World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(REVIEW ARTICLE)

CHEMISTRY BEHIND THE MICROBIAL HYDROGEN ECONOMY: FERMENTATION PATHWAYS, HYDROGENASE CATALYSIS AND ENERGY RECOVERY

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World Journal of Advanced Research and Reviews, 2026, 32(02), 1149–1160

Publication history: Received on 07 October 2026; revised on 16 November 2026; accepted on 18 November 2026

Article DOI: https://doi.org/10.30574/wjarr.2026.32.2.2932

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ABSTRACT

Biological hydrogen production through fermentation has attracted increasing attention as a potential route for sustainable hydrogen generation from renewable organic resources and waste materials. Compared with photobiological hydrogen production, fermentative systems offer several advantages, including relatively high hydrogen production rates, the ability to operate continuously under dark conditions, rapid microbial growth, and the capacity to utilize a broad range of organic substrates. Fermentative hydrogen production is primarily associated with anaerobic microorganisms that metabolize carbohydrates and other organic compounds through pathways involving pyruvate metabolism, reduced electron carriers, hydrogenases, and organic-acid formation. Hydrogenase enzymes play a central role in transferring reducing equivalents to protons, resulting in molecular hydrogen formation. The efficiency of the process depends strongly on substrate composition, microbial community structure, pH, temperature, oxidation–reduction conditions, hydrogen partial pressure, and the activity and stability of hydrogenases. Anaerobic digestion provides an important technological framework for converting complex organic wastes into useful energy products. Although conventional anaerobic digestion is predominantly directed toward methane production, integration of hydrogen-producing fermentation with subsequent methanogenesis can improve overall energy recovery through two-stage or sequential processes. This review discusses the biochemical basis of fermentative hydrogen production, the role of hydrogenases, substrate conversion pathways, microbial interactions, process parameters, advantages and limitations of anaerobic systems, and strategies for improving hydrogen productivity from organic wastes.

Keywords: Biohydrogen; Dark Fermentation; Hydrogenase; Anaerobic Digestion; Organic Waste; Fermentative Bacteria; Hydrogen Production; Renewable Energy; Microbial Biotechnology

1 INTRODUCTION

Hydrogen is increasingly recognized as an important energy carrier in the transition toward sustainable and low-carbon energy systems [1]. Unlike conventional fossil fuels, hydrogen can be converted into useful energy through fuel cells, in which water is the principal electrochemical product when hydrogen reacts with oxygen. Hydrogen can also be used in

industrial processes, transportation, energy storage, and as a feedstock for the production of chemicals and synthetic fuels [2]. However, the environmental advantages of hydrogen are strongly dependent on its production pathway. At present, a substantial proportion of hydrogen is produced from fossil-based resources through processes such as steam reforming and gasification. These conventional routes are associated with significant consumption of non-renewable resources and, unless carbon capture and storage is incorporated, substantial greenhouse-gas emissions. Consequently, the development of renewable, low-carbon and resource-efficient hydrogen-production technologies has become an important area of research [3].

Among the alternative approaches, biological hydrogen production has attracted considerable scientific interest because microorganisms are capable of converting renewable substrates and organic wastes into hydrogen under comparatively mild operating conditions. Biological hydrogen production can utilize naturally occurring microbial metabolic pathways rather than requiring extremely high temperatures or pressures [4]. Several biological routes have been investigated, including direct and indirect photobiological hydrogen production, photo fermentation, dark fermentation, and microbial electrochemical systems. Each approach has specific advantages and limitations related to substrate requirements, energy input, microbial growth, hydrogen yield, reactor design and process stability. Dark fermentation, also referred to as fermentative hydrogen production, is particularly attractive because it does not depend on solar radiation. Therefore, unlike photosynthetic systems, fermentative hydrogen production can theoretically proceed continuously during both day and night [5]. This characteristic is important for industrial-scale operation because hydrogen production is not restricted by light availability, seasonal variations in solar intensity, or the requirement for transparent photobioreactors. In addition, fermentative microorganisms can generally grow relatively rapidly under suitable environmental conditions, allowing active biomass to be established and maintained in engineered reactors. These characteristics make dark fermentation a promising platform for continuous biological hydrogen production [6].

Another important advantage of fermentative hydrogen production is its potential to utilize organic wastes as feedstocks. Large quantities of biodegradable organic matter are generated from municipal solid waste, food-processing industries, agricultural activities, livestock operations, wastewater treatment and other industrial processes. Conventional waste-management approaches may require substantial energy input and can generate secondary environmental problems. Converting the organic fraction of these wastes into hydrogen provides an opportunity to combine waste treatment with renewable-energy recovery [7]. Organic wastes can contain carbohydrates, proteins, lipids and other biodegradable components that can be transformed by microbial communities into soluble intermediates and subsequently into hydrogen and other fermentation products. Thus, dark fermentation can potentially contribute to both waste valorization and energy recovery [8].

Fermentative hydrogen production involves a series of interconnected biochemical reactions through which microorganisms convert organic substrates into hydrogen, carbon dioxide, organic acids and alcohols [9]. Carbohydrates, particularly glucose and other readily biodegradable sugars, are frequently used as model substrates because their metabolic pathways are comparatively well characterized. During carbohydrate metabolism, glycolysis converts glucose into pyruvate while generating ATP and reduced electron carriers such as NADH. Pyruvate can subsequently undergo anaerobic conversion to acetyl-CoA and other intermediates, with the generation of additional reducing equivalents [10]. The electrons generated during substrate oxidation must ultimately be transferred to appropriate acceptors in order to maintain cellular redox balance.

Hydrogenase enzymes play a central role in this process by catalyzing the reversible conversion between molecular hydrogen and protons with electrons. In hydrogen-producing microorganisms, hydrogenases can utilize reducing equivalents generated during substrate metabolism and transfer electrons to protons, resulting in molecular hydrogen formation [11]. The simplified reaction can be represented as:

$$2H^+ + 2e^- \rightarrow H_2$$

The efficiency of this reaction is closely associated with the availability of reducing equivalents and the activity of the hydrogenase system. However, not all electrons generated during substrate degradation are necessarily converted into hydrogen. Microbial metabolism can divert reducing equivalents toward alternative products such as lactate, ethanol, butyrate and other reduced metabolites [12]. Consequently, the metabolic distribution of fermentation products is an important determinant of the final hydrogen yield. The nature of the fermentation pathway also influences hydrogen production. Acetate-oriented metabolism generally allows a greater proportion of substrate reducing equivalents to be recovered as hydrogen, whereas pathways producing highly reduced end products can consume a greater fraction of the available electrons. Therefore, improving hydrogen production requires not only maintaining an active hydrogenase but also controlling the metabolic environment so that a favorable proportion of substrate carbon and electrons is directed toward hydrogen-producing pathways [13].

The hydrogen-production process is further influenced by the composition and activity of the microbial community. Hydrogen-producing bacteria may interact with other microorganisms that consume fermentation intermediates or hydrogen. In complex anaerobic systems, syntrophic relationships can strongly influence the thermodynamic feasibility of individual reactions. Hydrogen accumulation, for example, can affect the energetics of certain oxidation reactions and may consequently alter the distribution of fermentation products. Maintaining appropriate hydrogen partial pressure is therefore an important consideration in reactor operation. Environmental and operational parameters also exert major effects on fermentative hydrogen production. Parameters such as pH, temperature, substrate concentration, organic loading rate, hydraulic retention time, inoculum characteristics, oxidation–reduction conditions and hydrogen partial pressure can influence microbial growth and metabolic activity. Excessive substrate loading may result in rapid acid accumulation and inhibition, whereas insufficient substrate availability can limit hydrogen productivity. Similarly, unsuitable pH or temperature conditions can reduce enzyme activity and alter the composition of the microbial community [14].

Fermentative hydrogen production is closely related to anaerobic digestion because acidogenesis represents one of the major stages of anaerobic organic-matter conversion. Conventional anaerobic digestion involves hydrolysis, acidogenesis, acetogenesis and methanogenesis. Hydrogen can be generated during the acidogenic and acetogenic stages, but in a conventional methanogenic digester it may subsequently be consumed by hydrogen-utilizing methanogens. As a result, conventional anaerobic digestion generally directs the final energy product toward methane rather than hydrogen. Separating hydrogen-producing fermentation from subsequent methanogenesis therefore provides an opportunity to recover hydrogen as an intermediate energy product before the remaining organic compounds are converted to methane [15].

The integration of dark fermentation and anaerobic digestion is consequently an important research direction. In a two-stage system, the first reactor can be optimized for hydrogen production, while the organic-acid-rich effluent from the first stage can be transferred to a second reactor for methane production. Such a configuration can improve overall utilization of the organic substrate and potentially recover energy in two forms. It may also reduce the residual organic load of the fermentation effluent and provide an integrated approach for the treatment and valorization of organic wastes [16].

Despite these advantages, several challenges continue to limit the large-scale implementation of fermentative hydrogen production. Hydrogen yields from complex organic wastes can be lower than theoretical values because of incomplete substrate conversion, competing metabolic pathways, inhibition, hydrogen accumulation and instability of microbial communities. In addition, the composition of real wastes can vary substantially with source, season and processing conditions. Reactor start-up, microbial retention, hydrogen separation and purification, process stability and economic feasibility must also be considered when moving from laboratory experiments to industrial-scale systems. Therefore, a comprehensive understanding of the biochemical mechanisms and engineering factors controlling fermentative hydrogen production is essential. Particular attention must be given to the relationship between substrate degradation, electron transfer, hydrogenase activity and fermentation-product formation. Advances in microbial characterization, metabolic engineering, reactor design and process monitoring may provide new opportunities for improving hydrogen productivity and stability [17].

This review focuses on fermentative hydrogen production from organic compounds, with particular emphasis on the microbial and biochemical pathways responsible for hydrogen evolution, the role of hydrogenase enzymes, electron-transfer mechanisms, fermentation-product distribution and the influence of major operating parameters. The review also examines the relationship between dark fermentation and anaerobic digestion, the advantages and limitations of anaerobic systems, strategies for improving hydrogen production, and the potential integration of hydrogen and methane production for enhanced energy recovery from organic wastes[18].

2 FERMENTATIVE HYDROGEN PRODUCTION

Fermentative hydrogen production is commonly referred to as dark fermentation because the process does not require illumination. Anaerobic and facultative anaerobic microorganisms convert organic substrates into hydrogen and reduced fermentation products.

Organic substrate → reducing equivalents → H_2 + organic acids/alcohols + CO_2

For glucose, hydrogen formation is closely associated with the metabolic conversion of pyruvate. Pyruvate can be converted to acetyl-CoA through pyruvate:ferredoxin oxidoreductase, generating reduced ferredoxin. Hydrogenase can then oxidize reduced ferredoxin and transfer electrons to protons: $2H^+ + 2e^- \rightarrow H_2$.

The metabolic route taken by microorganisms determines the amount of hydrogen produced. Acetate-producing pathways generally have a higher theoretical hydrogen yield than pathways leading predominantly to reduced products such as lactate or ethanol [19]. Anaerobic process mechanism has shown in Figure1.

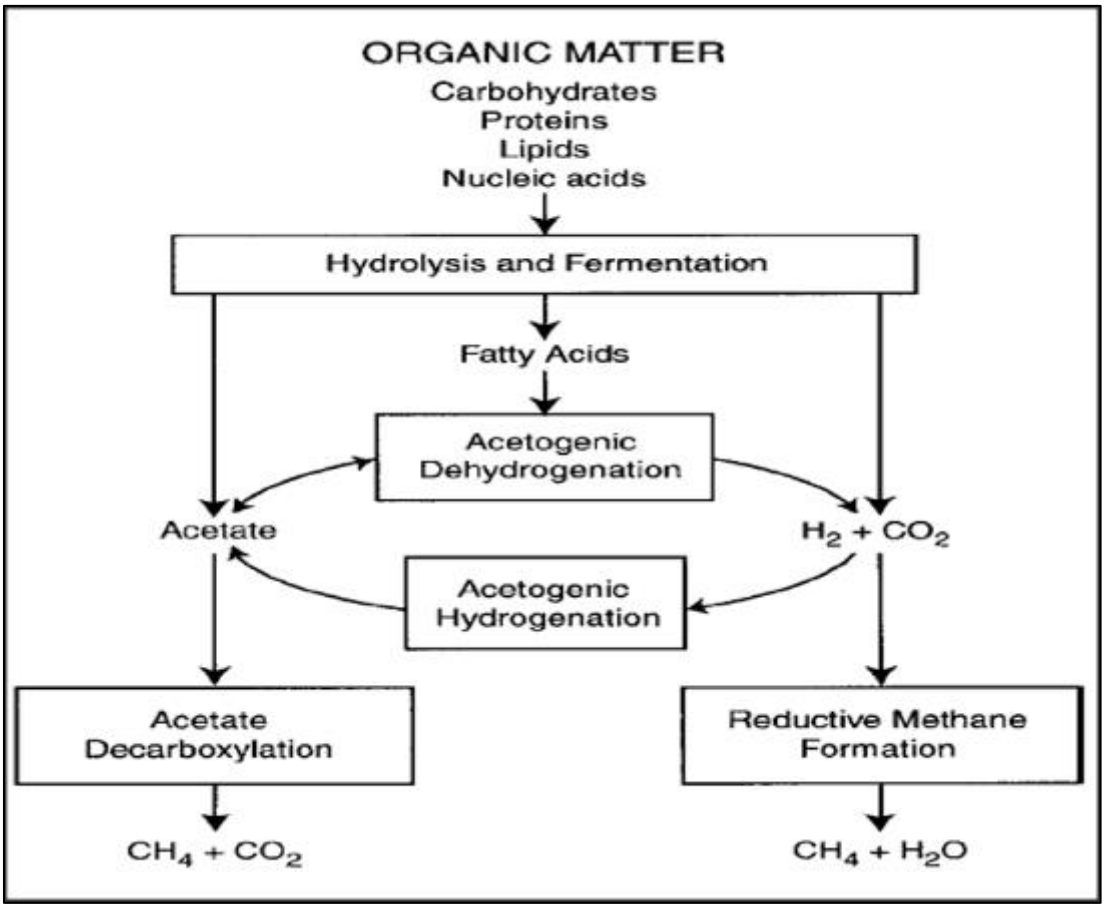


Figure 1: Anaerobic process mechanism

3 ADVANTAGES OF FERMENTATIVE HYDROGEN PRODUCTION

3.1 High hydrogen evolution rates

The high hydrogen evolution rate is mainly associated with the direct conversion of organic substrates through fermentative metabolic pathways. During substrate degradation, reducing equivalents are generated and can be transferred through hydrogen-producing reactions involving hydrogenase enzymes. A high volumetric production rate is advantageous because it can allow greater hydrogen production from a given reactor volume and may support the development of smaller and more efficient fermentation systems. However, hydrogen production rate depends on substrate concentration, microbial activity, pH, temperature, hydrogen partial pressure and the metabolic pathway followed by the microorganisms [20].

3.2 Continuous operation

The ability to operate under dark conditions provides an important operational advantage because hydrogen production is not restricted by the availability of sunlight. Continuous operation can allow fermentative systems to receive organic substrates regularly from wastewater or industrial waste streams and convert them into hydrogen without interruption. This characteristic can improve compatibility with industrial processes in which organic wastes are generated continuously. Stable continuous operation, however, requires appropriate control of pH, temperature, substrate loading and microbial biomass to maintain consistent hydrogen production [21].

3.3 Rapid microbial growth

Rapid growth of hydrogen-producing microorganisms can contribute to the establishment of an active microbial population within the fermentation reactor. Once suitable environmental conditions are established, active biomass can continuously metabolize organic substrates and generate reducing equivalents required for hydrogen formation. Rapid

microbial growth may also facilitate recovery following moderate disturbances in operating conditions. However, maintaining the desired hydrogen-producing population is important because other microorganisms may compete for the same substrates or consume hydrogen, thereby reducing the net hydrogen yield.

3.4 Utilization of waste substrates

The ability to use organic wastes as substrates provides both economic and environmental advantages. Many food-processing residues, agricultural materials and wastewater streams contain biodegradable organic compounds that can serve as carbon and energy sources for fermentative microorganisms. Their conversion into hydrogen can reduce the amount of organic material requiring conventional disposal while simultaneously recovering useful energy. The composition of waste substrates can vary considerably, however, and complex materials may require suitable pretreatment or hydrolysis to make the organic compounds more accessible to hydrogen-producing microorganisms [22].

3.5 Simultaneous waste treatment and energy recovery

The combination of waste treatment and hydrogen recovery makes fermentative hydrogen production particularly attractive within a circular bioeconomy framework. During fermentation, biodegradable organic matter is converted into hydrogen and soluble fermentation products, thereby reducing part of the organic load of the original waste. The remaining organic acids can subsequently be utilized in downstream anaerobic digestion for methane production. Consequently, integration of hydrogen-producing fermentation with subsequent biological treatment can improve overall resource utilization and increase the amount of useful energy recovered from organic wastes [23].

4 MICROBIAL AND BIOCHEMICAL BASIS OF HYDROGEN FORMATION

4.1 Substrate hydrolysis and uptake

Hydrolysis is particularly important when complex organic wastes are used as substrates because many organic compounds are not directly available for uptake by fermentative microorganisms. Extracellular enzymes produced by hydrolytic microorganisms break down large molecules into smaller, soluble compounds that can be transported into microbial cells. The efficiency of this step can strongly influence the overall hydrogen-production process because incomplete hydrolysis limits the amount of substrate available for subsequent fermentation. Factors such as particle size, substrate composition, temperature, pH and microbial enzyme activity can therefore affect the rate of hydrolysis and substrate uptake [24].

4.2 Glycolysis

Glycolysis is a central metabolic pathway that provides both energy and reducing equivalents for fermentative hydrogen production. During the conversion of glucose to pyruvate, substrate-level phosphorylation generates ATP, while NAD^+ is reduced to NADH. The subsequent utilization of NADH is important for maintaining cellular redox balance and can influence the distribution of fermentation products. The efficiency of glycolytic metabolism therefore affects the amount of pyruvate available for downstream hydrogen-producing pathways and ultimately influences the overall hydrogen productivity of the microbial culture.

4.3 Pyruvate oxidation

Pyruvate represents an important metabolic branch point in anaerobic fermentation. Its conversion to acetyl-CoA provides substrates for the formation of acetate and other fermentation products while simultaneously generating reduced electron carriers. In hydrogen-producing microorganisms, reduced ferredoxin generated during pyruvate metabolism can provide electrons for hydrogenase-mediated hydrogen evolution. The direction and rate of pyruvate conversion are influenced by the redox state of the cell and by the accumulation of fermentation products. Efficient pyruvate oxidation is therefore important for maintaining the flow of electrons toward hydrogen production [25].

4.4 Hydrogen formation

Hydrogen formation represents the final step in the transfer of reducing equivalents toward molecular hydrogen. Hydrogenases receive electrons from reduced cellular electron carriers and facilitate their transfer to protons, producing H_2. The reaction can be represented in simplified form as $2H^+ + 2e^- \rightarrow H_2$. Hydrogen production can be affected by the availability of electron donors, hydrogenase activity, pH, temperature and hydrogen partial pressure. Accumulation of hydrogen may also influence the thermodynamic favorability of upstream reactions, making effective hydrogen removal and gas management important considerations in fermentative hydrogen-production systems.

5 ROLE OF HYDROGENASE IN FERMENTATIVE HYDROGEN PRODUCTION

Hydrogenases are central enzymes in biological hydrogen metabolism. They catalyze reversible reactions involving molecular hydrogen: $2H^+ + 2e^- \rightleftharpoons H_2$.

Different classes of hydrogenases have been identified based on their metal composition and structural characteristics, including [NiFe]-hydrogenases, [FeFe]-hydrogenases and [Fe]-hydrogenases. In many anaerobic fermentative microorganisms, [FeFe]-hydrogenases are particularly important for hydrogen evolution.

The activity of hydrogenase is influenced by pH, temperature, redox potential, hydrogen partial pressure, availability of electron donors, metal availability, enzyme expression and the metabolic state of the microorganism.

Hydrogenase activity can therefore become a major determinant of hydrogen productivity. A major challenge is the sensitivity of many hydrogenases to oxygen. Consequently, maintaining appropriate anaerobic conditions is essential [26-27].

6 ELECTRON TRANSFER AND HYDROGEN PRODUCTION

Efficient hydrogen production requires an effective connection between substrate oxidation and proton reduction. During fermentation, electrons generated from substrate oxidation must be transferred to appropriate electron carriers and ultimately to hydrogenase.

Organic substrate → NADH/reduced ferredoxin → hydrogenase → H^+ → H_2

Electrons may also be consumed through competing pathways. Microorganisms may use reducing equivalents for the production of lactate, ethanol, butanol and other reduced metabolites. Consequently, hydrogen productivity depends on directing a sufficiently large fraction of the available reducing power toward hydrogen formation [28].

7 EFFECT OF FERMENTATION PRODUCTS ON HYDROGEN YIELD

Fermentation products provide an important indication of the metabolic pathway operating within a hydrogen-producing culture. Principal products may include acetate, butyrate, ethanol, lactate, propionate, CO_2 and H_2.

Acetate and butyrate formation are commonly associated with hydrogen-producing fermentation, whereas increased formation of reduced products such as lactate and ethanol can indicate diversion of reducing equivalents away from hydrogen production. Propionate formation is generally considered unfavorable for hydrogen recovery because it consumes reducing equivalents.

Monitoring the volatile fatty acid profile can therefore provide valuable information about the metabolic state and hydrogen-production potential of a fermentation system [29].

8 ANAEROBIC DIGESTION AND ITS RELATIONSHIP WITH HYDROGEN PRODUCTION

Anaerobic digestion is a complex microbial process through which organic matter is converted into biogas, principally methane and carbon dioxide. The process generally involves four interconnected stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Hydrogen is primarily generated during the acidogenic stage. Consequently, hydrogen production can be enhanced by manipulating operating conditions to favor acidogenic microorganisms while suppressing or separating methanogenic activity [30]. In conventional anaerobic digestion, hydrogen produced during acidogenesis is subsequently consumed by hydrogenotrophic methanogens. Therefore, the hydrogen concentration in a conventional methane-producing digester is usually low. This creates an important opportunity for two-stage anaerobic digestion, in which hydrogen-producing fermentation is separated from methane production [31].

9 ADVANTAGES OF ANAEROBIC DIGESTION

9.1 Low oxygen requirement

Anaerobic processes do not require continuous aeration. Because aeration can represent a major energy requirement in wastewater treatment, eliminating or reducing oxygen supply can lower operational energy demand.

9.2 Lower biomass production

Anaerobic microorganisms generally have lower biomass yields than aerobic microorganisms. Consequently, anaerobic treatment can generate substantially less excess sludge.

9.3 Energy recovery

The production of methane provides an opportunity for energy recovery. Methane-rich biogas can be used for heating or electricity generation after appropriate treatment.

9.4 High organic loading capability

Anaerobic reactors can be designed to treat relatively concentrated organic waste streams and can operate at high organic loading rates when appropriate reactor configurations and microbial communities are maintained [32].

9.5 Treatment of industrial wastewater

Anaerobic digestion has been successfully applied to several high-strength industrial wastewaters containing biodegradable organic matter.

9.6 Biodegradation of selected recalcitrant compounds

Under suitable conditions, anaerobic microbial communities can contribute to the transformation of certain xenobiotic and recalcitrant compounds [33].

9.7 Smaller reactor requirements

High-rate anaerobic reactor configurations can achieve substantial organic removal with relatively small reactor volumes compared with conventional treatment systems [34].

10 LIMITATIONS OF ANAEROBIC DIGESTION

10.1 Slow start-up

Establishment of a stable anaerobic microbial community can require considerable time, particularly in systems with low inoculum activity.

10.2 Sensitivity to toxic compounds

Anaerobic microorganisms can be sensitive to toxic substances, including certain heavy metals, sulfide, solvents and other inhibitory compounds [35].

10.3 Temperature sensitivity

Microbial growth and reaction rates are strongly influenced by temperature. Low temperatures can significantly reduce biological conversion rates.

10.4 pH and alkalinity requirements

Methanogenic systems are particularly sensitive to pH changes. Adequate alkalinity and buffering capacity are therefore important for maintaining process stability.

10.5 Odor and corrosive gases

Anaerobic treatment can generate compounds such as hydrogen sulfide and other malodorous gases. Appropriate gas handling and treatment systems are therefore required.

10.6 Nutrient-removal limitations

Anaerobic treatment alone generally cannot provide the same biological nitrogen and phosphorus removal capabilities as specifically designed aerobic or anoxic treatment processes [36].

11 FACTORS AFFECTING FERMENTATIVE HYDROGEN PRODUCTION

11.1 pH

The pH of the fermentation medium influences the activity of hydrogen-producing microorganisms as well as the function of enzymes involved in substrate degradation and hydrogen evolution. Changes in pH can alter the distribution of fermentation products, microbial community structure and the availability of nutrients. Excessive acidification may occur when volatile fatty acids accumulate during fermentation, potentially inhibiting microbial activity and reducing hydrogen productivity. Therefore, appropriate pH control and buffering capacity are important for maintaining stable hydrogen production, particularly during continuous operation [37].

11.2 Temperature

Temperature affects the rates of biochemical reactions, microbial growth and substrate degradation and therefore has a direct influence on hydrogen productivity. Mesophilic systems generally operate at moderate temperatures, whereas thermophilic systems operate at higher temperatures and may provide enhanced hydrolysis and reaction rates under suitable conditions. However, each microbial community has an optimum temperature range, and temperatures outside this range can decrease enzyme activity and inhibit microbial growth. Maintaining a stable temperature is therefore essential for consistent fermentative hydrogen production [38].

11.3 Substrate concentration

The concentration and composition of the substrate determine the amount of carbon and reducing power available to the microbial community. At an appropriate concentration, increasing substrate availability can enhance microbial activity and hydrogen production. However, excessive concentrations may cause rapid accumulation of organic acids, decrease the pH and create osmotic or metabolic stress. Substrate concentration should therefore be optimized together with the organic loading rate and reactor retention time to maintain efficient conversion without causing inhibition [39-40].

11.4 Hydrogen partial pressure

Hydrogen partial pressure is an important thermodynamic factor in fermentative hydrogen production. When hydrogen accumulates in the reactor headspace or dissolved phase, certain oxidation reactions involved in fermentation may become less favourable, which can redirect metabolism toward alternative reduced products. Maintaining a relatively low hydrogen partial pressure can help sustain favourable conditions for hydrogen-producing reactions. Appropriate gas removal, headspace management or continuous gas collection can therefore contribute to improved hydrogen production and process stability [41].

11.5 Hydraulic retention time

Hydraulic retention time determines how long the substrate and microbial biomass remain within the fermentation reactor and therefore influences substrate conversion and microbial selection. An appropriate retention time provides sufficient opportunity for microorganisms to degrade the substrate and produce hydrogen. If the retention time is too short, active microorganisms may be washed out before establishing a stable population, whereas excessively long retention times may reduce volumetric productivity and provide opportunities for competing microorganisms to become established. Optimization of retention time is therefore important for balancing hydrogen yield, productivity and microbial stability [42-43].

11.6 Organic loading rate

Organic loading rate represents the quantity of organic substrate supplied to the reactor relative to its operating volume and time. Increasing the loading rate can improve hydrogen productivity when the microbial community has sufficient capacity to convert the incoming substrate. However, excessive loading may result in rapid acid production, accumulation of volatile fatty acids, pH reduction and microbial inhibition. An optimized organic loading rate should therefore provide sufficient substrate availability while maintaining stable microbial activity and effective conversion of organic matter into hydrogen [44-45].

11.7 Inoculum

The inoculum provides the initial microbial population responsible for substrate degradation and hydrogen production and can therefore have a major influence on reactor performance. The origin of the inoculum, microbial diversity, physiological condition and pretreatment method can affect hydrogen yield and production rate. In some experimental systems, inoculum pretreatment is used to reduce hydrogen-consuming microorganisms and enrich hydrogen-

producing populations. Selection of an appropriate inoculum and adequate adaptation to the selected substrate can improve start-up performance, microbial stability and reproducibility of hydrogen production [46].

12 STRATEGIES FOR IMPROVING HYDROGEN PRODUCTION

Several approaches can be employed to improve fermentative hydrogen production: selection of efficient hydrogen-producing microorganisms; optimization of pH and temperature; control of hydrogen partial pressure; optimization of substrate concentration; inoculum pretreatment; enrichment of hydrogen-producing microbial communities; genetic and metabolic engineering; immobilization of microbial biomass; use of continuous-flow reactors; and integration with secondary methane production. Metabolic engineering represents an emerging strategy for redirecting intracellular reducing equivalents toward hydrogen formation. Similarly, reactor engineering can improve mass transfer, microbial retention and substrate conversion [47-48]. The Key strategies for enhancing fermentative hydrogen production through microbial, biochemical, and reactor-level approaches has shown in Figure 2.

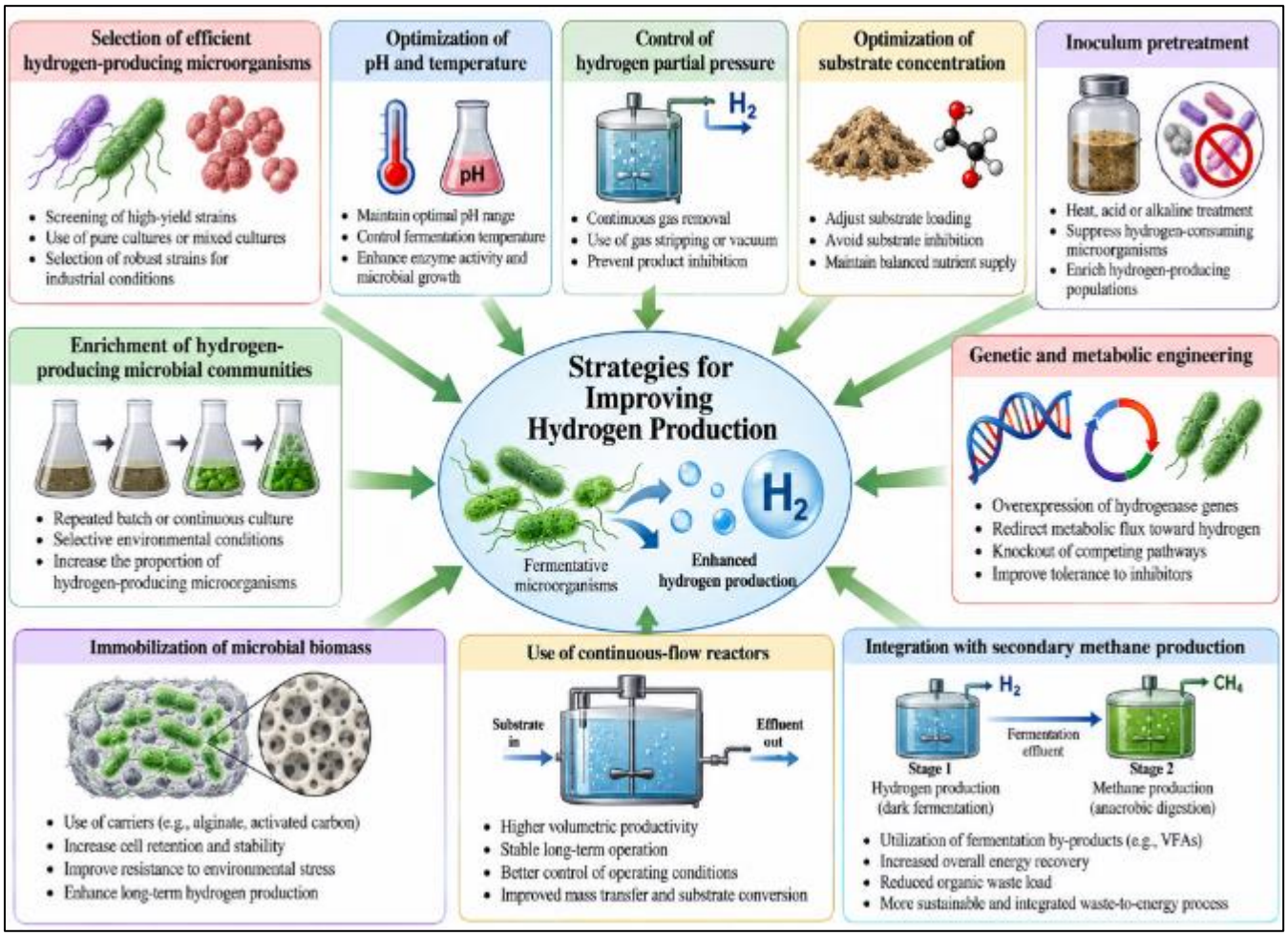


Figure 2: Key strategies for enhancing fermentative hydrogen production through microbial, biochemical, and reactor-level approaches.

13 INTEGRATION OF DARK FERMENTATION AND METHANE PRODUCTION

Although dark fermentation can generate hydrogen, significant quantities of organic acids remain in the fermentation effluent. Direct disposal of this residual organic matter reduces the overall energy recovery potential. A promising solution is the integration of dark fermentation with subsequent methanogenesis. The first stage produces H_2, CO_2 and volatile fatty acids, while the second stage converts the remaining organic acids into methane. This two-stage approach can therefore recover energy in two forms hydrogen followed by methane while simultaneously reducing the organic load of the waste stream. This configuration is particularly attractive for food waste, agricultural residues, municipal organic waste and high-strength industrial wastewater [49-50].

14 RESEARCH GAPS AND FUTURE PERSPECTIVES

Despite considerable progress, several challenges must be addressed before fermentative hydrogen production can achieve widespread industrial application. Hydrogen yield from complex organic wastes remains below theoretical values in many systems because of competing metabolic pathways and incomplete substrate conversion. Microbial community dynamics are also difficult to control, particularly in mixed-culture systems. Future studies should combine metagenomics, Meta transcriptomics, proteomics and metabolomics to identify microorganisms and metabolic pathways associated with high hydrogen productivity. Greater attention should also be given to long-term reactor stability; continuous operation; hydrogen purification; substrate pretreatment; inhibition by ammonia, sulfide and toxic metals; mixed-substrate fermentation; microbial community engineering; hydrogen partial-pressure control; process scale-up; techno-economic assessment; and life-cycle assessment. The integration of dark fermentation with methane production is particularly promising because it can improve the overall energy recovery from organic waste while reducing residual organic matter.

15 CONCLUSION

Fermentative hydrogen production represents a promising biological pathway for converting renewable organic substrates and wastes into a valuable energy carrier. The major advantages of the process include relatively rapid hydrogen evolution, operation without light, microbial growth under controlled conditions, and the ability to utilize diverse organic feedstocks. Hydrogenase enzymes play a central role in the conversion of microbial reducing equivalents into molecular hydrogen, while fermentation-product distribution determines the efficiency with which substrate electrons are recovered as hydrogen.

Anaerobic digestion provides an important technological framework for biological waste conversion, although conventional methanogenic systems consume much of the hydrogen generated during acidogenesis. Separating hydrogen-producing fermentation from subsequent methanogenesis can therefore provide a strategy for recovering both hydrogen and methane. Future research should focus on improving hydrogen yield, microbial stability, reactor design, substrate utilization and process economics. Integration of microbiological, biochemical, metabolic-engineering and reactor-engineering approaches will be essential for developing commercially viable fermentative hydrogen-production systems.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Acknowledgments

The author expresses sincere gratitude to Almighty God and his beloved parents for their blessings, guidance, encouragement, and unwavering support throughout the preparation of this manuscript. The author also gratefully acknowledges Dr. M. Sasidhar, Principal, Dr. K. Sai Manoj, CEO, Sri K. Rama Mohana Rao, Secretary and Correspondent, Sri K. Lakshmi Karthik, President, and Sri K. Ramesh Babu, Industrialist and Chairman of Amrita Sai Institute of Science and Technology, for their visionary leadership, constant encouragement, and commitment to academic excellence and research. Special thanks are extended to all faculty members, colleagues, and staff of the Science and Humanities (S&H) Department and the (CRT) Department for their valuable support, cooperation, and constructive suggestions. Finally, heartfelt appreciation is extended to all friends, well-wishers, and professional associates who directly or indirectly contributed to the successful completion of this manuscript.

Disclosure of conflict of interest

The authors declare no conflicts of interest.

REFERENCES

[1] Hallenbeck PC, Ghosh D. Advances in fermentative biohydrogen production: the way forward? Trends Biotechnol. 2009;27(5):287-297.

[2] Levin DB, Pitt L, Love M. Biohydrogen production: prospects and limitations to practical application. Int J Hydrogen Energy. 2004;29(2):173-185.

[3] Das D, Veziroğlu TN. Advances in biological hydrogen production processes. Int J Hydrogen Energy. 2008;33(21):6046-6057.

[4] Kapdan IK, Kargi F. Bio-hydrogen production from waste materials. Enzyme Microb Technol. 2006;38(5):569-582.

[5] Show KY, Lee DJ, Tay JH, Lin CY, Chang JS. Biohydrogen production: current perspectives and the way forward. Int J Hydrogen Energy. 2012;37(20):15616-15631.

[6] Wang J, Wan W. Factors influencing fermentative hydrogen production: a review. Int J Hydrogen Energy. 2009;34(2):799-811.

[7] Wang JL, Wan W. Experimental design methods for fermentative hydrogen production: a review. Int J Hydrogen Energy. 2009;34(1):235-244.

[8] Levin DB, Chahine R. Challenges for renewable hydrogen production from biomass. Int J Hydrogen Energy. 2010;35(10):4962-4969.

[9] Hawkes FR, Dinsdale R, Hawkes DL, Hussy I. Sustainable fermentative hydrogen production: challenges for process optimisation. Int J Hydrogen Energy. 2002;27(11-12):1339-1347.

[10] Nath K, Das D. Improvement of fermentative hydrogen production: various approaches. Appl Microbiol Biotechnol. 2004;65:520-529.

[11] Hallenbeck PC. Fundamentals of the fermentative production of hydrogen. Water Sci Technol. 2005;52(1-2):21-29.

[12] Levin DB, Islam R, Cicek N, Sparling R. Hydrogen production by Clostridium thermocellum 27405 from cellulosic biomass substrates. Int J Hydrogen Energy. 2006;31(11):1496-1503.

[13] Ghimire A, Frunzo L, Pirozzi F, Trably E, Escudié R, Lens PNL, et al. A review on dark fermentative biohydrogen production from organic biomass: process parameters and use of by-products. Appl Energy. 2015;144:73-95.

[14] Soares JF, Confortin TC, Todero I, Mayer FD, Mazutti MA. Dark fermentative biohydrogen production from lignocellulosic biomass: technological challenges and future prospects. Renew Sustain Energy Rev. 2020;117:109484. doi:10.1016/j.rser.2019.109484.

[15] Sivagurunathan P, Kumar G, Park JH, Park JH, Kim SH. Optimization of various parameters influencing dark fermentative hydrogen production from renewable biomass. Renew Sustain Energy Rev. 2016;64:121-131.

[16] Mohan SV, Pandey A, eds. Biohydrogen Production: Routes and Relations. Springer; 2014.

[17] Muñoz-Páez KM, Juárez-García M, Rojas-Valencia MN, et al. Dark fermentation for biohydrogen production: substrates, microorganisms and process parameters. Renew Sustain Energy Rev. 2021.

[18] Sharmila VG, Tamilarasan K, Dinesh Kumar M, Kumar G, Varjani S, Adish Kumar S, et al. Trends in dark biohydrogen production strategy and linkages with transition towards low carbon economy: an outlook, cost-effectiveness, bottlenecks and future scope. Int J Hydrogen Energy. 2022;47(34):15309-15332. doi:10.1016/j.ijhydene.2021.12.139.

[19] Lopez-Hidalgo AM, Smoliński A, Sanchez A. A meta-analysis of research trends on hydrogen production via dark fermentation. Int J Hydrogen Energy. 2022;47(27):13300-13339. doi:10.1016/j.ijhydene.2022.02.106.

[20] Moussa RN, Moussa N, Dionisi D. Hydrogen production from biomass and organic waste using dark fermentation: an analysis of literature data on the effect of operating parameters on process performance. Processes. 2022;10(1):156. doi:10.3390/pr10010156.

[21] Albuquerque MM, Sartor GDB, Martinez-Burgos WJ, Scapini T, Edwiges T, Soccol CR, et al. Biohydrogen produced via dark fermentation: a review. Methane. 2024;3(3):500-532. doi:10.3390/methane3030029.

[22] Li J, Zheng G, He J, Jiang R, Shen W, Jiang H. Hydrogen-producing capability of bacterial strains isolated from anaerobic sludge and their hydrogenase activity. Bioresour Technol. 2008;99:105-111.

[23] Levin DB, Zhu H, Beland M, Cicek N, Holbein BE. Potential for hydrogen and methane production from biomass. Int J Hydrogen Energy. 2007;32(13):2028-2036.

[24] Lay JJ, Lee YJ, Noike T. Feasibility of biological hydrogen production from organic fraction of municipal solid waste. Water Res. 1999;33(11):2579-2586.

[25] Chen CC, Lin CY, Chang JS. Kinetics of hydrogen production with continuous anaerobic cultures utilizing sucrose as the limiting substrate. Appl Microbiol Biotechnol. 2001;57:56-64.

[26] Fang HHP, Liu H. Effect of pH on hydrogen production from glucose by a mixed culture. Bioresour Technol. 2002;82(1):87-93.

[27] Kotay SM, Das D. Biohydrogen as a renewable energy resource—prospects and potentials. Int J Hydrogen Energy. 2008;33(1):258-263.

[28] Choi JD, Ahn Y, Sang BI. Microbial hydrogen production from food waste by anaerobic fermentation. Bioresour Technol. 2011;102:7463-7468.

[29] Krake S, Tovar GEM, Zibek S. Microaerobic dark fermentation by purple bacteria as an emerging perspective for biohydrogen production—a review. Biotechnol Biofuels Bioprod. 2026;19:32. doi:10.1186/s13068-026-02747-5

[30] Khanna N, Das D. Biohydrogen production by dark fermentation. WIREs Energy Environ. 2013;2(4):401-421. doi:10.1002/wene.15.

[31] Krake S, Tovar GEM, Zibek S. Microaerobic dark fermentation by purple bacteria as an emerging perspective for biohydrogen production—a review. Biotechnol Biofuels Bioprod. 2026;19:32. doi:10.1186/s13068-026-02747-5.

[32] Lee DJ, Show KY, Su A. Dark fermentation on biohydrogen production: pure culture. Bioresour Technol. 2011;102(18):8393-8402. doi:10.1016/j.biortech.2011.03.041.

[33] Wang JL, Wan W. Factors influencing fermentative hydrogen production: a review. Int J Hydrogen Energy. 2009;34(2):799-811.

[34] Hallenbeck PC, Ghosh D. Advances in fermentative biohydrogen production: the way forward? Trends Biotechnol. 2009;27(5):287-297.

[35] Levin DB, Pitt L, Love M. Biohydrogen production: prospects and limitations to practical application. Int J Hydrogen Energy. 2004;29(2):173-185.

[36] Das D, Veziroğlu TN. Advances in biological hydrogen production processes. Int J Hydrogen Energy. 2008;33(21):6046-6057.

[37] Ghimire A, Frunzo L, Pirozzi F, Trably E, Escudié R, Lens PNL, et al. A review on dark fermentative biohydrogen production from organic biomass: process parameters and use of by-products. Appl Energy. 2015;144:73-95. doi:10.1016/j.apenergy.2015.01.045.

[38] Sarangi PK, Nanda S. Biohydrogen production through dark fermentation. Chem Eng Technol. 2020;43(9):1747-1760. doi:10.1002/ceat.201900452.

[39] Rittmann S, Herwig C. A comprehensive and quantitative review of dark fermentative biohydrogen production. Microb Cell Fact. 2012;11:115. doi:10.1186/1475-2859-11-115.

[40] Ahmad A, Rambabu K, Hasan SW, Show PL, Banat F. Biohydrogen production through dark fermentation: recent trends and advances in transition to a circular bioeconomy. Int J Hydrogen Energy. 2024;52:335-357. doi:10.1016/j.ijhydene.2023.05.161.

[41] Moussa RN, Moussa N, Dionisi D. Hydrogen production from biomass and organic waste using dark fermentation: an analysis of literature data on the effect of operating parameters on process performance. Processes. 2022;10(1):156. doi:10.3390/pr10010156.

[42] Albuquerque MM, Sartor GB, Martinez-Burgos WJ, Scapini T, Edwiges T, Soccol CR, et al. Biohydrogen produced via dark fermentation: a review. Methane. 2024;3(3):500-532. doi:10.3390/methane3030029.

[43] Cheng D, Ngo HH, Guo W, Chang SW, Nguyen DD, Deng L, et al. Advanced strategies for enhancing dark fermentative biohydrogen production from biowaste towards sustainable environment. Bioresour Technol. 2022;351:127045. doi:10.1016/j.biortech.2022.127045.

[44] Sasaki K, Sasaki D, Tsuge Y, et al. Changes in the microbial consortium during dark hydrogen fermentation in a bioelectrochemical system increases methane production during a two-stage process. Biotechnol Biofuels. 2018;11:173. doi:10.1186/s13068-018-1175-z.

[45] Singh T, Alhazmi A, Mohammad A, et al. Integrated biohydrogen production via lignocellulosic waste: opportunity, challenges & future prospects. Bioresour Technol. 2021;338:125511. doi:10.1016/j.biortech.2021.125511.

[46] Balachandar G, et al. Biological hydrogen production via dark fermentation: a holistic approach from lab-scale to pilot-scale. Int J Hydrogen Energy. 2020;45:5202-5225. doi:10.1016/j.ijhydene.2019.09.006.

[47] Dahiya S, et al. Renewable hydrogen production by dark-fermentation: current status, challenges and perspectives. Bioresour Technol. 2021;321:124354. doi:10.1016/j.biortech.2020.124354.

[48] Mohanakrishna G, Sneha NP, Rafi SM, Sarkar O. Dark fermentative hydrogen production: potential of food waste as future energy needs. Sci Total Environ. 2023;888:163801. doi:10.1016/j.scitotenv.2023.163801.

[49] Vidal A, Mohiuddin O, Chance E, Serrano-Blanco S, Howard TP, Muñoz-Muñoz J, et al. Biohydrogen production through dark fermentation of agricultural waste: novel strain and feedstock characterisation. Bioresour Technol. 2025;434:132839. doi:10.1016/j.biortech.2025.132839.

[50] Binchebo TL, Narra S, Ancha VR, Asefa SK, Mengesha TT, Jiru EB, et al. Recent developments in biohydrogen production through dark fermentation of biomass waste: feedstocks, microbial population dynamics, and system integration. Front Energy Res. 2026;14. doi:10.3389/fenrg.2026.1819152