involves using lignocellulosic biomass blends, such as corn stover and rice straw [72]. These agricultural residues are often abundant and underutilized. While pretreatment is required to enhance the release of fermentable sugars, this cost is justified by the resulting benefits. However, the study highlights that without government subsidies or centralized biorefinery setups, the capital expenditures and logistics required to handle bulk biomass can hinder economic scalability.

Hybrid systems that combine dark fermentation with acetone–butanol–ethanol (ABE) fermentation for treating organic municipal waste offer an economic advantage by co-producing biohydrogen alongside biofuels [54]. This multi-product approach diversifies revenue streams, enhancing the overall financial attractiveness of the process despite its complexity. Nonetheless, high production costs and the need for advanced downstream separation technologies can still limit economic feasibility, especially in developing urban areas that lack integrated waste management infrastructure.

Microbial fuel cells (MFCs) demonstrate economic practicality for treating fruit and vegetable peels sourced from household or restaurant waste streams, which often require no preprocessing and are readily available. A study has shown that MFCs designed to utilize this waste can generate electricity cost-effectively to power small devices, using inexpensive anode materials such as graphite felt and cloth, making them viable for low-resource or decentralized applications [45].

In the case of co-fermentation, research involving a mixture of buffalo slurry, cheese whey, and crude glycerol showed how economically viable strategies could emerge from optimizing substrate composition [98]. This mixture not only provided a nutrient-rich feedstock without the need for costly additives but also reduced reliance on synthetic media by 50%. This approach is efficient in regions with strong agricultural or dairy industries, where a variety of organic residues are co-produced year-round.

Microbial electrolysis cells (MECs) that treat rice mill wastewater represent an efficient, value-driven solution. According to Keruthiga et al. [78], the incorporation of artificial light-assisted MECs, powered by low-cost electrodes made from sugar industry waste, resulted in significant energy gains while offsetting wastewater treatment costs. These systems are economically justified in regions where rice and sugar processing industries are prevalent, mainly when wastewater treatment regulations impose financial penalties for non-compliance.

Table 14 synthesizes the estimated levelized cost of hydrogen (LCOH) across seven principal waste-to-biohydrogen pathways, ranging

from \$2.50/kg H₂ for optimized dark fermentation of sugar-rich waste such as pineapple peel and kitchen waste [37,45] to \$48.96/kg for early-stage microbial electrolysis cell systems [77]. Three cost drivers dominate the biohydrogen cost structure: (i) pretreatment intensity, accounting for 30–50% of OPEX in lignocellulosic pathways where enzymatic or acid hydrolysis is required [7,72]; (ii) electrode and membrane replacement costs in electrochemical systems, representing 30–50% of MEC/MFC OPEX [75,76] and (iii) photobioreactor infrastructure for two-stage DF–PF configurations, which adds substantial CAPEX [54,86]. Critically, sugar-rich waste feedstocks, particularly pineapple waste and POME, achieve the lowest LCOH range (\$2.50–6.80/kg) because they bypass the most expensive component: chemical pretreatment. The DF + ABE biorefinery approach [54,86] offers an intermediate pathway (\$5.60–18.00/kg) where multi-product revenue from butanol and ethanol co-production offsets higher system complexity.

Table 15 extends this economic analysis to regional cost variations across five tropical countries where the feedstocks reviewed in this article are primarily generated. Malaysia's combination of low industrial electricity tariffs (\$0.085–0.097/kWh), negative-cost POME feedstock, palm oil mills currently pay for waste disposal, effectively subsidizing biohydrogen feedstock acquisition [40,42], and dedicated hydrogen policy support through three complementary roadmaps (HETR, NETR, SHER) positions it among the most favorable locations for waste-to-biohydrogen deployment, particularly for POME- and pineapple-based pathways [110]. Brazil offers the lowest sugarcane bagasse cost (\$3–8/ton) co-located at sugar mills [22], while India's National Green Hydrogen Mission sets the most aggressive price target supported by the lowest labor and electricity costs in this comparison. These regional differences confirm that LCOH competitiveness is pathway- and geography-dependent: POME-based systems favor Malaysia and Indonesia; SCB pathways favor Brazil; rice straw routes favor India and Thailand [25,29].

5.4. Technology readiness levels (TRLs)

The Technology Readiness Level (TRL) framework is a 9-point scale that measures the maturity of a technology, ranging from basic research (TRL 1) to full commercial deployment (TRL 9). Understanding where a biohydrogen system stands along this scale helps researchers, investors, and policymakers assess risk, scalability, and funding needs.

In the case of pineapple peel waste, the study by Dorta et al. [35] exemplifies a TRL 3 technology. Their research focused on producing biohydrogen from pineapple biomass through fermentation, with additional value from bromelain enzyme extraction. While the concept was successfully demonstrated in a laboratory setting, the system remained at the proof-of-concept stage. This study aligns with TRL 3, where experimental studies confirm technical viability in principle, but no integrated system or prototype has been developed yet.

A more advanced study by Chu et al. [38] investigated pineapple peel juice in a two-stage anaerobic digestion system to optimize hydrogen and methane yields. Operating under continuous stirred tank reactors (CSTRs), this system underwent extended lab-scale operation, producing stable hydrogen yields. It represents TRL 4–5, where components are validated in a laboratory environment (TRL 4) and then tested in a relevant simulated environment (TRL 5). However, it lacked field validation, which limits advancement to higher TRLs.

For lignocellulosic waste, such as sugarcane bagasse and corn stover, Bhatia et al. [72] described a dark fermentation approach conducted under controlled, lab-scale conditions. The study emphasized that most biohydrogen systems from lignocellulosic waste remain at TRL 3–4, due to complexities in pretreatment, hydrolysis, and microbial inhibition. Although hydrogen was successfully generated, these systems need substantial design optimization to progress toward TRL 5 or beyond.

An example of a study reaching TRL 6 is Segundo et al. [111], which tested pineapple pulp in a microbial fuel cell (MFC) configuration. The

research used nine modular MFC units (each 1.5 L) to demonstrate integration, electrical output, and substrate conversion. While still lab-based, the modular system's scalability and repeatability indicate it is ready for prototype demonstration in relevant environments, characteristic of TRL 6.

Finally, the work by Kamusoko et al. [112] explored a hybrid dark-photofermentation system using pineapple waste, including pomace, peel, and crown. Their study uniquely combined mature anaerobic digestion (TRL 9) with emerging biohydrogen modules, placing the hybrid system at TRL 7. This stage involves demonstrating the system prototype in operational environments, suggesting that the integrated technology is nearly field-ready, pending validation in real-world contexts such as waste treatment plants or decentralized energy hubs.

5.5. Integration with power systems and grid compatibility

Integration into power systems is crucial for transitioning biohydrogen from an experimental novelty to a meaningful component of the renewable energy mix. Although most studies remain at the lab or pilot scale, several offer real insights into how waste-derived hydrogen and biogas can complement electricity grids, decentralized systems, or hybrid energy setups.

Morya et al. [29] present a compelling case for rice straw as a substrate for dark fermentation. Their study highlighted that the hydrogen produced could be blended with natural gas or used directly in fuel cells, signaling both distributed and centralized power applications. While still at pilot scale, the ability to interface with gas pipelines or proton exchange membrane fuel cells makes it a promising approach for grid-compatible green energy.

In another study, Guo et al. [24] investigated crop residues, such as corn and wheat straw, through dark fermentation, highlighting their coupling with methane production for biogas-fired cogeneration. The dual output (hydrogen and methane) was envisioned for biogas plants supplying electricity to rural grids, especially in China's agricultural zones. Their approach underscores the significance of biohydrogen systems in facilitating rural electrification through hybrid bioenergy strategies.

Xu et al. [113] investigated the utilization of agricultural and forestry residues (such as corn stover and wheat straw) through a combination of photo- and dark fermentation, as well as water photolysis. Their study not only confirmed hydrogen production but also emphasized that the system is recommended for decentralized energy systems. The modularity and flexibility of these systems make them suitable for small-scale power grids, remote communities, or disaster relief setups, where energy resilience is a top priority.

Nizzy et al. [114] presented a model for an integrated biorefinery utilizing rice husk, wheat straw, and agro-industrial residues. In this model, hydrogen was co-produced with biochar and power, creating a circular output stream. The hydrogen was used for fuel cell integration, while biochar was envisioned for soil conditioning or for generating carbon credits. This comprehensive valorization approach supports both clean power and agricultural sustainability, with clear regional energy potential.

Lastly, Florio et al. [79] addressed the organic fraction of municipal solid waste (OFMSW) using a two-stage system: microbial fuel cells (MFCs) followed by hydrogen recovery. Their system was explicitly tested for small-scale energy supply, demonstrating actual integration with power outputs in the milliwatt range, making it suitable for self-powered sensors, biosensors, or LED devices. Although limited in production, this demonstrates that even low-yield bioelectrochemical systems can be useful for targeted power delivery in innovative waste systems or urban microgrids.

5.6. Scalability and regional relevance

The success of any biohydrogen initiative depends heavily on regional factors, particularly the availability of waste feedstock, local infrastructure, and the energy needs of surrounding communities. These studies present diverse case contexts that illustrate the potential for scalability across different geographical areas.

In Indonesia, pineapple waste has been examined for its strong biohydrogen potential, especially given the country's high fruit-processing volume and tropical climate. The study noted that fermentation-based hydrogen production could be deployed in agro-industrial zones, such as Java and Lampung, where raw material availability is steady and logistical chains for agricultural exports are already in place. This setup allows for modular, localized energy systems that can serve food processing facilities and nearby communities [115].

Another regionally relevant example is the use of fruit and vegetable market waste, including peels, leaves, and discarded pulp, in biohydrogen synthesis through dark fermentation. This process shows strong potential in urban food market hubs across South Asia, where organic waste volumes are high but underutilized. The study emphasized that municipal cooperation and decentralized digesters could help manage waste while providing a clean energy source for surrounding peri-urban settlements [116].

Sugarcane bagasse, one of the most abundant agricultural residues in countries like Brazil, India, and Thailand, also offers significant scalability for biohydrogen systems. However, the study stressed that the logistics of biomass transportation and pretreatment remain a bottleneck. Regional implementation would therefore benefit from collocating biohydrogen units near sugar mills, where both steam and waste are readily accessible for integrated thermochemical-biological systems [22].

In rice-producing countries such as Vietnam and Bangladesh, rice straw is a viable feedstock for hydrogen production through dark fermentation. The study cited seasonal straw surpluses and the environmental urgency to curb field burning as two critical factors supporting the adoption of this technology. While feedstock availability is high, the authors suggested that scalability may require mobile pretreatment and fermentation units to adapt to rural distribution patterns [25].

Finally, pineapple peel waste was also explored in another context, where small-to medium-scale operations were considered practical in pineapple-rich regions such as the Philippines and Ghana. Although not industrially scaled, the study emphasized the local suitability of these systems in supporting community microgrids, remote school electrification, or agri-processing hubs, especially in areas with limited grid connectivity [39].

6. Life cycle and sustainability considerations

Life-cycle and sustainability considerations assess the impacts of products, processes, or organizations throughout their lifespans, from

raw material extraction to disposal. This approach, known as Life Cycle Sustainability Assessment (LCSA), as illustrated in Fig. 12, enhances traditional Life Cycle Assessment (LCA) by incorporating life cycle costing (LCC) and social life cycle assessment (S-LCA), with a focus on environmental, economic, and social dimensions. LCSA provides a framework for evaluating sustainability, guiding businesses and policymakers in alignment with global goals, such as the UN Sustainable Development Goals (SDGs). It emphasizes the importance of recognizing trade-offs and unintended consequences to prevent shifting burdens between stages or impact areas. However, LCSA faces challenges such as integrating various indicators, managing data gaps, and harmonizing methodologies. Ultimately, considering life cycle and sustainability factors is crucial for creating strategies that support long-term well-being for people and the planet (see Section 8).

6.1. LCA inclusion across reviewed studies

Many studies highlight sustainability as a primary goal in biohydrogen research; however, a closer examination reveals significant gaps and inconsistencies in the substantiation of these claims. Most projects emphasize short-term energy outputs without considering the full environmental, economic, and social dimensions of sustainability. A notable gap is the lack of comprehensive Life Cycle Assessments (LCA). While some papers mention reduced greenhouse gas (GHG) emissions or energy return on investment (EROI) values, only a few conduct a complete LCA or clearly define system boundaries. For instance, a study on hydrogen production from pineapple waste highlights its potential for climate mitigation. Still, it lacks an LCA model to account for upstream emissions from feedstock transport, pretreatment, and reactor energy input. Without such assessments, it is impossible to verify whether the system genuinely reduces overall environmental impact [117]. Similarly, Jin et al. [1] report significant reductions in CO₂ emissions from dark-photofermentation using wheat straw; however, they omit critical categories, such as water use, land impacts, and energy consumption, in the post-treatment stages. These oversights limit the comparability and credibility of sustainability claims.

Another major gap is the fragmented application of circular economy (CE) principles, particularly concerning how waste is converted into energy without thorough consideration for co-product or by-product valorization. Many studies successfully demonstrate hydrogen production from agro-industrial or municipal residues, but fail to address what happens to the leftover biomass or fermentation sludge. For example, Morya et al. [118,119] process rice straw for hydrogen generation, demonstrating its potential to reduce chemical use; however, they do not explore how to integrate the residual solids into composting or secondary applications. In another case, Rojas-Flores et al. [82] used pineapple pulp in microbial fuel cells to effectively generate electricity; however, they missed the opportunity to extract enzymes such as bromelain or to reuse the fiber-rich pulp, thereby undermining circular integration. Similarly, Guo et al. [24] combine corn and wheat straw for dark fermentation, achieving good hydrogen yields. Still, neglects to

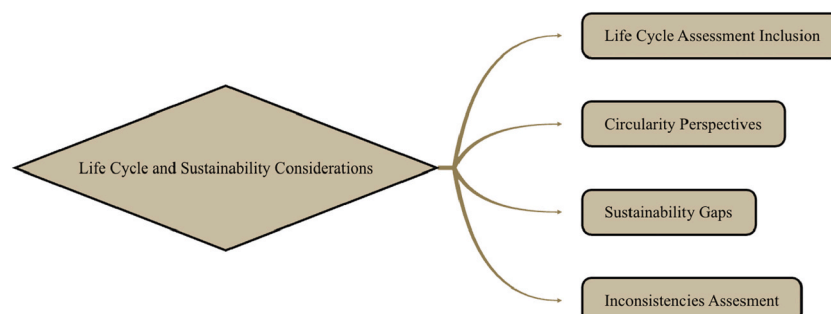


Fig. 12. Life cycle and sustainability considerations.

address the fate of the nutrient-rich digestate, which limits the circular flow of materials back into agricultural systems. These examples illustrate how systems that start with circular potential often lose momentum because residual streams are not reintegrated into the bioeconomy.

Moreover, inconsistencies extend to the economic and social dimensions, where most papers narrowly define sustainability in terms of technical feasibility or laboratory-scale yields; only a handful attempt to evaluate long-term affordability, cost recovery, or social acceptance. For instance, a study has been done on hydrogen production from pineapple waste. It demonstrates low-cost substrate utilization, yet it provides cost-benefit analysis or capital expenditure estimation to assess economic viability at scale [37]. Similarly, Kafaei et al. [80] utilize oily kitchen waste to generate hydrogen and electricity, promoting energy diversification. Still, they omit any assessment of how this system would scale or perform under real-world municipal waste variability. Additionally, some studies are effective in optimizing pretreatment for wheat straw fermentation; however, they overlook whether the technology can be adopted by rural communities or linked with policy support, both of which are crucial indicators of social sustainability [6]. Without understanding how these technologies integrate into local livelihoods, regulatory frameworks, or funding mechanisms, their broader impact remains uncertain.

Another inconsistency is the lack of standardized metrics and system boundaries, which makes it challenging to compare findings across studies. Some papers report GHG reductions as percentages, while others report them in kilograms of CO₂ per unit of hydrogen, and many do not specify units at all. In the work by Lu et al. [120], for example, apple waste is utilized to produce hydrogen, and environmental benefits are proposed. However, there is no clear baseline or reference system for comparison. This ambiguity weakens the robustness of the results and complicates cross-comparisons. Similarly, in the study using dairy wastewater, while improvements in chemical oxygen demand (COD) and pH are reported, the link between these improvements and broader sustainability goals, such as nutrient recycling and water reuse, remains speculative [77]. Without harmonized reporting, it is challenging to construct a comprehensive picture of which systems are truly sustainable and which are not.

In summary, while the reviewed studies contribute valuable insights into biohydrogen production from waste, many fall short of providing a comprehensive sustainability assessment. Common issues include incomplete or missing LCAs, underutilization of fermentation by-products, and the absence of economic and social feasibility metrics.

6.2. Circularity perspectives

A circular economy reconceptualizes waste as a resource to be valorized rather than a problem. In the realm of biohydrogen production, this entails transforming organic residues into renewable energy while generating complementary by-products, such as compost, electricity, or biochar, to complete the resource loop. The following examples, derived from five major waste streams, illustrate how biohydrogen systems are progressively adopting circularity, although gaps persist in achieving full lifecycle integration. Pineapple residues, which are rich in carbohydrates and cellulose, are highly suitable for dark fermentation and microbial electrolysis; however, most studies do not fully close the material loop. Pineapple and cassava waste were used for hydrogen production via dark fermentation, resulting in reduced organic waste volumes and moderate energy recovery. Still, the study did not investigate the downstream reuse of fermentation sludge or fiber residues [121]. Pineapple juice waste was used in microbial fuel cells to generate bioelectricity, providing proof of concept for direct waste-to-power conversion; however, the process was limited to powering small devices and lacked circular applications for by-products [82]. Whole pineapple biomass was processed using dark fermentation followed by anaerobic digestion, yielding energy-dense biogas and reducing GHG

emissions. However, the fibrous residues were not valorized for compost or material uses [117]. Pineapple pulp waste diverted from landfills was converted into hydrogen, effectively reducing methane emissions; however, the remaining slurry was not addressed as a secondary value stream [110]. Hydrogen was also produced from pineapple cores and peels, contributing to decarbonization, though opportunities to extract enzymes such as bromelain were missed [37]. Fermentation of pineapple peel resulted in GHG reductions of up to 98% compared to fossil fuel use; however, the nutrient-rich by-products were not integrated into soil restoration or feed applications [36]. Avoiding landfill of pineapple residues demonstrated clear climate benefits, but the potential to convert fibers into packaging or bio-based materials remained unexplored [35]. Overall, pineapple waste shows significant promise as a renewable energy feedstock; however, full circularity through co-product extraction and reintegration into agricultural systems is seldom achieved.

Hydrogen and biofuels were simultaneously generated from food waste, contributing to energy diversification and enhancing overall conversion efficiency, although the model did not account for composting the remaining biomass [54]. Utilizing municipal biowaste in mechanical-biological treatment (MBT) alongside anaerobic digestion reduced lifecycle emissions and produced partially stabilized compost; however, the pathways for compost application were not monitored [55]. The combination of microbial fuel cells with anaerobic digestion enhanced H₂ production while stabilizing urban organic waste, indicating a viable approach to urban circularity [79]. Hydrogen derived from municipal waste has been suggested as a diesel alternative for public transport, offering a direct climate benefit; however, no data were provided on the management of residual bioslurry [122]. Hydrogen production from kitchen waste was tested in laboratory settings; however, it was not integrated into broader waste management systems or used to reuse fermented residues [123]. These instances highlight the potential of MSW to bolster renewable energy systems in urban areas, though achieving complete circularity would require improved integration of nutrient cycling and urban agriculture.

Industrial wastewater, rich in organic matter, presents both environmental and economic benefits for energy recovery. Municipal wastewater was combined with microalgae and dark fermentation to generate hydrogen while simultaneously removing nitrogen and phosphorus, thus facilitating energy recovery and nutrient reclamation [108]. Brewery effluent underwent fermentation and microbial electrolysis, achieving both hydrogen extraction and COD reduction, although downstream sludge reuse was not incorporated [84]. Rice mill wastewater was treated in artificial photo-assisted electrolysis cells, producing hydrogen and demonstrating a hybrid system that reduced water treatment costs and energy consumption. However, nutrient reuse was not evaluated [78]. Dairy wastewater processed in microbial fuel cells enabled hydrogen production and pH stabilization, but the handling of the final digestate was not modeled [77]. Textile wastewater was used to generate hydrogen via MFCs, providing a decentralized waste-to-energy solution. However, the feasibility of scaling or integrating into factory treatment systems was not thoroughly assessed [97]. Acetate-rich wastewater was processed in electro-fermentation units, resulting in an improved yield and reduced reliance on fossil fuels; however, the study did not investigate the fate of carbon-rich effluents [124]. A review of various industrial effluents proposed biohydrogen production as a means to simultaneously reduce COD and GHGs, though circularity endpoints were often speculative [68]. These instances demonstrate how industrial wastewater can pose a treatment challenge while also presenting an opportunity for energy generation, particularly when integrated with circular economy models.

Microalgal biomass can be fermented into hydrogen under anaerobic conditions, thereby supporting sustainability through the use of renewable aquatic feedstocks. However, the digestate was not utilized [125]. Comparisons of microalgae and other residues revealed that fermentation provided the best carbon balance and energy output;

however, nutrient recycling was only proposed in theory [126]. Fermentation of mixed waste with glycerol and urban residues reduced carbon emissions and sludge volume; however, the composting of the digestate was not modeled [34]. Algal co-digestion with organic waste improved hydrogen yield and reduced emissions, but the potential for nutrient and water reuse was not examined [127]. These studies emphasize the potential of unconventional waste to contribute to sustainability if integrated into value chains, highlighting the need for further lifecycle analyses, especially in rural areas [128].

6.3. Sustainability gaps and inconsistencies in assessments

While many studies emphasize sustainability as a key objective in biohydrogen research, a closer examination reveals significant gaps and inconsistencies in the support for these claims. Most projects focus on short-term energy outputs, failing to capture the full environmental, economic, and social dimensions of sustainability. A clear gap exists due to the lack of a comprehensive Life Cycle Assessment (LCA). Although some papers mention reduced greenhouse gas (GHG) emissions or Energy Return on Investment (EROI) values, only a few conduct a complete LCA or clearly define system boundaries. For instance, the study on hydrogen production from pineapple waste highlights its potential for climate mitigation. Still, it lacks an LCA model to account for upstream emissions from feedstock transport, pretreatment, and reactor energy input. Without such assessments, it is impossible to verify whether the system truly reduces overall environmental impact [117].

Another major gap is the inconsistent application of circular economy (CE) principles, particularly in the conversion of waste into energy, with inadequate follow-through on the valorization of co-products or by-products. Many studies have successfully demonstrated hydrogen production from agro-industrial or municipal residues, but they often fail to consider the fate of the leftover biomass or fermentation sludge. For example, a study on using processed rice straw for hydrogen generation demonstrates its potential to reduce chemical use. Still, it does not examine how to integrate the residual solids into composting or secondary applications [29]. In another case, Rojas-Flores et al. [82] utilize pineapple pulp in microbial fuel cells, effectively recovering electricity. However, they miss the opportunity to extract enzymes such as bromelain or to reuse the fiber-rich pulp, which undermines circular integration. Likewise, Guo et al. [24] combine corn and wheat straw for dark fermentation, achieving good hydrogen yields, but leave the fate of the nutrient-rich digestate unaddressed, thereby limiting the circular flow of materials back into agricultural systems. These examples illustrate how circular potential systems often lose momentum because residual streams are not effectively reintegrated into the bioeconomy.

Moreover, the inconsistency extends to the economic and social dimensions, where most papers narrowly define sustainability in terms of technical feasibility or laboratory-scale yields; only a minority attempt to evaluate long-term affordability, cost recovery, or social acceptance. For instance, Ahmad et al. [37] investigate hydrogen production from pineapple waste and demonstrate low-cost substrate utilization; however, they provide no cost-benefit analysis or capital expenditure estimation to assess economic viability at scale. Similarly, Kafaei et al. [80] utilize oily kitchen waste to generate hydrogen and electricity, promoting energy diversification; however, they omit any assessment of how this system would scale or perform under real-world municipal waste variability. Additionally, studies such as those by Zhu et al. [6] are effective in optimizing pretreatment for wheat straw fermentation; however, they overlook whether the community can adopt the technology and whether it is supported by policy, two crucial indicators of social sustainability. Without understanding how these technologies fit into local livelihoods, regulatory frameworks, or funding mechanisms, their broader impact remains uncertain.

Another inconsistency is the lack of standardized metrics and system boundaries, making it difficult to compare findings across studies. Some papers report GHG reductions as percentages, others as kilograms of CO₂

per unit of hydrogen, and many do not specify units at all. In the work by Lu et al. [120], for example, apple waste is used to produce hydrogen, suggesting environmental benefits. However, there is no clear baseline or reference system for comparison. This ambiguity weakens the robustness of the results and complicates cross-comparison. Similarly, in the study by Chaurasia et al. [77], which utilized dairy wastewater, improvements in chemical oxygen demand (COD) and pH were noted. Still, the connection between these improvements and broader sustainability goals, such as nutrient recycling or water reuse, remains speculative. Without harmonized reporting, it is challenging to build a coherent picture of which systems are truly sustainable.

In summary, while the reviewed studies contribute valuable insights into biohydrogen production from waste, many fall short of a holistic sustainability assessment. The most common issues include partial or missing LCAs, underutilization of fermentation by-products, and the absence of economic and social feasibility metrics.

7. Research gaps and future directions

7.1. Unresolved technical barriers

Despite growing interest in biohydrogen as a clean, renewable energy source, several technical barriers continue to hinder its large-scale deployment, especially when transitioning from controlled laboratory experiments to industrial applications. One of the most pressing challenges across various waste types, including municipal solid waste, agro-industrial wastewater, microalgal biomass, and co-digested waste streams, is the inefficient conversion of substrates into hydrogen. Many waste materials, particularly those high in lignocellulosic or complex organic content, require extensive pre-treatment to destabilize recalcitrant structures and release fermentable sugars. For example, using mixed agricultural residues such as sugarcane bagasse and corn stalks has shown limited conversion efficiency during standard dark fermentation due to their complex structures, which not only reduce hydrogen yields but also increase the energy burden of the pre-treatment process [72]. This situation highlights a broader challenge: the energy input required for pre-treatment often exceeds the net energy gained, undermining the sustainability advantage of biohydrogen production.

Another persistent barrier is the instability and suboptimal performance of microbial systems, particularly in non-sterile, real-world waste environments. While dark fermentation remains the most common biological pathway for hydrogen production, its effectiveness heavily relies on the resilience and activity of microbial consortia. For instance, in co-fermentation studies using substrates like buffalo slurry and cheese whey, maintaining microbial synergy and suppressing competitive hydrogen-consuming pathways proved challenging, especially under unregulated conditions [98]. Similarly, microbial electrolysis systems using acetate or algal biomass, such as *Scenedesmus obliquus*, experienced performance fluctuations due to inconsistent microbial-electrode interactions and variable organic loading rates [109,125]. These cases illustrate that microbial adaptability is a weak point, particularly when scaling up or processing waste with inconsistent composition.

Reactor design and process integration present additional technical challenges. Although many biohydrogen systems show potential at the laboratory scale, their scalability is often limited by poor reactor optimization, insufficient process integration, and inadequate control of operational variables. For example, a study using rice mill wastewater demonstrated that photo-assisted microbial electrolysis cells (APAMEC) were technically feasible but lacked integration with renewable energy systems and required further validation under scaled-up conditions [78]. Similarly, two-stage hybrid systems that couple dark fermentation with microanalysis cells have shown promise in controlled experiments but have encountered difficulties in synchronizing fermentation kinetics and energy recovery efficiency when tested in real-world scenarios [84]. Additionally, single-chamber microbial fuel cells (MFCs) have suffered from high internal resistance and electrode instability, which reduces

net hydrogen output and makes long-term operation less viable. These technological shortcomings are magnified in larger systems, where maintaining uniform substrate flow, pH, temperature, and anaerobic conditions becomes more complex and costly.

Moreover, limited automation and real-time process control significantly impact system performance and reliability. Many existing studies operate in batch or semi-continuous modes without incorporating dynamic feedback loops to regulate crucial fermentation parameters. For example, biohydrogen production from kitchen waste using hybrid fermentation pathways has been hindered by inefficiencies and reduced hydrogen yields due to a lack of automated pH control and monitoring of intermediate metabolites, such as volatile fatty acids (VFAs) [45]. The absence of intelligent process control systems represents a critical bottleneck, particularly in settings that require long-duration or continuous operation, where system stability is essential.

Lastly, a significant yet often overlooked barrier is the discrepancy between theoretical and actual energy recovery. While theoretical hydrogen yields, such as those predicted from organic fractions of municipal solid waste or algal biomass, appear favorable, actual yields are often lower due to heat losses, substrate conversion, and system inefficiencies. For instance, although theoretical yields from microalgal substrates can reach up to 33 kWh/kg H₂, the recoverable energy is significantly reduced when operational inefficiencies and energy inputs (such as aeration, stirring, or inoculum preparation) are considered [108]. This gap between theoretical modeling and practical implementation underscores the urgent need for more realistic energy and life-cycle assessments that account for all inputs and outputs across the production chain.

In conclusion, the technical barriers to biohydrogen production are complex and include feedstock variability, microbial instability, inefficient reactor design, and limited scalability. These issues hinder the efficiency, economic feasibility, and environmental viability of hydrogen production.

To address these challenges, coordinated research in advanced bioreactor design, synthetic microbial engineering, and automated control systems is essential. Such integrated approaches are necessary for biohydrogen to become a practical and scalable energy solution.

7.2. Microbial optimization needs

Microbial optimization is a significant research gap in the field of biohydrogen production. Biological methods, such as dark fermentation, photofermentation, and microbial fuel cells (MFCs), show promise for converting organic waste into hydrogen; however, the stability, efficiency, and adaptability of microbial communities remain limiting factors across various real-world waste streams. Studies on municipal solid waste, urban wastewater, and agricultural residues rich in starch and lignocellulose highlight the urgent need for resilient, well-adapted, and metabolically active microbial systems.

One example involves using urban wastewater in a hybrid system that combines microalgae cultivation and dark fermentation. In this system, microalgal biomass grown in wastewater was used to produce hydrogen, demonstrating initial promise in nutrient recovery and waste valorization. However, the microbial communities responsible for fermentation were not robust under scale-up conditions. The study identified a major gap in optimizing microbial consortia for large photobioreactor systems, as the microbial strains had not adapted to the high ammonium and phosphate content, resulting in lower-than-expected hydrogen yields. The study suggested targeted selection or genetic enhancement of microalgae-compatible hydrogen producers to improve metabolic efficiency under nutrient-rich wastewater conditions [108].

Another critical example of microbial limitations is observed in biohydrogen production from rice straw, a common agricultural waste. Although this lignocellulosic biomass has a high theoretical hydrogen yield, the microorganisms used are largely mixed anaerobic consortia

and struggle with the toxicity of pretreatment byproducts and the low bioavailability of sugars. The study recommended enhancing the enzymatic activity and resilience of microbial strains through environmental adaptation or genetic engineering to tolerate harsh post-hydrolysis conditions [6]. Without optimized microbes that can survive these inhibitors, the benefits of biomass pretreatment are compromised, leading to low yields and unstable hydrogen production.

Another aspect of microbial inefficiency is observed in the processing of food leftovers from municipal solid waste via two-stage dark fermentation. Although the thermophilic microbial population was well-suited to the organic substrate, it experienced significant performance declines due to the fluctuating pH and nutrient loads typical of kitchen waste. The authors concluded that long-term operation of thermophilic hydrogen-producing systems will require controlled co-cultures, improved pH tolerance, and possibly the inclusion of pH-buffering microbes to stabilize reactor environments [54].

In another promising study, sorghum starch, a starchy agricultural feedstock, was utilized to produce hydrogen through a combination of dark fermentation and enzymatic hydrolysis. While the enzymatic breakdown successfully made sugars accessible, the microbial community used for fermentation exhibited slow adaptation rates and low metabolic conversion efficiency, especially under variable starch content. The researchers emphasized the need for microbial cultures with faster fermentation kinetics and amylase compatibility, thereby improving synchronization between the enzymatic and microbial phases [3]. This study highlights that even in high-carbon substrates, microbial response and metabolic alignment can become bottlenecks.

Microbial fuel cells (MFCs) that utilize oily kitchen waste, such as used vegetable oils and peels, also highlight the importance of microbial optimization. Although the system avoided costly oil-separation steps, the microbial population in the MFCs displayed low electrochemical activity and struggled with long-chain fatty acids, thereby inhibiting current generation and limiting hydrogen recovery. The authors called for selective enrichment of electroactive microbes and the development of inocula capable of efficiently metabolizing high-lipid substrates [80]. It is especially relevant for decentralized applications in the food processing or hospitality sectors, where oily waste is abundant.

7.3. LCA-standardization and techno-economic models

When it comes to advancing biohydrogen technologies, one of the most consistent and overlooked issues reflected in the cross-analysis table is the lack of standardization in Life Cycle Assessment (LCA) and the limited use of techno-economic models. While many studies have demonstrated that converting organic waste into hydrogen is technically feasible, far fewer have taken the next crucial step: evaluating the full environmental and economic impacts of these systems. It creates a significant disconnect between lab-scale innovation and real-world application, where policymakers and industries require solid, quantifiable data to inform their decisions.

Take, for example, the study using liquid pineapple waste (LPW) in a hybrid dark and photo-fermentation system. Technically, the process worked: hydrogen was successfully produced, and the pathway even opened the door to other byproducts. However, the researchers acknowledged that no complete life-cycle assessment was conducted. They recommended future studies to measure greenhouse gas emissions, energy inputs and outputs, and overall resource use throughout the process. Without this kind of cradle-to-grave data, it is difficult to determine whether these systems are truly more sustainable than existing methods. Another similar case involved cassava peels and pineapple pulp waste, where, despite promising hydrogen yields, no attempt was made to quantify environmental benefits, such as carbon reduction or EROI, making it difficult to justify long-term investment in such systems [121].

The challenge is not just environmental, but it is also economic. For instance, a study that explored a multi-stage process using pineapple

peels, cores, and leaves attempted to combine dark fermentation for hydrogen with bromelain enzyme recovery. It showed impressive potential for producing both fuel and value-added products. However, it struggled to address economic feasibility. There was no clear cost model, no investment forecast, and no benchmarking for operational costs [117]. This is a recurring trend: many studies highlight technical performance but stop short of applying techno-economic analysis that could translate these findings into industry-scale ventures. Supporting this, another paper examining sorghum starch-based hydrogen production also lacked detailed economic modeling, despite showing potential scalability. Without cost-benefit insights, the pathway from lab bench to business case remains unclear [3].

Even when researchers acknowledge the importance of these frameworks, implementation often remains incomplete. For example, the study involving pineapple peels, crown, and stem proposed using LCA in future work, but did not include any current evaluation of energy recovery, emissions, or resource savings [82]. Likewise, a study using urban wastewater and microalgae fermentation reported promising hydrogen yields and nutrient recovery but failed to validate these results with a standard LCA or financial analysis [108]. These partial assessments make it hard to compare technologies across projects and regions.

In summary, the lack of standardized LCA and techno-economic models is a significant barrier to biohydrogen research, particularly for systems that utilize agricultural waste. Without a consistent way to measure environmental impact, energy efficiency, and economic feasibility, we risk losing sight of the bigger picture. This gap also prevents meaningful policy development and makes industrial uptake far more difficult. In the future, researchers need to integrate modular, waste-specific LCA tools and develop regionally adaptable techno-economic simulations that account for real-world variables such as feedstock availability, energy pricing, carbon credits, and infrastructure needs. Only with this holistic perspective can biohydrogen transition from an experimental innovation to a genuinely sustainable solution in the circular economy.

7.4. Policy, incentives, and regional deployment strategies (global)

Globally, interest in hydrogen as a clean and versatile energy carrier is rising rapidly. However, much of the focus in current national policies and strategic roadmaps remains narrowly concentrated on electrolysis-based green hydrogen, often excluding an equally essential and practical pathway: biohydrogen derived from organic waste. Despite its clear potential to address both energy needs and environmental burdens, there is a glaring absence of dedicated policy frameworks to support waste-to-hydrogen pathways, such as dark fermentation, photo-fermentation, or microbial electrolysis cells (MECs). Regions like the European Union and Japan, for instance, have made substantial progress in hydrogen planning, yet they seldom acknowledge the value of waste-based biohydrogen production [102]. This policy blind spot results in the systematic underrepresentation of biohydrogen from agricultural residues and municipal solid waste in climate strategies, circular economy goals, and clean energy policies.

The research gaps identified are based on the research studies, particularly the lack of life cycle assessments (LCA), low technology readiness levels (TRLs), weak economic modeling, poor power system integration, and unclear scalability, highlighting critical areas where current policies and incentives can be more effectively aligned to support biohydrogen development. In Asia, for instance, studies on pineapple and rice straw waste emphasize lab-scale success but struggle with commercialization due to underdeveloped techno-economic frameworks and the absence of LCA inclusion [1]. Policies like Malaysia's Hydrogen Economy and Technology Roadmap and India's National Green Hydrogen Mission could introduce targeted incentives, such as TRL grants and LCA support, to help bridge this gap. Similarly, in Europe, although frameworks like the EU Green Deal support low-carbon innovation, many studies still fail to meet funding criteria

due to the absence of environmental accounting or integration models [72].

Mandating the inclusion of LCA and offering integration subsidies can help projects utilizing wheat straw or municipal solid waste qualify for EU funding. In the United States, promising systems like microbial electrolysis cells (MECs) using oily kitchen waste remain underfunded due to unclear economic feasibility and low TRL; extending the Hydrogen Production Tax Credit (45 V) to biohydrogen systems with measurable EROI could improve their uptake [80,109]. Meanwhile, in South Africa and other African nations, rural-appropriate technologies such as co-fermentation of livestock and agro-waste face scalability challenges due to limited policy support. Integrating these systems into national electrification or waste valorization plans, coupled with micro-financing or carbon offset schemes, could unlock their deployment [45,98]. Overall, transforming research limitations into actionable policy instruments ensures that waste-to-hydrogen pathways not only evolve in laboratories but also translate into impactful real-world solutions.

8. Propose a framework for the Life Cycle Assessment and Techno-economic analysis

Building on the LCA gaps and methodological inconsistencies and the research priorities, this section proposes a standardized Life Cycle Assessment and Techno-Economic Analysis (LCA-TEA) framework for evaluating waste-to-biohydrogen systems. Fig. 13 operationalizes this framework as a four-phase workflow: (1) Goal and Scope Definition, (2) Life Cycle Inventory, (3) Life Cycle Impact Assessment, and (4) Interpretation and TEA Integration, with explicit decision nodes at each phase boundary that allow practitioners to adapt the methodology to the feedstock-specific parameters catalogued in Tables 2–13.

8.1. Goal and Scope Definition

The goal of the LCA is to quantify and compare the environmental and economic performance of biohydrogen production from waste feedstocks. The framework adopts the following scope parameters:

- (a) Functional Unit (FU): 1 kg of purified hydrogen gas (H_2) at (assumption: 99.97% purity, delivered at 1 atm and 25 °C. This FU enables direct comparison with established hydrogen production benchmarks.
- (b) System Boundary: Cradle-to-grave, encompassing six stages: (i) feedstock collection and transport; (ii) pretreatment (mechanical, thermal, chemical, or enzymatic, depending on feedstock); (iii) biological conversion via dark fermentation, photofermentation, MEC, MFC, or hybrid configurations; (iv) gas purification and compression (pressure swing adsorption or membrane separation); and (v) waste management and disposal of spent biomass, digestate, and process effluents.
- (c) Allocation Method: System expansion (avoided burden approach) is the preferred allocation method, consistent with ISO 14044 Clause 4.3.4.2. Co-products such as volatile fatty acids (VFAs), biogas (CH_4), and digestate (fertilizer substitute) are credited with displacing their conventional equivalents. Where system expansion introduces excessive complexity, economic allocation based on market value may be applied as a sensitivity variant.
- (d) Temporal and Geographic Scope: The framework must be designed with selected regions as the primary geographic context. Transport distances and feedstock availability data reflect the selected region's conditions, though the modular structure allows adaptation to other tropical contexts. The selection of the temporal scope depends on how many years of operational lifetime with annual discounting.

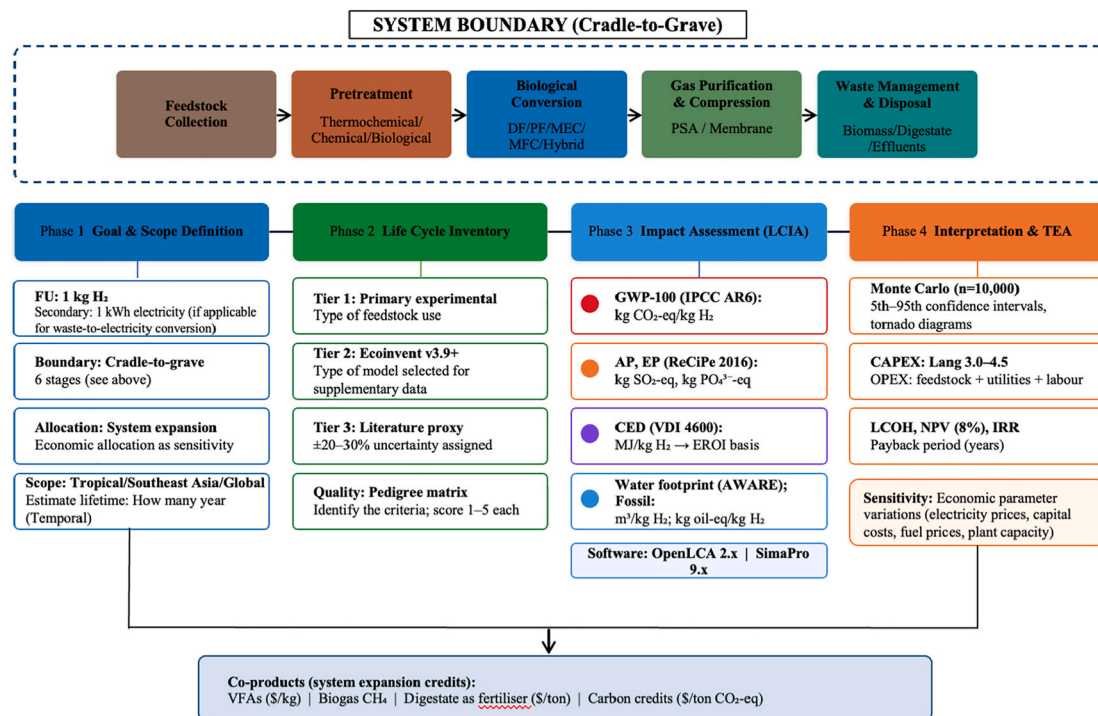


Fig. 13. Proposed LCA-TEA framework for waste-to-biohydrogen.

8.2. Life Cycle Inventory (LCI)

The LCI phase compiles all material and energy inputs and outputs for each life cycle stage. Data sources are stratified by quality tier:

- Tier 1 (Primary Data):** Identify what experimental data are involved (such as from the collection of data through raw materials or secondary review data), including substrate composition (VS, COD, TS, sugar content), hydrogen yields under specified conditions, energy consumption for pretreatment and reactor operation, and co-product quantities.
- Tier 2 (Background Data):** Ecoinvent v3.9+ (allocation, cut-off system model), chemical production (H_2SO_4 , NaOH, enzyme preparations), transport, and waste treatment. Identify what type of model can provide supplementary data, especially for hydrogen compression, storage, and fuel cell operation, enabling benchmarking against grey and green hydrogen pathways.
- Tier 3 (Proxy Data):** For parameters not available from Tier 1 or Tier 2 sources, literature proxy values are used with an assigned uncertainty of $\pm 20\text{--}30\%$. All Tier 3 data points are flagged in the inventory and subjected to sensitivity analysis in the interpretation phase.
- Data Quality Assessment:** A pedigree matrix adapted from Ecoinvent is applied to all inventory data, scoring reliability, completeness, temporal correlation, geographical correlation, and technological correlation on a 1–5 scale. Aggregate data quality scores are reported alongside results to enable transparent assessment of confidence levels [118,119].

8.3. Life Cycle Impact Assessment (LCIA)

Impact assessment translates the LCI into environmental impact indicators using established characterization methods. The following impact categories are selected based on their relevance to biohydrogen systems, as shown in Table 16.

The selection of the software is applied to identify which model is suitable for developing the framework. **Software:** OpenLCA 2.x (open-

Table 16

Impact Assessment based on the categories, unit selections, type of method used to identify the impact category, and how the impact category is relevant towards biohydrogen conversion.

Impact Category	Unit	Method	Relevance to BioH ₂
Global Warming Potential (GWP-100)	kg CO ₂ -eq/kg H ₂	IPCC AR6 (2021)	Primary climate metric; enables comparison with grey H ₂ (10–12 kg CO ₂ -eq/kg H ₂) and electrolysis H ₂ (1–3 kg CO ₂ -eq/kg H ₂)
Acidification Potential (AP)	kg SO ₂ -eq/kg H ₂	ReCiPe 2016 Midpoint (H)	Captures emissions from pretreatment chemicals and energy inputs
Eutrophication Potential (EP)	kg PO ₄ ³⁻ -eq/kg H ₂	ReCiPe 2016 Midpoint (H)	Relevant for wastewater-based pathways (nutrient discharge)
Cumulative Energy Demand (CED)	MJ/kg H ₂	VDI 4600	Quantifies total energy input; basis for EROI calculation (EROI = energy in H ₂ /CED)
Water Footprint (WF)	m ³ /kg H ₂	AWARE	Critical for water-intensive pretreatment and fermentation processes
Fossil Resource Depletion	kg oil-eq/kg H ₂	ReCiPe 2016 Midpoint (H)	Measures dependence on fossil inputs across the life cycle

source) is recommended as the primary LCA software, enabling full transparency and reproducibility. In contrast, SimaPro 9.x may be used as a validation platform. All characterization factors, inventory datasets, and model parameters should be archived as supplementary data to support future meta-analysis.

ISO Compliance: This framework complies with ISO 14040:2006 (Principles and Framework) and ISO 14044:2006 (Requirements and Guidelines). Critical review by an independent LCA practitioner is recommended for studies intended for publication or policy application, consistent with ISO 14044 Clause 6.

8.4. Interpretation and techno-economic evaluation

The interpretation phase integrates environmental and economic results to identify the most sustainable and commercially viable waste-to-biohydrogen pathways.

8.4.1. Environmental interpretation

Contribution analysis identifies the life cycle stages with the highest environmental burden across all impact categories. Hotspot identification enables targeted process improvement: for example, if pretreatment dominates GWP, the framework recommends evaluating milder alternatives (enzymatic vs acid hydrolysis). Scenario comparison evaluates the environmental performance of different feedstock–pathway combinations (e.g., pineapple waste + DF vs POME + MEC hybrid vs OFMSW + two-stage DF-PF), to enable identifying what is still lacking in terms of the analysis in biohydrogen pathways.

Uncertainty Analysis: Monte Carlo simulation (10,000 iterations) is applied to all Tier 2 and Tier 3 data parameters, with probability distributions (lognormal for emissions, triangular for process parameters) derived from the pedigree matrix scores. Results will be reported as median values with 5th–95th percentile confidence intervals. Tornado diagrams identify the parameters with the greatest influence on each impact category.

8.4.2. Techno-economic analysis (TEA)

The TEA module is integrated within the LCA framework to enable simultaneous environmental and economic optimization (see Table 17). By integrating LCA and TEA within a standardized, cross-sectoral framework, this review addresses the methodological fragmentation observed in previous biohydrogen assessments and establishes a reproducible benchmarking structure for future comparative studies: the disconnect between environmental claims and economic reality that pervades the current biohydrogen literature. The framework's modular design allows researchers to apply it to any waste-to-hydrogen pathway reviewed in this article, using the standardized FU, system boundary, and impact categories specified above, and adapting feedstock-specific inventory data from experimental data or literature review-based data.

9. Conclusion

Scaling a sustainable waste-to-hydrogen economy requires more than just promising technology; it demands a coordinated, multi-layered approach that brings together policy, innovation, infrastructure, and community action. This cross-sectoral review of waste feedstock categories reveals three overarching findings. First, sugar-rich agro-industrial wastes, such as pineapple peel/juice and palm oil mill effluent (POME), consistently achieve the highest molar yields (up to 2.3 mol H₂/mol sugar and 1.88 mol H₂/mol sugar, respectively) with minimal

pretreatment, positioning them as the most deployment-ready feedstocks for tropical biohydrogen systems. On the second finding, no waste-to-biohydrogen pathway reviewed has surpassed Technology Readiness Level 7 (see Section 5.4), with most systems clustered at TRL 3–5, confirming that the field remains pre-commercial and requires targeted scale-up investment. The third finding, the levelized cost of biohydrogen (LCOH) spans a 20-fold range, from \$2.50/kg H₂ for optimized dark fermentation of sugar-rich waste to \$48.96/kg for early-stage MEC systems (see Table 14), indicating that pathway selection, not feedstock availability alone, is the primary cost determinant.

The first research priority is advancing microbial consortia engineering for the three highest-impact conversion pathways. For dark fermentation of pineapple and food waste, future studies should target C5 sugar (xylose, arabinose) co-utilization by engineering pentose-fermenting *Clostridium* or *Enterobacter* co-cultures, since pentose sugars from hemicellulose remain largely unconverted in all reviewed studies (Section 3.1.7, gap ii). For POME-fed systems, research should quantify the long-term stability of Fe²⁺-supplemented thermophilic consortia beyond the 72-h batch cycles reported in the current literature (as in Table 7), with a specific target of achieving >1000 h of continuous operation at >80% of peak yield. For MEC-assisted systems, electrode biofilm resilience under fluctuating organic loading rates must be characterized, as the current highest-performing nickel-cobalt cathode configurations (Section 4.4) have only been validated under steady-state conditions.

The second priority is closing the methodological gap in sustainability assessment. The near-total absence of standardized life cycle assessment across reviewed studies (Section 3.1.7, gap iv; Section 7) undermines cross-study comparability and investor confidence. Future studies should adopt the cradle-to-grave LCA–TEA framework proposed in Section 8 (Fig. 13) as a minimum reporting standard, including: (a) explicit system boundary declaration (cradle-to-gate vs. cradle-to-grave), (b) functional unit harmonization to 1 kg H₂ at purity ≥99.97%, (c) impact assessment using at minimum GWP₁₀₀, acidification, and eutrophication categories (Table 16), and (d) integrated LCOH calculation with transparent CAPEX/OPEX disaggregation. The target is to reduce the current LCOH of dark fermentation systems from \$2.50–5.80/kg H₂ (Table 14) to below the grey hydrogen benchmark of \$1.50–2.00/kg H₂ within a 10-year horizon, primarily through pretreatment cost reduction and co-product revenue (biochar, volatile fatty acids) credited within the LCA system boundary.

Third, the transition from laboratory validation (TRL 3–5) to pilot demonstration (TRL 6–7) requires three specific engineering milestones: (a) continuous-flow reactor validation at ≥10 L working volume for >2000 h, as no reviewed study exceeds bench-scale batch operation (Tables 2–13); (b) hydrogen purification to PEM fuel cell grade (≥99.97%) integrated within the fermentation–separation process train, since all reviewed yields are reported as raw biogas requiring downstream cleaning; and (c) demonstration of grid-compatible power output through biohydrogen-fed fuel cell or gas-blending systems in at least one Southeast Asian agro-industrial site, building on the grid integration readiness assessment in Section 5.5. Malaysia's Sarawak state, with its convergence of abundant POME feedstock, low electricity tariffs (\$0.085–0.097/kWh), and dedicated hydrogen policy support (SHER, NETR, HETR), offers the most favorable conditions for this pilot deployment (Table 16).

Finally, three policy adjustments would accelerate the commercialization of biohydrogen. First, national hydrogen strategies (NETR, HETR) should create a dedicated biohydrogen feed-in tariff category distinct from electrolysis-based green hydrogen, recognizing the dual waste-diversion and energy-generation value that the LCOH analysis (Table 14). Second, carbon credit frameworks should be extended to waste-to-hydrogen pathways based on the avoided methane emissions from diverted organic waste, which the environmental metrics show can yield negative net GHG emissions when landfill avoidance is credited. Third, Sarawak's PCDS 2030 should include specific biohydrogen pilot

Table 17
Specification of techno-economic analysis (TEA) component.

TEA Component	Specification
CAPEX	Estimates total capital investment (TCI) for construction, equipment, and installation, often using the "major equipment factored approach".
OPEX	Feedstock cost (if any), utilities (electricity, water), enzyme/chemical costs, operating labor, and maintenance (per year).
Revenue	H ₂ selling price (\$/kg H ₂ , sensitivity range); VFA co-products (\$/kg H ₂); digestate as fertilizer (\$/ton); carbon credits (\$/ton CO ₂ -eq avoided).
Streams	
Financial Metrics	Project's financial performance: Levelized Cost of Hydrogen (LCOH, \$/kg H ₂); Net Present Value (NPV); Internal Rate of Return (IRR); Simple Payback Period (years).
Sensitivity Analysis	Assessment of economic parameter variations (electricity prices, capital costs, fuel prices, plant capacity) on project profitability; identifies key cost drivers and feasibility constraints.

targets, for example, commissioning at least two POME-to-hydrogen demonstration plants (>100 kg H₂/day) by 2028, leveraging the existing palm oil mill infrastructure identified in the scalability analysis (Section 5.6).

Unit diversity inventory.

mL H ₂ /g-VS	Volatile solids
mL H ₂ /g-TS	Total solids
mL H ₂ /g-sugar	Sugar (hydrolysate)
mL H ₂ /g-reducing sugar	Reducing sugar
mL H ₂ /g-COD	Chemical oxygen demand
mL H ₂ /g (basis not specified)	Not specified
mL H ₂ /L	Volume of reactor liquid
mL/g (basis not specified)	Not specified
mL/g-COD	Chemical oxygen demand
g H ₂ /g glucose	Glucose mass
mol H ₂ /mol glucose	Glucose (molar)
mol H ₂ /mol sugar	Sugar (molar)
mol H ₂ /mol substrate	Substrate (molar)
mol H ₂ /mol COD	COD (molar)
mol H ₂ /mol (basis not specified)	Not specified

Inter-conversion factors for hydrogen yield units.

Conversion	Factor	Notes/Assumptions
1 mol H ₂ at STP	= 22,414 mL	Ideal gas law; STP = 0 °C, 101.325 kPa
1 L H ₂ /kg VS	= 1 mL H₂/g VS	Dimensional equivalence (no conversion needed)
1 mol H ₂ /mol glucose	× 11,191 = mL H₂/g glucose	M(H ₂) = 2.016 g/mol; M (glucose) = 180.16 g/mol; assumes complete substrate conversion
Theoretical max (DF, acetate)	4 mol H₂/mol glucose	= 497.6 mL H ₂ /g glucose at STP; butyrate pathway = 2 mol H ₂ /mol glucose
1 mol H ₂ /mol sucrose	× 6558 = mL H₂/g sucrose	M(sucrose) = 342.30 g/mol; theoretical max = 8 mol H ₂ /mol sucrose
Energy content (LHV)	1 kg H₂ = 120 MJ	Lower heating value; used for efficiency calculations
Energy content (HHV)	1 kg H₂ = 141 MJ	Higher heating value; includes latent heat of water vapor
VS → sugar approximation	Feedstock-dependent	Requires compositional analysis; not applied in this review without source data

CRediT authorship contribution statement

Andrica Claudia Henry: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft. **Mohd Farid Atan:** Conceptualization, Data curation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Mohamad Afiq Mohd Asrul:** Project administration, Supervision, Writing – review & editing. **Josephine Chang Hui Lai:** Project administration, Supervision, Writing – review & editing. **Shanti Faridah Salleh:** Project administration, Supervision, Writing – review & editing. **Rafeah Wahi:** Project administration, Supervision, Writing – review & editing. **Hafizah Abdul Halim Yun:** Project administration, Supervision, Writing – review & editing. **Ivy Ai Wei Tan:** Project administration, Supervision, Writing – review & editing. **Florianna Lendai Michael Mulok:** Project administration, Supervision, Writing – review & editing. **Rosnah Abdullah:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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