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Integrating dark fermentation and electrohydrogenesis for enhanced biohydrogen production from food waste

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Biohydrogen production from food waste offers a sustainable and carbon-neutral alternative to fossil fuels. However, its large-scale application is limited by the rapid hydrolysis of biodegradable organics, resulting in the accumulation of inhibitory byproducts such as ammonia and volatile fatty acids (VFAs), especially lactic acid. These compounds suppress hydrogen-producing bacteria and reduce system efficiency. Integrating dark fermentation (DF) with microbial electrolysis cells (MECs) has emerged as a promising approach to overcome these limitations by converting residual organics into additional hydrogen *via* electrohydrogenesis. Optimization of operational parameters such as pH, hydraulic retention time (HRT), and organic loading rate (OLR) further enhances hydrogen yield by minimizing VFA accumulation and improving system stability. Integrated DF–MEC systems have achieved hydrogen yields of up to 1608.6 ± 266.2 mL H_2 per g COD consumed and COD removal efficiencies of 78.5 ± 5.7%. Heat pretreatment and the use of genetically engineered microbial strains have been shown to further enhance hydrogen production. Engineered strains have delivered hydrogen yields ranging from 0.47 to 1.88 mol H_2 per mol glucose. MEC integration has also demonstrated a 30–40% increase in hydrogen production compared to standalone DF systems. The digestate from lactate-driven DF, enriched with VFAs such as acetate and lactate, provides an excellent substrate for MECs, thereby enhancing electrohydrogenesis. Despite high initial capital costs, the long-term benefits, such as waste valorization, greenhouse gas reduction, and renewable energy recovery, make the DF–MEC system a viable and scalable solution for sustainable hydrogen production from food waste.

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1. Introduction

The global energy crisis and increasing environmental degradation have intensified the need for clean, affordable, and sustainable energy systems. Achieving Sustainable Development Goal 7 (SDG 7) requires transitioning from fossil fuels to renewable sources that are efficient and environmentally friendly.[1,2] Fossil fuels, while still the dominant global energy source, contribute significantly to greenhouse gas (GHG) emissions and resource depletion. In this context, biowaste has emerged as a promising renewable energy feedstock due to its high organic content, availability, and biodegradability. It currently accounts for approximately 70% of global renewable energy generation.[3–6] Among emerging alternatives, hydrogen (H_2) has gained significant attention due to its high energy content almost three times that of hydrocarbon fuels and its clean combustion profile, generating only water as a byproduct.[7] Hydrogen plays a critical role in sectors such as petroleum refining, ammonia synthesis, metal processing, and food manufacturing.[8,9] However, traditional hydrogen production methods, including steam methane reforming (SMR), coal gasification, and electrolysis, are often energy-intensive, costly, and associated with CO_2 emissions. Biological hydrogen production offers a more sustainable alternative, but is still constrained by low yields and slow kinetics.[10–12]

Food waste has been known as an ideal substrate for biohydrogen production due to its high carbohydrate content, biodegradability, and global abundance.[13,14] According to the Food and Agriculture Organization (FAO), food waste is predicted to reach 138 million tonnes annually by 2025, representing a significant and underutilized bioresource.[4,15] Anaerobic digestion (AD) is widely used to convert food waste into biogas; however, it suffers from limitations such as ammonia inhibition and suboptimal methane yield.[16,17] Enhancing biohydrogen production from food waste requires

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optimizing feedstock characteristics, reactor design, and process parameters.[18]

Dark fermentation (DF) is a promising anaerobic process for hydrogen production that operates without the need for light and can utilize diverse substrates, including vegetable peels, dairy residues, and brewery waste.[19–21] However, DF is limited by several factors, including substrate complexity, microbial community dynamics, pH, temperature, and the accumulation of inhibitory metabolites such as volatile fatty acids (VFAs).[22] Recent advances, such as lactate-driven dark fermentation (LD-DF) have shown potential to increase hydrogen yields by enabling cross-feeding interactions between lactic acid bacteria (LAB) and hydrogen-producing bacteria.[23–27] Although progress has been made using pure cultures, metabolic engineering, and nanoparticle supplementation, large-scale LD-DF systems remain under development.[28–31]

Typically, DF achieves only 20–30% of the theoretical hydrogen yield.[32] Integrating it into a two-stage process with anaerobic digestion (DF–AD) has been proposed to improve energy recovery by converting DF effluent into methane.[33] Until now, food waste's complex composition and thermodynamic barriers have often limited the complete conversion of VFAs. Pretreatment strategies such as hydrothermal processing improve substrate solubilization and microbial accessibility. More recently, the integration of microbial electrolysis cells (MECs) with DF has shown promise in overcoming these limitations. MECs use electroactive bacteria to oxidize VFAs and organic acids into protons, electrons, and CO_2, producing additional hydrogen through electrohydrogenesis at a low applied voltage.[33,34] Despite these advancements, the combined application of DF, LD-DF, and MECs remains at a conceptual or pilot scale. Further research is needed to evaluate system performance, microbial interactions, and process stability under realistic operating conditions.

This review examines the integration of dark fermentation and microbial electrolysis cells (DF–MECs) as a sustainable method for hydrogen production from food waste. It critically examines pretreatment strategies, the role of lactic acid bacteria (LAB), key operational parameters, and metabolic intermediates that influence system performance. In addition, we highlight the current technological bottlenecks, microbial limitations, and optimization strategies necessary to improve the industrial scalability of DF–MEC systems. While previous reviews have addressed DF or MEC technologies separately, very few have provided a mechanistic and integrated perspective on how microbial communities, electron flow, metabolic shifts (*e.g.*, lactate/acetate dynamics), and electrode performance interact across DF–MEC systems, particularly under food waste conditions. This includes an evaluation of electrode materials and their role in enhancing bioelectrochemical interactions, hydrogen recovery, and system robustness. This review fills that gap by offering a multi-dimensional analysis that links microbial ecology, process engineering, and electrochemical dynamics. We aim to provide a conceptual framework and forward-looking roadmap that will support the scaling up and integration of circular bioeconomy technologies in DF–MEC.

1.1 Literature search strategy

To ensure a comprehensive and unbiased review of the integration between dark fermentation (DF) and microbial electrolysis cells (MECs) for hydrogen production from food waste, a systematic literature search was conducted across major scientific databases including Web of Science, Scopus, ScienceDirect, and SpringerLink. The search focused on peer-reviewed journal articles published between 2016 and 2024, using Boolean combinations such as:

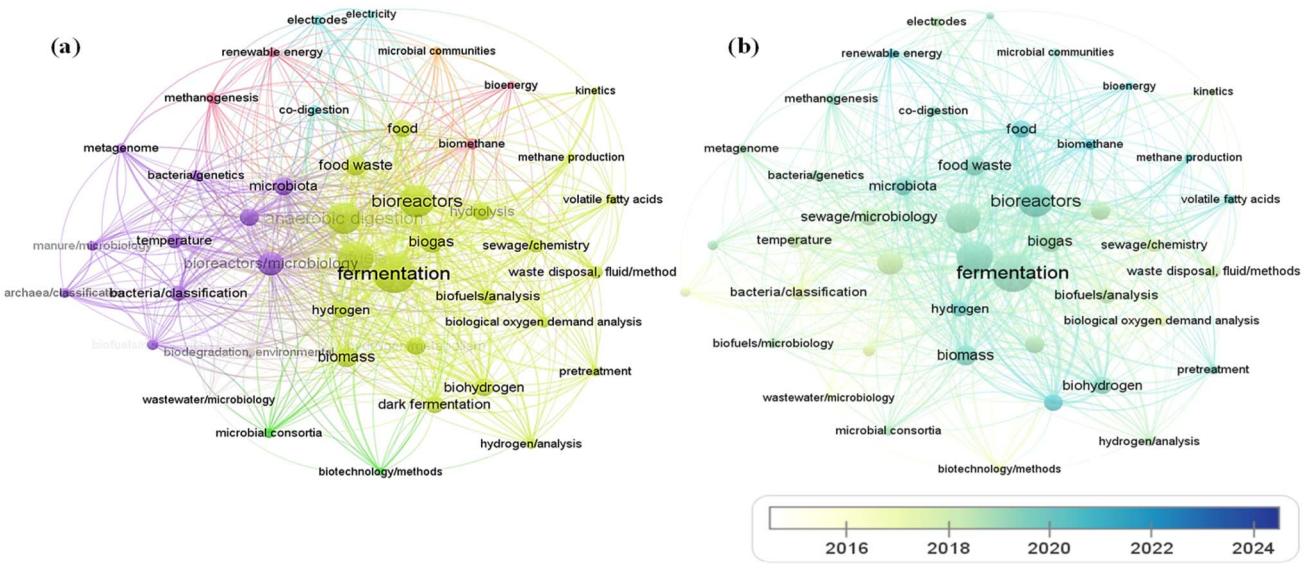

Fig. 1 Progression of keywords and titles in related references. (a) Keyword clustering analysis displays all keywords in a grouped trend, with the keyword with the highest weight indicating the highest frequency of occurrence within each category. (b) Temporal evolution sequence of research and review article keywords from 2016–2024.

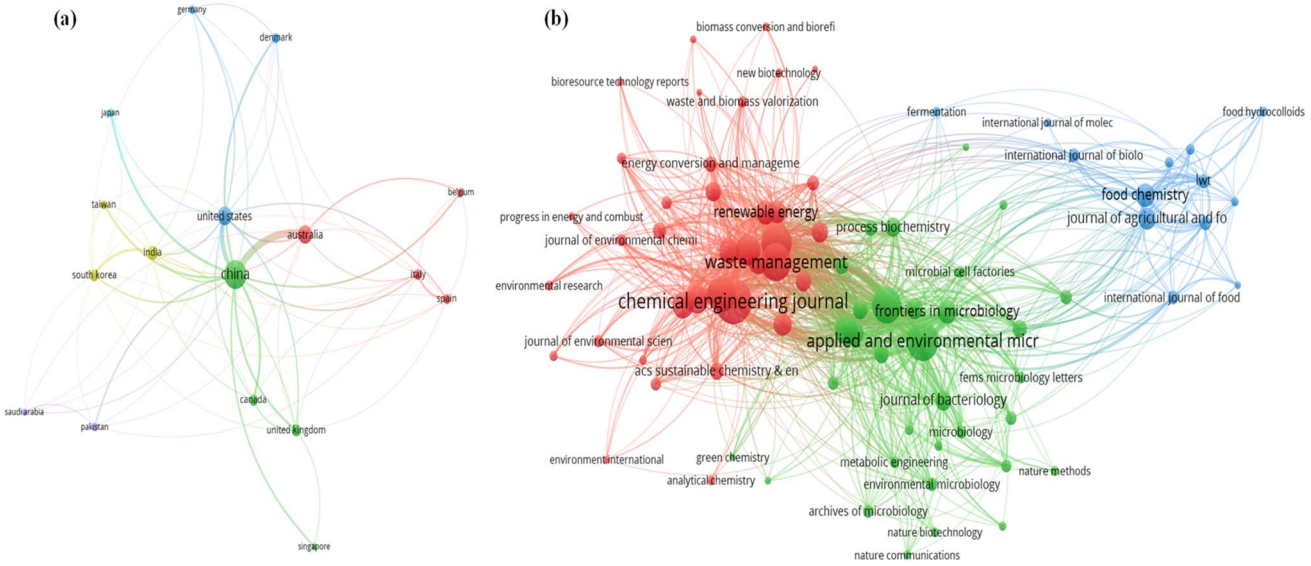

Fig. 2 Meta-analysis of the data related to biohydrogen production from food waste using dark fermentation and microbial electrolysis cells: (a) country-based analysis and (b) most popular *SCI Journals* that published the data on the current topic.

- "Dark fermentation" AND "microbial electrolysis"
- "DF–MEC" AND "biohydrogen" AND "food waste"
- "Volatile fatty acids" OR "lactate" AND "bioelectrochemical systems"

Articles were included if they:

(1) Involved laboratory or pilot-scale experimentation on DF, MECs, or integrated DF–MEC systems using food waste or organic-rich substrates;

(2) Addressed microbial community dynamics, metabolic pathways, or system performance metrics (*e.g.*, H_2 yield).

Exclusion criteria:

(1) Conference proceedings or non-peer-reviewed literature

(2) Studies focused solely on methane production or unrelated bioproducts

(3) Reviews lacking original experimental insights

Approximately 130 articles were initially identified, out of which 76 were selected for detailed review after full-text screening. The keyword network and temporal evolution of the DF–MEC literature were visualized using VOS viewer (version 1.6.20) to extract thematic clusters and identify publication trends (Fig. 1). Additionally, a meta-analysis of journal distribution and country-wise research activity was performed to contextualize the scope of contributions (Fig. 2).

2. Dark fermentation for hydrogen production from FW

Dark fermentation (DF) is an anaerobic biological process that converts organic matter into hydrogen (H_2), carbon dioxide (CO_2), and short-chain organic acids, without the need for light, as shown in Fig. 3. It has received significant attention as a sustainable method for hydrogen production, particularly from organic wastes such as food waste (FW).[35] Food waste is a desirable feedstock due to its high biodegradability, rich composition of carbohydrates, proteins, and lipids, and high moisture content (72–85%), as shown in Table 1. It also contains elevated levels of volatile solids and chemical oxygen demand, ranging from 19.3g L^{-1} to 346 g L^{-1}, depending on the source and season. The favorable carbon-to-nitrogen (C/N) ratio (typically 9–12) further supports microbial fermentation, while its cellulose and hemicellulose contents provide fermentable sugars after hydrolysis.[36,37] Food waste has demonstrated remarkable potential across various hydrogen production strategies. A two-stage system combining dark fermentation and photo-fermentation achieved cumulative yields of 671 mL H_2 per g food waste with an 80.2% COD removal efficiency, showcasing its suitability for scalable hydrogen bioconversion.[14]

During DF, fermentative and acidogenic bacteria metabolize simple sugars into hydrogen and various byproducts such as metabolites (VFAs) and alcohols. This process commonly involves three stages (i) hydrolysis, (ii) acidogenesis, and (iii) acetogenesis, facilitated by microbial consortia dominated by *Clostridium*, *Enterobacter*, *Bacillus*, and *Thermoanaerobacterium* species.[44] The theoretical hydrogen yield from glucose fermentation can reach up to 12 mol H_2 per mol glucose, but practical yields are much lower, typically around 3.67 to 3.8 mol H_2 per mol due to the diversion of electrons toward acetate and butyrate formation, which serve as electron sinks.[45–47] Proton-releasing pathways (*e.g.*, acetate production) favor hydrogen generation, while alternative pathways (*e.g.*, ethanol and lactate) reduce hydrogen yields. The following representative equations illustrate the metabolic routes during DF.[48,49] Despite its simplicity and scalability, DF is constrained by several limitations, including low hydrogen yield, accumulation of inhibitory byproducts (*e.g.*, VFAs, ethanol, and lactic acid), and incomplete substrate utilization. To address these challenges, researchers have explored various pretreatment strategies, codigestion

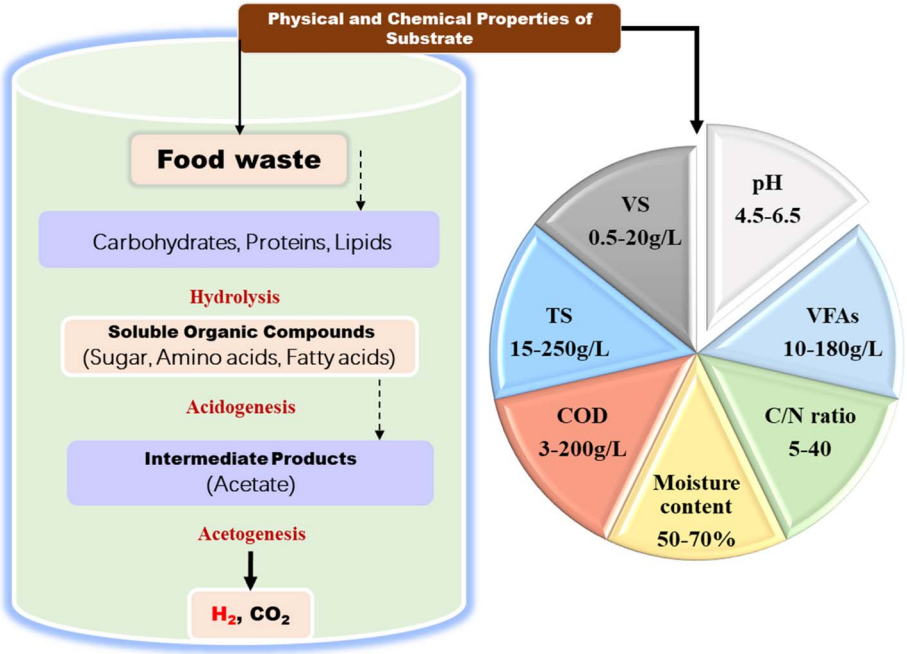

Fig. 3 Illustration of the primary steps in dark fermentation-assisted microbial electrolysis cells for hydrogen production and the key physicochemical properties of food waste. (VS = Volatile Solids, TS = Total Solids, COD = Chemical Oxygen Demand, C/N = Carbon-to-Nitrogen Ratio, VFAs = Volatile Fatty Acids, and g L^{-1} = Gram per Liter).

approaches, and hybrid integrations with systems such as microbial electrolysis cells and photofermentation.[50–52]

2.1 Fermentation pathways and microbial roles

Hydrogen production in DF systems is determined based on the balance between hydrogen-producing and hydrogen-consuming metabolic routes.[53,54] Among the various pathways, the acetate and butyrate pathways are the most favourable for hydrogen generation due to their higher theoretical H_2 yields. In contrast, the lactate and propionate pathways typically result in little or no hydrogen production due to reduced electron flow toward H_2 evolution.[55,56]

Acetate pathway (high H_2 yield – 4 mol per mol glucose):

$$C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CH_3COOH + 2CO_2 \quad (1)$$

This pathway is the most thermodynamically favourable and is commonly observed in *Clostridium acetobutylicum* and *C. butyricum*. It represents the optimal route for biohydrogen production in DF systems.

Butyrate pathway (moderate H_2 yield – 2 mol per mol glucose):

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2H_2 + CH_3CH_2CH_2COOH + 2CO_2 \quad (2)$$

This pathway predominates under slightly acidic conditions and is prevalent in real-world waste applications with moderate hydrogen partial pressures.

Ethanol pathway (no net H_2 – stress conditions):

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \quad (3)$$

Ethanol fermentation yields no net hydrogen and occurs under elevated hydrogen partial pressure or nutrient limitations.

Lactate pathway (zero H_2 balance):

$$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH \quad (4)$$

Lactate is a metabolic dead-end for hydrogen production, contributing to electron diversion under suboptimal redox conditions.

Propionate pathway (H_2-consuming – inhibitory):

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \quad (5)$$

This pathway actively consumes hydrogen and is a significant inhibitor of the net hydrogen yield. It is favoured under conditions of low pH and high hydrogen partial pressure. These symmetrical representations clarify the energetic and microbial implications of each pathway and serve as the biochemical foundation for optimising DF–MEC systems.

The microbial community plays a pivotal role in directing these pathways. Species from the *Firmicutes* phylum, particularly *Clostridium*, dominate hydrogenogenic reactions, while *Bacteroidetes* and *Proteobacteria* contribute to hydrolysis and acidogenesis (Table 2). Methanogens such as *Methanosaeta* and *Methanosarcina* consume hydrogen and must be suppressed through pretreatment or selective inhibition.[57,58] Effective hydrogen production requires managing microbial dynamics to favor acetate–butyrate fermentation over lactate and ethanol pathways, which produce little or no hydrogen. Unlike conventional anaerobic systems, the integrated DF–MEC

Table 1 General characteristics of food waste[a]

Carbohydrates	Proteins	Lipids	Moisture	Cellulose	Hemicellulose	Starch	Lignin	Ash	Volatile solids	Total solids	Ref.
35.5–69.0	3.9–21.9	—	—	—	—	—	—	1.0–2.0	—	—	38
35.5	14.4	24.1	81.7	—	—	—	—	—	87.5	18.3	39
55.0	16.9	14.0	81.5	16.9	—	24.0	—	5.9	94.1	18.5	40
48.3	17.8	—	81.9	—	—	42.3	—	—	98.2	14.3	41
52.57–59.69	7.93–9.57	5.58–7.29	—	15.4–20.8	3.31–3.75	33.47–36.53	—	—	—	—	42
53.96–56.04	16.21–17.59	—	—	16.54–17.26	7.69–7.71	—	16.43–17.57	5.88–5.92	—	—	40
55.0 ± 1.04	16.9 ± 0.693	—	81.5 ± 0.663	16.9 ± 0.36	7.7 ± 0.010	24.0 ± 1.06	17.0 ± 0.566	5.9 ± 0.022	94.1 ± 0.350	18.5 ± 0.715	36
58.9 ± 4.0	16.8 ± 4.7	8.0	—	3.3 ± 0.3	3.0 ± 0.2	30.2 ± 3.4	15.8 ± 1.7	—	1.2 ± 0.1	199.9 ± 21.3	43

[a] No unit required; all are in % out of 100 from total food waste.

Table 2 Bacteriological communities involved in DF from different organic biowastes (adapted from ref. 62 with permission from Taylor & Francis Online copyright 2022)

Process	Domain	Phylum	Genus	Species examples	Reference
Hydrolysis and acidogenesis	Bacteria	Bacteroidetes, Proteobacteria, Actinobacteria, Chloroflexi, Firmicutes, and Euryarchaeota	*Aminobacterium*, *Anaerobacter*, *Atopostipes*, *Bacillus*, *Bacteroides*, *Bifidobacterium*, *Campylobacter*, *Candidatus*, *Cloacibacillus*, *Clostridium*, *Enterococcus*, *Escherichia*, *Fervidobacterium*, *Fibrobacter*, *Fusobacterium*, *Gracilibacter*, *Halocella*, *Lactobacillus*, *Lutispora*, *Pectinatus*, *Propionibacteria*, *Pseudomonas*, *Ralstonia*, *Shewanella*, *Streptococcus*, *Thermomonas*, *Thermotoga*, and *Trichococcus*	*Bacillus cereus*, *Candidatus cloacimonas*, *Clostridium difficile*, *Clostridium carboxydivorans*, *Escherichia coli*, *Pseudomonas mendocina*, and *Thermomonas haemolytica*	63 and 64
	Fungi	Ascomycota, Basidiomycota, and Rozellomycota	*Aspergillus*, *Issatchenkia*, *Gibberella*, *Neocallimastigomycota*, *Paraphoma*, *Penicillium*, *Pseudogymnuascus*, and *Trichoderma*	*Neocallimastix piromyces* and *Trichoderma reesei*	65 and 66
Acetogenesis	Bacteria	Firmicutes	*Acetobacterium*, *Anaerovorax*, *Clostridium*, *Eubacteria*, *Ruminococcus*, and *Treponema*	*Clostridium carboxydivorans*, *Eubacterium limosum*, and *Thermoanaerobacter kivui*	67 and 68

platform enables real-time control of metabolic bottlenecks by continuously lowering hydrogen partial pressure *via* electrohydrogenesis. This electrochemical removal of H_2 thermodynamically favours hydrogenogenic pathways and reduces feedback inhibition.[59,60] Additionally, the MEC environment enriches electroactive and hydrogen-producing bacteria while selectively inhibiting methanogens and lactate producers due to altered redox conditions and competitive substrate utilization.[61] Pretreatment strategies, such as thermal shock or antibiotic application, further skew the microbial composition toward fermentative and electroactive species. Together, substrate characteristics, pH, temperature, and system configuration shape a metabolic landscape that enhances the overall hydrogen yield in DF–MEC systems by suppressing hydrogen-consuming organisms and optimizing electron flow.

2.2 Role of lactic acid bacteria (LAB) and challenges in lactate-driven dark fermentation

Lactic acid bacteria (LAB), including species from the *Lactobacillus*, *Streptococcus*, and *Pediococcus* genera, play an essential role in food waste fermentation due to their ability to convert carbohydrates into lactic acid under anaerobic conditions rapidly.[64,69] LAB is prevalent in bioreactors processing food waste because of the substrate's high carbohydrate and protein contents. These bacteria typically utilize either homolactic fermentation *via* the Embden–Meyerhof–Parnas (EMP) pathway or heterolactic fermentation *via* the pentose phosphate pathway.[70,71] While LAB contribute to the initial stages of food waste breakdown, their overabundance poses a challenge to the production of hydrogen. LAB compete with hydrogen-producing bacteria (HPB) for sugars and can secrete antimicrobial compounds that inhibit key hydrogenogenic species such as *Clostridium* and *Thermoanaerobacterium*.[68,72,73] Moreover, lactic acid, as a fermentation end-product, has a zero-hydrogen yield and can lower the reactor pH, further suppressing hydrogen production efficiency.

Experimental studies have shown that LAB dominance often corresponds with low hydrogen yields. For example, in DF systems operated at pH 4.0, hydrogen production dropped significantly, with *Lactobacillus* and *Streptococcus* dominating the microbial population.[74,75] Conversely, when pH levels were reduced to between 1.0 and 3.0 during pretreatment, *Clostridium* species became the dominant species, resulting in

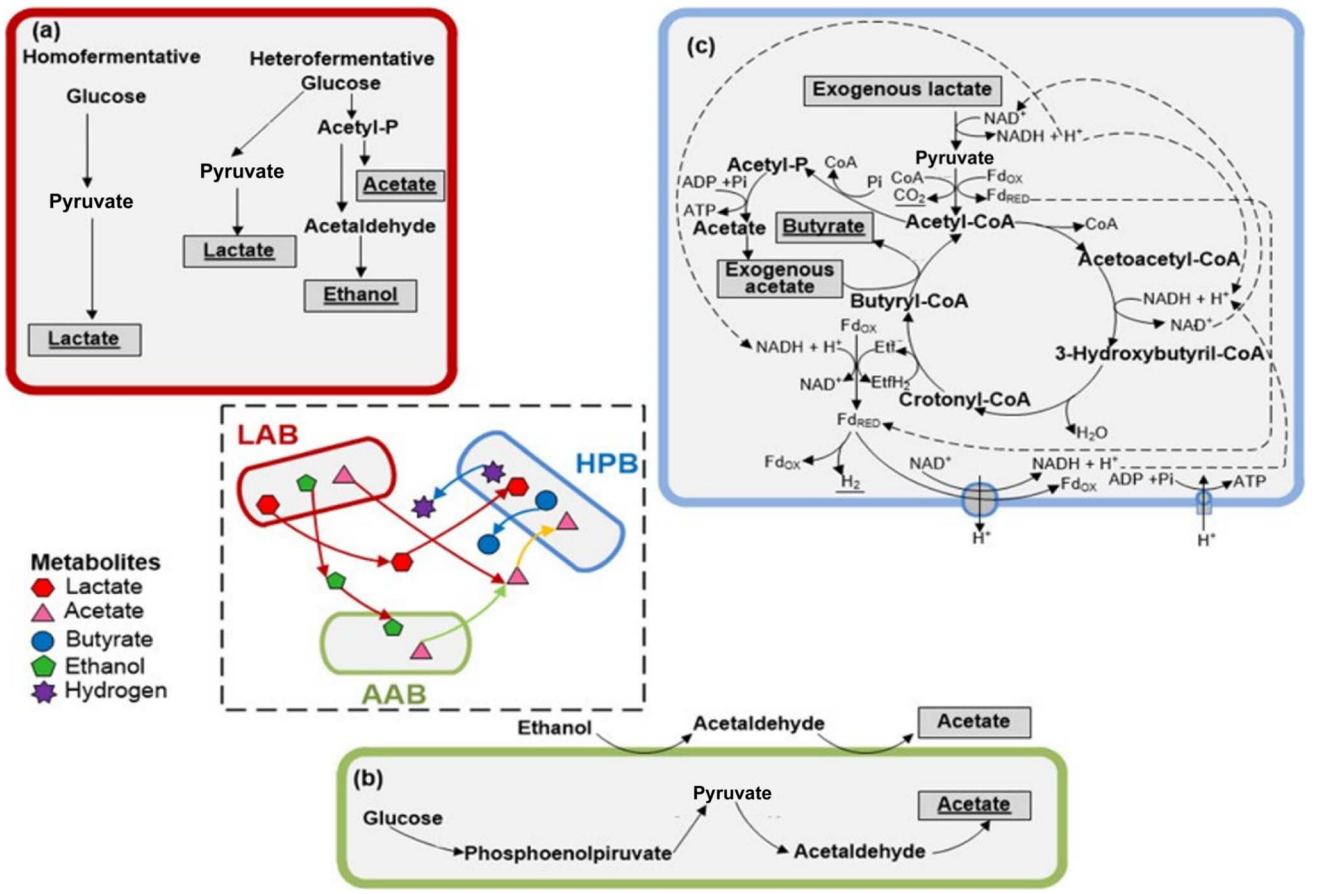

Fig. 4 Bacteriological processes are shown schematically: (a) homo- and heterolactic fermentation by lactic acid bacteria (LAB) and (b) acetic acid bacteria (AAB) oxidizing glucose and ethanol, and (c) hydrogen synthesis from lactate oxidation by hydrogen-producing bacteria (HPB). Based on lactate and acetate within the dotted rectangle, we can see the putative cross-feeding interactions of LAB, AAB, and HPB. (LAB = Lactic Acid Bacteria, HPB = Hydrogen-Producing Bacteria, and AAB = Acetic Acid Bacteria) (Adapted from ref. 69 with permission of MDPI. Copyright 2023).

increased hydrogen production. At pH 2.0, hydrogen yields reached up to 158 mL H_2 per g VS, compared to only 54 mL H_2 per g VS in the control.[74,76] To mitigate LAB-related inhibition, several control strategies have been explored. These include pH control and shock treatments, which selectively inhibit LAB while maintaining hydrogen-producing populations; thermal or acid pretreatments, which target LAB and methanogens by disrupting cell membranes and enzyme activity;[77,78] cold storage (*e.g.*, at 4 °C) of feedstock to reduce LAB proliferation before fermentation.[75,79]

Despite their drawbacks, LAB can also be harnessed for positive contributions in a process known as lactate-driven dark fermentation (LD-DF). In LD-DF, lactate produced by LAB is subsequently metabolized by acidogenic bacteria, such as *Clostridium butyricum*, into hydrogen and butyrate.[64] The microbial roles and syntrophic interactions of LAB, acetic acid bacteria (AAB), and HPB involved in this pathway are schematically represented in Fig. 4.[75] This two-step fermentation process allows for indirect hydrogen production and has been explored using food waste, sludge, and agricultural residues.[25,27,80,81] LD-DF offers benefits such as enhanced substrate utilization, tolerance to mixed feedstocks, and potential for integration with biorefineries and waste valorization platforms.[82,83] However, LD-DF systems are sensitive to operational factors such as pH, temperature, and organic loading rate. One major challenge is the reduction in the carbon-to-nitrogen (C/N) ratio due to lactic acid accumulation, which can limit microbial diversity and reduce hydrogen generation.[80,84] In addition, large-scale control of LAB populations remains economically and technically difficult, making reactor stability a concern. Thus, while LAB poses challenges in conventional DF, they may also offer an opportunity through LD-DF strategies if effectively managed. Future work should focus on balancing LAB populations, optimizing lactate conversion, and engineering microbial consortia that synergistically integrate LAB and hydrogen producers.

2.3 Strategies to enhance lactate-driven dark fermentation (LD-DF)

Lactate-driven dark fermentation (LD-DF) is a promising extension of conventional dark fermentation that leverages lactic acid as an intermediate for hydrogen production.[85] While LD-DF presents challenges related to microbial competition and pathway efficiency, several strategies have been developed to enhance its hydrogen-generating potential. These include bioaugmentation, metabolic engineering, and the use of synthetic microbiomes. Bioaugmentation involves introducing specific microbial strains to improve the overall performance of the fermentation system. In LD-DF, adding facultative anaerobes or hydrogen-producing bacteria (HPB) such as *Bacillus*, *Paenibacillus*, *Enterobacter*, and *Escherichia* has been shown to enhance hydrolysis and increase hydrogen yields.[86–89] These bacteria can outcompete lactic acid bacteria (LAB) for available substrates, shifting the microbial balance in favor of hydrogenogenesis. For example, hydrogen yields in mixed cultures have been significantly improved when bioaugmentation was combined with sludge or food waste substrates under optimized conditions. Facultative anaerobes typically achieve hydrogen yields of 1.0–2.0 mol H_2 per mol glucose, while obligate (strict) anaerobes like *Clostridium* can theoretically reach up to 4 mol H_2 per mol glucose.[90] This

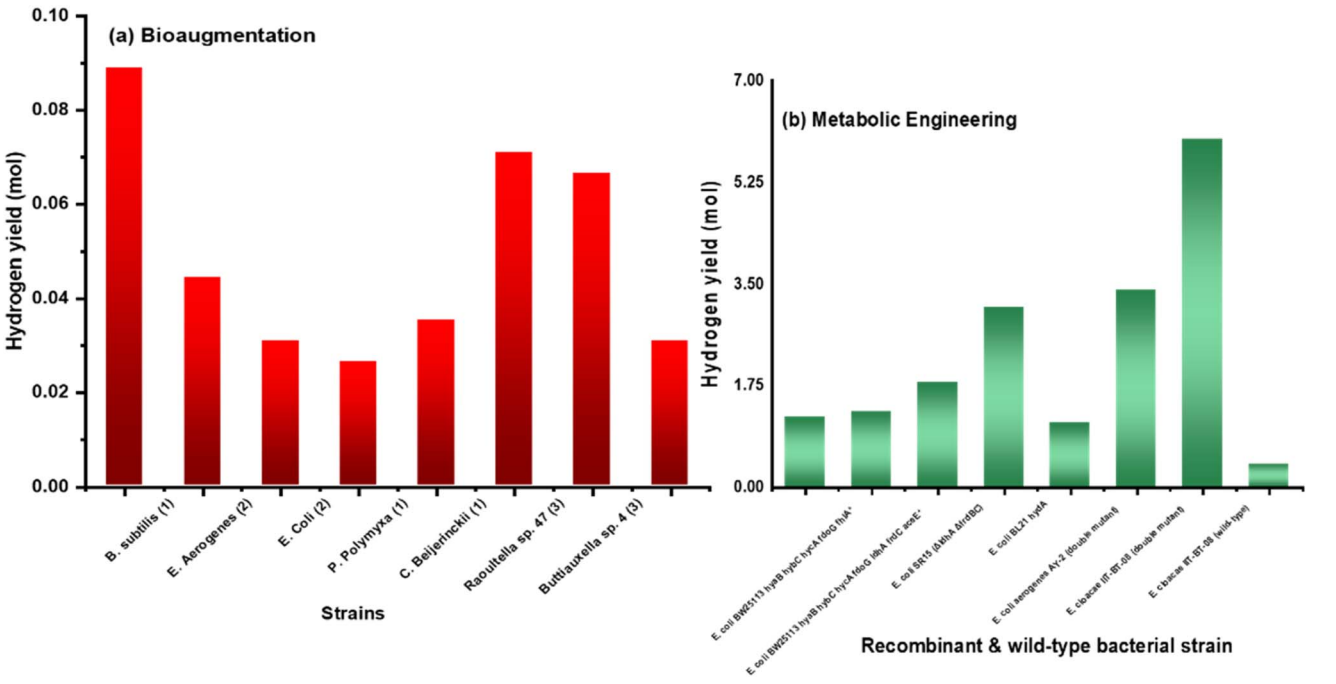

Fig. 5 Bioaugmentation with different strains (a) and metabolic engineering of recombinant and wild-type bacterial strains (b) for hydrogen yield using lactate driven dark fermentation. (Data extracted from ref. 93 and 94 with permission from Elsevier and Wiley Copyright 2023; 2008).

highlights the importance of microbial selection and reinforcement for high-yield LD-DF operations.

Metabolic engineering focuses on modifying microbial metabolic pathways to enhance hydrogen production efficiency, as shown in Fig. 5. This can include redirecting carbon flux away from lactic acid and ethanol pathways, overexpressing key hydrogenase enzymes, and knocking out genes responsible for inhibitory byproducts. Metabolically engineered strains of *Clostridium* and *Enterobacter* have demonstrated improved hydrogen yields (0.47–1.88 mol H_2 per mol glucose) under LD-DF conditions compared to wild-type strains.[90] Synthetic microbiomes offer another promising avenue to improve LD-DF. These are deliberately constructed microbial communities composed of axenic or enriched cultures, either native or genetically engineered, that work synergistically to degrade complex substrates and produce hydrogen. By carefully designing these consortia, researchers can reduce microbial competition, enhance lactate-to-hydrogen conversion rates, and improve process stability at high organic loading rates. Initial studies combining metabolic engineering with synthetic microbiomes have shown significant improvements in hydrogen yield, particularly under stress conditions such as elevated organic loads or acidic pH.[91,92]

2.3.1 Outlook for LD-DF integration. LD-DF also shows strong potential for integration into biorefinery and waste-to-energy platforms, especially when paired with microbial electrochemical technologies. Previous studies have demonstrated enhanced hydrogen production when LD-DF is followed by microbial electrolysis, enabling more complete utilization of fermentation byproducts such as lactate and VFAs.[76,93] To further improve system efficiency, several targeted techniques have been explored including bioaugmentation with lactate-utilizing hydrogenogenic strains, metabolic engineering of LAB, and development of synthetic microbiomes that redirect lactate metabolism toward increased hydrogen production and energy recovery (Fig. 6). Although LD-DF still faces challenges, such as sensitivity to pH, reduced C/N ratios, and microbial instability, these enhancement strategies represent viable pathways for its scale-up and optimization. Continued research into microbial selection, reactor design, and genetic manipulation will be critical for unlocking the full potential of LD-DF for sustainable hydrogen production.

2.4 Integration with microbial electrolysis cells (MECs) for bioelectrochemical enhancement

While dark fermentation (DF) is a promising biological process for hydrogen production from food waste, it is inherently limited by low hydrogen yields and the accumulation of inhibitory intermediates such as VFAs and ethanol. To overcome

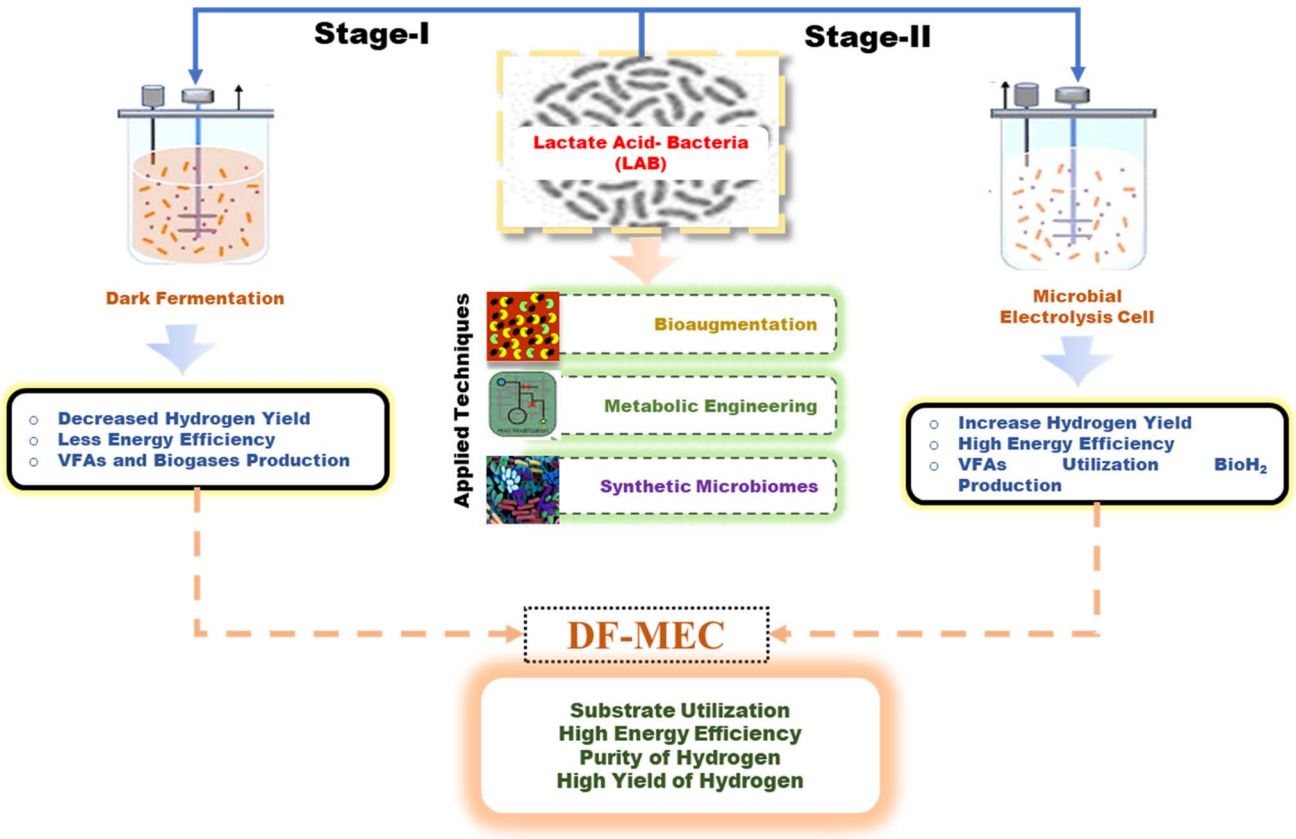

Fig. 6 Enhancement strategies targeting lactic acid bacteria (LAB) in lactate-driven dark fermentation (LD-DF) integrated with microbial electrolysis cells (MECs). Bioaugmentation, metabolic engineering, and synthetic microbiomes are applied to shift lactate metabolism toward hydrogenogenic pathways, improving VFA utilization, hydrogen yield, and energy efficiency in DF–MEC systems.

these constraints, microbial electrolysis cells (MECs) have been integrated with DF systems to improve substrate utilization and recover additional hydrogen through electrochemical means.[33,34,95]

2.4.1 Principle of MEC operation. MECs are bioelectrochemical systems in which electroactive bacteria oxidize organic matter at the anode, releasing electrons and protons. With a small external voltage (typically ≥0.114 V), these electrons are driven to the cathode, where they associate with protons to form H_2 gas.[96] The key anodic and cathodic reactions using acetate as the substrate are:

$$\text{Anode: } CH_3COOH + 2H_2O \rightarrow 2CO_2 + 8e^- + 8H^+ \quad (6)$$

$$\text{Cathode: } 8H^+ + 8e^- \rightarrow 4H_2 \quad (7)$$

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3 + 9H^+ + 8e^- \quad (8)$$

This process achieves greater feedstock conversion than DF alone. While DF is thermodynamically constrained to produce only 2–4 mol H_2 per mol glucose, MECs can recover residual energy from fermentation byproducts, potentially converting up to 90–95% of the organic matter into hydrogen.[34,97] A visual comparison between microbial electrolysis cells, microbial fuel cells, and microbial electrosynthesis cells highlighting differences in energy input, product generation, and application scope is presented in Fig. 7. This schematic clarifies how MECs, unlike MFCs and MESs, require an applied voltage and are primarily designed for hydrogen recovery from waste streams. Beyond hydrogen production, MECs have been successfully applied for downstream valorization of fermentation effluents. Recent studies have demonstrated that microbial electrolysis cells, when optimized for feed conductivity and COD concentration, can achieve over 95% organic removal and methane yields of up to 1.1 mmol per g COD consumed. These systems not only improve hydrogen or methane yield but can also achieve net positive energy balances, making them promising waste-to-energy platforms.[98]

2.4.2 Thermodynamics of electrohydrogenesis. The theoretical minimum voltage required for electrohydrogenesis under standard conditions (25 °C, pH 7, 1 atm H_2 pressure) is calculated using the Nernst equation:

$$E_{an} = E^{\circ}_{an} - \frac{RT}{8F}\ln\frac{[CH_3CO\cdot O^-]}{[HCO_3^-]^2[H^+]^9} \leftrightarrow 0.187 - \frac{8.31 \times 298.15}{8 \times (9.65 \times 10^4)}\ln\frac{[0.0169]}{[0.005]^2[10^{-7}]^2} = -0.3\ \text{V} \quad (9)$$

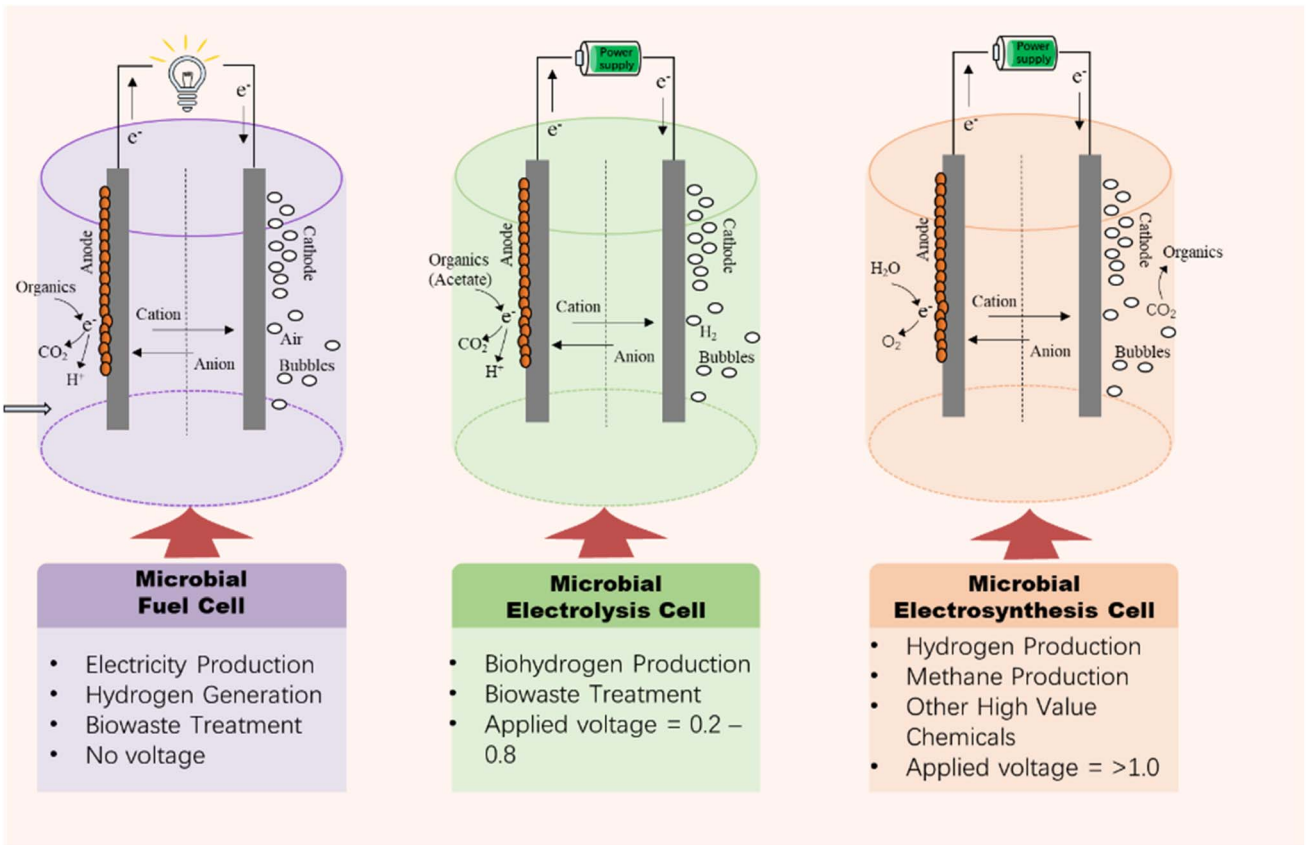

Fig. 7 Comparative schematic of microbial electrochemical systems: a microbial fuel cell (MFC) produces electricity without external voltage; a microbial electrolysis cell (MEC) requires applied voltage (typically 0.2–0.8 V) to generate hydrogen from organic waste; and a microbial electrosynthesis cell (MES) requires higher voltages (>1.0 V) for synthesis of chemicals like methane or acetate. MECs are particularly suited for dark fermentation effluent due to their ability to convert VFAs into hydrogen.

Standard ideal gas constant = 8.31 J mol^{-1} K^{-1}, Faraday's constant = 9.65 × 10^4 C mol^{-1}, and standard electrode potential, E_0 = 0.187 V, were used in the calculations. The temperature was taken as 298.15 K (25 °C, standard condition). H_2 was produced at the cathode electrode. Because E_{eq} is negative, there is no inherent tendency for the leftover acetate quantity to be spontaneously converted to biohydrogen using eqn (10), and the hypothetical cathode potential at temperature = 298.15 K, pH = 7.0, and hydrogen partial pressure = 1 atm was calculated using eqn (11).[97]

$$2H^+ + 2e^- \rightarrow H_2\,(g) \quad (10)$$

$$E_{cat} = E^{\circ}_{cat} - \frac{RT}{8F}\ln\frac{P_{H_2}}{[H^+]^2} \leftrightarrow 0 - \frac{8.31 \times 298.15}{8 \times (9.65 \times 10^4)}\ln\frac{1}{[10^{-7}]^2} = -0.414\ \text{V} \quad (11)$$

However, the standard cathode potential (E°_{cat}) is defined as 0 V under standard conditions (H_2 partial pressure = 1 atm, $[H^+]$ = 1 M, T = 298.15 K). Thus, eqn (12) for the equilibrium voltage is as follows:[97]

$$E_{eq} = E^{\circ}_{cat} - E_{an} = (-0.414) - (-0.300) = -0.114\ \text{V} \quad (12)$$

Due to practical energy losses (*e.g.*, ohmic resistance and activation energy), an applied voltage (E_{ap}) between 0.2 and 0.8 V is generally required.[97] Compared to traditional water electrolysis (1.23 V), MECs operate at much lower energy inputs while producing biohydrogen from complex organic waste streams.

2.4.3 DF–MEC system synergy. Coupling DF with MECs creates a two-stage system where DF converts carbohydrates into hydrogen, CO_2, and VFAs, and a MEC uses residual VFAs (especially acetate and butyrate) to produce additional hydrogen through bioelectrochemical conversion. This integration mitigates hydrogen inhibition by removing H_2 more efficiently and enables the recovery of energy from otherwise recalcitrant byproducts.[96,99] Studies show that MECs fed with DF effluent containing acetate can achieve higher current densities and coulombic efficiencies compared to butyrate or propionate.[100] Moreover, recent applications of dual-chamber MECs have shown that such systems can effectively reduce the COD content of DF effluents while instantaneously recovering energy through the production of value-added biofuels such as CH_4, indicating broader potential for DF–MEC configurations in waste valorization.[98]

Moreover, coupling MECs with LD-DF systems offers additional advantages by targeting lactate as a precursor. Lactate produced during DF can be metabolized into acetate by electroactive bacteria such as *Geobacter sulfurreducens* and *Desulfovibrio*, further contributing to hydrogen generation at an applied voltage.[24,99] Although DF–MEC systems offer improved hydrogen recovery and effluent quality, their performance depends heavily on operational parameters such as pH and temperature stability (optimal: 6.5–7.5, 35–55 °C), electrode material and surface area (*e.g.*, carbon felt and graphite brushes), substrate composition and VFA profile (acetate-rich streams preferred), reactor configuration and applied voltage.[34,97] Rozendal *et al.* reported an anode voltage loss of 0.04 V due to internal resistance in a MEC system operating with sodium acetate, yielding a daily hydrogen recovery of 0.02 m^3 H_2 per m^3 reactor volume.[101] These findings highlight the importance of system design and energy input efficiency in maximizing biohydrogen production.

3. Boosting bio-H_2 from FW through DF–MEC coupling

Dark fermentation (DF) has been widely studied for hydrogen production from food waste (FW) due to its low energy input, simple reactor design, and compatibility with various substrates. However, DF alone suffers from limited hydrogen yield because of the accumulation of inhibitory metabolites such as lactate and alcohols.[102] To address these limitations, integrating DF with MECs has emerged as a promising hybrid approach for enhancing hydrogen yield and waste valorization. MECs use electroactive bacteria at the anode to oxidize organic intermediates, while a small external voltage drives proton reduction at the cathode, thereby producing additional hydrogen. Compared to microbial fuel cells (MFCs), MECs can achieve up to 100% higher hydrogen production under optimized conditions.[103] When coupled with DF, MECs can utilize the effluent containing residual VFAs and organic acids, significantly improving overall substrate utilization and biohydrogen recovery.

Unlike traditional anaerobic digestion or photofermentation systems, DF–MEC integration offers dual advantages: rapid hydrogen generation in the DF stage and prolonged hydrogen recovery from leftover substrates in the MEC stage.[104] For example, in a two-stage system using palm oil mill effluent, *Thermoanaerobacterium* species dominated the DF stage, while *Geobacter* and *Desulfovibrio* were prevalent in the MEC stage, yielding 73 mL-H_2 per g COD and 236 mL-H_2 per g COD, respectively (Table 3).[105,106] Pretreatment methods also play a crucial role in improving the performance and energy efficiency of DF–MEC systems. Alkaline-ultrasonic pretreatment has been reported to enhance hydrogen production by 350% in DF and 400% in MEC,[103] while microbial enrichment (*e.g.*, *Acetobacterium*, *Geobacter*, and *Desulfovibrio*) can improve COD reduction and microbial stability.[107] However, co-produced metabolites and suspended particles in DF effluent can reduce MEC efficiency, which highlights the importance of pretreatment and biofilm engineering.

Temperature, pH, and hydraulic retention time are also critical factors. Thermophilic conditions tend to enhance microbial diversity and reactor kinetics, but must be carefully controlled to prevent the formation of inhibitory byproducts. Some systems have incorporated pH-resistant methanogens or applied selective inhibition (*e.g.*, chloroform) to suppress methane and increase biohydrogen selectivity.[55,112] Furthermore, integrating DF–MECs with other waste-to-energy

Table 3 Comparison of H_2 yield, production rates, and MEC operational parameters across various substrates in integrated dark fermentation (DF) and microbial electrolysis cell (MEC) systems[a]

	Dark fermentation (DF)		Microbial electrolysis cell (MEC)				
Substrate	H_2 yield	H_2 production rate	H_2 yield	H_2 production rate	Energy efficiency (%)	Applied voltage (V)	Ref.
Food waste	0.049 L H_2 per g VS	1.55 ± 0.00 L per L per day	0.511 L H_2 per g VS	3.48 ± 0.48 L per L per day	175.4 ± 5.8	−0.2	108
Industrial by-products (cheese, sugar, fruit processing, fruit juice, spirit, and paper)	0.018 ± 0.004 L	0.00081 ± 2.73 L H_2 per h	0.219 ± 0.139 to 1.48 ± 0.267 L H_2 per g COD	1.61 ± 266 L per L per day	NA	0.2 *vs.* SCE	109
Palm oil mill	0.073 L H_2 per g COD	NA	0.236 L H_2 per g COD	7.81 L per L per day	89–471	0.7	106
Cassava starch wastewater	0.223 L H_2 per g COD	NA	0.245 L H_2 per g COD	0.061 L H_2 per g COD per day	90.09	0.6	110
Cellulose	14.3 mmol H_2 per g COD	0.24 L H_2 per m^3 per day	33.2 mmol H_2 per g COD	0.48 L H_2 per m^3 per day	23	0.43	59
Water hyacinth	0.110 ± 0.43 L H_2 per g VS	0.056 ± 0.12 L h^{-1}	0.565 ± 0.019 L H_2 per g VS	0.078 ± 1.1 L L^{-1} h^{-1}	112 ± 4	0.8	47
Domestic wastewater	135.15* mL H_2 per g COD	NA	1200.00 mL H_2 per g COD	1335.15* mL H_2 per g COD	51.56*	0.8	111

[a] H_2 yield is expressed in L H_2 per g COD; H_2 production rate (HPR) in L H_2 per L per day, unless otherwise specified. * = Calculated; NA = not available.

platforms such as hydrothermal gasification can further enhance hydrogen yield from wet biomass. Potassium-based catalysts in hydrothermal processes have yielded up to 1.88 mol H_2 per kg with a 35% H_2 mole fraction at 360–450 °C, providing a downstream valorization option for excess moisture in FW.[113] Despite these advances, challenges persist, particularly in maintaining microbial synergy, ensuring reactor stability, and achieving cost-effective scale-up. Nevertheless, the DF–MEC configuration offers significant promise as a flexible, modular platform for high-yield H_2 production from food waste and other organic residues. Further investigation into microbial–electrochemical interactions, reactor configurations, and techno-economic assessments will be crucial for real-world implementation.

4. Operational parameters driving DF–MEC biohydrogenation

Stable environmental conditions are critical for sustained hydrogen production in integrated DF–MEC systems, as they help suppress hydrogen-consuming microorganisms and promote the selective growth of electroactive, hydrogen-producing bacteria. Compared to standalone DF or MEC operations, DF–MEC integration introduces complex interactions between biological and electrochemical processes, making operational parameter control even more critical. Factors such as pH, temperature, hydrogen partial pressure (HPP), hydraulic retention time (HRT), organic loading rate (OLR), and oxidation–reduction potential (ORP) not only influence microbial metabolism but also affect electrode performance, electron flow, and gas recovery. This section explores how these critical parameters govern hydrogen yield and system stability in DF–MEC configurations. Table 4 summarizes influential DF studies, while Fig. 8 compares trends between DF, MECs, and their integration. The following subsections evaluate the role of each parameter, with an emphasis on the synergistic or antagonistic effects they have on the coupled DF–MEC performance.

4.1 pH

pH is a critical operational parameter that influences enzymatic activity, microbial metabolic pathways, and hydrogen yield in dark fermentation (DF) and MEC systems.[125,126] Hydrogen-producing bacteria typically thrive in a slightly acidic to neutral pH range (5.5–7.0), while methanogens and hydrogen-consuming microbes are favoured under near-neutral to alkaline conditions (6.3–7.8).[127,128] In DF–MEC systems, maintaining an optimal pH range (6.5–7.0) becomes even more crucial, as proton availability significantly affects cathodic hydrogen evolution and electrochemical efficiency.[22,129] Maintaining an initial pH between 6.0 and 7.0 has consistently been shown to optimize hydrogen production. At lower pH values (<6.0), the activity of hydrogen-producing microbes is inhibited, reducing both substrate conversion and gas yields.[22] For example, fermentation of coconut milk wastewater at pH 6.5 produced a maximum of 0.28 L H_2 per L.[130] In contrast, acidic conditions

Table 4 Bioreactor's performance under different dark fermentation conditions and hydrogen production[a]

Dark fermentation conditions							
Bioreactor type	Substrate concentration	pH	Temp./HRT/OLR	Substrate pretreatment	Hydrogen yield (YH_2)	Approach to enhance performance	Ref.
SBR 3 Batch per day	—	5.3	HRT = 30 h, 24 h	Control Acid, pH = 2 35 °C, 1 day Alkali, pH = 12.5, 1 day	Max 25 decreased to 7.1 Max 48 decreased to 5 24.5 stable for 25 days 62.6 stable for 50 days	The CFU per g VS was reduced by 4.9 logs after alkali pretreatment and by less than 1 log after acid pretreatment	114
BR	30 g carbo. COD per L	—	35 °C	Control (no pretreatment) thermal 90 °C, 20 min Acid, pH = 1, 1 = day Alkali, pH = 13, 1 = day	153.5 mL H_2 per g VS	Heat-(90 °C for 20 m), acid-(pH 1 for 1 day), and alkali-treatment (pH 13 for 1 day)	115
BR	30 g carbo. COD per L	7	35 °C	Control Acid, pH = 1, 12 h, 20 °C Acid, pH = 2, 12 h, 20 °C Acid, pH = 3, 12 h, 20 °C Acid, pH = 4, 12 h, 20 °C	1.74 mol H_2 per mol hexose	Lactic acid bacteria were suppressed by pH 1–3 pretreatment. *Clostridium* sp. emerged as the dominating species. The genera *Lactobacillus* and genus *Streptococcus* become predominant after pH 4	76
ABR		—	OLR = 29.0–47.0 g COD per L per day HRT = 1.6 days Temp. = 35 °C	—	12.9 mL H_2 per g COD	OLR change (29, 36, 47 g COD per L per day)	116
i-CSTR		—	OLR = 19, 28 g COD per L per day HRT = 4 days Temp. = 55 °C	—	38.1 mL H_2 per g COD	OLR change (19, 28 g COD per L per day)	117
Membrane bioreactor		—	OLR = 70.2–125.4 g COD per L per day HRT = 18.7, 14.0, 10.5 h Temp. = 55 °C	—	111.1 mL H_2 per g VS	OLR change (70.2, 89.4, 125.4 g COD per L per day)	118
CSTR		—	OLR = 19.0–57.0 g VS per L per day HRT = 24–8 h Temp. = 35 °C	—	11.2 mL H_2 per g VS	OLR change (19–57 g VS per L per day)	119
BR	30 g carbo. COD per L	5	Temp. = 35 °C	—	1.92 mol H_2 per mol hexose	Initial pH change (5.0–9.0)	22
CSTR, BR = 1 day	—	6, 5.5	Temp. = 37 °C HRT = 0.7	Alkali, pH = 11, 6 h Control Sonication 79 kJ per g TS Heat 70 °C 30 min Acid pH = 3, 4 °C, 24 h Alkali pH = 11, 4 °C, 24 h Sonication + heat sonication + acid sonication + alkali	Decreased after 3 days 41 (−) 97 (+136%) 70 (+70%) 55 (+34%) 46 (+12%) 78 (+136%) 118 (+90%) 67 (+63%)	Most of the hydrogen is produced when the amount of soluble carbohydrates is maximized (through sonication and using acid). The most significant reduction in H_2 output occurred under the combination of high COD and protein solubilization (sonication + alkali)	120

Table 4 (*Contd.*)

Dark fermentation conditions							
Bioreactor type	Substrate concentration	pH	Temp./HRT/OLR	Substrate pretreatment	Hydrogen yield (YH_2)	Approach to enhance performance	Ref.
ASBR	—	12	OLR = 15.4–27.0 g COD per L per day HRT = 42–24 h Temp. = 35 °C	—	61.7 mL H_2 per g VS	HRT change (42–24 h) SRT change (160–24 h)	121
CSTR	—	—	OLR = 1.2 g VS per L per day HRT = 5 days Temp. = 35–55 °C	—	1.8 mol H_2 per mol hexose	VS concentration change (3–10 g VS per L), Temp. comparison	122
ASBR	—	12	OLR = 20 g carbo. COD per L per day HRT = 36 days Temp. = 35 °C	—	0.9 mol H_2 per mol hexose	C/N ratio change (10–30)	123
CSTR	—	—	OLR = 17.7–106 g VS per L per day HRT = 48–4 h Temp. = 35 °C	—	—	HRT 48–8 h, brown water codigestion	124

[a] COD per L per day = chemical oxygen demand per liter per day; BR = Batch Reactor; CSTR = Continuously Stirred Tank Reactor; ABR = Anaerobic Baffled Reactor; SBR = Sequence Batch Reactor; HRT = Hydraulic Retention Time; OLR = Organic Loading Rate; ASBR = Anaerobic Sequencing Batch Reactor; i-CSTR = Intermittent-Continuously Stirred Tank Reactor.

below pH 5.5 tend to favor lactic acid production and suppress hydrogen yield.[131]

During DF, organic acid accumulation can lead to pH drops (*e.g.*, from ~6.5 to ~4.5), further inhibiting hydrogen-producing pathways. To counter this, buffering agents such as NaOH, KOH, or $CaCO_3$ are often added to maintain optimal pH.[128,132] Studies suggest that initiating fermentation at a slightly higher pH (6.8–7.0) can offset this acidification and sustain microbial activity throughout the process.[76] While acid pretreatment (*e.g.*, adjusting FW to pH 3) can enhance hydrolysis, it may underperform compared to sterilization or controlled thermal pretreatment in supporting hydrogen production.[133,134] Hence, balancing the initial pH and pretreatment strategy is vital for maintaining microbial membrane stability and nutrient transport, ultimately improving biohydrogen yields in DF–MEC systems.

4.2 Temperature

Temperature has a strong influence on the microbial community structure, substrate degradation rate, and hydrogen yield in DF–MEC systems. The mesophilic range of 35–40 °C and thermophilic range of 50–60 °C are commonly explored, with thermophilic conditions (around 55 °C) often yielding higher biohydrogen production due to enhanced microbial metabolism and suppression of hydrogen-consuming organisms like methanogens and homoacetogens.[135,136] The electrochemical activity in MECs is also temperature sensitive.[137,138] Thermophilic DF–MEC systems have demonstrated enhanced hydrogen evolution due to improved electron transfer rates and microbial resilience; however, they required tighter thermal control for stable MEC operation. Thermophilic fermentation also promotes the dominance of heat-tolerant hydrogen-producing bacteria such as *Thermoanaerobacterium* spp., while simultaneously inhibiting lactate-producing microbes that dominate under mesophilic conditions.[139,140] For instance, a study reported optimal yields at 55 °C, where microbial selection favoured efficient hydrogenogenesis with minimal competing pathways.[136]

Nevertheless, thermophilic systems are more susceptible to operational instability. Sudden temperature shifts can disturb microbial communities and reduce hydrogen output. Bioaugmentation using specialized hydrogen-producing strains has been effective in stabilizing bioreactor performance after temperature disturbances.[141] In one case, bioaugmentation applied after a return to 55 °C yielded better recovery than when applied during the temperature shift.[141] Lactate-driven dark fermentation (LD-DF), in contrast, operates optimally under mesophilic conditions (35–45 °C).[142] While LD-DF has potential for integration with MECs *via* intermediate substrates (*e.g.*, lactate or VFAs), its thermophilic limitations must be considered in DF–MEC system design. In summary, thermophilic DF–MEC systems offer higher hydrogen yields and microbial resilience, but require tighter control of temperature and microbial community stability for sustained performance.

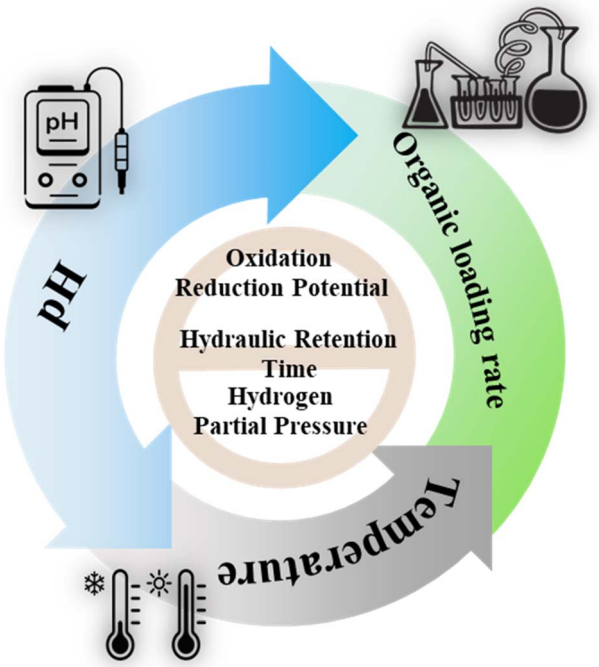

Fig. 8 Major operational parameters for biohydrogen production from food waste. pH and temperature with their dependent variable factors.

4.3 Hydrogen partial pressure (HPP)

HPP is a critical thermodynamic factor that directly impacts hydrogen production in dark fermentation (DF) systems.[143] During fermentation, as hydrogen accumulates in the reactor headspace, it creates feedback inhibition on hydrogenase enzymes, altering the redox potential of the system. This leads to reduced hydrogen yields and a shift in microbial metabolic pathways toward non-hydrogenic products such as ethanol, lactate, or propionate.[138,144] Studies have shown that lowering hydrogen partial pressure enhances biohydrogen yield and fermentation kinetics. For example, reducing HPP under thermophilic conditions (55 °C) increased the maximum hydrogen yield to 30.69 mL H_2 per g COD added, with a butyrate-to-acetate (B/A) ratio of 1.97, indicating more favourable conditions for hydrogen production.[145] Lower HPP also improves kinetic parameters, increasing maximum hydrogen production (P_{max}) and the production rate (R_{max}), and reducing lag time (λ), while promoting ethanol-type fermentation under specific conditions.[146,147] MECs significantly reduce HPP by converting accumulated H_2 and VFAs at the cathode, thus sustaining favorable thermodynamics for continuous biohydrogen generation in DF reactors.[148,149] In DF–MEC systems, microbial electrolysis cells (MECs) indirectly contribute to HPP control by consuming hydrogen-inhibiting intermediates and maintaining low hydrogen levels in the reactor environment.[137,148] MECs continuously remove hydrogen and utilize residual VFAs for electrochemical conversion, further enhancing hydrogen recovery and preventing gas accumulation that inhibits fermentation.[150]

Moreover, reduced HPP has been associated with changes in the microbial community structure and soluble microbial products (SMPs), favoring hydrogen-producing species while suppressing hydrogen consumers such as methanogens.[19] Thus, effective management of hydrogen partial pressure is essential for maintaining favorable fermentation thermodynamics, stabilizing microbial communities, and enhancing overall system efficiency in integrated DF–MEC configurations.

4.4 Hydraulic retention time (HRT)

Hydraulic Retention Time (HRT) is a critical operational parameter that influences substrate conversion, microbial population dynamics, and hydrogen yield in dark fermentation-assisted microbial electrolysis cell (DF–MEC) systems.[151] In DF–MEC systems, HRT affects not only substrate biodegradation but also the residence time of electroactive intermediates available for MEC recovery, requiring fine-tuned coordination between microbial and electrochemical processes.[152,153] HRT refers to the typical residence time of the substrate in the bioreactor and has a direct impact on reactor performance and stability.[154,155] Short HRTs (*e.g.*, <6 days) favour faster-growing hydrogen-producing bacteria and enhance hydrogen production rates. However, excessively short retention times can lead to biomass washout, incomplete substrate degradation, and accumulation of inhibitory compounds such as volatile fatty acids (VFAs).[156] On the other hand, longer HRTs promote microbial diversity and more complete breakdown of complex organics like cellulose and hemicellulose, but they can also favour the growth of hydrogen-consuming organisms and lead to product shifts (*e.g.*, from acetate to propionate) that lower the net hydrogen yield.[155,157]

One study showed that extending HRT from 40 to 60 days improved degradation of cellulose from 52.1% to 55.4%, and hemicellulose from 71.4% to 76.8%.[157,158] However, when HRT exceeded 12 days, propionic acid became the dominant VFA, while acetic acid was more prevalent at shorter HRTs, highlighting the need to balance retention time to maintain favorable metabolic conditions for hydrogen production.[159] In DF–MEC systems, optimal HRT ensures sufficient contact time for both fermentative and electroactive microbial processes. Inadequate HRT may limit the availability of bioavailable VFAs for MEC utilization, reducing hydrogen recovery at the cathode. Conversely, too long a retention time may result in metabolite accumulation or methanogen proliferation, decreasing coulombic efficiency. Therefore, determining the optimal HRT is essential for balancing substrate degradation, hydrogen yield, microbial community stability, and reactor operating costs. While DF alone may benefit from shorter HRTs to enhance productivity, MEC integration often requires fine-tuning retention time to maximize energy recovery and effluent quality.

4.5 Organic loading rate (OLR)

The organic loading rate is a critical parameter influencing the efficiency and stability of hydrogen production in dark fermentation-assisted microbial electrolysis cell (DF–MEC) systems.[160] High OLRs in DF–MEC systems increase VFA

production, which must be balanced by MEC efficiency to prevent acid accumulation and electrode inhibition.[118,161] Thus, load optimization must consider both fermentative conversion and electrochemical VFA scavenging. The OLR defines the amount of organic substrate (typically measured as COD or volatile solids) fed per unit reactor volume per day (*e.g.*, g COD per L per day), and is mathematically represented as:

$$\mathrm{OLR} = \frac{Q \times S_o}{V}$$

where Q is the influent flow rate (L per day), S_0 is the substrate concentration (g COD per L), and V is the working volume of the reactor (L).[162]

An optimal OLR ensures sufficient substrate availability for microbial activity without overloading the system, thereby maintaining optimal conditions for microbial growth and proliferation. At low OLRs, microbial metabolism may be underutilized, while high OLRs can lead to substrate accumulation, VFA build-up, pH drops, and inhibition of hydrogen-producing bacteria.[145] In DF systems, increasing OLR has been linked to elevated propionate and ethanol formation, particularly when the system is under strain.[163] For DF–MEC systems, maintaining an appropriate OLR is even more critical. High OLRs may produce more VFAs, which can serve as electron donors in MECs, but excessive accumulation can inhibit electrogenic activity and reduce coulombic efficiency. Studies have shown that applying silicone oil to reduce hydrogen partial pressure under high OLR conditions (60–160 g TC per L per day) enhanced hydrogen yields and upregulated genes related to homoacetogenesis, a key hydrogen-producing pathway.[162] A semi-continuous DF reactor treating municipal solid waste demonstrated that increasing the OLR from 7.5 to 14 g VS per L per day led to a 49.2% increase in VFA production. However, propionate (a hydrogen-suppressing acid) accounted for over 86% of total VFAs.[163] These findings underscore the importance of maintaining an appropriate balance between the OLR and VFA profiles to optimize hydrogen production in DF–MEC systems. Adjusting HRT in coordination with the OLR can help control this balance for sustained performance.

4.6 Oxidation–reduction potential (ORP)

The oxidation–reduction potential (ORP) is a vital parameter that reflects the electron transfer environment in a fermentation system and directly affects microbial metabolism, enzymatic activity, and product distribution. In DF–MEC systems, ORP is closely linked to shifts in microbial communities and dynamics of biohydrogen generation. In dark fermentation, maintaining a strongly reducing environment (typical ORP between −250 and −400 mV) favors hydrogen-producing pathways. ORP influences the direction of metabolic flows: at more negative values, hydrogenogenic reactions dominate, while less reducing conditions lead to a shift toward solventogenesis or methanogenesis.[164] In lactate-driven DF, precise ORP control has been shown to regulate gene expression and optimize hydrogen yields. Techniques such as using bioelectrochemical reactors, redox reagents, and gas sparging can be employed to stabilize ORP and improve metabolite profiles.[61]

In DF–MEC systems, maintaining an appropriate ORP is essential not only for microbial metabolism but also for optimal electrode performance and electrochemical efficiency. The anode operates as an electron sink, and maintaining a sufficiently low ORP enhances electron transfer from fermentative bacteria to the electrode surface.[165] Studies show that ORP values between −300 and −450 mV are ideal for maximizing coulombic efficiency and hydrogen evolution at the cathode. Moreover, ORP affects biofilm activity and the enrichment of exoelectrogenic bacteria such as *Geobacter* and *Shewanella*, which are crucial for MEC performance.[166] Thus, fine-tuning ORP in DF–MEC systems is vital for synchronizing microbial fermentation and electrohydrogenesis.

MECs further benefit from controlled ORP conditions. In one study, an initial ORP of −350 mV in a butyrate-based reactor resulted in a hydrogen yield of 5.951 L H_2 per g, while a more reducing potential of −400 mV in an ethanol-fed system increased the H_2 yield to 8.357 L H_2 per g.[167] These findings highlight the importance of fine-tuning the redox conditions in DF–MEC configurations to enhance substrate conversion and electrochemical efficiency. ORP is also influenced by reactor design, electrode material, applied voltage, and substrate type, all of which contribute to shaping the electrochemical environment and microbial community structure. Thus, monitoring and adjusting ORP offers a powerful tool to maximize hydrogen production in integrated DF–MEC systems. Overall, ORP serves as a unifying operational parameter that links microbial dynamics, electron flow, and electrochemical hydrogen recovery in integrated DF–MEC platforms.

To date, DF–MEC systems exhibit distinct operational sensitivities compared to standalone DF or MEC processes. Optimising parameters in coordination, rather than in isolation, is essential for enhancing hydrogen yield, microbial stability, and electrochemical efficiency. Future DF–MEC designs must incorporate adaptive control strategies for key factors such as pH, HRT, ORP, and OLR to ensure scale-up viability. The integration of DF and MECs imposes new demands on system tuning, requiring precise synchronization of microbial activity with electrochemical conditions. Managing parameters like pH, HPP, and ORP not only influences microbial hydrogenogenesis but also dictates electron flow and cathodic hydrogen recovery. A systems-level optimization framework is therefore crucial for unlocking the full biohydrogen potential of DF–MEC configurations.

5. Electrode materials and bioelectrochemical performance in DF–MEC systems

Electrodes represent an essential component in the inclusive performance of integrated dark fermentation–microbial electrolysis cell systems, directly mediating interfacial electron transfer, microbial colonization dynamics, and hydrogen (H_2) evolution efficiency.[168,169] Their physicochemical properties significantly influence system kinetics, coulombic efficiency, and long-term operational stability.

5.1 Anodic materials and an electroactive microbial interface

The anode serves as the terminal electron acceptor for electroactive bacteria oxidizing organic substrates.[153,165] Carbonaceous materials, such as carbon cloth, carbon felt, graphite rods, and reticulated vitreous carbon (RVC), are commonly employed due to their high conductivity, corrosion resistance, and microbial compatibility.[169–171] These surfaces support the attachment and biofilm development of key exoelectrogens such as *Geobacter*, *Shewanella*, and fermentative *Clostridium* species.[166] However, inter-study variability in biofilm architecture and electrochemical activity is frequently attributed to differences in surface functionalization, redox potential distribution, and porosity. Advanced modification strategies, such as metal nanoparticle deposition (*e.g.*, Ni, Fe_3O_4, and Cu), heteroatom doping, and plasma activation, have been applied to enhance extracellular electron transfer (EET) and increase electrochemical surface area.[172,173]

5.2 Cathodic catalysts and hydrogen evolution efficiency

The cathode enables the hydrogen evolution reaction, in which protons and electrons recombine to form molecular hydrogen.[170] While platinum (Pt)-based electrodes exhibit superior catalytic performance with low overpotential requirements, their susceptibility to poisoning by sulfur species and high material cost limit their scalability. Alternative cathode materials such as stainless-steel mesh, nickel-molybdenum alloys, molybdenum disulfide (MoS_2), and carbon-based composites offer moderate hydrogen evolution reaction (HER) activity while providing a favorable balance between cost and performance. A Co–Mo catalyst coated on stainless steel demonstrated low overpotential (~92 mV at 10 mA cm^{-2}) and a boosted H_2 production rate by over 30% compared to that of bare steel.[174] Studies also confirm nickel-molybdenum alloys and MoS_2-CNT composites as robust, scalable options.[175] Nevertheless, these substitutes often require higher overpotentials and may exhibit limited long-term electrocatalytic stability under fluctuating reactor conditions typical of DF–MEC systems.

5.3 Electrode-biofilm synergy and functional limitations

The mutual compatibility between electrode surface properties and microbial consortia is crucial for sustained bioelectrochemical performance.[153,176] Electrode characteristics, such as hydrophilicity, surface roughness, and conductivity govern biofilm formation, redox mediator diffusion, and overall charge transfer resistance.[177,178] Suboptimal surface–biofilm interactions can result in reduced current densities, increased internal resistance, and lower coulombic efficiencies.[179,180] Furthermore, long-term operation often encounters challenges such as cathodic passivation, biofouling, and shifts in the microbial community, all of which degrade hydrogen recovery rates and process stability.

5.4 Toward scalable and functional electrodes

Current advances focus on the development of structurally engineered electrodes such as 3D carbon foams, graphene aerogels, and metal–organic framework (MOF)-derived scaffolds aimed at enhancing volumetric current density and mass transport.[176,178] Biogenic and conductive polymeric materials (*e.g.*, polyaniline and polypyrrole) are also being explored for their dual benefits of microbial affinity and cost-effectiveness.[181] Despite laboratory-scale success, upscaling these systems remains constrained by trade-offs between material durability, manufacturing cost, and system integration.[182] Holistic optimization encompassing material properties, reactor hydrodynamics, and microbial electrokinetics is imperative for translating DF–MEC technologies into industrially viable platforms.

6. Impact of pretreatment and metabolic pathways on DF–MEC H_2 yield

The efficiency of dark fermentation integrated with microbial electrolysis cells (DF–MECs) for hydrogen production from food waste strongly depends on upstream pretreatment strategies and the resulting profile of metabolic intermediates, particularly volatile fatty acids (VFAs).[91,183] Pretreatment enhances hydrolysis, solubilizes organic matter, and shapes the microbial pathways that govern hydrogen yields in both fermentation and electrochemical stages. Food waste contains complex polymers such as cellulose, hemicellulose, and lignin, which require pretreatment to increase microbial accessibility and fermentability.[54,184] Physical methods like heat and ultrasound, chemical treatments using acids or alkalis, and biological interventions including antibiotic-assisted microbial suppression have all been studied to improve biohydrogen production (Table 6). Heat pretreatment at 90 °C for 20 minutes has shown greater efficiency in enhancing hydrogen production compared to acid or alkali hydrolysis.[115] Ultrasonic pretreatment improves substrate solubilization and microbial accessibility, leading to hydrogen yield improvements of up to 80%.[120,185] Alkali pretreatment applied under optimized volatile solid loading conditions has also demonstrated increased hydrogen generation.[186,187] However, highly acidic or alkaline treatments often require post-neutralization and may disrupt microbial stability. Biological pretreatments using antibiotics such as chloramphenicol, amoxicillin, and oxytetracycline have been shown to inhibit methanogens and lactic acid bacteria (LAB), selectively enhancing hydrogen-producing communities.[188,189]

On the other hand, operational conditions, organic matter removal, VFA profiles, and hydrogen yields from food waste and related substrates are shown in Table 5. These pretreatment strategies directly influence the VFA composition in fermentation effluent. Acetate and butyrate are the most favorable VFAs for hydrogen production, while lactate and propionate are typically associated with lower hydrogen yields.[161] Thermal and alkali pretreatments often favor the formation of acetate and butyrate, which align with hydrogen-producing metabolic routes. In contrast, acidic or LAB-dominated conditions result in elevated lactic acid and propionate levels, which inhibit

Table 5 Summary of operational conditions, organic matter removal, and VFA profiles for hydrogen yields or production from food waste and related substrates (compiled from ref. 46 and 155)[a]

Type of FW	Operational conditions	VS or TVS or carbohydrate removal (%)	VFA production/yield	H_2 yield/production	Ref.
Food waste	Low pH-6 BR, 30 °C, pH 6.0	NA	TVFAs = 34.05 g L^{-1} Ac, Pr, Bu, Va	NA	156
Food waste	Mixed culture, ASBR, 35 °C, pH 5.5–6, HRT 48 h	Carbohydrate removal = 80.6%	TVFA = 6578 mg L^{-1} Acetate: 1.80 mg L^{-1} Butyrate: 1.45 mg L^{-1}	NA	191
OFMSW (anaerobic digestion plant)	BR, 35 °C, pH 5.5	NA	NA	152–237 mL H_2 per g VS	192
Food waste	Fed-batch, 37 °C, low HRT = 6.67, high OLR 2 g-VS per L per day, pH uncontrolled	VS = 15.41 ± 0.94% TS = 16.11 ± 0.98%	TVFA = 0.54 g-VFAs per g-VS Acetic acid = 20–30 Propionic acid = 3–10 Butyric acid = 14–23 Others = 35–65	14.66 mL H_2 per g VS_{added}	193
Fruit, vegetable, and fish	CSTR, 34 °C, pH 5.5, HRT 12 h	47.3 ± 2	NA	13.13 ± 1.04	194
Food waste	LBR, pH 7, 22 °C, S : I ratio of 25 : 1 fermentation time six days	NA	0.65 g-COD per g-VS Acetic acid = 27.9 Propionic acid = 12.9 Butyric acid = 33.7 Others = 25.5	NA	195
Cafeteria waste	ASBR, 35 °C, pH 5.5, HRT 12 h	NA	Acetate: 2.37 ± 2.8 g L^{-1} Propionic: 0.90 ± 0.18 g L^{-1} Butyrate: 0.90 ± 0.13 g L^{-1} Isobutyric: 0.02 ± 0.01 g L^{-1} Isovaleric: 0.03 ± 0.01 g L^{-1} Lactate: 9.41 ± 0.59 g L^{-1}	103.6 ± 19.8 (mL H_2 per g COD)	74
Fruit, vegetable, and cheese whey	BR, 37 °C, pH 5.5, HRT 3–11 days	16.36%	Fruit, vegetable lactate = 0.142 ± 0.014 (g L^{-1}) Cheese whey lactate = 6.18 ± 0.59 (g L^{-1})	449.89 (mL H_2 per g COD)	196
Food waste (University Cafeteria)	BR, UASB, pH = 5.5–4.1	NA	Butyric acid = 1167–940 mg L^{-1} Acetic acid = 509–459 mg L^{-1} Propionic acid = 168–2 mg L^{-1} Lactic acid = 121–77 mg L^{-1}	14.6–103.6 mL H_2 per g VS_{added}	79

[a] BR = Batch Reactor; CSTR = Continuously Stirred Tank Reactor; ASBR = Anaerobic Sequencing Batch Reactor; LBR = leach bed reactor; UASB = Upflow Anaerobic Sludge Blanket; TVFA = Total Volatile Fatty Acids; VS = Volatile Solids, TS = Total Solids, TVS = Total Volatile Solids; COD = Chemical Oxygen Demand.

Table 6 Comparison of pretreatment methods for food waste: mechanisms and operational impacts on DF–MEC hydrogen production

Pretreatment methods	Measure	Operational conditions	Substrate	Outcome	Mechanism	Ref.
Physical treatment	Hydrothermal	Temperature: 160 °C Time: 10 min	FW	Soluble COD has increased by 65%	Solubilization of food waste has been increased by 65%	197
	Microwave	Temperature: 145 °C	FW	Increased biogas production	Disrupted sludge and increased solubilization	198
	Thermal	Temperature: 120 °C Time: 30 min	FW	Biogas production increased by 11%	Increasing the solubilization	199
	Ultrasonication	Batch anaerobic Temperature: 30 °C	FW	Promoted the release of carbohydrates and proteins into the liquid phase and H_2 gas increased by 77%	Organic matter solubilization and VFAs promote anaerobic digestion efficiency	185
Chemical treatment	Acid	With 10 mol per L HCl at room temperature (18 ± 2 °C) until pH 2 for 24 h	FW	Biogas production decreased by 66%	Forming inhibitors	199
	Alkaline	Chemical: NaOH pH: 11 temperature: 4 °C Time: 24 h	FW	Soluble COD increased by 28%		120
	Acid	Chemical: HCl pH: 3 temperature: 4 °C Time: 24 h	FW	Soluble COD increased by 28%		120
Biological treatment	Biological solubilization	FW + water	FW	Decreased organic concentration in the effluent	Increasing the solubilization	200
	Enzyme	Enzyme: glucoamylase concentration: 2 g L^{-1} Temperature: 60 °C Contact time: 24 h	FW	Soluble COD has increased by 25%		201–203
Physical-chemical or thermo-chemical treatment	Thermo-acid	With ten mol per L HCl at room temperature (18 ± 2 °C) until pH 2 for 24 h and then 120 °C + 30 min	FW	Biogas production increased by 18%	Increasing the solubilization	199
	Thermo-chemical liquidation	175 °C, 4 MPa, 1 h	FW	24% higher COD solubilization and 6% higher biogas production		204

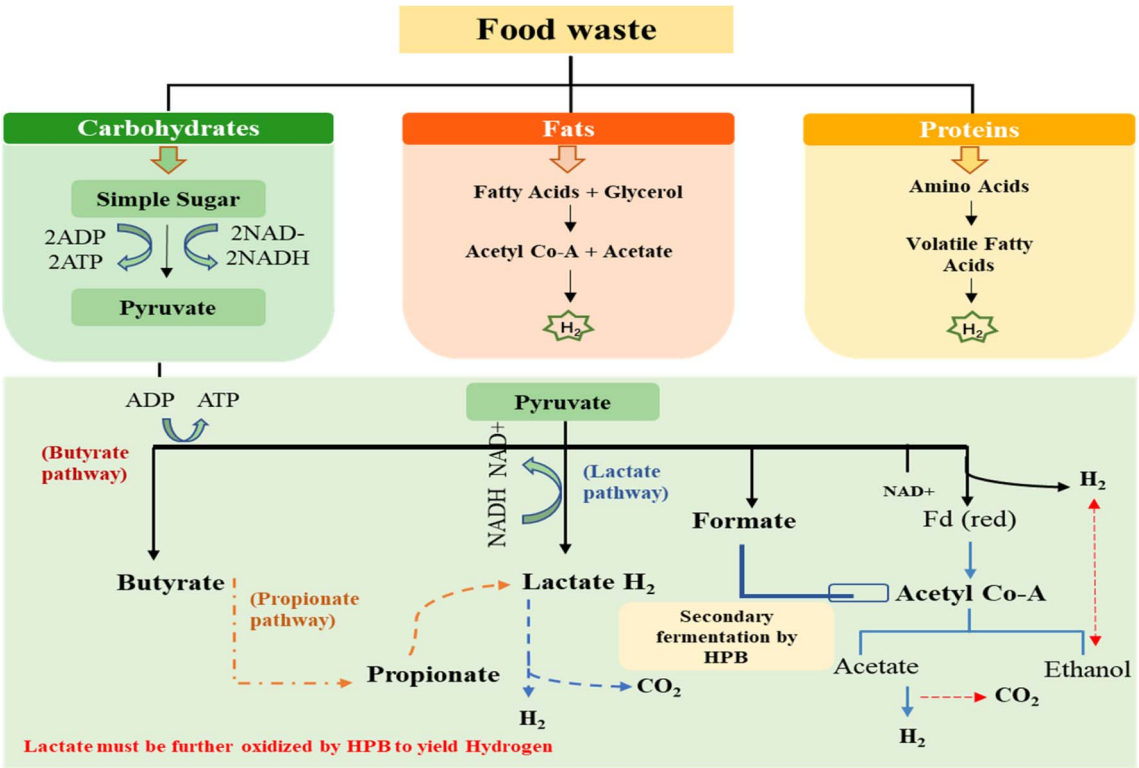

Fig. 9 Simplified metabolic pathways of food waste during dark fermentation and microbial electrolysis. Food waste undergoes hydrolysis and acidogenesis to produce intermediates like acetate, butyrate, lactate, and propionate. Acetate and butyrate are key for hydrogen production in both dark fermentation and electrohydrogenesis. Lactate can be further converted to hydrogen *via* lactate-driven pathways in DF–MEC systems.

hydrogen-producing bacteria. In lactate-driven dark fermentation (LD-DF), lactate produced by LAB is converted into hydrogen and butyrate by acidogenic bacteria such as *Clostridium butyricum*, offering an alternative route for energy recovery.[80] In addition to physicochemical pretreatments, the choice and adaptation of the microbial inoculum significantly affect fermentation outcomes. Recent studies have shown that using autochthonous microbial consortia enriched from specific biomass sources, such as *Ulva* spp., can enhance substrate-specific fermentation performance, even under thermophilic conditions.[190]

VFAs serve as critical substrates in MECs. Among them, acetate has been identified as the most efficient electron donor for anode-respiring bacteria, resulting in higher current density and coulombic efficiency.[100,159,205] Butyrate and propionate are less effective due to their complex oxidation mechanisms and lower electron yields.[100,159] Optimizing pretreatment to generate acetate-rich effluents significantly improves MEC performance in DF–MEC configurations.[205,206] In typical MECs, acetate-based biofilms support better electrode colonization and hydrogen recovery.[100] However, excessive accumulation of VFAs, particularly propionate and lactate, can inhibit microbial activity, lower pH, and reduce hydrogen output.[55,207] When total VFA concentrations exceed 4000 mg L^{-1} or propionate levels surpass 1500 mg L^{-1}, fermentation efficiency declines.[159,208,209] Managing VFA accumulation is essential for maintaining system stability and hydrogen productivity. This can be achieved by adjusting hydraulic retention time (HRT), applying pH control, integrating gas stripping or electrochemical recovery to reduce hydrogen partial pressure, or using codigestion strategies to balance substrate composition.[210] Bioaugmentation with VFA-degrading bacteria has also shown promise in improving reactor performance under high VFA loads.[126,211,212]

In DF–MEC systems, the downstream MEC stage plays a vital role in VFA conversion. Residual VFAs from the fermentation stage are consumed by electroactive bacteria at the anode, enabling additional hydrogen production.[159,205] This integrated design not only enhances energy recovery but also reduces organic load in the final effluent, supporting more sustainable and circular waste-to-energy processes.[213] However, pretreatment methods significantly affect the breakdown of food waste and the generation of VFAs, which in turn determine the efficiency of hydrogen production in DF–MEC systems. Optimizing pretreatment to favor acetate and butyrate formation, while minimizing inhibitory acids like lactate and propionate, is essential for maximizing hydrogen yield and ensuring stable system performance (Fig. 9).

7. Challenges and optimization of DF–MEC systems

This section discusses the primary biological, technical, and integration challenges associated with DF–MEC systems, and highlights recent advances and future directions for system optimization and commercial deployment.

7.1 Biological and substrate-related challenges in DF–MEC systems

Food waste contributes significantly to global greenhouse gas emissions, with approximately 3.3 billion tonnes produced annually due to inefficiencies in supply chains, storage, and transportation.[3,214] Despite its high moisture content, complex composition, and variable biodegradability, food waste remains an attractive and low-cost feedstock for hydrogen production *via* dark fermentation (DF) and microbial electrolysis cells (MECs). However, several technological and biological limitations continue to hinder the scalability, efficiency, and long-term sustainability of DF–MEC systems.

One of the primary challenges is the requirement for extensive pretreatment to break down carbohydrates, lipids, and proteins into fermentable intermediates. The slow hydrolysis rate and the tendency of lipids to cause flotation and inhibit microbial contact further reduce substrate accessibility. Optimizing key operational parameters, such as HRT, OLR, and pH, is critical to enhance hydrogen yield and minimize the formation of inhibitory byproducts, such as acetic, propionic, and butyric acids. These volatile fatty acids (VFAs) act as electron sinks, lowering hydrogen production and overall process efficiency.[94,215] Although theoretical hydrogen yields from glucose can reach 12 mol H_2 per mol, practical values are often limited to 3.8–4 mol H_2 per mol due to VFA accumulation. Under controlled thermophilic conditions, yields as high as 11.5 mol H_2 per mol glucose have been achieved, but replicating such results in real-world systems remains difficult.[216] Increasing substrate concentration can lead to excessive production of non-gaseous byproducts, shift microbial metabolism toward lactate or ethanol pathways, and destabilize fermentation. However, maintaining an alkaline pH (8–9) has been found to suppress VFA accumulation, promoting better microbial activity and improved hydrogen generation. Co-digestion strategies, combining food waste with nutrient-rich or lignocellulosic substrates, and solid-state fermentation have proven useful in reducing VFA toxicity and balancing nutrient content. Additionally, innovative approaches such as membrane bioreactors, gas stripping, or headspace recirculation are being employed to lower hydrogen partial pressure and increase hydrogen yield (YH_2).[80,82,210] These strategies help shift microbial metabolism away from reduced end-products and toward more efficient hydrogen-producing pathways, especially in lactate-driven DF (LD-DF) systems.

7.2 Technical constraints and microbial management in DF–MEC integration

The integration of DF and MEC technologies introduces additional complexity. Key technical challenges include striking a balance between microbial hydrogen production and consumption, minimising external energy input for MEC operation, and maintaining process stability. Hydrogen loss due to methanogenic activity, variations in the reactor configuration, electrode fouling, and substrate composition all affect system performance.[154,155] Further complications arise from the need to maintain strict anaerobic conditions and to suppress methanogens while promoting electroactive and hydrogen-producing bacteria. Microbial community dynamics represent another critical factor influencing the performance of DF–MEC systems. The coexistence of *Clostridium* species, electroactive bacteria such as *Geobacter*, and lactic acid bacteria (LAB) requires careful microbial management. In LD-DF–MEC systems, the cross-feeding of lactate between LAB and hydrogen producers must be optimized to maintain energy-efficient conversions. Metabolic competition and electron drain within microbial networks can also limit overall H_2 yield. Biological optimization approaches, including bioaugmentation and metabolic engineering, have been employed to overcome these limitations, improving hydrogen yields to between 0.47 and 1.88 mol H_2 per mol glucose in engineered strains.[90]

7.3 Scale-up challenges and operational blockades

To scale DF–MEC systems toward industrial implementation, operational optimisation must be combined with wastewater quality management. This includes maintaining acceptable levels of COD, BOD, TOC, nitrogen, and phosphorus. Although MECs offer improved removal of organic pollutants and extended energy recovery, consistent operation under variable feedstock conditions and reactor fouling remains a barrier to adoption. Recent studies have shown that DF–MEC systems can achieve maximum hydrogen yields of 1608.6 $\pm$ 266.2 mL H_2 per g COD consumed, along with COD removal efficiencies of up to 78.5 $\pm$ 5.7%.[109,213] However, these outcomes are highly dependent on the reactor setup, microbial synergy, and the composition of the feedstock. Long-term stability and reproducibility under operational stress remain challenges to be addressed. Moreover, the energy requirements for external voltage application in MECs, as well as the risk of methane generation from residual substrates, impact both the environmental footprint and the net energy output. Heat pretreatment remains one of the most widely adopted methods for enhancing microbial accessibility and suppressing methanogenesis due to its simplicity and cost-effectiveness. Nonetheless, the need for tailored microbial consortia and precise trophic interactions persists, particularly in LD-DF–MEC systems. Enhancing the understanding of microbial behaviour, syntrophic partnerships, and substrate conversion kinetics is vital for optimising overall system performance.

7.4 CO_2 control and carbon recovery strategies in DF–MEC systems

CO_2 is an inevitable byproduct of substrate oxidation in both dark fermentation and microbial electrolysis stages of DF–MEC systems. Its accumulation can contribute to elevated headspace pressure, pH imbalance, and inhibition of key hydrogen-producing enzymes.[59,217] Effective CO_2 control is thus vital for maintaining reactor stability and optimizing biohydrogen yield. Several strategies have been explored to mitigate CO_2 build-up and enhance carbon recovery. One approach involves the use of gas-permeable membranes or headspace gas stripping to selectively remove CO_2, thereby improving hydrogen purity and reducing gas-phase inhibition. Alternatively, alkaline cathodic environments in MECs can facilitate CO_2 absorption as carbonate or bicarbonate, providing passive mitigation.[111,218,219]

More advanced systems have incorporated biocathodes with autotrophic microorganisms that fix CO_2 into biomass or short-chain fatty acids. Additionally, emerging concepts such as microbial electrosynthesis offer the potential to convert CO_2 into acetate, methane, or other value-added compounds using renewable electricity and engineered microbial consortia.[218,220] These CO_2 control strategies not only enhance the performance of DF–MEC systems but also align with broader goals of carbon neutrality and circular resource use, reinforcing the role of DF–MEC technology in sustainable waste-to-energy platforms.

7.5 Future outlook: toward circular bioeconomy integration

In conclusion, although DF–MEC systems offer a sustainable approach to hydrogen production and waste management, their implementation at an industrial scale requires overcoming technical, economic, and environmental barriers. Continued research into microbial engineering, integrated reactor design, and dynamic process control is essential to unlock the full potential of these systems. A multidisciplinary approach that combines biotechnology, electrochemistry, environmental engineering, and systems optimisation will be crucial in transforming DF–MECs into commercially viable hydrogen generation technologies.

8. Conclusion and prospects

Biohydrogen (Bio-H_2) represents a sustainable and renewable alternative to fossil fuels, with food waste offering a cost-effective and abundantly available feedstock for its production. Rich in biodegradable organic matter, particularly carbohydrates, food waste can be effectively valorized through dark fermentation (DF), especially when coupled with microbial electrolysis cells (MECs). However, the scalability and stability of such systems hinge on optimizing microbial performance, substrate utilization, and reactor conditions. This review highlights heat pretreatment as one of the most effective and economically viable strategies for enhancing hydrogen production. It improves substrate solubilization, suppresses methanogens, and enriches hydrogen-producing bacterial populations, resulting in greater conversion of complex organics into hydrogen-favorable intermediates such as acetate and butyrate. Nevertheless, the accumulation of lactate during lactate-driven dark fermentation (LD-DF) remains a key bottleneck that reduces hydrogen yields over time.

Addressing this challenge requires integrated strategies including microbial consortia selection, process control, and genetic modification to improve enzymatic hydrogen production. The digestate from LD-DF, typically rich in volatile fatty acids such as lactate and acetate, serves as an ideal substrate for MECs. By applying a small external voltage, MECs utilise electroactive bacteria to oxidise these compounds, enabling additional hydrogen generation at the cathode. This DF–MEC integration maximizes energy recovery by converting residual intermediates into hydrogen, and significantly increases the overall hydrogen yield (YH_2), with reports indicating 30–40% improvements compared to DF alone.

For industrial applications, achieving long-term efficiency and scalability requires a well-balanced microbial community and optimized system design. The coupling of LD-DF with MECs offers a viable waste-to-energy pathway for sectors such as agriculture, food processing, and municipal waste management, transforming organic residues into clean hydrogen fuel. Moreover, the environmental and economic benefits of this system are substantial. The DF–MEC approach reduces greenhouse gas emissions, diverts food waste from landfills, and supports the production of renewable hydrogen. Although the initial capital investment may be significant, the long-term advantages, including higher hydrogen yields, improved effluent quality, and enhanced energy recovery, demonstrate strong potential for industrial-scale deployment. Future studies should also incorporate techno-economic analyses to assess not only capital investment but also operational costs, maintenance requirements, and return on investment (ROI), thereby validating the financial feasibility of DF–MEC systems.

In conclusion, the coupling of dark fermentation with microbial electrolysis cells presents a scalable, sustainable, and efficient route for biohydrogen production from food waste. Further advancements in microbial consortia engineering, reactor configurations, and integrated system control will be essential to fully realize the potential of DF–MEC platforms. This integrated strategy aligns with the principles of the circular economy and supports global goals for renewable energy development and waste minimization.

Author contributions

Anam Jalil: conceptualization, investigation, formal analysis, writing – original draft, software, and validation. Hikmatullah Ahmadi, Fabrice Ndayisenga, and Sohail Khan: formal analysis & review and editing. Atif Ahmad and Xiangyang Wang: resources, software, validation; visualization, data curation. Zhisheng Yu: supervision, funding acquisition, project administration, writing – review & editing. All authors read and approved the final manuscript.

Conflicts of interest

There are no conflicts to declare.

Data availability

No primary data were generated or analyzed in this study. All data discussed in this review are derived from previously published sources, which are cited appropriately throughout the article.

Abbreviations

AD	Anaerobic Digestion
E_{an}	Anodic Potential
E_{ap}	Applied Voltage
$BioH_2$	Biohydrogen

CH_4	Methane
C/N	Carbon to Nitrogen Ratio
COD	Chemical Oxygen Demand
DF	Dark Fermentation
FAO	Food and Agriculture Organisation
FW	Food Waste
GHG	Greenhouse Gases
H_2	Hydrogen
HLa	Lactic Acid Fermentation
HPP	Hydrogen Partial Pressure
HRT	Hydraulic Retention Time
LAB	Lactate Acid-Producing Bacteria
LD-DF	Lactate Driven Dark Fermentation
LD-DF–	Lactate Driven Dark Fermentation assisted
MECs	Microbial Electrolysis Cells
MECs	Microbial Electrolysis Cells
MFCs	Microbial Fuel Cells
OLR	Organic Loading Rate
ORP	Oxidation–Reduction Potential
SDG 7	Sustainable Development Goal 7
VFAs	Volatile Fatty Acids
VS	Volatile Solids
YH_2	Hydrogen Yield

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References

1 D. D. T. Ferraren-De Cagalitan and M. L. S. Abundo, *Renew. Sustain. Energy Rev.*, 2021, **151**, 111413.
2 C. Umunnawuike, S. Q. A. Mahat, P. I. Nwaichi, B. Money and A. Agi, *Biomass Bioenergy*, 2024, **188**, 107345.
3 M. M. Habashy, E. S. Ong, O. M. Abdeldayem, E. G. Al-Sakkari and E. R. Rene, *Trends Biotechnol.*, 2021, **39**, 1274–1288.
4 O. Ali Qamar, F. Jamil, M. Hussain, A. H. Al-Muhtaseb, A. Inayat, A. Waris, P. Akhter and Y.-K. Park, *Chem. Eng. J.*, 2023, **454**, 140240.
5 M. Gupta, N. Savla, C. Pandit, S. Pandit, P. K. Gupta, M. Pant, S. Khilari, Y. Kumar, D. Agarwal, R. R. Nair, D. Thomas and V. K. Thakur, *Sci. Total Environ.*, 2022, **825**, 153892.
6 M. Sharma, E.-S. Salama, N. Thakur, H. Alghamdi, B.-H. Jeon and X. Li, *Chem. Eng. J.*, 2023, **465**, 142546.
7 E. Elbeshbishy, B. R. Dhar, G. Nakhla and H.-S. Lee, *Renew. Sustain. Energy Rev.*, 2017, **79**, 656–668.
8 S. Singh, S. Jain, P. S. Venkateswaran, A. K. Tiwari, M. R. Nouni, J. K. Pandey and S. Goel, *Renew. Sustain. Energy Rev.*, 2015, **51**, 623–633.
9 S. Venkata Mohan and A. Pandey, in *Biohydrogen*, Elsevier, 2019, pp. 1–23.
10 I. Dincer and C. Acar, *Int. J. Hydrogen Energy*, 2015, **40**, 11094–11111.
11 N. Ade, A. Alsuhaibani, M. M. El-Halwagi, H. Goyette and B. Wilhite, *Int. J. Hydrogen Energy*, 2022, **47**, 6404–6414.
12 B. Amini Horri and H. Ozcan, *Curr. Opin. Green Sustainable Chem.*, 2024, **47**, 100932.
13 A. Le Pera, M. Sellaro, F. Sicilia, R. Ciccoli, B. Sceberras, C. Freda, E. Fanelli and G. Cornacchia, *Sci. Total Environ.*, 2023, **880**, 163240.
14 W. Zong, R. Yu, P. Zhang, M. Fan and Z. Zhou, *Biomass Bioenergy*, 2009, **33**, 1458–1463.
15 S.-H. Kim, G. Kumar, W.-H. Chen and S. K. Khanal, *Bioresour. Technol.*, 2021, **331**, 125024.
16 Y. Zhou, V. Kumar, S. Harirchi, V. S. Vigneswaran, K. Rajendran, P. Sharma, Y. Wah Tong, P. Binod, R. Sindhu, S. Sarsaiya, D. Balakrishnan, M. Mofijur, Z. Zhang, M. J. Taherzadeh and M. Kumar Awasthi, *Bioresour. Technol.*, 2022, **360**, 127565.
17 S. Feng, H. Hao Ngo, W. Guo, S. Woong Chang, D. Duc Nguyen, X. Thanh Bui, X. Zhang, X. Y. Ma and B. Ngoc Hoang, *Chem. Eng. J.*, 2023, **471**, 144669.
18 Z. Li, A. Fang, H. Cui, J. Ding, B. Liu, G. Xie, N. Ren and D. Xing, *Chem. Eng. J.*, 2021, **417**, 127986.
19 V. Hovorukha, O. Havryliuk, G. Gladka, O. Tashyrev, A. Kalinichenko, M. Sporek and A. Dołhańczuk-Śródka, *Energies*, 2021, **14**, 1831.
20 C. Cavinato, A. Giuliano, D. Bolzonella, P. Pavan and F. Cecchi, *Int. J. Hydrogen Energy*, 2012, **37**, 11549–11555.
21 X. Qu, H. Zeng, Y. Gao, T. Mo and Y. Li, *Front. Chem.*, 2022, **10**, 978907.
22 D.-H. Kim, S.-H. Kim, K.-W. Jung, M.-S. Kim and H.-S. Shin, *Bioresour. Technol.*, 2011, **102**, 8646–8652.
23 L. J. Martínez-Mendoza, R. Lebrero, R. Muñoz and O. García-Depraect, *Bioresour. Technol.*, 2022, **364**, 128070.
24 L. Regueira-Marcos, O. García-Depraect and R. Muñoz, *Fuel*, 2023, **338**, 127238.
25 L. T. Fuess, A. D. N. Ferraz, C. B. Machado and M. Zaiat, *Bioresour. Technol.*, 2018, **247**, 426–433.
26 V. F. Diaz-Cruces, O. García-Depraect and E. León-Becerril, *BioEnergy Res.*, 2020, **13**, 571–580.
27 A. Ghimire, V. Luongo, L. Frunzo, F. Pirozzi, P. N. L. Lens and G. Esposito, *Int. J. Hydrogen Energy*, 2017, **42**, 4861–4869.
28 E. Munier, H. Licandro, E. Beuvier and R. Cachon, *Int. Microbiol.*, 2023, **26**, 501–511.

29 P. Sivagurunathan, P. C. Sahoo, M. Kumar, R. Prakash Gupta, D. Bhattacharyya and S. S. V. Ramakumar, *Bioresour. Technol.*, 2023, **367**, 128260.
30 A. Detman, D. Mielecki, A. Chojnacka, A. Salamon, M. K. Błaszczyk and A. Sikora, *Microb. Cell Fact.*, 2019, **18**, 36.
31 Ö. B. Gökçek, F. Baş, H. Muratçobanoğlu and S. Demirel, *Fuel*, 2023, **339**, 127475.
32 J. L. Varanasi, R. Veerubhotla, S. Pandit and D. Das, in *Microbial Electrochemical Technology*, Elsevier, 2019, pp. 843–869.
33 B. E. Logan, R. Rossi, A. Ragab and P. E. Saikaly, *Nat. Rev. Microbiol.*, 2019, **17**, 307–319.
34 P. T. Sekoai, K. O. Yoro, M. O. Bodunrin, A. O. Ayeni and M. O. Daramola, *Rev. Environ. Sci. Biotechnol.*, 2018, **17**, 501–529.
35 R. Łukajtis, I. Hołowacz, K. Kucharska, M. Glinka, P. Rybarczyk, A. Przyjazny and M. Kamiński, *Renew. Sustain. Energy Rev.*, 2018, **91**, 665–694.
36 A. I. Vavouraki, V. Volioti and M. E. Kornaros, *Waste Manage.*, 2014, **34**, 167–173.
37 F. Piadeh, I. Offie, K. Behzadian, J. P. Rizzuto, A. Bywater, J.-R. Córdoba-Pachón and M. Walker, *J. Environ. Manage.*, 2024, **349**, 119458.
38 N. I. S. Muhammad and K. A. Rosentrater, *Energies*, 2020, **13**, 436.
39 M. He, Y. Sun, D. Zou, H. Yuan, B. Zhu, X. Li and Y. Pang, *Procedia Environ. Sci.*, 2012, **16**, 85–94.
40 A. I. Vavouraki, E. M. Angelis and M. Kornaros, *Waste Manage.*, 2013, **33**, 740–745.
41 L. Zhang and D. Jahng, *Waste Manage.*, 2012, **32**, 1509–1515.
42 M. Bibra, N. K. Rathinam, G. R. Johnson and R. K. Sani, *Renew. Energy*, 2020, **155**, 1032–1041.
43 A. Gallipoli, C. M. Braguglia, A. Gianico, D. Montecchio and P. Pagliaccia, *J. Environ. Sci.*, 2020, **89**, 167–179.
44 M. L. Chong, V. Sabaratnam, Y. Shirai and M. A. Hassan, *Int. J. Hydrogen Energy*, 2009, **34**, 3277–3287.
45 S. K. S. Patel, J.-K. Lee and V. C. Kalia, *Indian J. Microbiol.*, 2018, **58**, 529–530.
46 A. K. Pandey, S. Pilli, P. Bhunia, R. D. Tyagi, R. Y. Surampalli, T. C. Zhang, S.-H. Kim and A. Pandey, *Chemosphere*, 2022, **288**, 132444.
47 T. Thu Ha Tran and P. Khanh Thinh Nguyen, *Bioresour. Technol.*, 2022, **357**, 127340.
48 J. Lacroux, M. Llamas, K. Dauptain, R. Avila, J.-P. Steyer, R. van Lis and E. Trably, *Sci. Total Environ.*, 2023, **865**, 161136.
49 V. Narisetty, L. Zhang, J. Zhang, C. Sze Ki Lin, Y. Wah Tong, P. Loke Show, S. Kant Bhatia, A. Misra and V. Kumar, *Bioresour. Technol.*, 2022, **358**, 127381.
50 K. Bolatkhan, B. D. Kossalbayev, B. K. Zayadan, T. Tomo, T. N. Veziroglu and S. I. Allakhverdiev, *Int. J. Hydrogen Energy*, 2019, **44**, 5799–5811.
51 R. Miandad, M. Rehan, O. K. M. Ouda, M. Z. Khan, K. Shahzad, I. M. I. Ismail and A. S. Nizami, in *Biohydrogen Production: Sustainability of Current Technology and Future Perspective*, Springer India, New Delhi, 2017, pp. 237–252.
52 F. Khosravitabar, *J. Appl. Phycol.*, 2020, **32**, 277–289.
53 G. Sołowski, I. Konkol and A. Cenian, *Biomass Bioenergy*, 2020, **138**, 105576.
54 K. Kucharska, P. Rybarczyk, I. Hołowacz, D. Konopacka-Łyskawa, E. Słupek, P. Makoś, H. Cieśliński and M. Kamiński, *Biomass Bioenergy*, 2020, **141**, 105691.
55 C. Bian, X. Chen, J. Wang, B. Xiao, R. Liu, L. Li and J. Liu, *J. Clean. Prod.*, 2023, **420**, 138370.
56 H. Liu, P. Han, H. Liu, G. Zhou, B. Fu and Z. Zheng, *Bioresour. Technol.*, 2018, **260**, 105–114.
57 S. Rawat, A. Rautela, I. Yadav, S. Misra and S. Kumar, *BioEnergy Res.*, 2023, **16**, 2131–2154.
58 A. Kadier, M. S. Kalil, K. Chandrasekhar, G. Mohanakrishna, G. D. Saratale, R. G. Saratale, G. Kumar, A. Pugazhendhi and P. Sivagurunathan, *Bioelectrochemistry*, 2018, **119**, 211–219.
59 A. Wang, D. Sun, G. Cao, H. Wang, N. Ren, W.-M. Wu and B. E. Logan, *Bioresour. Technol.*, 2011, **102**, 4137–4143.
60 S. Chen, Z. Tao, F. Yao, B. Wu, L. He, K. Hou, Z. Pi, J. Fu, H. Yin, Q. Huang, Y. Liu, D. Wang, X. Li and Q. Yang, *Bioresour. Technol.*, 2020, **316**, 123901.
61 C.-G. Liu, J.-C. Qin and Y.-H. Lin, in *Fermentation Processes*, InTech, 2017.
62 R. Kumar, R. Kumar, S. K. Brar and G. Kaur, *Bioengineered*, 2022, **13**, 14987–15002.
63 Y. Li, X. Zhang, H. Xu, H. Mu, D. Hua, F. Jin and G. Meng, *J. Biosci. Bioeng.*, 2019, **128**, 50–55.
64 X. Shi, L. Wu, W. Wei and B.-J. Ni, *Crit. Rev. Environ. Sci. Technol.*, 2022, **52**, 3787–3812.
65 X. Yang, Z. Zhang, S. Li, Q. He, X. Peng, X. Du, K. Feng, S. Wang and Y. Deng, *Environ. Res.*, 2022, **212**, 113298.
66 S. G. Langer, C. Gabris, D. Einfalt, B. Wemheuer, M. Kazda and F. R. Bengelsdorf, *Microb. Biotechnol.*, 2019, **12**, 1210–1225.
67 Y.-X. Fan, J.-Z. Zhang, Q. Zhang, X.-Q. Ma, Z.-Y. Liu, M. Lu, K. Qiao and F.-L. Li, *Adv. Appl. Microbiol.*, 2021, **117**, 1–34.
68 Q. Wu, H. Zheng, Y. Chen, M. Liu, X. Bao and W. Guo, *J. Clean. Prod.*, 2021, **289**, 125765.
69 B. Aranda-Jaramillo, E. León-Becerril, O. Aguilar-Juárez, R. Castro-Muñoz and O. García-Depraect, *Fermentation*, 2023, **9**, 644.
70 C. Anagnostopoulou, K. N. Kontogiannopoulos, M. Gaspari, M. S. Morlino, A. N. Assimopoulou and P. G. Kougias, *Chemosphere*, 2022, **296**, 133871.
71 P. Tsapekos, M. Alvarado-Morales, S. Baladi, E. F. Bosma and I. Angelidaki, *Front. Sustain.*, 2020, **1**, 4.
72 B. Teusink and D. Molenaar, *Curr. Opin. Syst. Biol.*, 2017, **6**, 7–13.
73 O. García-Depraect, R. Castro-Muñoz, R. Muñoz, E. R. Rene, E. León-Becerril, I. Valdez-Vazquez, G. Kumar, L. C. Reyes-Alvarado, L. J. Martínez-Mendoza, J. Carrillo-Reyes and G. Buitrón, *Bioresour. Technol.*, 2021, **324**, 124595.
74 I. Moreno-Andrade, J. Carrillo-Reyes, S. G. Santiago and M. C. Bujanos-Adame, *Int. J. Hydrogen Energy*, 2015, **40**, 17246–17252.

75 J. H. Jo, C. O. Jeon, D. S. Lee and J. M. Park, *J. Biotechnol.*, 2007, **131**, 300–308.
76 D.-H. Kim, S. Jang, Y.-M. Yun, M.-K. Lee, C. Moon, W.-S. Kang, S.-S. Kwak and M.-S. Kim, *Int. J. Hydrogen Energy*, 2014, **39**, 16302–16309.
77 S. Duan, J. He, X. Xin, L. Li, X. Zou, Y. Zhong, J. Zhang and X. Cui, *Bioresour. Technol.*, 2023, **384**, 129245.
78 D. Jiang and S. Zhu, in *Waste to Renewable Biohydrogen*, Elsevier, 2021, pp. 123–137.
79 C. Sreela-or, T. Imai, P. Plangklang and A. Reungsang, *Int. J. Hydrogen Energy*, 2011, **36**, 14120–14133.
80 C. Martínez-Fraile, R. Muñoz, M. Teresa Simorte, I. Sanz and O. García-Depraect, *Bioresour. Technol.*, 2024, **403**, 130846.
81 O. García-Depraect and E. León-Becerril, *Fermentation*, 2023, **9**, 787.
82 J. A. Magdalena, L. Perat, L. Braga-Nan and E. Trably, in *Wastewater Exploitation: From Microbiological Activity to Energy*, Springer Nature, Switzerland, 2024, pp. 67–90.
83 E. L. N. Dzulkarnain, J. O. Audu, W. R. Z. Wan Dagang and M. F. Abdul-Wahab, *Bioresour. Bioprocess.*, 2022, **9**, 16.
84 J.-H. Park, S.-H. Lee, H.-J. Ju, S.-H. Kim, J.-J. Yoon and H.-D. Park, *Renew. Energy*, 2016, **86**, 889–894.
85 O. García-Depraect, R. Muñoz, E. Rodríguez, E. R. Rene and E. León-Becerril, *Int. J. Hydrogen Energy*, 2021, **46**, 11284–11296.
86 A. Nzila, *Anaerobe*, 2017, **46**, 3–12.
87 L. Cabrol, A. Marone, E. Tapia-Venegas, J.-P. Steyer, G. Ruiz-Filippi and E. Trably, *FEMS Microbiol. Rev.*, 2017, **41**, 158–181.
88 P. Sharma and U. Melkania, *Energy Convers. Manag.*, 2018, **163**, 260–267.
89 S. G. Santiago, E. Trably, E. Latrille, G. Buitrón and I. Moreno-Andrade, *Lett. Appl. Microbiol.*, 2019, **69**, 138–147.
90 P. Majidian, M. Tabatabaei, M. Zeinolabedini, M. P. Naghshbandi and Y. Chisti, *Renew. Sustain. Energy Rev.*, 2018, **82**, 3863–3885.
91 H. Wei, T. Junhong and L. Yongfeng, *Phys. Sci. Rev.*, 2016, **1**(10), 20160050.
92 R. Tamaian, in *ECM 2023*, MDPI, Basel Switzerland, 2023, p. 14.
93 E. Villanueva-Galindo, M. Vital-Jácome and I. Moreno-Andrade, *Int. J. Hydrogen Energy*, 2023, **48**, 9957–9970.
94 G. Vardar-Schara, T. Maeda and T. K. Wood, *Microb. Biotechnol.*, 2008, **1**, 107–125.
95 B. E. Logan, B. Hamelers, R. Rozendal, U. Schröder, J. Keller, S. Freguia, P. Aelterman, W. Verstraete and K. Rabaey, *Environ. Sci. Technol.*, 2006, **40**, 5181–5192.
96 T. Fudge, I. Bulmer, K. Bowman, S. Pathmakanthan, W. Gambier, Z. Dehouche, S. M. Al-Salem and A. Constantinou, *Water*, 2021, **13**, 445.
97 G. Zhen, X. Lu, G. Kumar, P. Bakonyi, K. Xu and Y. Zhao, *Prog. Energy Combust. Sci.*, 2017, **63**, 119–145.
98 G. Kanellos, T. Zonfa, A. Polettini, R. Pomi, A. Rossi, A. Tremouli and G. Lyberatos, *Biomass Bioenergy*, 2024, **189**, 107335.
99 Z. Yu, X. Leng, S. Zhao, J. Ji, T. Zhou, A. Khan, A. Kakde, P. Liu and X. Li, *Bioresour. Technol.*, 2018, **255**, 340–348.
100 R. Cardeña, I. Moreno-Andrade and G. Buitrón, *J. Chem. Technol. Biotechnol.*, 2018, **93**, 878–886.
101 R. A. Rozendal, H. V. M. Hamelers, G. J. W. Euverink, S. J. Metz and C. J. N. Buisman, *Int. J. Hydrogen Energy*, 2006, **31**, 1632–1640.
102 Z. M. A. Bundhoo, *Int. J. Hydrogen Energy*, 2017, **42**, 26667–26686.
103 D. Call and B. E. Logan, *Environ. Sci. Technol.*, 2008, **42**, 3401–3406.
104 F. Rezaeitavabe, S. Saadat, N. Talebbeydokhti, M. Sartaj and M. Tabatabaei, *Biomass Bioenergy*, 2020, **143**, 105846.
105 C. Mamimin, A. Jehlee, S. Saelor, P. Prasertsan and S. O-Thong, *Int. J. Hydrogen Energy*, 2016, **41**, 21692–21701.
106 P. Khongkliang, A. Jehlee, P. Kongjan, A. Reungsang and S. O-Thong, *Int. J. Hydrogen Energy*, 2019, **44**, 31841–31852.
107 X. Jia, M. Li, Y. Wang, Y. Wu, L. Zhu, X. Wang and Y. Zhao, *Environ. Sci. Ecotechnology*, 2020, **1**, 100006.
108 J. Huang, H. Feng, L. Huang, X. Ying, D. Shen, T. Chen, X. Shen, Y. Zhou and Y. Xu, *Waste Manage.*, 2020, **103**, 61–66.
109 A. Marone, O. R. Ayala-Campos, E. Trably, A. A. Carmona-Martínez, R. Moscoviz, E. Latrille, J.-P. Steyer, V. Alcaraz-Gonzalez and N. Bernet, *Int. J. Hydrogen Energy*, 2017, **42**, 1609–1621.
110 P. Khongkliang, P. Kongjan, B. Utarapichat, A. Reungsang and S. O-Thong, *Int. J. Hydrogen Energy*, 2017, **42**, 27584–27592.
111 W. Liu, S. Huang, A. Zhou, G. Zhou, N. Ren, A. Wang and G. Zhuang, *Int. J. Hydrogen Energy*, 2012, **37**, 13859–13864.
112 T. P. Phan, T. L. Nguyen and P. K. T. Nguyen, *Biomass Bioenergy*, 2023, **175**, 106885.
113 H. Su, D. Hantoko, M. Yan, Y. Cai, E. Kanchanatip, J. Liu, X. Zhou and S. Zhang, *Int. J. Hydrogen Energy*, 2019, **44**, 21451–21463.
114 S.-H. Kim and H.-S. Shin, *Int. J. Hydrogen Energy*, 2008, **33**, 5266–5274.
115 D.-H. Kim, S.-H. Kim and H.-S. Shin, *Enzyme Microb. Technol.*, 2009, **45**, 181–187.
116 A. Tawfik and M. El-Qelish, *Bioresour. Technol.*, 2012, **114**, 270–274.
117 Z.-K. Lee, S.-L. Li, P.-C. Kuo, I.-C. Chen, Y.-M. Tien, Y.-J. Huang, C.-P. Chuang, S.-C. Wong and S.-S. Cheng, *Int. J. Hydrogen Energy*, 2010, **35**, 13458–13466.
118 D.-Y. Lee, K.-Q. Xu, T. Kobayashi, Y.-Y. Li and Y. Inamori, *Int. J. Hydrogen Energy*, 2014, **39**, 16863–16871.
119 A. Castillo-Hernández, I. Mar-Alvarez and I. Moreno-Andrade, *Int. J. Hydrogen Energy*, 2015, **40**, 17239–17245.
120 E. Elbeshbishy, H. Hafez, B. R. Dhar and G. Nakhla, *Int. J. Hydrogen Energy*, 2011, **36**, 11379–11387.
121 S.-H. Kim, S.-K. Han and H.-S. Shin, *Process Biochem.*, 2008, **43**, 213–218.
122 H. S. Shin, J. H. Youn and S. H. Kim, *Int. J. Hydrogen Energy*, 2004, **29**, 1355–1363.
123 D.-H. Kim, S.-H. Kim, K.-Y. Kim and H.-S. Shin, *Int. J. Hydrogen Energy*, 2010, **35**, 1590–1594.

124 S. Paudel, Y. Kang, Y.-S. Yoo and G. T. Seo, *Waste Manage.*, 2017, **61**, 484–493.
125 Y. Kawagoshi, N. Hino, A. Fujimoto, M. Nakao, Y. Fujita, S. Sugimura and K. Furukawa, *J. Biosci. Bioeng.*, 2005, **100**, 524–530.
126 L. Wu, W. Wei, Z. Chen, X. Shi, D. Wang, X. Chen and B.-J. Ni, *Chem. Eng. J.*, 2023, **472**, 144824.
127 K. Khatami, M. Atasoy, M. Ludtke, C. Baresel, Ö. Eyice and Z. Cetecioglu, *Chemosphere*, 2021, **275**, 129981.
128 J. Pan, R. Zhang, H. Elmashad, H. Sun and Y. Ying, *Int. J. Hydrogen Energy*, 2008, **33**, 6968–6975.
129 Z.-K. Lee, S.-L. Li, J.-S. Lin, Y.-H. Wang, P.-C. Kuo and S.-S. Cheng, *Int. J. Hydrogen Energy*, 2008, **33**, 5234–5241.
130 J. Wongthanate, K. Chinnacotpong and M. Khumpong, *Int. J. Energy Environ. Eng.*, 2014, **5**, 76.
131 N. Dong-Jie, W. Jing-Yuan, W. Bao-Ying and Z. You-Cai, *Int. J. Hydrogen Energy*, 2011, **36**, 5289–5295.
132 M. Domińska, R. Ślęzak, J. Świątkiewicz, K. Paździor and S. Ledakowicz, *Energies*, 2024, **17**, 974.
133 S. Jodhani, J. Sebastian, J. Lee, K. Venkiteshwaran, H.-S. Lee, V. Singh, B. Ormeci and A. Hussain, *Fermentation*, 2024, **10**, 162.
134 C. Linyi, Q. Yujie, C. Buqing, W. Chenglong, Z. Shaohong, C. Renglu, Y. Shaohua, Y. Lan and L. Zhiju, *Environ. Res.*, 2020, **188**, 109743.
135 J. Wongthanate and K. Chinnacotpong, *Environ. Eng. Res.*, 2015, **20**, 121–125.
136 L. Sillero, R. Solera and M. Perez, *J. Clean. Prod.*, 2023, **382**, 135237.
137 F. Ndayisenga, Z. Yu, B. Wang, G. Wu and H. Zhang, *Energy Convers. Manage.: X*, 2024, **22**, 100541.
138 F. Ndayisenga, Z. Yu, B. Wang, G. Wu, H. Zhang, I. A. Phulpoto, J. Zhao and J. Yang, *Process Saf. Environ. Prot.*, 2022, **167**, 213–224.
139 D.-H. Kim, J. Wu, K.-W. Jeong, M.-S. Kim and H.-S. Shin, *Int. J. Hydrogen Energy*, 2011, **36**, 10666–10673.
140 A. I. Osman, T. J. Deka, D. C. Baruah and D. W. Rooney, *Biomass Convers. Biorefin.*, 2023, **13**, 8383–8401.
141 O. Okonkwo, R. Escudie, N. Bernet, R. Mangayil, A.-M. Lakaniemi and E. Trably, *Appl. Microbiol. Biotechnol.*, 2020, **104**, 439–449.
142 R. M. M. Ziara, D. N. Miller, J. Subbiah and B. I. Dvorak, *Int. J. Hydrogen Energy*, 2019, **44**, 661–673.
143 E. A. Cazier, E. Trably, J. P. Steyer and R. Escudie, *Bioresour. Technol.*, 2015, **190**, 106–113.
144 F. M. S. Silva, C. F. Mahler, L. B. Oliveira and J. P. Bassin, *Waste Manage.*, 2018, **76**, 339–349.
145 I. Martins, E. Surra, M. Ventura and N. Lapa, *Appl. Sci.*, 2022, **12**, 4240.
146 J. Ding and X. L. Zhao, *IOP Conf. Ser. Earth Environ. Sci.*, 2018, **188**, 012021.
147 M. Yahya, C. Herrmann, S. Ismaili, C. Jost, I. Truppel and A. Ghorbal, *Biomass Bioenergy*, 2022, **161**, 106449.
148 B. Laurent, H. Serge, M. Julien, H. Christopher and T. Philippe, *Energy Procedia*, 2012, **29**, 34–41.
149 J. Ding and X. L. Zhao, *IOP Conf. Ser. Earth Environ. Sci.*, 2018, **188**, 012021.
150 A. P. Bernal, C. A. de Menezes and E. L. Silva, *Int. J. Hydrogen Energy*, 2021, **46**, 12758–12770.
151 L. Sillero, R. Solera and M. Pérez, *Biomass Bioenergy*, 2022, **167**, 106643.
152 N. Qi, X. Zhao, X. Hu, J. Wang and C. Yang, *Renew. Energy*, 2023, **219**, 119492.
153 S. Rafaqat, M. Khalid and N. Ali, *Chem. Eng. J.*, 2025, **519**, 165156.
154 G. Yang and J. Wang, *Int. J. Hydrogen Energy*, 2019, **44**, 25542–25550.
155 P. Srivastava, E. García-Quismondo, J. Palma and C. González-Fernández, *Int. J. Hydrogen Energy*, 2024, **52**, 223–239.
156 J. Yin, K. Wang, Y. Yang, D. Shen, M. Wang and H. Mo, *Bioresour. Technol.*, 2014, **171**, 323–329.
157 N. A. ElNaker, A. F. Yousef and S. W. Hasan, *Microbiologyopen*, 2018, **7**, e00590.
158 X.-S. Shi, J.-J. Dong, J.-H. Yu, H. Yin, S.-M. Hu, S.-X. Huang and X.-Z. Yuan, *Biomed Res Int.*, 2017, **2017**, 1–6.
159 P. Sukphun, S. Sittijunda and A. Reungsang, *Fermentation*, 2021, **7**, 159.
160 R. M. W. Ferguson, F. Coulon and R. Villa, *Water Res.*, 2016, **100**, 348–356.
161 A. Jalil and Z. Yu, *Sustainability*, 2024, **16**, 10755.
162 S. C. Santos, P. R. F. Rosa, I. K. Sakamoto, M. B. A. Varesche and E. L. Silva, *Bioresour. Technol.*, 2014, **159**, 55–63.
163 D. Yellezuome, X. Zhu, X. Liu, R. Liu, C. Sun, M. H. Abd-Alla and A.-H. M. Rasmey, *Chem. Eng. J.*, 2024, **480**, 148055.
164 V. Hovorukha, O. Tashyrev, O. Havryliuk and L. Iastremska, *Open Agric. J.*, 2020, **14**, 174–186.
165 G. Yang, Y. Luo, Y. Bian, X. Chen, L. Chen and X. Huang, *Water Res.*, 2025, **268**, 122585.
166 K.-Y. Kim, in *Microbial Electrolysis Cells for Biohydrogen Production*, Springer Nature Switzerland, Cham, 2025, pp. 139–151.
167 J. Song, D. An, N. Ren, Y. Zhang and Y. Chen, *Bioresour. Technol.*, 2011, **102**, 10875–10880.
168 J. Tang, Y. Bian, S. Jin, D. Sun and Z. J. Ren, *ACS Environ. Au*, 2022, **2**, 20–29.
169 C. Zhang, X. Zeng, X. Xu, W. Nie, B. K. Dubey and W. Ding, *Chemosphere*, 2024, **355**, 141764.
170 T. Zhang, Y. Chen, Y. Li, P. Chen, H. Ma, P. Han, C. Wang, W. Liu, Y. Wang, R. Qing and F. Xu, *Fuel*, 2024, **357**, 129648.
171 F. C. Walsh, L. F. Arenas, C. Ponce de León, G. W. Reade, I. Whyte and B. G. Mellor, *Electrochim. Acta*, 2016, **215**, 566–591.
172 X. Zheng, S. Hou, C. Amanze, Z. Zeng and W. Zeng, *Bioprocess Biosyst. Eng.*, 2022, **45**, 877–890.
173 M. Mashkour, M. Rahimnejad, F. Raouf and N. Navidjouy, *Biofuel Res. J.*, 2021, **8**, 1400–1416.
174 G. Lei, Y. Wang, G. Xiao and H. Su, *Catalysts*, 2025, **15**, 439.
175 M. B. Bahari, C. R. Mamat, A. A. Jalil, N. S. Hassan, M. H. Sawal, S. Rajendran and M. N. H. Z. Alam, *Process Saf. Environ. Prot.*, 2024, **191**, 1633–1647.
176 J. Li, G. Liu, D. Chen, C. Li, D. Liang, F. Wang, J. Wu, W. He, Y. Yu and Y. Feng, *ACS ES&T Eng.*, 2022, **2**, 263–270.

177 F. Ndayisenga, Z. Yu, B. Wang and D. Zhou, *Chem. Eng. J.*, 2023, 144002.
178 J. Li, Y. Qiu, D. Li, J. Wu, Y. Tian, G. Liu and Y. Feng, *Chem. Eng. J.*, 2023, **464**, 142736.
179 Y. Lu, X. He, H. Li, H. Chen, L. Li, J. Zhu, K. Chen, Z. Ding, S. Sun and S. Cheng, *Chem. Eng. J.*, 2025, **518**, 164514.
180 Y. Li, Y. Zong, C. Feng and K. Zhao, *Microorganisms*, 2025, **13**, 631.
181 C. Bi, Q. Wen, Y. Chen and H. Xu, *J. Appl. Electrochem.*, 2024, **54**, 2729–2743.
182 Y. Qiu, Y. Feng, Z. Yan, J. Li, D. Li, C. Yan and G. Liu, *Sci. Total Environ.*, 2023, **865**, 161289.
183 Z.-T. Zhao, J. Ding, B.-Y. Wang, M.-Y. Bao, B.-F. Liu, J.-W. Pang, N.-Q. Ren and S.-S. Yang, *Chem. Eng. J.*, 2024, **481**, 148444.
184 Y. Zhou, X. Zhang, Y. Wang and H. Liu, *Fermentation*, 2024, **10**, 179.
185 E. Elbeshbishy, H. Hafez and G. Nakhla, *Int. J. Hydrogen Energy*, 2012, **37**, 2960–2964.
186 A. Menon, F. Ren, J.-Y. Wang and A. Giannis, *J. Mater. Cycles Waste Manag.*, 2016, **18**, 222–230.
187 S. Sahil, R. Singh, S. K. Masakapalli, N. Pareek, A. A. Kovalev, Y. V. Litti, S. Nanda and V. Vivekanand, *Environ. Chem. Lett.*, 2024, **22**, 1665–1702.
188 Y. Wang, H. Zhang, Y. Feng, B. Li, M. Yu, X. Xu and L. Cai, *Biosens. Bioelectron.*, 2019, **136**, 8–15.
189 S. Li, T. Hua, C. S. Yuan, B. Li, X. Zhu and F. Li, *Bioresour. Technol.*, 2020, **298**, 122501.
190 A. F. M. Braga and P. N. L. Lens, *Biomass Bioenergy*, 2023, **176**, 106902.
191 O. Sarkar, J. Kiran Katari, S. Chatterjee and S. Venkata Mohan, *Fuel*, 2020, **276**, 117794.
192 L. Alibardi and R. Cossu, *Waste Manage.*, 2015, **36**, 147–155.
193 S. Wainaina, M. Parchami, A. Mahboubi, I. S. Horváth and M. J. Taherzadeh, *Bioresour. Technol.*, 2019, **274**, 329–334.
194 V. Redondas, X. Gómez, S. García, C. Pevida, F. Rubiera, A. Morán and J. J. Pis, *Waste Manage.*, 2012, **32**, 60–66.
195 W. A. Shewa, A. Hussain, R. Chandra, J. Lee, S. Saha and H.-S. Lee, *J. Clean. Prod.*, 2020, **261**, 121170.
196 J. Gomez-Romero, A. Gonzalez-Garcia, I. Chairez, L. Torres and E. I. García-Peña, *Int. J. Hydrogen Energy*, 2014, **39**, 12541–12550.
197 D. Shen, K. Wang, J. Yin, T. Chen and X. Yu, *Waste Manage.*, 2016, **51**, 65–71.
198 H. Shahriari, M. Warith, M. Hamoda and K. Kennedy, *J. Environ. Manage.*, 2013, **125**, 74–84.
199 J. Ma, T. H. Duong, M. Smits, W. Verstraete and M. Carballa, *Bioresour. Technol.*, 2011, **102**, 592–599.
200 H. B. Gonzales, K. Takyu, H. Sakashita, Y. Nakano, W. Nishijima and M. Okada, *Chemosphere*, 2005, **58**, 57–63.
201 J. Yin, X. Yu, K. Wang and D. Shen, *Int. J. Hydrogen Energy*, 2016, **41**, 21713–21720.
202 J. Yin, X. Yu, Y. Zhang, D. Shen, M. Wang, Y. Long and T. Chen, *Bioresour. Technol.*, 2016, **216**, 996–1003.
203 Y. Yin, Y.-J. Liu, S.-J. Meng, E. U. Kiran and Y. Liu, *Appl. Energy*, 2016, **179**, 1131–1137.
204 S. Sawayama, S. Inoue, T. Minowa, K. Tsukahara and T. Ogi, *J. Ferment. Bioeng.*, 1997, **83**, 451–455.
205 H. Karne, U. Mahajan, U. Ketkar, A. Kohade, P. Khadilkar and A. Mishra, *Mater. Today Proc.*, 2023, **72**, 775–786.
206 J. Sun, L. Zhang and K.-C. Loh, *Bioresour. Bioprocess.*, 2021, **8**, 68.
207 A. Naresh Kumar, A. K. Bandarapu and S. Venkata Mohan, *Chem. Eng. J.*, 2019, **374**, 1264–1274.
208 R. Slezak, J. Grzelak, L. Krzystek and S. Ledakowicz, *Environ. Technol.*, 2021, **42**, 4269–4278.
209 K. Xiao, Y. Zhou, C. Guo, Y. Maspolim and W. J. Ng, *J. Environ. Sci.*, 2016, **42**, 196–201.
210 A. Detman, D. Laubitz, A. Chojnacka, E. Wiktorowska-Sowa, J. Piotrowski, A. Salamon, W. Kaźmierczak, M. K. Błaszczyk, A. Barberan, Y. Chen, E. Łupikasza, F. Yang and A. Sikora, *Front. Microbiol.*, 2021, **11**, 612344.
211 Y.-B. Sim, J. Yang, S. M. Kim, H.-H. Joo, J.-H. Jung, D.-H. Kim and S.-H. Kim, *Bioresour. Technol.*, 2022, **366**, 128181.
212 I. Valdez-Vazquez, M. T. Ponce-Noyola and H. M. Poggi-Varaldo, *Int. J. Hydrogen Energy*, 2009, **34**, 4291–4295.
213 J. A. Magdalena, M. F. Pérez-Bernal, N. Bernet and E. Trably, *Bioresour. Technol.*, 2023, **374**, 128803.
214 G. Duman, K. Akarsu, A. Yilmazer, T. Keskin Gundogdu, N. Azbar and J. Yanik, *Int. J. Hydrogen Energy*, 2018, **43**, 10595–10604.
215 P. Mishra, S. Krishnan, S. Rana, L. Singh, M. Sakinah and Z. Ab Wahid, *Energy Strategy Rev.*, 2019, **24**, 27–37.
216 R. F. Susanti, L. W. Dianningrum, T. Yum, Y. Kim, B. G. Lee and J. Kim, *Int. J. Hydrogen Energy*, 2012, **37**, 11677–11690.
217 S. Liu, G. Ding, R. Gu, J. Hao, P. Liu, W. Qin, Y. Yu, Y. Han, J. Huang and W. He, *Resour. Conserv. Recycl.*, 2025, **212**, 107931.
218 A. Ghaderikia and Y. D. Yilmazel, *ACS Sustain. Chem. Eng.*, 2024, **12**, 1437–1445.
219 O. Khan, M. Z. Khan, I. Habib, M. Parvez, A. Alhodaib, Z. Yahya and M. Tripathi, *Int. J. Hydrogen Energy*, 2025, **137**, 1223–1234.
220 W. Hu, S. Zheng, J. Wang, X. Lu, Y. Han, J. Wang and G. Zhen, *Chemosphere*, 2024, **358**, 142119.