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Recent trends in optimization and integration process on biohydrogen production from food waste: A review

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ABSTRACT

The rapid accumulation of food waste and the associated release of greenhouse gases have intensified the urgency to develop sustainable waste-to-energy strategies. Among renewable options, biohydrogen has emerged as a promising clean fuel due to its high energy density and zero-carbon emissions upon utilization. This review synthesizes recent advances in the optimization and integration of dark fermentation processes for biohydrogen production from food waste. Key considerations include substrate and inoculum selection, pretreatment methods, reactor configurations, and operational parameters such as pH, temperature, and retention time, all of which critically influence hydrogen yield and process stability. Current evidence highlights the effectiveness of carbohydrate-rich food residues combined with targeted thermal, chemical, and biological pretreatments in enhancing biodegradability and microbial activity. Similarly, inoculum pretreatment strategies selectively enrich hydrogenogenic consortia while suppressing methanogens, thereby stabilizing fermentation. Furthermore, integrated bioprocesses such as coupling dark fermentation with photo-fermentation, microbial electrolysis cells, lactic acid fermentation, or biomethane production have demonstrated significant improvements in energy recovery and system resilience. Computational approaches, including kinetic modeling, machine learning, and artificial intelligence, further enhance process predictability and scalability. Despite these advancements, challenges remain regarding substrate heterogeneity, economic feasibility, and large-scale stability. This review underscores the transformative potential of optimized and integrated dark fermentation systems, positioning biohydrogen production from food waste as a viable pathway toward sustainable energy transition and circular bioeconomy implementation.

Keywords: biohydrogen production, dark fermentation, optimization, integration process, organic waste, food waste, waste-to-energy.

INTRODUCTION

Background on biohydrogen production

Climate change has emerged as one of the most critical global challenges in recent decades, largely driven by human activities. The buildup of greenhouse gases (GHGs), including carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), in the atmosphere has resulted in a substantial rise in global temperatures, precipitating pervasive environmental and social consequences. These impacts encompass the melting of polar ice caps and glaciers, rising sea levels, and altered weather patterns. Such changes pose significant risks to biodiversity, disrupt ecosystems, and endanger key sectors such as agriculture, water security, and human health on a global scale. The severity of these effects underscores the urgent need for effective strategies to mitigate climate change and protect vulnerable communities (Miller, 2020; Sonwani and Saxena, 2022).

The primary factors contributing to elevated greenhouse gas (GHG) emissions include industrial expansion, the combustion of fossil fuels, deforestation, and inadequate waste management. According to the findings of the Intergovernmental Panel on Climate Change (IPCC) in 2023, industrial processes contributed approximately 34% of global carbon emissions in 2021, thereby underscoring their considerable environmental impact. Among the various waste streams, organic waste – particularly food residues, fruit, and vegetable scraps – exerts a substantial influence on GHG emissions. Globally, an estimated 1.3 billion tons of food are wasted annually, which corresponds to roughly one-third of all food produced for human consumption (Kinanti et al., 2021). The decomposition of organic waste in landfills under anaerobic conditions results in the generation of methane, a potent greenhouse gas with a global warming potential that is 25 times greater than that of CO_2. In countries such as Indonesia, where food waste constitutes over 40% of municipal solid waste, inadequate management exacerbates methane emissions, soil and water contamination, and public health concerns (Afrianto et al., 2021; Angelina et al., 2024).

In order to address these environmental challenges, many countries have adopted policies promoting renewable energy sources and sustainable waste management. Anaerobic digestion technologies are capable of transforming organic waste into biogas, which is composed primarily of methane and carbon dioxide. This process offers two key benefits: it reduces waste and produces renewable energy. Anaerobic digestion is a process in which organic material is broken down by microbes through a series of stages, including hydrolysis, acidogenesis, acetogenesis, and methanogenesis. This process has been shown to reduce landfill volume, mitigate GHG emissions, and produce biogas that can be used for heat, electricity, or fuel (Kwok et al., 2019; Pati et al., 2019). Biogas systems embody a zero-waste approach, wherein residuals can be further utilized as organic fertilizers, thereby contributing to circular bioeconomy models. Despite these advantages, challenges persist in the optimization of feedstock variability, operational parameters, and economic feasibility (Afridi and Qammar, 2020).

Biohydrogen has recently garnered attention as a promising alternative energy source produced from the anaerobic digestion of organic waste, including food residues. In contrast to biogas, biohydrogen boasts a higher energy content, with an approximate value of 142 MJ/kg, and produces only water upon combustion. This characteristic positions biohydrogen as a clean and sustainable fuel option (Biswal et al., 2023). The enzyme is predominantly produced during the hydrolysis and acidogenesis phases of dark fermentation, a process that is characterized by its simplicity, broad substrate applicability, and cost-effectiveness. The optimization of biohydrogen production can be achieved through the co-digestion of diverse organic waste streams, the adjustment of operational parameters such as pH and temperature, and the utilization of microbial consortia or genetic engineering approaches (Aldaby, 2023; Jayachandran and Basak, 2023a). While predominantly at the laboratory or pilot scale, biohydrogen possesses considerable potential to contribute to energy transition efforts and climate change mitigation by offering a renewable, clean fuel derived from waste valorization, thereby aligning with global sustainability objectives.

As illustrated in Figure 1, the result of the keyword analysis from VOSviewer related to biohydrogen production from food waste from articles published in the SCOPUS index journal from 2015 to 2025 demonstrates significant findings. The VOSviewer mapping illustrates the centrality of keywords ”dark fermentation”, ”food waste”, and ”organic waste” in the research landscape of biohydrogen production. The use of keywords such as ”substrate pretreatment”, ”microbial community”, ”photofermentation”, and ”lactic acid fermentation” underscores ongoing endeavors to enhance process optimization and technological integration. This bibliometric visualization underscores the multidisciplinary orientation of recent studies, reflecting a growing emphasis on efficiency, sustainability, and circular bioeconomy applications. Moreover, Figure 2 presents data regarding the number of articles published on the subject of bihydrogen production from food waste during the period from 2015 to 2025. It is evident from the figure that there has been an increase in the number of published articles from 2022 to 2024.

Challenges in biohydrogen production from food waste

The process of biohydrogen production from food waste is confronted by numerous technical challenges, which directly impact its efficiency

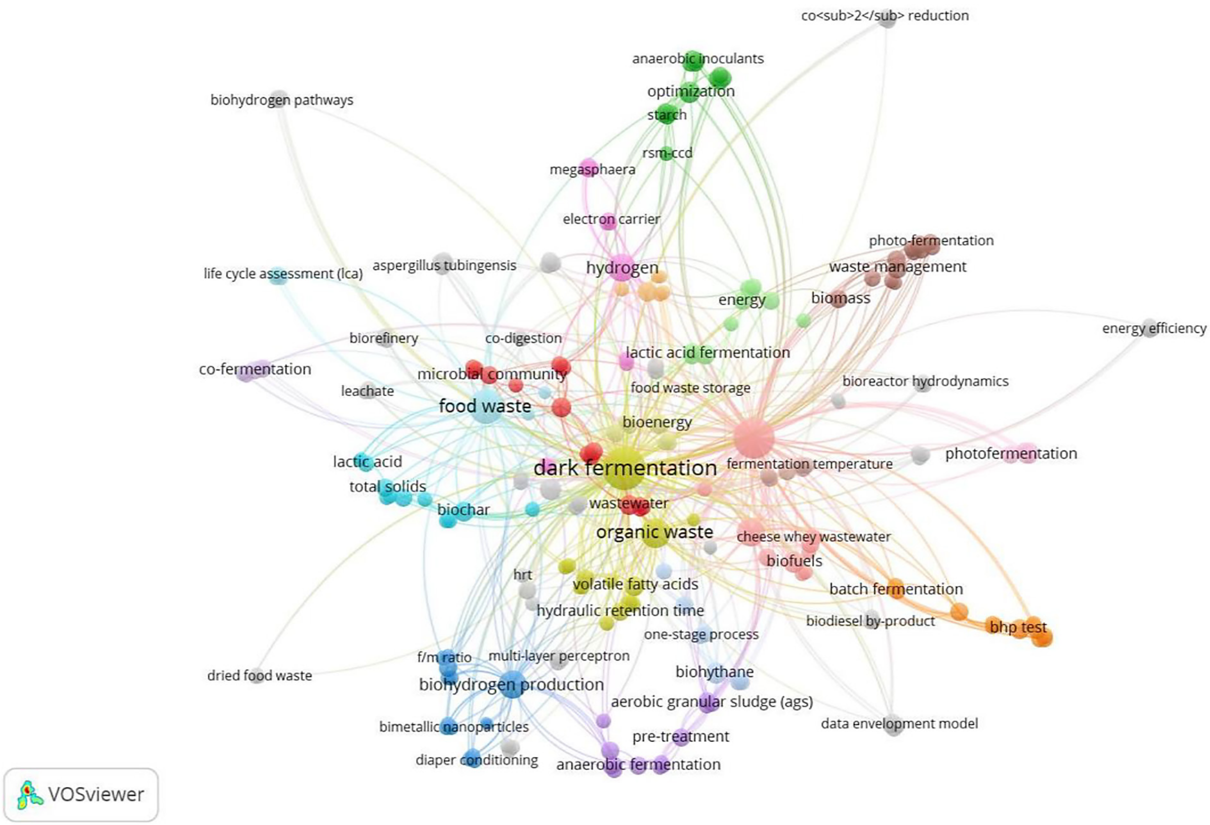

Figure 1. Keywords analysis from VOSviewer related to biohydrogen production from food waste from articles published in the span of 2015–2025

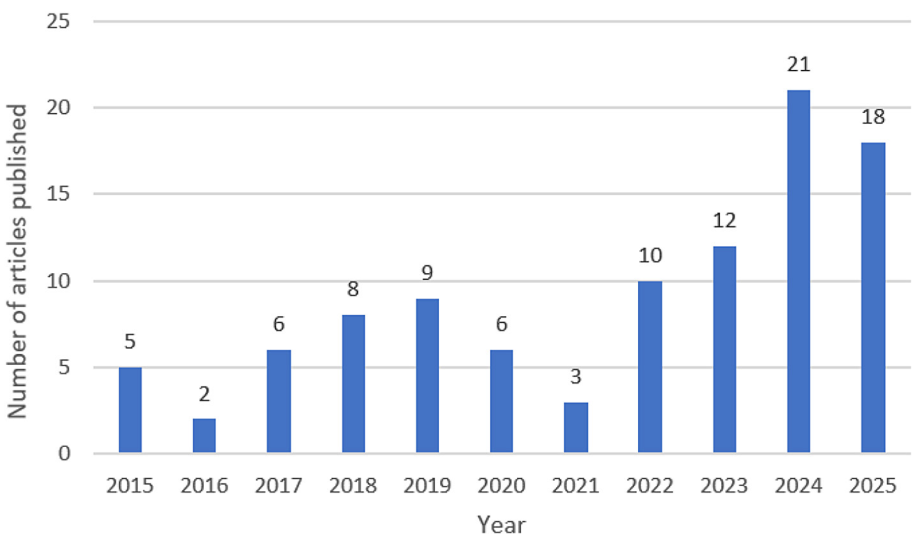

Figure 2. Number of articles published related to biohydrogen production from food waste from articles published in the span of 2015–2025

and consistency. A primary concern is the heterogeneity of food waste, which consists of varying proportions of carbohydrates, proteins, and fats. These components require different enzymatic pathways for microbial breakdown. This inherent complexity complicates the optimization of fermentation processes, as microbial communities must adapt to fluctuating substrate compositions, often resulting in inconsistent hydrogen yields (Husaini et al., 2023; Pradhan et al., 2024). Furthermore, food waste may contain inhibitory compounds, such as heavy metals and antimicrobial agents, which have been shown to suppress the microbial activity essential for hydrogen generation (Husaini et al., 2023). During the fermentation process, by-products such as volatile fatty acids accumulate, leading to a decrease in pH and further inhibition of microorganisms. This, in turn, results in a reduction in biohydrogen output (Hasibar et al., 2020).

The challenges associated with production efficiency and scalability represent significant obstacles in the realm of biohydrogen development. Notwithstanding technological advancements, the actual hydrogen yields remain considerably lower than the theoretical maximums. This discrepancy can be attributed to various factors, including suboptimal microbial strains, limitations in reactor design, and inadequate process control (Pradhan et al., 2024). Genetic engineering approaches, including CRISPR-Cas9, have demonstrated potential in enhancing microbial performance; however, they remain in the preliminary experimental stages and encounter regulatory challenges (Husaini et al., 2023). Furthermore, the process of increasing the scale of biohydrogen production necessitates continuous monitoring and precise adjustment of variables such as temperature, pH, and nutrient balance. This process can be both resource-intensive and technically complex, impeding a smooth transition from laboratory to industrial scale (Pradhan et al., 2024).

Another critical challenge that hinders the large-scale adoption of biohydrogen technologies is economic feasibility. The substantial capital expenditures required for the acquisition of specialized anaerobic digesters and bioreactors, in conjunction with the persistent operational expenses necessary to sustain optimal fermentation conditions, give rise to considerable financial impediments (Vishali et al., 2023). Additionally, the biohydrogen market is in its nascent stages of development, characterized by limited demand and underdeveloped distribution networks. This market environment presents significant challenges in achieving cost reductions through economies of scale (Pradhan et al., 2024). The present state of affairs reveals an ongoing concentration on the integration of biohydrogen production with existing waste management infrastructures and the development of cost-effective bioreactor designs. Nevertheless, the maturation and economic viability of these solutions will continue to constrain widespread implementation (Vishali et al., 2023).

This review aims to provide a comprehensive synthesis of recent advances in optimization processes and integration mechanisms in biohydrogen production via dark fermentation with food waste as substrate, which is this topic has not been explored in previous studies. Given the urgent global need to reduce greenhouse gas emissions and promote sustainable energy sources, biohydrogen presents a promising alternative fuel due to its high energy content and environmentally friendly combustion. However, the heterogeneous nature of food waste, the complexity of microbial fermentation processes, and the technical and economic challenges in scaling up biohydrogen production require a comprehensive understanding of the various factors that affect efficiency and feasibility. Therefore, this paper seeks to collate the latest knowledge from various biohydrogen testing results through dark fermentation. This will provide additional insights into optimization strategies through substrate and inoculum selection, effective pretreatment methods, bioreactor design, operational parameters, and explore efforts to integrate dark fermentation with other bioprocesses such as photofermentation, microbial electrolysis cells (MEC), lactic acid fermentation, and biomethane production. The main objectives are to increase biohydrogen yield, process effectiveness and stability, and identify key areas for improvement and potential future research.

This review explores innovative optimization strategies, including anaerobic co-digestion of mixed organic waste, advanced pretreatment techniques for substrates and inoculum, and emerging genetic and metabolic engineering approaches aimed at increasing microbial hydrogen yield. Furthermore, this review covers the application of kinetic and computational models, such as the Gompertz and Monod models, and the integration of machine learning and artificial intelligence to better predict and control fermentation dynamics. Beyond standalone dark fermentation, this research highlights integrated bioenergy systems that combine dark fermentation with complementary technologies such as photofermentation, MECs, lactic acid fermentation, and biomethane production. These integrated efforts demonstrate the potential to maximize energy recovery from food waste while addressing operational stability and environmental sustainability. Finally, this review discusses the techno-economic aspects of biohydrogen production, emphasizing the importance of evaluating capital and operational costs alongside environmental benefits to assess industrial-scale feasibility. By unifying technical, economic, and sustainability dimensions, this review provides an original and holistic roadmap for scaling up biohydrogen production toward industrial applications and a circular bioeconomy.

Biohydrogen production from dark fermentation process

Biohydrogen from dark fermentation

Biohydrogen production is emerging as a sustainable energy solution to reduce greenhouse gas emissions. Various biotechnological processes, including dark fermentation, photofermentation, and microbial electrolysis cells, are being investigated for hydrogen generation. Dark fermentation, in particular, is gaining attention due to its simplicity, cost-effectiveness, and ability to utilize a wide range of organic substrates like food waste and agricultural residues. This process relies on anaerobic microorganisms to convert organic matter into hydrogen gas without the need for light, making it energy-efficient and suitable for large-scale applications (Aziz et al., 2022; Kucharska et al., 2021). It is particularly attractive for waste-to-energy solutions, as it can utilize abundant organic waste while avoiding high energy inputs (Yahmed et al., 2021). Additionally, the use of thermophilic bacteria enhances hydrogen production by efficiently breaking down carbohydrates, producing both hydrogen and valuable by-products (Aziz et al., 2022). Recent innovations, such as the inclusion of magnetic nanoparticles, have further improved the efficiency of dark fermentation (Kucharska et al., 2021). With ongoing research, dark fermentation holds significant potential for advancing sustainable energy solutions, especially when combined with other biotechnological processes (Ncube et al., 2023).

Dark fermentation is one of the key processes that occurs during anaerobic digestion and is particularly relevant for biohydrogen production. This process involves the breakdown of organic matter in the absence of light, where microorganisms decompose complex compounds to generate biohydrogen, among other by-products. Anaerobic digestion occurs through four primary stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis, with each stage contributing to the overall process. Initially, hydrolysis breaks down complex organic materials, such as carbohydrates, fats, and proteins, into simpler compounds, including soluble sugars, amino acids, and fatty acids, facilitated by hydrolytic bacteria (Vanyan et al., 2022). During the subsequent acidogenesis stage, acidogenic bacteria ferment these soluble compounds into volatile fatty acids (VFAs), hydrogen gas, and carbon dioxide. The production of biohydrogen is particularly significant in this phase, as hydrogen is produced alongside VFAs during fermentation (Iragavarapu et al., 2023; Sahota et al., 2024). In the acetogenesis stage, acetogenic bacteria convert VFAs into hydrogen and acetate, which are essential substrates for methanogens. Finally, in methanogenesis, methanogenic archaea utilize hydrogen and acetate to produce methane (CH_4) and carbon dioxide (Czatzkowska et al., 2022; Ruiz-Aguilar et al., 2022). Thus, biohydrogen is primarily generated during the acidogenesis and acetogenesis stages of dark fermentation in anaerobic digestion (Figure 3).

A multitude of factors have been identified as influential in the context of biohydrogen yields during dark fermentation. The substrate type and composition play a crucial role in this process, as the nutrient balance, especially the carbon-to-nitrogen ratios, affects microbial metabolism. Operational parameters such as pH, temperature, and substrate-to-inoculum ratio have been demonstrated to significantly impact microbial activity and hydrogen production efficiency. For instance, a range of studies have identified thermophilic conditions (approximately 50–55 °C) and slightly acidic to neutral pH values as optimal for biohydrogen yield optimization. Furthermore, the structure of microbial communities, including the utilization of co-cultures or genetically engineered strains, has been demonstrated to enhance substrate degradation and hydrogen output. Pretreatment of substrates and process optimization through co-digestion strategies have also been demonstrated to improve stability and scalability of hydrogen production (Hasibar et al., 2020; Kim et al., 2022; Tagne et al., 2024).

Advantages and challenges of biohydrogen production from the dark fermentation process

The process of biohydrogen production through dark fermentation has been shown to offer several distinct advantages when compared with other biohydrogen generation methods. A salient benefit of this approach is its capacity to employ

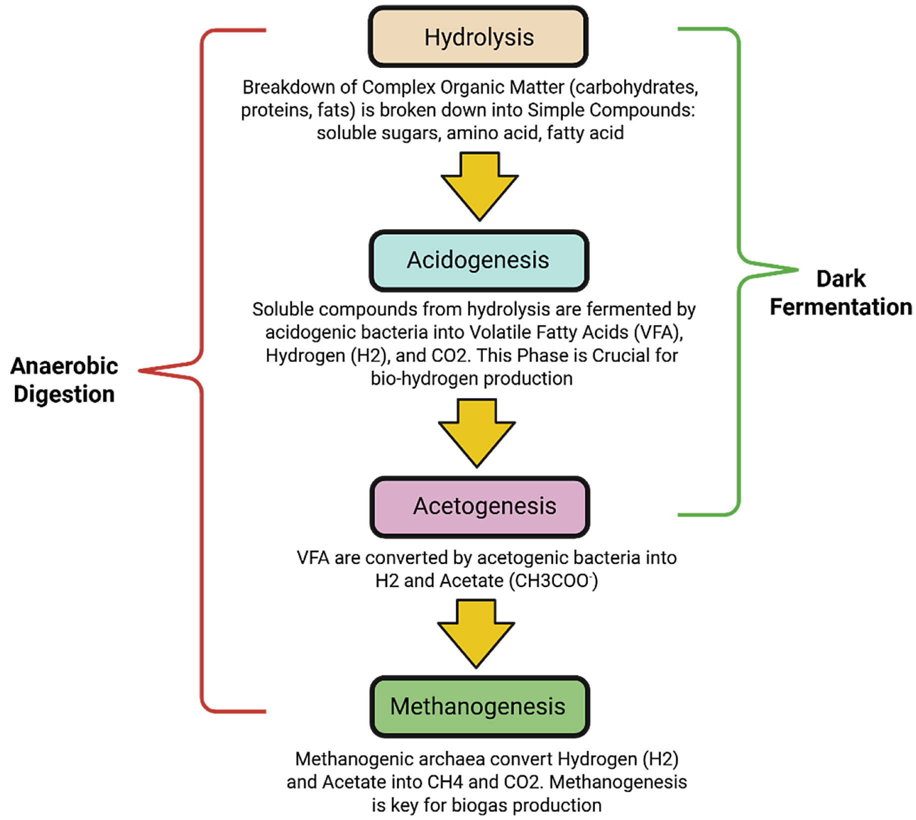

Figure 3. Main stages of biohydrogen production via dark fermentation

a diverse array of organic substrates, including agricultural residues, food waste, and industrial by-products. These substrates are plentiful and inexpensive, thereby ensuring the economic viability of the process (Pascualone et al., 2019). In contrast to the process of photo-fermentation, dark fermentation does not require light, thereby streamlining operational requirements and reducing energy expenditures. Consequently, this method is well-suited for large-scale applications and can tolerate a wide range of environmental conditions (Domińska, 2024). Furthermore, dark fermentation has been demonstrated to achieve high hydrogen yields due to the efficient metabolism of microbial communities involved in the process. Concurrently, dark fermentation minimizes greenhouse gas emissions by converting organic waste into clean energy rather than methane or carbon dioxide (Domińska, 2024; Li et al., 2019; Pascualone et al., 2019). This positions dark fermentation as a promising sustainable technology that supports circular bioeconomy goals.

Notwithstanding the aforementioned benefits, dark fermentation is confronted with numerous substantial challenges that hinder its extensive implementation and efficiency. A significant challenge pertains to substrate inhibition, wherein elevated concentrations of organic material can result in metabolite accumulation, including organic acids, consequently leading to diminished microbial activity and reduced hydrogen production (Gorgec and Karapinar, 2019). The intricate dynamics inherent within microbial communities also present challenges, as competing organisms, such as methanogens and lactic acid bacteria, have the capacity to consume hydrogen or produce inhibitory compounds, thereby reducing overall yields (Canto-Robertos et al., 2023; Domińska, 2024). Furthermore, the accumulation of toxic by-products, such as hydrogen sulfide, has the potential to adversely affect the viability of hydrogen-producing microbes, thereby exacerbating process instability (Asman et al., 2024). Operational parameters such as pH, temperature, and retention time require precise control; fluctuations in these parameters can shift microbial metabolism toward less desirable pathways like methane production (Aldaby, 2023). Economic considerations remain critical, as pretreatment, reactor costs, and process optimization must be addressed to ensure competitiveness with other hydrogen production technologies (Soares et al., 2020).

The utilization of food waste as a substrate in the context of dark fermentation has been demonstrated to yield significant advantages and substantial challenges. Food waste is a rich source of fermentable carbohydrates and nutrients, making it an excellent feedstock that not only supports renewable energy production but also helps mitigate environmental problems related to waste disposal (Jayachandran et al., 2022; Pradhan et al., 2024). Pre-treatment methods, such as hydrolysis, have been shown to enhance sugar availability, thereby improving hydrogen yields when fermentation parameters are optimized (Ghosh, 2022). However, the heterogeneous nature of food waste complicates process consistency, as variable compositions require adaptable microbial communities and tailored operational conditions (Husaini et al., 2023). Furthermore, inhibitors present in some food wastes and the acidic by-products generated can impede microbial performance, while the management of residual effluents remains an environmental concern (Hasibar et al., 2020; Vishali et al., 2023). Achieving high, stable hydrogen yields and economic scalability thus necessitates ongoing research in microbial engineering, process control, and integrated waste-to-energy system design (Husaini et al., 2023; Pradhan et al., 2024).

Substrate and inoculum preparation (selection and pretreatment)

Substrate selection from food waste

The characteristics of suitable substrates play a pivotal role in optimizing biohydrogen production via dark fermentation. The ideal substrates are characterized by an abundance of readily biodegradable organic matter, particularly carbohydrates, which function as the primary energy source for hydrogen-producing anaerobic bacteria. The high carbohydrate content of the substrate favors metabolic pathways such as the butyrate fermentation route, which is closely linked to high hydrogen yields (Guo et al., 2014). For instance, starch-rich waste materials, such as cassava, demonstrate optimal hydrogen production under controlled pH conditions (Tagne et al., 2024). Biomass with complex lignocellulosic structures, such as sugarcane bagasse or aquatic plants, also holds potential but requires pretreatment, like enzymatic hydrolysis, to convert cellulose and hemicellulose into fermentable sugars (Bekbayev et al., 2021). Proper pretreatment and substrate preparation have been shown to enhance substrate accessibility and microbial utilization, which are essential for maximizing biohydrogen yields (Chen et al., 2010).

Various types of food waste types have been identified as particularly promising substrates for biohydrogen production, owing to their abundant and diverse organic composition. Fruit peels (e.g., banana, mango, pineapple), vegetable residues (e.g., onion, sweet potato), and leftover cooked rice are abundant carbohydrate sources that support microbial fermentation. For instance, the yield of H2/gVS from mashed onions has been reported to reach up to 151.67 mL under optimized fermentation conditions (Panin et al., 2021). Similarly, combined fruit and vegetable waste treated by mild heat has been shown to yield 63.0 mL H2/gVS (Pascualone et al., 2019). The synergy present in mixed substrates has been shown to balance nutrients and improve microbial activity, often resulting in superior performance compared to single-substrate fermentation (Rodríguez-Valderrama et al., 2020). Conversely, lignin-rich substrates necessitate pretreatment methods such as microwave-assisted hydrolysis to enhance enzymatic digestibility and hydrogen production (Rokhati et al., 2023). Consequently, the judicious selection and preparation of suitable substrate types is paramount for ensuring the efficacy of dark fermentation.

Substrate concentration is a critical factor in ensuring efficient biohydrogen production, as it directly affects microbial metabolism and pathway selection. Optimal concentrations provide sufficient carbon and energy for microbial growth without causing substrate inhibition due to excess metabolite accumulation, such as volatile fatty acids, which can lower pH and suppress hydrogenase activity (Ghosh, 2022). For instance, the fermentation of food waste at approximately 60 gVS/L resulted in a peak hydrogen production of 120.78 mL H2/gVS (Xue et al., 2024). However, concentrations that exceed this range may lead to osmotic stress and microbial inhibition. Furthermore, substrate concentration exerts a significant influence on metabolic flux, promoting hydrogen-producing pathways at balanced levels but diverting to non-hydrogenic routes such as propionate formation when overloaded (Hasibar et al., 2020). Strategies such as fed-batch feeding and co-digestion of complementary substrates have been demonstrated to assist in maintaining optimal concentrations, thereby fostering stable microbial consortia and maximizing hydrogen yield (Pradhan et al., 2024).

Pretreatment methods on substrates

Pretreatment of substrates constitutes a pivotal step in optimizing biohydrogen production from dark fermentation. This process enhances the availability of easily degradable organic matter, reduces inhibitory compounds, and promotes the selection of hydrogen-producing microorganisms. Pretreatment methods are typically classified into three categories: physical, chemical, and biological. Physical methods, including thermal treatment, have been demonstrated to disrupt complex structures and enhance substrate solubility. Chemical pretreatments frequently entail the use of acid or alkali hydrolysis, a process that disrupts the structure of lignocellulosic biomass, thereby enhancing the accessibility of fermentable sugars. Biological pretreatments employ enzymes or microbial consortia to degrade complex polymers into simpler compounds. The selection of pretreatment modalities is contingent upon substrate characteristics and operational conditions, thereby ensuring efficient conversion during the fermentation process (Monlau et al., 2012; Pau et al., 2023).

Among these techniques, thermal pretreatment is widely applied to food waste substrates due to its effectiveness and simplicity. For instance, heating food waste at moderate temperatures (approximately 63–121 °C) for a predetermined duration has been shown to enhance biohydrogen yield by increasing organic solubility and reducing native microbial competitors (Pascualone et al., 2019; Rashidi et al., 2024). The combination of acid hydrolysis and heating has been shown to enhance sugar availability from fruit and vegetable waste, thereby significantly increasing hydrogen production (Rodríguez-Valderrama et al., 2020). Thermophilic fermentation subsequent to thermal pretreatment has been demonstrated to yield hydrogen production rates 5–10 times higher than those observed under mesophilic conditions. This enhancement has been attributed to an augmented microbial activity and substrate breakdown (Lin et al., 2020). In essence, the selection of an optimal pretreatment, customized to the substrate type, is imperative for achieving maximum biohydrogen production efficiency and sustainability (Monlau et al., 2012; Rashidi et al., 2024).

Inoculum selection

The selection of an appropriate inoculum constitutes a critical step in the optimization of biohydrogen production via dark fermentation. The inoculum serves as the source of active microbial communities that convert organic substrates into hydrogen gas. A suitable inoculum must harbor a robust population of hydrogen-producing bacteria, such as Clostridium species, while minimizing hydrogen-consuming microbes, like methanogens. Thermal pretreatment of inoculum, particularly anaerobic sludge derived from wastewater treatment facilities (WWTF), is a widely implemented method for the selective suppression of methanogens and the enrichment of spore-forming hydrogen producers. For instance, heating anaerobic sludge at 100 °C for 30 minutes has been shown to significantly improve biohydrogen yields by favoring Clostridium dominance (Hidalgo et al., 2023). The implementation of pretreatment strategies has been demonstrated to enhance microbial activity and optimize process efficiency.

Various inoculum types have been employed with success in the context of dark fermentation. Anaerobic sludge from WWTP remains the most prevalent due to its microbial diversity and availability. Alternative inocula include vermicompost, which is rich in Clostridium spp. and has demonstrated promising hydrogen production of up to 63 mL H2/gVS with mild substrate pretreatment (Pascualone et al., 2019). Sediments from forest wetlands and lakes have also been explored, with thermal pretreatment sometimes being required to optimize performance (Kim et al., 2024; Xue et al., 2024). It has been demonstrated that combining inocula from multiple sources, such as digesters treating cattle manure, tofu waste, and fruit residues, can produce synergistic effects, yielding significantly higher hydrogen volumes up to 231 mL H2/gVS than single inocula (Amekan et al., 2018).

The microbial consortium present within the inoculum is a critical factor in achieving optimal biohydrogen production. The presence of diverse microbes fosters the expression of complementary metabolic pathways and a phenomenon known as cross-feeding, thereby enhancing substrate breakdown and hydrogen yield. Clostridium species utilize the acetyl-CoA pathway to produce hydrogen, while Enterobacter species employ mixed acid fermentation, frequently yielding less hydrogen due to by-products such as ethanol and lactic acid (Hasibar et al., 2020; Sun et al., 2019).

The co-culturing of Clostridium acetobutylicum and Enterobacter aerogenes has been demonstrated to enhance yields by reducing inhibitory compounds (Martinez-Burgos et al., 2019; Wang et al., 2021). The degradation of carbohydrates, proteins, and lipids is facilitated by microbial diversity, thereby ensuring stable production in the face of environmental changes (Singh et al., 2023; Vishali et al., 2023). Cross-feeding by acetogens maintains pH and reduces inhibition, further stabilizing fermentation (Ji and Shen, 2024; Zhang et al., 2020). The strategic selection of inoculum and pretreatment are of paramount importance for the efficient and sustainable biohydrogen production (Husaini et al., 2023).

Pretreatment methods on inoculum

Pretreatment of inoculum is a critical step in optimizing biohydrogen production through dark fermentation. This process aims to enhance the selectivity for hydrogen-producing microorganisms while inactivating hydrogen-consuming bacteria such as methanogens, thereby improving the biodegradability and metabolic efficiency of the inoculum. Various pretreatment methods including thermal, acidic, alkaline, and combined techniques have been widely applied to achieve this goal. Thermal pretreatment is among the most commonly used; heating inoculum at temperatures between 90 °C and 121 °C for durations ranging from minutes to an hour effectively suppresses methanogens without harming spore-forming hydrogen producers like Clostridium spp. For example, Hidalgo et al. (2023) demonstrated that thermal treatment at 100 °C for 30 minutes increased hydrogen production to 121 mL/g glucose by promoting Firmicutes dominance (Hidalgo et al., 2023). Similarly, Domińska et al. (2024) reported enhanced biohydrogen yield from food waste inoculum treated at 70 °C (Domińska, 2024). Other studies confirm that thermal pretreatment minimizes hydrogen losses through methanogenesis, supporting more efficient hydrogen generation (Wongthanate and Chinnacotpong, 2015).

In addition to thermal methods, acidic pretreatment has been demonstrated to be effective in suppressing hydrogen-consuming microbes by lowering inoculum pH. Amekan et al. (2018) demonstrated that reducing inoculum pH to 3 for 24 hours enriched microbial communities that favor higher biohydrogen production (Amekan et al., 2018). Butyric acid pretreatment has also been demonstrated to enhance hydrogen yield to a considerable extent (Noguer et al., 2022). Innovative combinations, such as sonication coupled with hydrogen peroxide treatment, enhance microbial cell permeability and further suppress competitors, achieving up to 125% increase in hydrogen production from inoculum derived from cattle manure and cheese whey (Hangri et al., 2024). In general, pretreatment methods such as thermal, acid, and sonication have been shown to selectively promote the growth of hydrogen-producing bacteria. These methods have also been observed to reduce inhibitory by-products and stabilize fermentation performance. The selection of pretreatment should take into account the inoculum type, substrate characteristics, and fermentation conditions to optimize biohydrogen production efficiency (Hangri et al., 2024; Hidalgo et al., 2023).

Recent experiment on selection and pretreatment to enhance biohydrogen

Substrate selection and pretreatment are crucial factor in achieving high biohydrogen yields, as organic composition directly affects fermentation and microbial accessibility. Table 1 provides information on substrate types and pretreatments commonly used in optimization strategies. Among the many options explored, food waste has emerged as the most frequently used substrate in recent research due to its abundance, high carbohydrate content, and ease of collection. For example, Roslan et al. (2025) utilized food waste stored under lactic acid fermentation for 15 days, reporting substantial improvements in substrate solubility and microbial availability (Roslan et al., 2025). Carbohydrate-rich materials such as cassava waste and fruit or vegetable by-products also show great potential. In a recent study, Ngamnurak et al. (2025) demonstrated that food waste subjected to autoclaving at 121 °C for 15 minutes yielded 1.137 mL H_2/L, showing the effectiveness of thermal sterilization in suppressing microbial competitors and improving solubility (Ngamnurak et al., 2025). Likewise, cassava and pineapple wastes that were dried at 105 °C for 24 hours and ground to particle sizes below 20 mm produced notable hydrogen outputs of 125.7 and 153.5 mL H_2/g VS, respectively (Tagne et al., 2024). Rodríguez-Valderrama et al. (2020) applied dilute acid hydrolysis with HCl or H_2SO_4 at 0.5%, combined with heating at 120 °C for 120 minutes, followed by overliming with $Ca(OH)_2$, and reported a hydrogen molar yield of 2.31 mol

H_2/mol glucose (Rodríguez-Valderrama et al., 2020). Mechanical disruption, such as grinding to a fine size or mild thermal treatment in a water bath at 63 °C for 30 minutes, has also been shown to enhance the release of fermentable sugars and reduce inhibitory native microbiota, as demonstrated in Pascualone et al. (2019). These examples from the several recent experiments in table 1 indicate that the interplay between substrate characteristics and targeted pretreatment defines the extent of hydrogen production.

Inoculum selection and preparation conditions are both crucial for maximizing hydrogen production efficiency. Table 1 shows the various inoculum types and pretreatment techniques commonly applied in optimization strategies. Wastewater treatment plant sludge, anaerobic digestate, and enriched microbial consortia are the most widely studied inoculum sources due to their microbial diversity and accessibility. For instance, Domińska et al. (2024) employed sludge from both a municipal wastewater treatment plant and an anaerobic dairy digester, applying thermal shocks at 70 °C, 90 °C, and 121 °C, which resulted in hydrogen yields of 96 and 93 cm^3 H_2/gTVSFW, respectively (Domińska, 2024). One of the most compelling recent findings is that of Kim and Cho (2025), who enriched a thermophilic bacterial consortium derived from freshwater sediment, wetland, and forest puddle environments, obtaining hydrogen production rates of 353–403 $mL\cdot L^{-1}\cdot h^{-1}$ with H_2 concentrations of 55–62%. The microbial community itself plays a decisive role: Clostridium species are dominant hydrogen producers via the acetyl-CoA pathway, while Enterobacter species perform mixed acid fermentation, sometimes yielding less hydrogen due to ethanol and lactic acid by-products. Coculturing these strains reduces inhibitory compounds and enhances hydrogen yields(Wang et al., 2021). Pretreatment of inoculum is commonly carried out using thermal shocks. For example, Ngamnurak et al. (2025) heat-treated anaerobic granules at 105 °C for 2 hours to inhibit methanogens, significantly enriching hydrogen-producing populations (Ngamnurak et al., 2025). Lin et al. (2020) showed that heating sewage sludge at 95 °C for one hour selectively inhibited hydrogen-consuming microbes while enhancing hydrogen production (Lin et al., 2020). Additionally, Vázquez-López et al. (2024) demonstrated that inoculum sourced from a mesophilic anaerobic digester treating flour industry waste, after thermal shock at 105 ± 5 °C for 24 hours, yielded up to 265 mL H_2/g VS, reinforcing the importance of source type and pretreatment intensity (Vázquez-López and Moreno-Andrade, 2024). More innovative strategies, such as combining hydrogen peroxide (0.06 g/gTS) with sonication at 1419.36 J/gTS and following with thermal pretreatment at 105 °C for 1.5 hours, have also been successfully applied to digestates, resulting in significant yield improvements (Hangri et al., 2024). These findings highlight how inoculum origin, microbial diversity, and precise pretreatment conditions are decisive in shaping microbial activity and optimizing hydrogen yields.

In conclusion, the studies reviewed demonstrate that biohydrogen production is most effective when substrate and inoculum strategies are integrated thoughtfully. Substrates with high carbohydrate content, whether derived from food waste, cassava, or fruit and vegetable residues, must be supported by pretreatments that improve solubility, release fermentable sugars, and suppress native microbial competitors. At the same time, inocula sourced from wastewater, compost, or environmental sediments require pretreatment especially thermal or combined approaches to enrich hydrogen producers such as Clostridium while inhibiting methanogens. The evidence indicates that it is the synergy between substrate accessibility and inoculum selectivity that governs hydrogen yield and process stability. This dual focus not only advances the technical efficiency of dark fermentation but also provides a pathway toward sustainable and scalable biohydrogen production.

Bioreactor design and operational parameters

Bioreactor design

Bioreactor design is fundamental to the effective production of biohydrogen through dark fermentation, with several reactor types commonly employed in research and industrial applications. Batch reactors are widely used in laboratory and pilot studies due to their operational simplicity and ease of control. In these systems, the substrate is loaded at the start, and fermentation proceeds without additional input until completion. This setup is ideal for kinetic studies and optimization of process parameters on a small scale (Anjum et al., 2023). Continuous stirred-tank reactors (CSTRs), by contrast, facilitate the continuous feeding of substrates

Table 1. Recent experiment results (2019–2025) refer to selection and pretreatment for substrates and inoculum

No	Author and year	Substrate	inoculum	Pretreatment substrate	Pretreatment inoculum	Biohydrogen production
1	Ngamnurak, et al 2025 (Ngamnurak et al., 2025)	Glucose, Lactic acid, Acetic acid, and Food waste	Anaerobic granules (non-enriched) + enriched hydrogen-producing consortium	Food waste: autoclaved (121 °C, 15 min) or non-autoclaved; model substrates (glucose, lactic acid, acetic acid) used directly	Anaerobic granules heat-treated at 105 °C for 2 h to inhibit methanogens	From glucose: 1,605 ± 161 mL-H_2/L; from food waste: 1,137 mL-H_2/L (56.85 mL-H_2/g-VSadded)
2	Kim and Cho (2025) (Kim and Cho, 2025)	Food waste (Seoul, Korea)	Mixed thermophilic bacterial consortium (freshwater sediment, wetland, forest puddle sediment)	Food waste blended, diluted (1:3 with water), supplemented with minerals, adjusted to pH 6.0, autoclaved (121 °C, 15 min)	Inoculum pre-cultured through successive batch enrichments at 50 °C, with N_2 purging; no mention of heat shock	H_2 production rate: 353–403 $mL \cdot L^{-1} \cdot h^{-1}$; H_2 concentration: 55–62% (v/v)
3	Roslan et al. (2025) (Roslan et al., 2025)	Food waste (France)	Activated sludge from urban wastewater treatment plant (Narbonne, France)	Food waste stored under lactic acid fermentation (LAF) for 15 days at 4–55 °C, 10% TS	Activated sludge centrifuged, freeze-dried, stored at −80 °C; no heat pretreatment applied	Max yield (Pm): 57–94 mL/gVS; Max rate (Rm): up to 183 ± 14 mL/gVS·d (250% higher with exogenous inoculum)
4	Tagne et al. (2024) (Tiegam Tagne et al., 2024)	Cassava waste (starch-rich), Pineapple waste (hemicellulose-rich)	6 inocula dari full-scale anaerobic digesters di Italia,	Cassava: washed, dried in a 105 °C oven for 24 hours, ground with a hammermill to a size of <20 mm. Pineapple: freeze-dried	Heat treatment 100 °C selama 4 jam	Pineapple; 153.5 mL H2/g VS. Cassava; 125.7 mL H2/g VS
5	Domińska et al., 2024 (Domińska, 2024)	Food waste (dari rumah tangga, terutama limbah sayuran, tanpa limbah hewani)	1) Digested sludge dari WWTP (Wastewater Treatment Plant) Lodz 2) Sludge dari anaerobic dairy industry wastewater (DIW)	Substrate food waste collected from households. Shredded less than 3 mm particle size and stored frozen at -20°C. No chemical or thermal pretreatment.	Inoculum pretreatment involved thermal shock at 70 °C, 90 °C, and 121 °C for sludge from both sources, applied in a steam sterilizer.	96 cm^3 H2/gTVSFW for WWTP sludge. 93 cm^3 H2/gTVSFW for dairy industry wastewater.
6	Vázquez-López et al. (2024) (Vázquez-López and Moreno-Andrade, 2024)	Food Waste (FW) from municipal market	Mixed culture inoculum from mesophilic anaerobic digester of flour industry	Food waste crushed to particle size <5 mm; cornWW stored at -4 °C	Thermal shock at 105 ± 5 °C for 24h to eliminate methanogens and hydrogen-consuming bacteria; disintegrated and sieved (850 µm mesh)	Hydrogen yield 241.8–265 mL H2/gVS removed
7	Kim et al. (2024) (Kim et al., 2024)	Food waste from biogas plant	5 inokulum sources; Anaerobic digestion sludge, Freshwater sedimen, Wetland, Forest soil, Forest puddle sediment (FP)	Food waste is ground, autoclaved at 121 °C for 15 minutes.	Pretreatment pemanasan 105 °C selama 2 jam untuk menghilangkan bakteri non-hidrogen	3900 mL/L Using Wetland as inoculum

8	S. Hangri, K. Derbal, et al. (2024) (Hangri et al., 2024)	Dairy cow manure (DCM) and cheese whey (CW)	Degassed anaerobic digestate obtained from a full-scale plant treating cow manure.	Combined pretreatment using H2O2 (0.06 g/gTS) with ultrasonic specific energy input (USEI) of 1419.36 J/gTS. Pretreatment improved carbohydrates solubilization.	Thermal pre-treatment at 105 °C for 1.5 hours to inhibit methanogens in the inoculum (degassed anaerobic digestate).	334.90 mL/L
9	Villanueva-Galindo et al. (2023) (Villanueva-Galindo et al., 2024)	Food Waste (FW) from cafeteria	a) Sludge from anaerobic wastewater treatment in flour industry b) Microorganisms naturally present in HLa effluent	Ground with an industrial grinder, stored at −4 °C	Pretreated sludge dried at 105 °C for 24 h and ground (<65 µm); native microbial community not pretreated	596.3 mL H2/ Lreactor
10	Nam (2023) (Nam, 2023)	Mixture of food waste and sewage sludge.	Seed sludge from an anaerobic digester in a publicly owned treatment facility,	Alkali pretreatment using KOH at pH 13.0 was applied to enhance solubilization and disinfection of the substrate.	Seed sludge was heated at 90 °C for 30 minutes to select for spore-forming bacteria	Hydrogen yield 152.1 mL H2/g VS
11	Jinman Cao et al. (2022) (Cao et al., 2022)	Potato peel waste, from a university canteen	Anaerobic digester sludge	Dried in an oven, milled to about 20-mesh prior to fermentation.	Four pretreatment methods: heat-shock (100 °C for 15 min), aeration (24 h), acid (pH 3.0 for 24 h), and base (pH 10.0 for 24	71.0 mL/g-VS added.
12	Panin et al. (2021) (Panin et al., 2021)	Vegetable residues: broccoli, onion, and sweet potato.	Enriched microflora from the effluent of continuous hydrogen production fermenter.	Vegetables were prepared in mashed and powdered forms. Mashed vegetables were grinded to about 3 mm, while powdered vegetables were dried at 105 °C for 24 hours and then grinded.	No specific pretreatment mentioned; inoculum was enriched microflora from continuous fermenter effluent	Cumulative hydrogen 424.1 mL H2. Hydrogen yield 151.67 mL H2/g VS added
13	Chiu-Yue Lin et al. (2020) (Lin et al., 2020)	Kitchen waste from a café restaurant	Anaerobic digester sludge collected from a sewage treatment plant.	Kitchen waste was ground using a juice mixer to prepare it as a substrate. The substrate was stored at 4°C before use.	The inoculum (digested sludge) was heat-treated at 95 °C for 1 hour to inactivate hydrogen-consuming microorganisms and activate hydrogen-producing ones.	Hydrogen yield 75.4 mL/g COD
14	Rodríguez-Valderrama et al. (2020) (Rodríguez-Valderrama et al., 2020)	Fruits-and-vegetables wastes (FVW) and corn stover (CS).	Anaerobic sludge heat-shock treated in boiling water.	Dilute acid hydrolysis with HCl or H2SO4 at 0.5%, heated at 120 °C for 120 minutes, followed by overliming with Ca(OH)2 to remove inhibitors; neutralized to pH 7	Inoculum was heat-shocked in boiling water at 96°C for 2 hours to inhibit methane-producing microflora.	Cumulative hydrogen 212 mL H2. Hydrogen molar yield 2.31 mol H2/mol glucose

15	Dauptain et al., 2020 (Dauptain et al., 2020)	Corn silage, sorghum, OFMSW, food waste, dates, microalgae, and sewage sludge.	Activated sludge from wastewater treatment plant. Also, tests without inoculum to evaluate indigenous bacteria activity.	Some batch tests included thermal pretreatment of substrate at 90°C for 15 minutes to evaluate effects on indigenous bacteria and hydrogen yield. Most substrates were mechanically shredded or homogenized without chemical pretreatment.	Inoculum was freeze-dried and stored. Pretreatment was thermal exposure at 90 °C for 15 minutes to select spore-forming hydrogen producers and inhibit hydrogen consumers.	Hydrogen yields 306 ± 14 mL H2/g VS initial
16	Pascualone et al. (2019) (Pascualone et al., 2019)	Fruit and vegetable waste (FVW) ; bananas, apples, pears, pineapples, potatoes, etc.	Mixed microbial culture from commercial vermicompost	The fruit and vegetable waste was ground with distilled water. Pretreated in a water bath at 63 °C for 30 minutes to eliminate the natural microbiota present in the substrate.	The vermicompost was subjected to a heat shock pretreatment consisting of submerging in a water bath at 100 °C for 15 minutes and then incubating at 35 °C for 24 hours.	Hydrogen yield 129.2 mL H2/g VS at 5 g/L reducing sugars
17	Parichat Wadjeam et al., 2019 (Wadjeam et al., 2019)	Cassava starch wastewater and buffalo dung.	Indigenous hydrogen producers present in cassava starch wastewater and buffalo dung.	Cassava starch wastewater and buffalo dung were used without extensive pretreatment, except for adjusting the pH to optimal levels.	No specific pretreatment; indigenous hydrogen producers present in the cassava starch wastewater and buffalo dung were utilized directly.	16.90 mL H_2/g-CODadded

and removal of products, allowing for steady-state operation. This continuous mode supports sustained microbial activity and stable hydrogen production, making CSTRs better suited for scale-up and industrial biohydrogen production (Salameh et al., 2022). Additionally, fixed-bed reactors, where microorganisms are immobilized on solid supports, are sometimes utilized to enhance biomass retention and hydrogen yield (Husaini et al., 2023).

Each reactor type offers distinct advantages and limitations. Batch reactors allow flexible control and ease of operation, making them perfect for experimentation and parameter optimization. However, their discontinuous operation necessitates downtime for product removal and substrate replenishment, limiting overall productivity. CSTRs provide the advantage of uninterrupted hydrogen production and higher volumetric productivity by continuously supplying substrates and removing inhibitors. Nevertheless, they demand rigorous monitoring and control systems to maintain stable microbial activity and avoid process fluctuations, which can challenge long-term stability (Jayachandran and Basak, 2023a) (Sun et al., 2019). Fixed-bed reactors promote high biomass concentrations due to immobilized microbes, resulting in efficient substrate utilization and increased hydrogen production. Despite this, issues like mass transfer limitations and biofilm overgrowth may reduce operational lifespan and complicate maintenance (Sun et al., 2019).

Considering industrial-scale biohydrogen production, continuous reactors such as CSTRs are generally the most suitable due to their ability to sustain constant operation and higher productivity over extended periods. Their steady-state operation minimizes downtime and allows for better process integration with downstream applications. However, the complexity of maintaining stable conditions and preventing contamination requires advanced process control technologies (Maluta et al., 2019). While batch reactors remain valuable for research and small-scale production, and fixed-bed reactors can be advantageous where high biomass retention is needed, the continuous reactor remains the preferred choice for scalable, efficient, and sustainable biohydrogen production (Ghosh, 2022; Vishali et al., 2023).

Key operational parameter

Optimizing operational parameters is crucial to enhancing biohydrogen production through dark fermentation, as microbial activity and metabolic efficiency directly affect hydrogen yield. Temperature significantly influences enzymatic functions and microbial growth rates. Mesophilic conditions (30–40 °C) are typically optimal for many substrates such as food and fruit waste. Martínez-Mendoza et al. (2022) reported that fermentation at 37 °C with near-neutral pH and low solids content produced 49.5 NmL-H_2/gVS with a rate of 976.4 NmL-H_2/L-hour (Martínez-Mendoza et al., 2022). However, thermophilic conditions (45–60°C) often enhance microbial activity and hydrogen yield by stimulating thermophilic bacteria. Kim et al. (2024) observed markedly higher hydrogen production at 50 °C, reaching 2112 mL H_2/L, attributed to increased metabolic rates (Kim et al., 2024). Nevertheless, thermophilic processes require careful control to avoid rapid substrate degradation and system instability (Jamaludin et al., 2023; Salameh et al., 2022).

pH is another critical factor governing microbial community stability and enzymatic activity. Optimal biohydrogen production generally occurs in a slightly acidic to neutral range (pH 5.5–7.5), which promotes hydrogen-producing bacteria while inhibiting methanogens that consume hydrogen and reduce yields (Jayachandran and Basak, 2023a; Singh et al., 2022). Carrillo-Reyes et al. (2019) and Khamtib et al. (2021) emphasize maintaining pH near 7 for maximal hydrogen output (Carrillo-Reyes et al., 2019; Khamtib et al., 2021). Substrate-specific optima have been reported by Tagne et al. (2024), with cassava waste favoring pH 7.72 and pineapple waste optimal at pH 6 (Tiegam Tagne et al., 2024). Deviations can lead to the accumulation of solvents and volatile fatty acids that inhibit fermentation (Jamaludin et al., 2023; Rashidi et al., 2024).

Hydraulic retention time (HRT) determines the duration substrates remain in the reactor, directly impacting microbial degradation and hydrogen production. Adequate HRT ensures complete substrate conversion; too short leads to lower yields, while overly long HRT causes accumulation of inhibitory by-products like volatile fatty acids, lowering pH and suppressing hydrogenogenesis (Bandpey et al., 2024; Goren et al., 2024). Santiago et al. (2019) found 48 hours optimal for food waste, with Clostridium dominance (Santiago et al., 2019). Wadjeam et al. (2019) reported 60 hours as optimal for cassava and buffalo dung mixtures (Wadjeam et al., 2019). The interdependence of pH, temperature, and HRT necessitates integrated optimization, often using advanced modeling like RSM or ANN, to maximize biohydrogen yields and ensure stable industrial processes (Burger et al., 2022; Ji and Shen, 2024).

Scale-up challenges in biohydrogen production

Scaling up biohydrogen production from laboratory to industrial scale presents several significant challenges, especially when using food waste as substrate. One major issue is the inherent variability in food waste composition, which in lab settings can be controlled but at industrial scale leads to fluctuations in nutrient availability, pH, and microbial activity. This heterogeneity causes inconsistent hydrogen yields and complicates process stability (Husaini et al., 2023; Jayachandran and Basak, 2023b) Additionally, larger bioreactors face difficulties in maintaining uniform temperature, pH, and mixing throughout the vessel, which can reduce microbial efficiency and hydrogen production. Problems such as insufficient gas-liquid mass transfer and substrate concentration gradients often emerge in scale-up, causing accumulation of inhibitory compounds like volatile fatty acids (VFAs) that further hinder microbial metabolism (Bandpey et al., 2024; Ghosh, 2022).

To address these scale-up challenges, several strategies have been proposed. Advanced bioreactor designs, such as stirred-tank reactors with optimized impellers or fluidized bed reactors, improve mixing and gas transfer, helping maintain homogeneous conditions that enhance hydrogen yields (Hasibar et al., 2020; Wang et al., 2021). Real-time monitoring and control systems are critical for continuously adjusting operational parameters, mitigating substrate variability effects, and preventing inhibitory by-product buildup. Pretreatment of food waste prior to fermentation is also effective, as it homogenizes substrate composition by breaking down complex organics into simpler, more fermentable compounds, improving process consistency and efficiency (Vishali et al., 2023; Zhang et al., 2020). Furthermore, co-digestion with agricultural residues or wastewater can balance nutrient profiles and stabilize fermentation, ultimately supporting more reliable and higher biohydrogen production at industrial scale. Together, these approaches offer promising pathways to overcome the complexities of scaling biohydrogen production from food waste (Jayachandran and Basak, 2023b; Wang et al., 2021).

Recent experiment on bioreactor and operational parameter

Reactor design continues to play a central role in shaping biohydrogen yields during dark fermentation, with recent investigations underscoring both the strengths and limitations of batch and continuous systems. For example, Ngamnurak et al. (2025) explored the application of small-scale batch serum bottles alongside a 2-L laboratory bioreactor for testing model substrates and food waste. Their work demonstrated promising yields, with 1,605 mL H_2/L from glucose and 1,137 mL H_2/L from food waste under mesophilic conditions (35 °C, pH 5.5) (Ngamnurak et al., 2025). In parallel, Kim and Cho (2025) highlighted the potential of continuous stirred-tank reactors (CSTRs), showing that a 5 L setup operating at 50 °C and pH 6.0 delivered stable hydrogen production rates of 353–403 $mL \cdot L^{-1} \cdot h^{-1}$ with hydrogen concentrations of 55–62% (Kim and Cho, 2025). These findings demonstrate the adaptability of batch systems for parameter optimization and the promise of CSTRs for long-term productivity. Supporting this view, Tagne et al. (2024) identified optimal pH conditions for cassava and pineapple waste fermentation, achieving yields of 125.7–153.5 mL H_2/gVS (Tagne et al., 2024). Likewise, Panin et al. (2021) reported cumulative hydrogen production of 424.1 mL H_2 in batch reactors at 35 °C with a balanced substrate-to-inoculum ratio, further reinforcing the value of batch experiments for controlled parameter studies (Panin et al., 2021).

Operational conditions particularly pH, temperature, and substrate-to-inoculum (S/I) ratios play a decisive role in optimizing biohydrogen production, as they directly affect microbial metabolism, community stability, and the balance between hydrogenogenic and competing pathways. pH is one of the most influential factors, as it governs the enzymatic activity of hydrogen-producing bacteria while suppressing methanogens that consume hydrogen. Vázquez-López et al. (2024) demonstrated that adjusting pH to 7.5 under mesophilic conditions increased hydrogen yields to 241.8–265 mL H_2/gVS (Vázquez-López and Moreno-Andrade, 2024), while Tagne et al. (2024) showed that cassava and pineapple waste achieved maximum yields at pH 5.7 and 6.0 respectively, highlighting substrate-specific optima (Tagne et al., 2024). These results indicate that maintaining pH in a slightly acidic to near-neutral range favors metabolic pathways that channel electron flow toward hydrogen.

Temperature is equally critical because it controls microbial growth rates and enzyme kinetics. Kim et al. (2024) reported that moving from mesophilic (37 °C) to thermophilic (50 °C) fermentation enhanced production up to 2.112 mL/L, reflecting the heightened metabolic activity of thermophilic microbes (Kim et al., 2024). Similarly, Ngamnurak et al. (2025) found that mesophilic operation at 35 °C supported stable yields from both glucose and food waste, underlining that moderate conditions can also sustain efficient hydrogenogenesis (Ngamnurak et al., 2025). These findings confirm that different microbial consortia thrive under distinct temperature regimes, and matching thermal conditions with microbial communities is key for productivity.

The S/I ratio regulates substrate availability relative to microbial biomass, influencing both conversion efficiency and accumulation of inhibitory by-products. Roslan et al. (2025) showed that a high S/I ratio near 10:1 improved yields and rates by up to 250%, but excessive substrate can cause acidification that suppresses hydrogen production (Roslan et al., 2025). In contrast, Panin et al. (2021) achieved cumulative hydrogen production of 424.1 mL H_2 at an S/I ratio of 1:1, demonstrating that balanced loading can maintain stable microbial activity (Panin et al., 2021). Together, these studies illustrate that optimizing pH, temperature, and S/I ratios in tandem is essential to create favorable environments for hydrogenogenic microbes while minimizing inhibitory processes, ultimately leading to higher and more sustainable hydrogen yields.

In conclusion, the evidence highlights that neither reactor choice nor operational settings alone can guarantee optimal hydrogen production; instead, their integration is essential. Batch reactors continue to serve as invaluable tools for experimental validation and process optimization, while continuous systems such as CSTRs represent the most realistic option for industrial applications given their ability to sustain production. Success in scaling up dark fermentation will depend on balancing conditions such as temperature, pH, and retention time to encourage hydrogenogenic pathways while limiting inhibitory processes. By aligning reactor design with finely tuned operational strategies, researchers and practitioners can move closer to realizing biohydrogen's potential as a sustainable energy source (Table 2).

Recent advances in optimization biohydrogen production

Optimization with anaerobic co-digestion

Anaerobic co-digestion of mixed substrates, particularly combining food waste with other organic residues, has emerged as a promising strategy to enhance biohydrogen production via dark fermentation. By integrating diverse organic wastes such as fruit peels, potato starch residues, vegetable scraps, and food industry wastewater, this approach provides a balanced nutrient profile that supports the growth and metabolic activity of hydrogen-producing microbes. Studies have demonstrated significant increases in hydrogen yield when substrates like rice waste are co-digested with potato residues, due to the improved carbon-to-nitrogen ratio and synergistic microbial interactions (Jayachandran and Basak, 2023b). Additionally, combining potato starch waste with industrial food effluents accelerates substrate conversion by supplying essential cofactors for fermentative bacteria, thereby boosting hydrogen generation compared to single substrates (Wang et al., 2021). Fruit peels rich in simple sugars, such as banana and orange skins, when co-fermented with domestic wastewater, have also been shown to enhance biohydrogen production by providing readily metabolizable sugars and organic acids that stimulate anaerobic microbial activity (Hasibar et al., 2020).

Utilizing mixed substrates with high carbohydrate content represents an innovative approach to optimize biohydrogen yields sustainably. These substrate mixtures typically include carbohydrate-rich wastes such as banana peels, pineapple skins, cassava peel, potato peel, and leftover cooked rice, which contain complex polysaccharides like cellulose, hemicellulose, starch, and pectin. Through enzymatic or chemical hydrolysis, these polysaccharides are broken down into fermentable sugars (glucose, fructose), serving as efficient energy sources for hydrogen-producing bacteria like *Clostridium*, *Enterobacter*, and *Bacillus* species (Pradhan et al., 2024). For example, banana peel can contain up to 60% carbohydrates by dry weight, including both soluble and insoluble fibers, which provide a sustained substrate supply during fermentation (Martgrita et al., 2022). The complementary carbohydrate profiles in mixed substrates create a nutritionally balanced environment that enhances microbial metabolism, leading to improved hydrogen yields and process stability. Moreover, this approach contributes to integrated organic waste management and greenhouse gas reduction by valorizing otherwise underutilized biomass streams (Nutrients et al., 2017; Vishali et al., 2023). Thus, co-digestion of high-carbohydrate mixed wastes is a key strategy for advancing efficient, sustainable biohydrogen production.

Optimization with pretreatment of substrate and inoculum

Optimizing substrate pretreatment plays a crucial role in enhancing biohydrogen yields from food waste during dark fermentation. Innovative physical methods such as mechanical shredding and ultrasound promote increased substrate surface area and improved mass transfer, facilitating microbial access and enzymatic activity (Hovorukha et al., 2021). Chemical pretreatments, including alkaline and acid hydrolysis, effectively solubilize lignin and complex polysaccharides, increasing fermentable sugar availability and favoring microbial metabolism, as shown by Nam (2023) (Nam, 2023). Biological pretreatments leverage specialized microbes or enzymes to degrade substrates gently and sustainably; for example, thermophilic bacteria like *Thermotoga neapolitana* enhance hydrogen production from enzymatically treated waste (Zhang et al., 2020). Moreover, nanotechnology-based approaches, such as nano-ferrihydrite application, have emerged to selectively enhance microbial communities particularly *Clostridium* species thereby boosting biohydrogen yields via improved microbial attachment and activity (Zhang et al., 2020). These diverse pretreatment techniques alone or in synergy provide multifaceted strategies to optimize substrate biodegradability, microbial accessibility, and overall fermentation efficiency.

In parallel, optimizing inoculum pretreatment is essential for maximizing biohydrogen production efficiency. Thermal pretreatment remains a widely adopted approach to suppress hydrogen-consuming methanogens while

Table 2. Recent experiment results (2019–2025) refer to selection of reactor type and operational condition for biohydrogen production

No	Author and year	Reactor type	Operational conditions	Substrat/Inoculum ratio	Biohydrogen production
1	(Ngamnurak et al., 2025)	Batch 60-mL serum bottles (model substrates); 2-L lab-scale bioreactor (food waste, 1.4 L working volume)	Temp: 35 ± 2 °C; pH: adjusted 5.5 (range 4.5–8.0 tested); agitation 150 rpm; buffer: $NaHCO_3$; anaerobic conditions	20 g-VS/L substrate + 5 g-VS/L non-enriched + 1.44 g-VS/L enriched (≈ 3–4:1)	From glucose: 1,605 ± 161 mL-H_2/L; from food waste: 1,137 mL-H_2/L (56.85 mL-H_2/g-VSadded)
2	(Kim and Cho, 2025)	5 L continuous stirred-tank reactor (CSTR), 3 L working volume	Temp: 50 °C; pH maintained at 6.0 (KOH adjustment); agitation 150 rpm; HRT: 10 h; anaerobic (N_2 purging); DO <0.1%	240 mL substrate + 60 mL inoculum (≈ 4:1, v/v)	H_2 production rate: 353–403 $mL \cdot L^{-1} \cdot h^{-1}$; H_2 concentration: 55–62% (v/v)
3	(Roslan et al., 2025)	600 mL glass bottles (batch BHP tests), 200 mL working volume	Temp: 37 °C (water bath); pH adjusted to 6.0 (NaOH); buffer: 20 mL MES; anaerobic (N_2 purging); batch duration 3–5 days	20 g substrate + 250 mg VS inoculum (≈ 10:1, VS basis)	Max yield (Pm): 57–94 mL/gVS; Max rate (Rm): up to 183 ± 14 mL/gVS·d (250% higher with exogenous inoculum)
4	(Tiegam tagne et al., 2024)	Reactor: 125 mL Pyrex batch reactors sealed	Temperature: 40 °C; pH Optimized via RSM, ranged 4.5–6, optimal ~5.7 (cassava), 6 (pineapple); flushed with N2.	Substrate/Inoculum (S/I) ratio: 1:1	Pineapple; 153.5 mL H2/g VS. Cassava; 125.7 mL H2/g VS
5	(Domińska, 2024)	Reactor: batch, 1 L glass bottles	Reactor: batch, 1 L glass bottles, suhu 37 °C, pH awal 6–8; shaking 140 rpm.	Substrate/Inoculum (S/I) ratio: 1:1	96 cm3 H2/gTVSFW for WWTP sludge. 93 cm3 H2/gTVSFW for dairy industry wastewater.
6	(Vázquez-López and Moreno-Andrade, 2024)	Batch reactors, Schott bottles 360 mL.	Mesophilic 37 ± 1 °C, initial pH variable or adjusted to 7.5, shaken intermittently, anaerobic atmosphere by nitrogen purge	Substrate/Inoculum ratio 2.7 (gVS/gVS)	Hydrogen yield 241.8–265 mL H2/gVS removed
7	(S. M. Kim et al., 2024)	Batch reactors, serum bottles 120 mL	Incubation at mesophilic (37 °C) or thermophilic (50 °C) conditions; pH natural (~6–7); anaerobic (N2 purged); agitation 100 rpm; incubation until gas ceased	2 g wet inoculum + 40 mL food waste medium	3900 mL/L Using Wetland as inoculum 2112 mL/L Using Forest Sedimen as inoculum
8	(Hangri et al., 2024)	Batch reactors, serum bottles 120 mL with working volume 90 mL	Mesophilic temperature 35 ± 0.5 °C; pH natural (~6-7); anaerobic conditions with argon flushing; shaking 100 rpm; photo fermentation at 25 ± 2 °C with illumination 4000 lux, stirring at 250 rpm.	S/I ratio 5 g VS substrate/g VS inoculum	334.90 mL/L
9	(Villanueva-Galindo et al., 2024)	Reactor: Batch glass bottles, 600 mL total volume, 350 mL working volume	Temperature: 37 °C; pH: Initially adjusted to 7.0 with NaOH; Stirring: 120 rpm (1 min every 3 min).	S/I ratio not explicitly	596.3 mL H2/Lreactor
10	(Nam, 2023)	Batch reactor: 415 mL serum bottles with 200 mL distilled water	Temperature 35 °C; pH controlled at 6.0; shaker at 100 rpm; anaerobic conditions by N2 flushing.	S/I ratio not explicitly	Hydrogen yield 152.1 mL H2/g VS
11	(Cao et al., 2022)	Reactor batch 150 mL bottles, working volume 100 mL.	Temperature 37°C; pH adjusted 7,0, Shaking speed: 120 rpm; Anaerobic environment created with N2 flushing.	S/I ratio not explicitly	71.0 mL/g-VS added.
12	(Panin et al., 2021)	Batch reactor 235 mL glass bottles with 70 mL working volume	Temperature 35 °C; shaking at 120 rpm; initial pH ~7; no pH control during fermentation; retention time 7 days	1:1 (based on total solids)	Cumulative hydrogen 424.1 mL H2. Hydrogen yield 151.67 mL H2/g VS added

13	(Lin et al., 2020)	Batch reactor serum vial 235 mL capacity 60 mL working volume	Temperatures tested: 35 and 55 °C; initial pH 6.8; anaerobic (argon flushing); agitation in shaker.	30 mL substrate (kitchen waste), 20 mL inoculum (seed sludge).	Hydrogen yield 75.4 mL/g COD
14	(Rodríguez-Valderrama et al., 2020)	Batch reactors, 120 mL serum bottles with 70 mL working volume	Temperature 35 °C; 150 rpm shaking; anaerobic (N2 flushing); initial pH around 6–7; fermentation time up to ~87 h	Inoculum to substrate ratio (ISR) tested at 0.8, 1.0, 1.2 (g VS inoculum /g VS substrate)	Cumulative hydrogen (Hmax) 212 mL H2. Hydrogen molar yield 2.31 mol H2/mol glucose
15	(Dauptain et al., 2020)	Reactor Batch in 550 mL glass bottles with 350 mL working volume	Temperature 37 °C (mesophilic), no stirring, pH 6, Anaerobic by N2 purging	Substrate to inoculum ratio (S/X) set to 22 ± 1	Hydrogen yields 306 ± 14 mL H2/g VS initial
16	(Pascualone et al., 2019)	Batch reactors 72 mL working volume	Temperature 35 °C, pH adjusted to 5.5 use NaOH 1M.	Reducing sugars 5–25 g/L, inoculated with 8 mL vermicompost inoculum	Hydrogen yield 129.2 mL H2/g VS at 5 g/L reducing sugars
17	(Wadjeam et al., 2019)	Batch reactor: 120 mL glass bottles with 70 mL working volume and continue with CSTR reactor: 8 L with 6 L working volume	In Batch; Temperature 30 °C; shaking at 150 rpm In CSTR; 8 L with 6 L working volume; temperature 30 °C; shaking at 400 rpm.	S/I ratio not explicitly	16.90 mL H_2/g-CODadded

enriching hydrogen-producing bacteria, with controlled temperature and exposure time being critical to avoid damaging beneficial microbes (Domińska, 2024). Chemical treatments, such as acid and alkaline hydrolysis, further enhance inoculum performance by solubilizing organic matter and selectively inhibiting competitor microbes (Cieciura-Włoch and Borowski, 2019). Hybrid methods that combine thermal and chemical pretreatments, or blend diverse sludge sources like vermicompost with anaerobic sewage sludge, have demonstrated synergistic improvements in microbial activity and hydrogen output (Karthikeyan and Gokuladoss, 2022). Advanced techniques such as hydrodynamic cavitation increase microbial surface area and biodegradability, enhancing fermentation kinetics. Emerging strategies like bioaugmentation, involving the addition of specialized microbial consortia or enzymatic additives, further stimulate inoculum functionality and biohydrogen yield (Villanueva-Galindo et al., 2025). Together, these innovative pretreatment approaches for both substrate and inoculum are fundamental to advancing sustainable and efficient biohydrogen production via dark fermentation.

Optimization with genetic and metabolic engineering

Genetic and metabolic engineering have become vital tools for enhancing biohydrogen production by optimizing the microbial pathways involved in dark fermentation. Genetic engineering enables the modification of key hydrogen-producing bacteria, such as Clostridium and Enterobacter species, to increase their hydrogen output by overexpressing genes involved in hydrogenase enzymes or redirecting metabolic fluxes towards hydrogen-generating pathways. For example, knocking out competing pathways that produce by-products like lactate or ethanol can channel more substrates towards hydrogen formation, thereby improving yield and productivity (Pradhan et al., 2024). Metabolic engineering approaches also focus on constructing microbial consortia with synergistic interactions, enhancing substrate utilization and reducing inhibitory metabolites. Advances in CRISPR-Cas and synthetic biology tools facilitate precise genome editing, enabling the development of robust strains with improved tolerance to inhibitors and optimized enzyme activities, which are crucial for industrial biohydrogen applications (Wang et al., 2021).

Moreover, metabolic engineering can optimize the biochemical pathways of microbes to better convert complex organic wastes into fermentable sugars and subsequently hydrogen. By engineering metabolic circuits to enhance the acetyl-CoA and butyrate pathways, microbes can achieve higher hydrogen yields and reduce by-product formation. Additionally, bioaugmentation strategies incorporating engineered microbial strains into inocula have shown promise in boosting fermentation efficiency and stability. Combining these engineered microbes with pretreatment technologies such as enzymatic hydrolysis and nanomaterial augmentation can further enhance substrate accessibility and microbial activity (Villanueva-Galindo et al., 2025; Zhang et al., 2020). Thus, integrating genetic and metabolic engineering innovations holds significant potential to overcome current limitations in biohydrogen production, paving the way for more sustainable and economically viable dark fermentation processes.

Optimization with kinetic model, machine learning and artificial intelligence

The application of kinetic models is pivotal in optimizing biohydrogen production by enabling the analysis and prediction of system performance under varying environmental and substrate conditions. Among these, the Gompertz model is widely utilized due to its ability to describe hydrogen production kinetics, accounting for lag time, maximum production rate, and cumulative yield, thereby allowing fine-tuning of fermentation parameters for diverse substrates (Domińska, 2024). Another critical model is the Monod model, which relates microbial growth rates to substrate concentration, proving essential for understanding and optimizing microbial community dynamics, especially in continuous or semi-continuous dark fermentation processes (Jarosz, 2024; Kanwal and Torriero, 2022). These kinetic frameworks facilitate simulations that predict hydrogen yields and by-product formation, thus guiding more efficient bioreactor design and process control (Jayachandran and Basak, 2023b; Salameh et al., 2022). For example, Karthikeyan and Gokuladoss (2022) reported that the modified Gompertz model provided an excellent predictive fit ($R^2 > 0.99$) in systems using combined vermicompost and anaerobic sludge as inocula, yielding up to 50 mL H_2 g^{-1} VS (Karthikeyan and Gokuladoss, 2022). Furthermore, the integration of a Gompertz-based kinetic framework with a statistical response surface methodology for co-digestion of food waste and sewage sludge allows for the precise identification of optimal volatile solids concentrations and mixing ratios (Nam, 2023). These results demonstrated that kinetic evaluation could accurately describe hydrogen potential, maximum rate, and lag time, thereby validating kinetic modeling as a reliable tool for optimizing fermentation conditions and enhancing biohydrogen productivity.

Beyond traditional kinetic modeling, machine learning (ML) and artificial intelligence (AI) offer advanced capabilities for optimizing biohydrogen production. ML algorithms process extensive experimental datasets to uncover complex patterns and correlations that conventional methods might overlook, enabling real-time optimization of operational parameters (Bandpey et al., 2024). AI techniques such as artificial neural networks and genetic algorithms further enhance process adaptability by integrating with kinetic models to create hybrid systems capable of responding to dynamic conditions in fermentation (Wang et al., 2021) confirmed that hybrid ANN–GA optimization outperformed classical response surface methodology (RSM) in predicting and maximizing hydrogen yields from food waste hydrolysates, owing to its ability to capture nonlinear fermentation dynamics. Additionally, computational fluid dynamics (CFD) simulations provide detailed insights into bioreactor fluid flow and mass transfer, facilitating the identification and mitigation of scale-up challenges (Ghosh, 2022; Maluta et al., 2019). Collectively, these computational approaches represent a powerful strategy to maximize biohydrogen yields from food waste, advancing sustainable energy production.

Additionally, computational approaches enhance resilience in complex fermentation systems. By validating predictive models through kinetic frameworks such as the Gompertz model, researchers have demonstrated improved accuracy in forecasting hydrogen yields, particularly in mixed-inoculum and food waste systems (Karthikeyan and Gokuladoss, 2022). This underscores that the synergy of kinetic models and AI-based optimization can sustain high hydrogen yields, streamline reactor design, and ensure performance under variable substrate and environmental conditions. Collectively, these computational strategies spanning kinetic modeling, ML, and AI constitute a powerful framework to maximize biohydrogen yields from food waste and related substrates, advancing sustainable energy production and waste valorization (Figure 4).

Integration dark fermentation with other process

Integration dark fermentation with photo fermentation

The integration of dark fermentation (DF) and photo-fermentation (PF) represents a strategic advancement in optimizing biohydrogen production

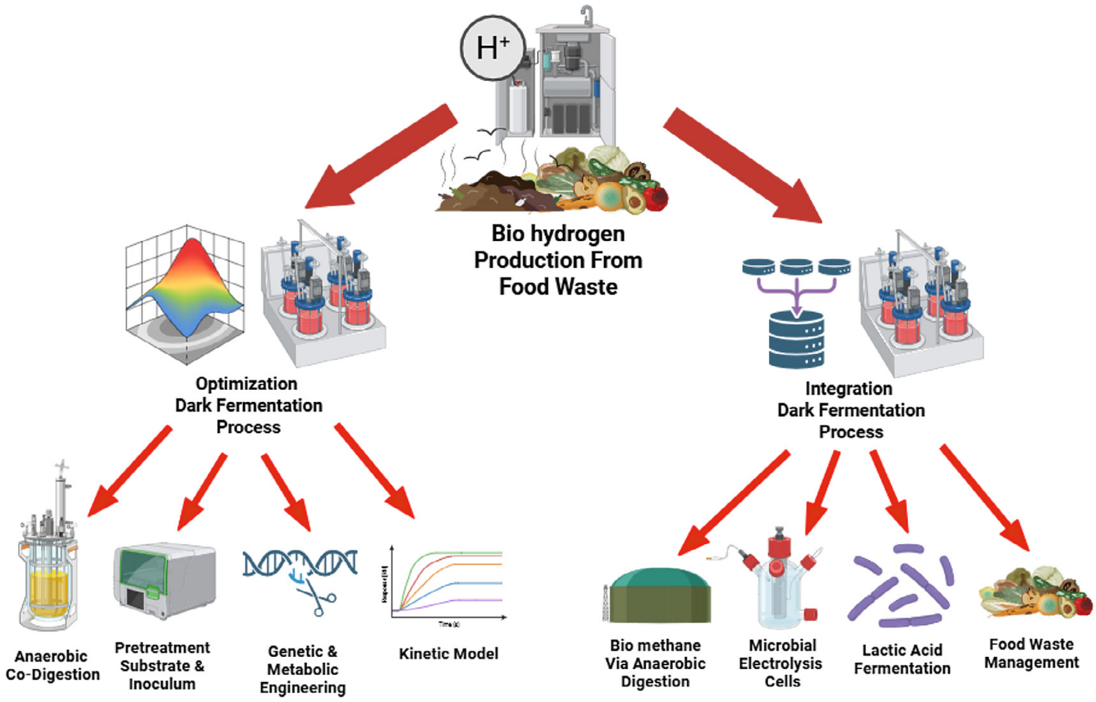

Figure 4. Enhancement strategy for biohydrogen production from food waste with optimization and integration process

by combining the complementary strengths of both processes. DF is known for its rapid initial hydrogen production through anaerobic degradation of carbohydrates but suffers from the accumulation of volatile fatty acids (VFAs) and the associated drop in pH, which limit yield (Das and Basak, 2021). PF, in contrast, employs photosynthetic bacteria such as *Rhodobacter sphaeroides* to metabolize VFAs and other residual organic acids, effectively converting inhibitory byproducts into additional hydrogen and thereby enhancing overall efficiency (Guo et al., 2024). This synergy enables integrated DF–PF systems to overcome individual process limitations, resulting in higher hydrogen yields, greater substrate conversion, and more stable performance.

Empirical studies consistently confirm the superiority of integrated DF–PF over single fermentation approaches. For example, sequential DF–PF of cheese whey achieved cumulative hydrogen yields of 6.65 mol H_2/mol lactose, significantly higher than the 2.43 mol H_2/mol lactose obtained by DF alone (Rao and Basak, 2022). Similarly, research on potato waste demonstrated that an optimized DF–PF combination produced 1580 mL H_2/L medium, corresponding to a yield of 6.31 mol H_2/mol carbohydrate and 53% substrate conversion, under conditions of 2 g/L carbohydrate, 0.2 g/L $FeCl_3$, and pH 7 (Das and Basak, 2021). Furthermore, another study using potato powder confirmed that PF consistently outperformed DF in terms of yield, with PF producing 240 mL H_2/g carbohydrate (1.92 mol H_2/mol glucose) compared to 175 mL H_2/g carbohydrate (1.41 mol H_2/mol glucose) in DF (Das and Basak, 2025). These findings collectively demonstrate that integrating the rapid hydrogen release of DF with the efficient VFA utilization of PF can substantially boost hydrogen recovery and waste valorization.

Overall, the integration of DF and PF proves effective in maximizing biohydrogen production because it unites the fast hydrogen generation of DF with the enhanced substrate utilization and byproduct recycling of PF. This combined strategy not only improves yields – achieving more than double the hydrogen recovery compared to DF alone – but also stabilizes pH, reduces the accumulation of inhibitory metabolites, and increases overall process efficiency (Das and Basak, 2025; Rao and Basak, 2022). The advantages extend beyond production performance, as integrated systems also support waste minimization by transforming agricultural residues like potato waste and cheese whey into valuable clean energy sources (Das and Basak, 2021; Guo et al., 2024). Thus, DF–PF integration stands as a sustainable approach that aligns with industrial scalability, environmental protection, and global clean energy transition goals.

Integration dark fermentation with microbial electrolysis cells (MECs)

The integration of dark fermentation and MECs offers a highly efficient two-stage approach to maximize biohydrogen production from organic waste. In the first stage, dark fermentation converts organic substrates into hydrogen along with organic acids such as acetate and butyrate as by-products. These organic acids then serve as substrates in the MEC, where electroactive microbes oxidize them at the anode to generate electrons, which are used at the cathode to produce additional hydrogen through an electrochemical reaction (Marone et al., 2017; Swaminathan et al., 2024). This combined process has demonstrated the potential to triple hydrogen production efficiency compared to dark fermentation alone. For example, integrated systems treating palm oil mill effluent achieved hydrogen conversion efficiencies up to 236 mL H_2/g COD, significantly surpassing single-stage dark fermentation results (Khongkliang et al., 2019; Marone et al., 2017). Optimal operation conditions, such as maintaining thermophilic temperatures, selecting appropriate electrode materials like platinum-coated carbon, and adjusting pH and voltage, play vital roles in enhancing microbial electron transfer and overall hydrogen yield (Cha et al., 2024; Swaminathan et al., 2024).

The enhanced efficiency of this integrated system is largely attributed to the activity of electroactive bacteria such as *Geobacter* and *Desulfovibrio* species, which dominate the MEC anode and facilitate effective electron transfer necessary for hydrogen production (Khongkliang et al., 2019; Swaminathan et al., 2024). MECs are particularly advantageous because they valorize volatile fatty acids (VFAs) and other organic acids, which would otherwise accumulate and inhibit dark fermentation, into high-yield hydrogen (Srivastava et al., 2024). Studies report that coupling DF with MEC can raise energy recovery from around 7–8% in single processes to over 60% in integrated systems (Srivastava et al., 2024). Moreover, comparative studies demonstrated that integrated DF–MEC systems yield significantly more hydrogen (up to 467 mL/g VS) than DF alone (148 mL/g VS) or MEC alone (123 mL/g VS), highlighting the superior efficiency of the combined process (Do et al., 2022).

Electrode materials like carbon coated with platinum further improve electron transfer kinetics, boosting hydrogen output (Cha et al., 2024; Dhar et al., 2015). MEC integration not only boosts hydrogen yield but also achieves high removal rates of VFAs and chemical oxygen demand (COD), often exceeding 70–90%, which contributes to both bioenergy recovery and environmental protection (Magdalena et al., 2023). Additional investigations emphasize that MECs enhance system performance by selectively favoring acetate-rich effluents from DF, which drive superior hydrogen yields and higher VFA removal rates compared with butyrate-dominated effluents (Magdalena et al., 2023). Similarly, studies using food wastewater demonstrated that pre-fermentation increases the abundance of *Geobacter* up to 78%, which in turn significantly improves MEC electron transfer efficiency and hydrogen productivity (Cha et al., 2024). These findings highlight the crucial role of MECs in not only exploiting the metabolic by-products of DF but also in reshaping microbial communities toward electroactive consortia optimized for biohydrogen production. While laboratory and small-scale studies demonstrate promising results, challenges remain in scaling up, including reactor design optimization and operational cost reduction. Nevertheless, the integration of dark fermentation and MEC technology holds great promise as a sustainable bioenergy and waste management solution (Marone et al., 2017; Swaminathan et al., 2024).

Integration dark fermentation with lactic acid fermentation

The integration of dark fermentation with lactic acid fermentation offers a promising strategy to optimize biohydrogen production from food waste. Lactic acid fermentation plays a crucial role in stabilizing organic matter within the substrate, preventing degradation and ensuring consistent substrate quality without the need for refrigeration. This stabilization maintains the biohydrogen potential of food waste across various storage conditions, with studies reporting stable hydrogen yields ranging from 88 to 94.6 mL/gVS after 15 days of lactic acid fermentation at temperatures between 4 °C and 55 °C (Roslan et al., 2024). Such stabilization is critical because lactic acid fermentation suppresses microbial activity that would otherwise result in carbon loss, thereby preserving the fermentable fraction for subsequent hydrogenogenesis (Martínez-Mendoza et al., 2023). Additionally, lactic acid fermentation produces substrates enriched in lactate that can be directly utilized in dark fermentation without compromising hydrogen yields. Maintaining total solids concentrations between 5% and 20% during storage supports substrate stability, while lower concentrations tend to trigger butyrate fermentation, which decreases biohydrogen potential (Roslan et al., 2024). Moreover, the controlled consumption of lactate during the integrated process correlates positively with hydrogen production rates, highlighting lactate's role as an energy-rich intermediate that supports efficient and rapid biohydrogen generation (Regueira-Marcos et al., 2024).

Microbial dynamics during lactic acid fermentation also contribute to the success of its integration with dark fermentation. Lactic acid bacteria (LAB) such as Lactobacillus, Lactococcus, and Weissella dominate the initial fermentation phase, producing metabolites that stimulate hydrogen-producing bacteria like Clostridium in the subsequent dark fermentation stage (Roslan et al., 2025). The coexistence and synergistic interaction between LAB and hydrogenogenic bacteria facilitate an efficient metabolic transition from lactate to hydrogen without the accumulation of inhibitory by-products like acetate (Ngamnurak et al., 2025). This cross-feeding mechanism enhances metabolic efficiency and system stability, resulting in hydrogen production rates significantly higher than single-stage fermentation processes. The combined approach not only improves substrate stability and biohydrogen yield by up to 57% but also offers scalability and sustainability by reducing energy demands for substrate storage (Roslan et al., 2024). Importantly, continuous LD-DF studies have shown that hydrogen production correlates strongly with lactate consumption and butyrate accumulation, confirming the centrality of lactate as a metabolic driver in biohydrogen pathways (Regueira-Marcos et al., 2023). Overall, integrating lactic acid and dark fermentation presents an innovative, environmentally friendly solution for converting food waste into renewable bioenergy.

Integration dark fermentation with biomethane production

The integration of biohydrogen production through dark fermentation with biomethane production represents a promising bioenergy strategy to maximize energy recovery from food waste. In this two-stage process, dark fermentation converts easily degradable organic compounds into biohydrogen, leaving residual substrates rich in energy yet to be utilized. These residuals are subsequently processed by methanogenic microorganisms under anaerobic digestion to produce methane. This sequential approach enables a more complete conversion of organic waste into renewable fuels, reducing the formation of inhibitory by-products and enhancing overall system efficiency (Husaini et al., 2023; Salameh et al., 2022). Empirically, DF enriches soluble intermediates (VFAs, alcohols) and simplifies complex organics, which methanogens subsequently convert with higher efficiency thereby boosting total biogas while relieving feedback inhibition on hydrogen producers. For example, in semi-continuous systems co-processing kitchen waste with hyperthermophilically pretreated grass, thermophilic operation of the methanogenic stage achieved 293 NmL $CH_4 \cdot gVS^{-1}$ compared to 131 NmL $CH_4 \cdot gVS^{-1}$ in grass mono-digestion, while the mesophilic DF regime favored hydrogenogenesis (Liczbiński et al., 2022).

Moreover, the integration offers distinct operational advantages over standalone processes. The initial dark fermentation stage simplifies complex organic matter into smaller, more accessible molecules, facilitating enhanced methane production downstream and reducing the hydraulic retention time needed in the methanogenic reactor. In a two-step system using domestic wastewater sludge and cow dung, DF at pH 5.5 maximized bioH_2 (108.04 $mL \cdot gVS^{-1}$), whereas AD at pH 7.5 maximized bioCH_4 (768.54 $mL \cdot gVS^{-1}$), with the combined stream (biohythane) reaching 811.12 $mL \cdot gVS^{-1}$ (Sufyan, 2023). This controllability minimizes VFA accumulation in the methanogenic reactor and curtails hydrogenotrophic backpressure on DF. This synergy not only boosts total biogas output but also stabilizes the digestion system by minimizing volatile fatty acid accumulation, which can inhibit microbial activity in both stages (Ghosh, 2022; Vishali et al., 2023).

Biochemical potential tests across mesophilic, thermophilic, and hyperthermophilic DF confirmed that thermophilic DF (55 °C), especially with nutrient co-substrates, yielded the highest bioH_2 (27.1 mL $H_2 \cdot gVS^{-1}$). Crucially, the DF effluent produced at elevated temperature was also a superior substrate for AD, with hyperthermophilic DF effluent generating the highest methane potential (117.36 mL $CH_4 \cdot gVS^{-1}$) under mesophilic AD (Sillero et al., 2023). This staged thermochemical tuning maximizes hydrogen without sacrificing the overall energy balance, reinforcing the complementarity between DF and AD. Empirical studies have demonstrated that combined biohydrogen and biomethane systems outperform single-stage anaerobic digestion in terms of energy recovery and waste management flexibility, making them highly suitable for scaling up to industrial bioenergy facilities. Such integrated approaches contribute significantly to sustainable energy production and organic waste valorization (Pradhan et al., 2024; Shetty et al., 2019). Reviews converge on the same rationale: co-digestion, pretreatment, and hybrid DF–AD configurations improve substrate degradability, nutrient balance, and process resilience compared to single-stage AD. These strategies consistently elevate gas yields and reduce inhibitory metabolites (Moradi et al., 2023).

Comparison of the various process integrations

The integration of DF with various complementary processes demonstrates distinctive advantages that address the inherent limitations of single-stage hydrogen production. Table 3 shows the coupling of DF with anaerobic digestion (AD) or "biohythane" production enables maximized energy recovery through the sequential generation of hydrogen and methane, while also enhancing system stability and reducing inhibitory metabolites. Similarly, DF–MEC integration significantly boosts hydrogen yield by valorizing VFAs and enhancing overall energy efficiency, offering an advanced technological approach to waste valorization. Meanwhile, DF–LAF ensures substrate stability without refrigeration and preserves hydrogen potential during storage, making it particularly attractive for practical applications involving food waste. DF–PF, in contrast, combines the rapid hydrogen generation of DF with the ability of photo-fermentation to utilize VFAs, thereby increasing hydrogen recovery and stabilizing system performance. Collectively, these integrations exemplify the complementarity between biological, electrochemical, and photobiological pathways in advancing renewable biohydrogen production.

Despite these advantages, several limitations challenge the scalability of these integrated systems. Table 3 shows the DF–AD approach requires strict pH management and complex reactor systems, which increase operational costs and risk microbial imbalances. DF–MEC, while efficient, faces high costs associated with electrode materials and technical barriers in scaling from laboratory to industrial applications. DF–LAF integration necessitates careful control of solids concentration to prevent metabolic shifts, and maintaining stable microbial communities at large scale remains difficult. For DF–PF systems, reliance on light availability, slower kinetics, and high operational costs pose significant barriers for industrial adoption. Thus, although each integration pathway demonstrates notable improvements over single-stage DF, the technological, economic, and operational drawbacks must be critically addressed before large-scale deployment can be realized.

In conclusion, the comparative analysis highlights a trade-off between performance gains and practical feasibility in integrated DF systems. While DF–AD and DF–PF offer substantial potential for maximizing energy recovery from organic waste, DF–MEC and DF–LAF provide unique niche advantages in energy efficiency and substrate stabilization. Nevertheless, all four integrations underscore the importance of system optimization, cost reduction, and microbial management to enable industrial scalability. Future research should prioritize hybrid designs, advanced reactor configurations, and techno-economic assessments to bridge the gap between laboratory success and commercial application. Ultimately, these integrated processes represent promising pathways toward sustainable bioenergy production, but their long-term viability will depend on overcoming current operational and economic constraints.

Techno economy analysis

The implementation of TEA in biohydrogen production from food waste has emerged as a critical tool for assessing the viability of sustainable energy solutions. As food residues represent one of the most abundant biomass resources globally, converting them into hydrogen provides a dual advantage: energy recovery and waste management. Melikoglu and Tekin (2024) have indicated that the potential annual yield of biohydrogen from household food waste in Turkey could reach 90,000 tons by the year 2030. This represents a significant economic opportunity, with an estimated potential value of USD 1.5 billion (Melikoglu and Tekin, 2024). Ganguly et al. (2025) demonstrated that integrating dark fermentation with microbial electrolysis cells for solid food waste enables negative carbon emissions of up to −8.0 kg

Table 3. Comparison of the advantages and disadvantages of various process integrations

NO	Process integration	Advantages	Disadvantages	Summary of results
1	Dark Fermentation + Anaerobic Digestion (DF–AD / Biohythane)	- Produces dual energy (H_2 + CH_4) with high efficiency - Reduces accumulation of inhibitory VFAs - Facilitates degradation of complex substrates - More stable compared to single-stage AD	- Requires different pH control for each stage - More complex and costly reactor system - Potential microbial imbalance between stages	Maximizes energy recovery by combining hydrogen and methane production, improves stability, and offers scalable waste-to-energy conversion.
2	Dark Fermentation + Microbial Electrolysis Cells (DF–MEC)	- Increases H_2 production 2–3 times - Utilizes VFAs for additional hydrogen - Energy recovery efficiency >60% - Reduces COD and organic pollutants	- High cost of electrode materials (e.g., Pt) - Technology still at lab/pilot scale - Challenges in reactor design and electricity demand	Greatly boosts hydrogen yield and energy efficiency, while also treating waste, though cost and scale-up remain key challenges.
3	Dark Fermentation + Lactic Acid Fermentation (DF–LAF)	- Stabilizes food waste without refrigeration - Preserves biohydrogen potential during storage - Lactate serves as an energy-rich substrate for DF - Synergy between LAB & Clostridium increases yield	- Requires strict control of solids concentration to avoid butyrate fermentation - Microbial dynamics difficult to maintain at large scale - Risk of substrate quality variability	Ensures stable substrates, reduces storage energy demands, and improves hydrogen yield through synergy between lactic acid and hydrogen-producing bacteria.
4	Dark Fermentation + Photo Fermentation (DF–PF)	- VFAs from DF converted into additional hydrogen - Higher H_2 yield (up to 2× compared to DF alone) - Stabilizes pH and reduces inhibitory metabolites - Maximizes conversion of agricultural & food waste	- Requires light and specific conditions (pH, nutrients, Fe, etc.) - PF process is relatively slow - Industrial scale-up faces cost and efficiency issues	Increases hydrogen yield by utilizing VFAs, stabilizes process conditions, and supports sustainable bioenergy from food and agricultural waste.

CO_2e per kilogram of hydrogen, while remaining competitive if current density is improved (Ganguly et al., 2025). These findings underscore the importance of TEA in elucidating the financial viability of food waste-based hydrogen systems and in assessing their environmental contributions.

Techno-economic assessments are essential for obtaining indispensable insights into the key parameters that drive profitability and sustainability in food waste biohydrogen pathways. As demonstrated by Mahmod et al. (2021), the economic viability of anaerobic digestion projects is significantly influenced by factors such as substrate quality and product selling price. Optimized scenarios have been shown to result in net present values (NPV) that exceed USD 4.6 million (Mahmod et al., 2021). However, as Deng et al. (2023) reported in their cassava stillage residue study, the inclusion of pretreatment steps may improve hydrogen yields but often increases costs, raising production expenses to as high as €2.33/m³ against a selling price of €0.68/m³ (Deng et al., 2024). This trade-off underscores the critical importance of TEA not only for evaluating raw profitability but also for identifying critical process bottlenecks, such as energy-intensive pretreatment, that jeopardize economic sustainability. The utilization of food waste as a substrate necessitates a judicious balance between maximizing yield and minimizing costs, with the TEA serving as a pivotal guide for optimal decision-making.

The broader implications of TEA extend beyond financial performance into policy design and sustainable energy transition. In Bangladesh, for instance, Nayeem et al. (2025) determined that biohydrogen production from biogenic residues could attain a 73.31% energy efficiency, with financial returns becoming appealing under tax incentives that reduced production costs from \$6.40 to \$4.95 per kilogram (Nayeem et al., 2025). These results demonstrate the potential of TEA to direct policymakers toward the incentivization of renewable hydrogen pathways, while concurrently enabling industrial entities to adopt circular economy practices. The extant literature emphasizes that techno-economic frameworks are indispensable in scaling biohydrogen production from food waste, as they integrate profitability, efficiency, and environmental benefits into a holistic assessment of sustainability (Deng et al., 2024; Mahmod et al., 2021).

Future research direction

Although significant advancements have been made in the production of biohydrogen from food waste, several unresolved issues persist, necessitating more precise and targeted research directions. Firstly, the inherent heterogeneity of food waste continues to impede process reliability at larger scales. Future studies must prioritize the development of adaptive microbial consortia and dynamic control systems that can automatically adjust to substrate variability. This calls for the integration of artificial intelligence and real-time monitoring to stabilize yields under fluctuating conditions.

Secondly, despite the advancement in pretreatment methods that have enhanced substrate solubility and inoculum selectivity, the majority of these methods are energy-intensive and economically unsustainable. It is imperative that research endeavors prioritize the development of cost-effective, eco-friendly alternatives, such as enzymatic, biological, or nanomaterial-assisted pretreatments. A particular emphasis should be placed on the scalability of these pretreatments and their environmental safety. Furthermore, there is an urgent need for more systematic techno-economic and life cycle assessments to ascertain the feasibility of such innovations at industrial scales.

Thirdly, microbial engineering signifies a pivotal domain of research. It is imperative that CRISPR-based genome editing not only focus on enhancing hydrogen yield but also on bolstering tolerance to inhibitors and by-products. The development of synthetic microbial consortia capable of coordinated substrate degradation and hydrogenogenesis presents a promising avenue for further research. Integration of dark fermentation with processes such as microbial electrolysis cells (MECs), photo-fermentation, or biomethane production remains largely confined to laboratory studies. It is imperative that pilot- and demonstration-scale projects be prioritized, accompanied by advanced kinetic modeling and AI-based predictive frameworks. This approach is designed to address the discrepancy between laboratory optimization and industrial application, thereby ensuring that biohydrogen production from food waste becomes both technically robust and commercially viable.

Policy implications and practical applications

The recent advancements in optimizing biohydrogen production from food waste carry significant implications for energy and environmental policy. Governments aiming to achieve decarbonization and circular economy goals may incorporate biohydrogen as a strategic component of renewable energy portfolios, complementing solar, wind, and biomethane pathways. Policies promoting sustainable waste valorization can incentivize municipalities to redirect organic residues from landfills to biohydrogen facilities, thereby reducing methane emissions while producing clean fuel. Techno-economic analyses highlight that such initiatives could be financially viable under supportive frameworks, including subsidies for green hydrogen, carbon credit trading, and tax incentives Moreover, standardizing regulatory guidelines for pretreatment processes, reactor technologies, and microbial engineering can accelerate technology transfer from laboratory to industry. International collaborations and public–private partnerships will be essential to harmonize infrastructure, support pilot-scale demonstrations, and create robust hydrogen distribution networks. Thus, policy design must holistically integrate enviro nmental, economic, and technological dimensions to enable large-scale adoption of biohydrogen.

The optimization of biohydrogen production provides pragmatic solutions for both the generation of renewable energy and the management of waste. The employment of advanced pretreatment, adaptive inocula, and real-time process control has been demonstrated to enhance the efficiency and stability of the conversion of food waste into hydrogen fuel. This hydrogen can be utilized in fuel cells for electricity generation, integrated into industrial heating systems, or stored for mobility applications, thereby reducing dependency on fossil fuels. Concurrent diversion of food residues into controlled fermentation processes has been demonstrated to reduce landfill burden, decrease methane emissions, and generate nutrient-rich by-products suitable for agricultural use. These applications directly contribute to sustainable urban development and climate mitigation strategies.

The integration of dark fermentation with complementary processes has been demonstrated to extend the practical scope of biohydrogen production. The implementation of DF-MEC systems has been demonstrated to enhance hydrogen yields while concomitantly removing organic pollutants from wastewater, thereby offering a dual benefit of energy recovery and wastewater treatment. DF–PF systems have been shown to maximize substrate utilization by converting volatile fatty acids into additional hydrogen. In contrast, DF–LAF ensures stable feedstock supply without the need for refrigeration, thereby reducing operational costs. The DF–AD pathways further valorize residuals into methane, yielding biohythane as a dual-energy carrier. These integrations result in the creation of flexible, resilient biorefineries capable of handling heterogeneous waste streams. In practice, such systems can be implemented in municipal waste treatment facilities and agro-industrial enterprises, thereby aligning with sustainable energy policies.

CONCLUSIONS

This review comprehensively examines the recent advancements in biohydrogen production from food waste via dark fermentation, emphasizing the optimization of various processes and the integration of complementary systems. The findings underscore the critical role of substrate selection and pretreatment strategies in maximizing hydrogen yields. Carbohydrate-rich food residues, when subjected to thermal, chemical, or biological pretreatments, significantly enhance biodegradability, thereby improving hydrogen production. Inoculum optimization through thermal pretreatment selectively enriches hydrogen-producing microbial communities while suppressing methanogens, ensuring process stability. Additionally, reactor designs, particularly continuous stirred-tank reactors (CSTRs), are essential for maintaining stable operational conditions by regulating key parameters such as pH, temperature, and retention time, thus achieving consistent hydrogen yields. Furthermore, computational models like Gompertz and Monod, integrated with machine learning and artificial intelligence, offer predictive control over process dynamics. Despite promising laboratory and pilot-scale outcomes, scaling up biohydrogen production faces challenges linked to food waste heterogeneity, economic constraints, and reactor stability, underscoring the need for ongoing innovation and optimization

Moreover, this review introduces a novel approach by combining process optimization with the integration of biotechnological systems, such as the coupling of dark fermentation with microbial electrolysis cells (MECs), photo-fermentation, and biomethane production. These integrated systems significantly enhance hydrogen yield and energy recovery, addressing the challenges of resource efficiency and process stability. The combination of optimization and process integration presented in this review is a significant innovation, as it has not been extensively explored in prior literature. This holistic approach, which merges technical optimization with integrated bioprocesses, represents a valuable contribution to the field of biohydrogen production. Despite these advancements, challenges such as substrate heterogeneity, economic feasibility, and reactor stability remain. Continued research is essential to refine these systems for industrial-scale applications. Ultimately, these innovations position biohydrogen as a promising alternative energy source, contributing to the realization of a circular bioeconomy and supporting efforts in climate change mitigation.

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