Recent progress on rational design of catalysts for fermentative hydrogen production

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Abstract
The increasingly severe energy crisis has strengthened the determination to develop environmentally friendly energy. And hydrogen has emerged as a candidate for clean energy. Among many hydrogen generation methods, biohydrogen stands out due to its environmental sustainability, simple operating environment, and cost advantages. This review focuses on the rational design of catalysts for fermentative hydrogen production. The principles of microbial dark fermentation and photo-fermentation are elucidated exhaustively. Various strategies to increase the efficiency of fermentative hydrogen production are summarized, and some recent representative works from microbial dark fermentation and photo-fermentation are described. Meanwhile, perspectives and discussions on the rational design of catalysts for fermentative hydrogen production are provided.

KEYWORDS
Catalysts, fermentation, hydrogen production, renewable energy

1 | INTRODUCTION

With the continuous expansion of social industrial development, the demand for fossil energy is also increasing. In addition to the positive significance for development, it also brings a series of negative effects, such as pollution to the environment and the resulting energy crisis. To deal with the above problems, nearly all countries have formulated policies to develop clean and renewable energy. Among them, hydrogen (H₂) has received...
TABLE 1 Comparison of parameters of different hydrogen production methods

| Hydrogen production method | Technology maturity | Energy conversion efficiency | Current market share | Economic cost ($/kg) | Reference |
|---------------------------|---------------------|-----------------------------|----------------------|---------------------|-----------|
| reforming of fossil fuels | Commercialized      | 60%–85%                     | 95%                  | 1.3–2.27            | 31,45,46  |
| Electrolysis of water     | Mature              | 40%–60%                     | 4%                   | 6.35–12.6           | 31,45,47  |
| Biological hydrogen production | Immature        | 10%–80%                     | <1%                  | 4.15–7.00           | 48–50     |

extensive attention and is considered to be a strong candidate for clean energy due to its environmental friendliness and practicality. 19–28

Currently, the main commercial hydrogen production methods include fossil fuels reforming and water electrolysis, accounting for 95% and 4% of the market, respectively. 29–31 Hydrogen production by reforming fossil fuels is the most proven hydrogen production method with the highest conversion rate and the lowest economic cost. 12,32 The specific parameters compared with other methods are shown in Table 1. However, new hydrogen production methods will be introduced with the depletion of fossil fuels and degradation of the environment. Eco-friendly hydrogen production methods have been widely concerned by scientists, including hydrogen production by electrolysis, photolysis of water, and biological hydrogen production. 33–41 Water electrolysis is an effective strategy, and it usually depends on a sufficient electricity supply and high-effective electrocatalyst. 42 However, the current recyclable electricity resources can not fully meet the needs for water electrolysis; thereby it is hard to reduce the cost. 31 At the same time, expensive electrode materials and reactors also increase their cost. Moreover, the development and the cost of high-efficiency catalysts also make the industrial application of this method facing certain challenges. 43 Although the photolysis of water avoids dependence on electricity resources to a certain extent, the actual reaction efficiency will be affected by the actual natural environment and catalysts, and currently the efficiency of this method is still relatively low, so large-scale use may not be universal. 44

Unlike the above methods, biological hydrogen production, as a pollution-free by-product method, could use various raw materials and natural bacteria under the environmental conditions of temperature and pressure. 51–53 At the same time, it also has relatively high energy conversion efficiency and low cost. After receiving attention, biological hydrogen production triggered a scientific research boom and ushered in a golden age of this field. Biological hydrogen production includes a variety of pathways involving biology, such as biological photolysis (direct and indirect), fermentation (such as dark fermentation and photo-fermentation) and microbial electrolysis, etc. 54 Different biological hydrogen production pathways depend on different operating conditions, for instance, substrate concentration, pH, and temperature. The optimal reaction conditions help maximize hydrogen production. Biophotolysis refers to the use of energy directly or indirectly generated from sunlight to generate hydrogen, so the reaction is environmentally friendly and easy to operate. As for microbial electrolysis, a small amount of electricity is required, but this is far less than the electricity demand for electrolysis of water. 54 The different microbial fermentation pathways are mainly the difference between bacteria and substrates. A variety of bacteria will produce hydrogen in normal physiological activities under light or dark conditions, respectively. To further stimulate the enzymatic activity of bacteria, the introduction of nanocatalysts is crucial. Nanocatalysts such as noble metals and transition metals are commonly used, and their introduction can make up for the deficiencies of some bacteria, thereby improving the efficiency of hydrogen production. Therefore, rational catalyst design has also aroused the interest of scientists, and we will also focus on the related work progress. As shown in Figure 1, rational catalysts should be selected and designed according to the required functions.

This review briefly summarizes the most instructive advances in the rational design of catalysts for fermentation hydrogen production. We elaborate on related work from several aspects. At first, the principles of microbial dark fermentation and photo-fermentation are elucidated.

FIGURE 1 Selection and design principle of catalysts

- Co-factors of enzymes
- Enhanced expression of the protein
- Enhance electron transfer
- Regulate metabolic pathways
- Enhance bacterial activity
- Enhance light utilization range and efficiency
- Produce less photogenerated free radicals
- Promote synthesis of photosynthetic pigments
Then, design principles of high-efficiency hydrogen production catalyst by fermentation are introduced and some recent representative works on microbial dark fermentation and photo-fermentation are summarized. Specially, different types of catalysts are classified here, including noble metal-based materials, no-noble metal-based materials, bio-derived materials, semiconductor nanomaterials, etc. Finally, the current challenge and future trends are also elaborated to inspire more efforts in this field.

2 DARK FERMENTATIVE HYDROGEN PRODUCTION

Dark fermentative hydrogen production (DFHP), is the process in which facultative and obligate anaerobic bacteria act on organic matter in the absence of oxygen and light to produce hydrogen through a series of reactions. The main bacteria in the DFHP process are Enterobacter and Clostridium, and carbohydrates are the preferred carbon source for them, so they can use substrates like dairy wastewater and kitchen waste lignocellulosic biomass. For example, acetate fermentation and Butyrate fermentation are two common hydrogen production pathways. Their specific pathways are shown in Figure 2. Herein, H₂ is formed by disposing of excess electrons. Under anaerobic conditions, protons (H⁺) act as the electron acceptor, thus producing H₂ by neutralizing the electrons produced by the oxidation of organic substrates. Whereas in aerobic respiration, oxygen is the electron acceptor and the end product is water. If it only produces acetate, the reaction can get 4 mol H₂/mol glucose, but if butyrate is the only final product, such a reaction can only get 2 mol H₂/mol glucose. The reaction process can be expressed by the following Equations (1) and (2),

Acetate fermentation

\[ C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 4H_2 + 2CO_2 \]  

Butyrate fermentation

\[ C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2CH_2COOH + 2H_2 + 2CO_2 \]  

The hydrogen production capacity of acidogenic bacteria is primarily used to degraded organic compounds into alcohols and volatile fatty acids (VFAs). Different microorganisms, hydrogenase activity, excessive substrate, trace elements, metal ions, temperature, pH, and toxic substances can inhibit the metabolism of fermentation microorganisms and thus affect hydrogen production in these systems. The traditional methods to improve hydrogen yield include pretreatment of the substrate, redesign/retrofitting of bioreactors, optimization of biological process parameters, etc. In recent years, more and more new technologies have been used to increase biohydrogen production by fermentation. Among these strategies, the utilization of nanomaterials has become attractive in improving biological processes due to their excellent physical and chemical properties like high specific surface area and catalytic activity. The application of nanomaterials in dark fermentative biohydrogen production from waste can positively accelerate the production rate and yield of the reaction even with a very low concentration. Some hydrogen-producing microorganisms can use nanoparticles, especially in anaerobic environments, to transfer electrons to receptors more efficiently, improving the dynamics of biological processes and enhancing microbial activity as biocatalysts through this ability to react rapidly with electron donors. The effect of nanoparticles on hydrogen production by dark fermentation is currently an active research field.

Up to now, various strategies have been used to enhance the efficiency of DFHP. At first, different catalysts are designed with different compounds and properties, like noble metal materials, non-noble metal materials, and bio-derived materials, etc. Specific illustrations of their performance in DFHP are described below (Table 2). Additionally, some technical combinations involving nanomaterials such as microbial fuel cells (MFCs) or microbial electrolysis cells (MECs) are also briefly summarized.

In terms of dark fermentation, catalysts are mainly required to have the following effects: enhancing the electron transfer rate during biological hydrogen production, acting as co-factors to enhance hydrogenase activity as well as the formation of certain proteins, and enhancing the
| Substrate                        | Microorganism                  | Nanoparticles                  | The optimal concentration of nanomaterials | Maximum H₂ yield/rate | H₂ yield increased by (%) | Fermentation conditions |
|----------------------------------|--------------------------------|--------------------------------|--------------------------------------------|------------------------|---------------------------|--------------------------|
| Distillery wastewater            | Mixed culture                  | Fe                             | 0.7 g/L                                    | 12.2 ml/h              | 69.4                      | 37 6                     | 71                       |
| Distillery wastewater            | Mixed culture                  | Fe₂O₃                          | 0.7 g/L                                    | 12.7 ml/h              | 71                        | 37 6                     | 71                       |
| Palm oil mill effluent           | Bacillus anthracis PUNAJAN 1   | NiO                            | 1.5 mg/L                                   | 0.563 L/g-COD          | 35.2                      | 37 7                     | 72                       |
| Palm oil mill effluent           | Bacillus anthracis PUNAJAN 1   | CoO                            | 1.0 mg/L                                   | 0.487 L/g-COD          | 22.9                      | 37 7                     | 72                       |
| Industrial wastewater            | Mixed culture                  | α-Fe₂O₃ + NiO + ZnO            | 200 mg/L (α-Fe₂O₃) + 20 mg/L (NiO) + 10 mg/L (ZnO) | 49 mL/g-COD           | 48.5                      | 50 5.5                   | 73                       |
| Distillery Wastewater            | Mixed culture                  | Hematite + NiO                 | 50 mg/L (Hematite) + 10 mg/L (NiO)         | 17.2 mmol/g COD        | 27.1                      | 37 5.5                   | 74                       |
| Distillery Wastewater            | Mixed culture                  | Hematite + NiO                 | 200 mg/L (Hematite) + 5 mg/L (NiO)         | 7.85 mmol/g COD        | 62                        | 37 5.5                   | 74                       |
| Rice straw hydrolysate           | Klebsiella sp. WL1316          | Ec-NiO                         | 20 mg/L                                    | 101.45 ± 3.32 mL/g substrate | 37.78                     | 25 8                     | 75                       |
| Glucose                          | Anaerobic sludge               | Fe                             | 0.5 mg/L                                   | 551 mL/g volatile solids | 8                         | 37 5.5                   | 76                       |
| Glucose                          | Mixed culture sludge           | Ptl                            | 5 mg/L                                     | 2.48 mol/mol glucose   | 6.4                       | 37 7                     | 77                       |
| Glucose                          | Clostridium butyricum          | Ag                             | 20 nmol/L                                  | 2.48 mol/mol substrate | 67.6                      | 35 8.5                   | 78                       |
| Sucrose                          | Mixed culture from cracked cereals | Hematite                  | 200 mg/L                                   | 3.57 mol/mol substrate | 32.64                     | 35 8.5                   | 79                       |
| Sucrose                          | Clostridium butyricum          | Au                             | 10 nmol/L                                  | 4.48 mol/mol substrate | 46                        | 35 7.2                   | 80                       |
activity of bacteria. Meanwhile, nanomaterials can transfer intermediate metabolites to higher proportions of acetate and butyrate. When combined with the bioelectric system, electrode materials should be conducive to electron diffusion and provide a sufficient microenvironment to maintain stability and prolong the activity of biocatalysts.

2.1 Application of noble metal-based nanocatalysts

Noble metal materials have been extensively studied in fermentative hydrogen production due to their excellent durability and catalytic performance. For the first time, Zang et al. claimed the excellent effect of adding gold nanoparticles (NPs) to artificial wastewater on hydrogen production by fermentation. Compared with the blank test, the hydrogen production rate can be increased by 61.7% after adding gold nanoparticles with the size of 5nm. They suggest that the quantum size effect of gold nanoparticles leads to a stronger affinity for electrons while metabolism away from alcohol and to VFAs production, which boosts the hydrogen production rate.80 Similarly, Zhao et al. reported the effects of silver nanoparticle concentration (0-200 nmol L–1) on bacterial growth and H2 production utilizing glucose-fed mixed culture dominated by Clostridium butyricum. The addition of silver NPs could effectively change the metabolism from alcohol and reduce acid to the production of VFAs, thus enhancing the hydrogen production efficiency of the mixed culture by improving the kinetics of biological hydrogen production. The maximum hydrogen yield of 2.48 mol/mol glucose was obtained at a silver NPs concentration of 20 nmol L–1.75 Although significantly enhanced hydrogen production efficiency, the high price of precious metal nanoparticles limits its wide application. Therefore, it is also promising to increase hydrogen production methods by combining nonprecious metal materials with bacteria.

2.2 Application of non-noble metal-based nanocatalysts

Hydrogenase plays a significant role in hydrogen production process during the fermentation, and there are [Fe-Fe]-hydrogenase and [Ni-Fe]-hydrogenase according to the type of metal atoms present in the active site.83 The iron and nickel ions are needed as co-factors to express and enhance the activity of these enzymes within the dark fermentation system to promote the rate of DFHP.82-85 Given the above conclusion, iron and nickel are the preferred research subjects, and their concentration in the reaction system will affect the synthesis of enzyme, thereby affecting the catalytic hydrogen production rate.86 Taherdanak et al. added different concentrations of Ni2+ and Fe2+ to the anaerobic reactor with glucose as the substrate to promote enzyme synthesis.76 The results show that the co-factor with a suitable concentration can promote hydrogen production by fermentation. Furthermore, the effect of Fe0 NPs and Ni0 NPs on hydrogen production was also compared in this study, and the effect of the metal supplementation on the biohydrogen yield was shown below: Ni2+ ions > Fe0 NPs > Fe2+ ions > Ni0 NPs.76 The improvement effect of the Ni2+ could be owed a great deal to the interaction of the Ni2+ ions and the [Ni-Fe]-hydrogenases, and the presence of Ni2+ ions significantly reduced the EtOH production. Moreover, the addition of Fe0 NPs promoted the activity of hydrogenase and electron transfer of ferredoxin and inhibited HBu-producing bacteria. In addition, the introduction of Fe2+ inhibited the production of EtOH and HPr, and the latter consumed hydrogen, so the hydrogen production was increased, while Ni0 NPs had little effect on biological hydrogen production.76

Iron oxide nanoparticles were also proved to be satisfactory co-factors in dark fermentation for hydrogen production.71 Malik et al. investigated the effects of two different iron compounds (Fe0 nanoparticles and Fe2O3 nanoparticles) on biohydrogen production by fermentation in molasses-based distillery wastewater. When adding 0.7 g L–1 Fe0 NPs and Fe2O3 NPs, the H2 yield was the best, increasing by 71% and 69.4%, respectively. In addition to enhancing hydrogenase activity, the addition of Fe0 NPs and Fe2O3 NPs accelerates the production of iron-sulfur protein in ferredoxin, thereby catalyzing the electron transfer of H2 produced by hydrogenase, leading to high hydrogen yield.71 Another study proved that in terms of sewage sludge pretreated by ionizing radiation, in addition to being a co-factor of hydrogenase, Fe2+ can also effectively convert H2O2 produced by ionizing radiation into more hydroxyl radical (•OH), improving the effect of sludge decomposition, inhibiting the hydrogen consumption pathway, and finally enhancing the substrate degradation rate and hydrogen production (Figure 3A,B).87
Similarly, Elreedy et al. investigated the effect of utilizing mixed culture bacteria (MCB) combined with single NPs, (α-Fe₂O₃, NiO, and ZnO), dual-NPs, and multi-NPs for hydrogen production from industrial wastewater containing mono-ethylene glycol (as shown in Figure 3C). The mechanism is very similar to the previous study. Other than the improved relative abundance of hydrogen-producing bacteria, the activity of hydrogenase, aldehyde dehydrogenase (ALDH) and alcohol dehydrogenase (ADH) enzymes were also increased. Moreover, hydrogen production was further improved when double or multiple nanoparticles were added to the MCB. These results suggested that adding multiple co-factors was more attractive in hydrogen production. However, the synthesis of the above-mentioned nanoparticles requires toxic or dangerous chemicals, and green synthesis will offer a more environmentally friendly and less toxic approach.

2.3 Application of bio-derived materials

There are abundant polyphenols, flavonoids, organic acids, alkaloids, and other active compounds in natural plants, which could stabilize and promote the interaction between plant extracts and metal ions. Zhang et al. synthesized green NiO NPs from the extract of Eichhornia crassipes (Ec) and evaluated its regulatory effect on hydrogen production by fermentation with Klebsiella WL1316 as inoculum and straw hydrolysate as substrate. Adding Ec-NiO-NP brought about a maximum increase of 623% in hydrogenase activity (Figure 4A–C). And in contrast to the previous research utilizing chemically synthesized Ni₀ and NiO NPs, a higher hydrogen production in this study was obtained. The main reason is that Ec-NiO-NP improves the utilization rate of reducing sugar of Klebsiella WL1316, accelerates the conversion of pyruvate, and promotes the expression of hydrogenase and formic acid-hydrolyase-related genes, ultimately increase hydrogen production.

As shown in Figure 4F, Sinharoy et al. described the synthesis and characterization of iron NPs from green tea extract (GT-INP) and the effect on hydrogen production from anaerobic biomass. The FETEM diagram indicated that most of the NPs are spherical, rough-faced, and exist in a chain aggregation structure (Figure 4D,E). Additionally, the XRD pattern showed that the material contains Fe₃O₄.
and FeO(OH). Then, they used carboxydotrophic bacteria that could use CO as the only carbon source and energy, and CO became a substitute substrate for biological hydrogen production. In the synthesis process, the presence of a great quantity of hydrophobic groups on the surface of the GT-INP enhanced the solubility of CO in water, which was encapsulated by polyphenols, proteins, and amines available in green tea extract, thus the amount of CO in the continuously operated gas lift bioreactor was increased. The maximum H₂ production rate notably increased to 0.0662 mmol/l/h at an optimum GT-INP concentration of 1000 mg L⁻¹. However, dark fermentation cannot completely degrade organic matter, and the removal liquid contains numerous VFAs and other organic matter, which requires other methods to be utilized.

### 2.4 Coupling with microbial fuel cells

To date, the industrial-scale dark fermentation process development has been limited by its low hydrogen yield and estimated costs associated with H₂ production, and incomplete substrate consumption leads to a large number of organic compounds in the effluent of dark fermentation. Therefore, it has been noted that more hydrogen production can be achieved by coupling processes, such as bioelectrochemical systems.

Bioelectrochemical systems (BESs) are novel systems with the ability to convert the chemical energy of organic waste, such as lignocellulosic biomass and low-strength wastewaters into electricity or hydrogen/chemical products in MFCs or MECs, respectively. As shown in Figure 5A, the typical MFC is composed of two electrodes, an anode receiving electrons from a microbial culture and a corresponding cathode transferring electrons to an electron acceptor, usually oxygen, and in the absence of oxygen, protons can act as electron acceptors to produce hydrogen.

Fermentation hydrogen production, currently theoretically at most 4 mol H₂/mol glucose, can bypass this biochemical barrier by producing hydrogen from acetate using a fully anaerobic microbial fuel cell.

In series, Sharma et al. connected hydrogen production bioreactor (HPB) and single-chamber MFC (SCMFC) and studied energy production and wastewater treatment capacity. Note that 71% COD removal rate and 2.05 mol H₂/mol glucose hydrogen production were obtained, and the previously reported organic loading rate of batch mode MFCs is much lower than that in continuous flow SCMFC. It shows that DFHP coupling with MFC can effectively recover organic matter in wastewater. Simultaneous process of DF and MFC (sDFMFC) is a promising strategy because it can ensure the strength of a single process and provide significant cost savings (Figure 5B). The anode chamber exhibited a diverse microbial community, both fermentative bacteria and electrogenic bacteria.
are present, so H₂ and electricity are generated at the same time. Meanwhile, the internal resistance of this hybrid system is lower than that of batch MFC, which is more favorable for electron transfer, and the overall energy recovery greatly exceeds the value reported in the literature. ¹⁰⁵

### 2.5 Coupling with microbial electrolysis cells

Increasing the cathode potential in the MFC circuit electrochemically generates hydrogen directly from the protons and electrons produced by the bacteria. This method dramatically reduces the required energy to produce hydrogen directly from organic substances compared to producing hydrogen through the electrolysis of water. ¹⁰⁷ A microbial electrolysis cell is usually composed of anodes, cathodes, and membranes. The membrane typically separates the anode where the oxidation processes (e.g., acetate oxidation or water oxidation) occur and the cathode where the reduction processes (e.g., O₂ reduction or H₂ release) occur (Figure 6). ⁹⁹–¹⁰¹

Cusick et al. concluded that in the case of the fed winery or domestic wastewater, energy recovery and pollutant degradation from wastewater could be more effective with MFCs than MECs. However, from an economic point of view, hydrogen production from wastewater fed MECs could also be low-budget on account of electrical energy requirements. ¹⁰² MEC studies often use pure compounds (mainly acetate) as substrates, and when utilizing other substrates (such as domestic and animal wastewater), hydrogen production is relatively poor or methane is produced in large quantities. ⁹₃,¹₀³

To study the practical application ability (Figure 7A–C), the feasibility of two-step hydrogen production of various wastewaters and industrial by-products was evaluated by combining the dark fermentation and microbial electrolysis, that is, the six kinds of wastewater were first fermented to produce hydrogen, and then MEC was used to degrade the metabolites to continue to produce hydrogen. The overall hydrogen production increased up to 13 times compared to fermentation alone, and approximately 9.82 mol H₂/mol glucose was obtained from juice wastewater, which is one of the best results compared to the previous studies. The final hydrogen yields are extraordinarily close to the theoretical maximum of 12 mol H₂/mol glucose. The results show that the combination of dark fermentation and microbial electrolysis can maximize the conversion of agricultural and industrial wastewater and by-products into biohydrogen, which is a promising option in practical
Li et al. developed a nitrogen-based core-shell catalyst named N-Fe/Fe$_3$C@C with iron-based composite (Fe/Fe$_3$C) nanorods as the core and graphite carbon as the shell. They successfully modified the cathode with N-Fe/Fe$_3$C@C, and the effect on fermentation process of the catalyst was studied (Figure 7D–G). The LSV curve showed that the modified cathode exhibited a higher cathode current than the CNT modified and unmodified cathode, and correspondingly, the rate of hydrogen production was the best of the three. In the end, they found that the rate and efficiency of hydrogen production were slightly lower than Pt/C cathode, but the cost was less than 5% of that, indicating good commercial application progress.
In addition, many recent studies have shown that nanostructured materials are ideal and attractive for biocatalytic systems, because these materials are more conducive to the diffusion of electrons in the electrodes, providing enough microenvironment to maintain the stability and prolong the activity of the biocatalysts.\textsuperscript{94,106,107} Although great breakthroughs have been made, few studies on combining these technologies with fermentative hydrogen production, which worth further study.

3 | PHOTO-FERMENTATIVE BIOHYDROGEN PRODUCTION

Photo-fermentation hydrogen production is the process in which organic substrates are converted into biohydrogen under light conditions, manifested as a group of different photosynthetic bacteria by a series of biochemical reactions. After the photo-fermentation hydrogen-producing bacteria capture the photon to the photosynthetic unit, and the energy is sent to the photosynthetic reaction center for charge separation, producing high-energy electrons, and causing a proton gradient, thus synthesizing ATP. Photo-fermentation bacteria feed on organic matter and use the energy molecule ATP under anaerobic conditions to break it down into simpler compounds of CO\textsubscript{2} and H\textsubscript{2}, through the action of nitrogenase. The overall process can be expressed as Equation (3).\textsuperscript{53,57}

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 12\text{H}_2\text{O} + \text{light energy} \rightarrow 12\text{H}_2 + 6\text{CO}_2 \quad (3)
\]

The metabolic pathway of hydrogen production by photo-fermentation bacteria using humic acid is shown in Figure 8. Photo-fermentation is generally more promising than dark fermentation because it achieves higher theoretical substrate conversion efficiency and relatively less by-production, wide spectrum utilization, and can utilize organic wastes that are not available to dark fermentation.\textsuperscript{55,109,110} Typically, photosynthetic purple nonsulfur bacteria (PNS) can convert carbon sources (e.g., sugars, organic acids) to H\textsubscript{2} in anoxic conditions, while dark fermentation bacteria cannot utilize volatile fatty acids such as acetic acid and butyric acid.\textsuperscript{111} Therefore, organic acids and VFAs produced during dark fermentation reactions can be used as natural substrates for photo-fermentation bacteria.

In terms of photo-fermentative hydrogen production (PFHP), in addition to the requirements similar to dark fermentation, catalysts are preferred to have these activities: promote the synthesis of photosynthetic pigments, enhance the light utilization range and efficiency of bacteria without generating too many photogenerated free radicals to harm bacterial activity. Table 3 below lists various parameters for the study of nanomaterials in photo-fermentative hydrogen production.

3.1 | Application of transition metal compounds

Several micronutrients, for instance, Fe, Mo, Co, and Ni, are indispensable minerals for cell growth and co-factors
## Previous study on photo-fermentative hydrogen production using nanomaterials

| Substrate                        | Microorganism                          | Nanoparticles                  | The optimal concentration of nanomaterials | Maximum H₂ yield/rate | H₂ yield increased by (%) | Fermentation conditions       | Reference |
|----------------------------------|----------------------------------------|--------------------------------|------------------------------------------|-----------------------|--------------------------|-----------------------------|-----------|
| Dark fermentation effluents      | Mixed culture                          | Fe₃O₄                          | 100 mg/L                                 | 12.37 ml/h            | 64.4                     | 30                          | 5.18      | 3000 lx   | 112       |
| Corn straw                       | Mixed culture                          | TiO₂                           | 300 mg/L                                 | 20.1 ml/h             | 27.9                     | 30                          | 7         | 6500 lx   | 113       |
| Corn stover                      | Mixed culture HAU-M1                   | SnO₂                           | 200 mg/L                                 | 147 ml/h              | 25.64                    | 30                          | 6.5       | 192 W/m²  | 114       |
| Brewery and restaurant effluent  | Rhodobacter sphaeroides DSM 158        | Fe + Mo                        | 70 µmol/L (Fe) + 14 µmol/L (Mo)          | 160 ml/h              | 93                       | 30                          | 7         | 126 W/m²  | 115       |
| Sugar hydrolyzate                | Rhodobacter sp. and Bacillus subtilis PF_1 | NiCo₂O₄                      | 100 mg/L                                 | 2978 ml/L             | –                        | 37                          | 7         | 3500 lx   | 116       |
| Dark fermentation liquid of activated sludge | Rhodopseudomonas palustris | TiO₂                          | 100 mg/L                                 | 1.01 mmol/g dried sludge | 46.1                     | 30                          | 8         | 200 W/m²  | 117       |
| Acetate                          | R. palustris strain CGA009              | Silica-core gold-shell NPs     | 10 mg/L                                  | 2.31 mmol/mmol acetate | 115                      | 30                          | 7         | 20 W/m²   | 118       |
| Sodium acetate                   | Rhodopseudomonas sp. nov. strain A7    | TiO₂                           | 300 mg/L                                 | 2.81 mol/mol acetate  | 8.08                     | 35                          | 7         | 150 W/m²  | 119       |
| Sodium acetate                   | Rhodopseudomonas sp. nov. strain A7    | SiC                            | 200 mg/L                                 | 2.99 mol/mol acetate  | 18.6                     | 35                          | 7         | 150 W/m²  | 119       |
| Malate                           | Mixed culture                          | Fe(SO₄)OH(H₂O)₃                | 300 mg/L                                 | 3.106 mol/mol malate  | 20                       | 30                          | 5.5       | 1800 lx   | 120       |
| Food wastes                       | Bioaugmentation of purple nonsulfur (PNS) bacteria | g-C₃N₄ nanosheets              | 16.5 mg/L                                 | 64.2 mol/mol sugar    | 435                      | 30                          | 7         | 3600 lm   | 121       |
of several enzymes in biological hydrogen metabolism of photosynthetic bacteria.\textsuperscript{50,122} Li et al. studied the effects L-cysteine and nano-Fe\textsubscript{3}O\textsubscript{4} NPs on the hydrogen production of dark fermentation effluents during the process of photo-fermentation hydrogen production by photosynthetic strain HAU-M1 (Figure 9).\textsuperscript{112} Fe is a significant component of nitrogenase, and L-cysteine residue and Fe are both essential components of Fe-protein, which act as the electron donor in biological nitrogen fixation.\textsuperscript{97} The addition of L-cysteine not only promotes bacterial aggregation and energy transfer of the substrate to H\textsubscript{2}, but also acts as a reducing agent in a favorable fermentation environment. Therefore, their addition promotes the nitrogenase activity without weakening flocculation and reduce electron transfer to soluble microbial products (SMPs), thus increasing hydrogen production. The lower organic compounds that remained in the fermentation effluents were beneficial to decrease biological wastewater treatment costs.

Furthermore, for the hybrid dark and photo-fermentative hydrogen production, the sugar hydrolyzate was directly utilized as a substrate, mixed strains of \textit{Bacillus subtilis} PF_1 and \textit{Rhodobacter} sp. were used for DF and PF, respectively, the synthesis of NiCo\textsubscript{2}O\textsubscript{4} NPs was carried out by fungus \textit{Emericella variecolor} NS3 through in-vitro way.\textsuperscript{116} As a co-factor, NiCo\textsubscript{2}O\textsubscript{4} NPs enhanced hydrogenase and nitrogenase activities, reducing the hindrance of ethanol production to hydrogen production during the light-dark reaction process. Moreover, the hybrid fermentation produces 2978 ml L\textsuperscript{-1} cumulative H\textsubscript{2} after 336 h, which indicated that the product under the mixed fermentation system had long sustainability and good productivity.

### 3.2 Application of semiconductor nanomaterials

In the PFHP process, the significant factors affecting the conversion efficiency of light energy are light saturation and utilization range. The photocatalytic nanomaterials with large specific surface area and bandgap can be added to the hydrogen production system to broaden the light utilization range of hydrogen-producing bacteria. The combination of photo-fermentation and photocatalysis indicated an obvious improvement in photohydrogen yield, meanwhile a novel approach to break through the limitation of the biochemical routes in molecular H\textsubscript{2} production and bacterial growth was provided, thus improving hydrogen production efficiency.\textsuperscript{110,123}

As early as the 1980s, Nikandrov et al. found that TiO\textsubscript{2} particles could promote the efficiency of photo-fermentative biohydrogen production.\textsuperscript{124} Recently, another study showed the relationship between the addition of TiO\textsubscript{2} nanoparticles and the photo-fermentation hydrogen production from corn straw (Figure 10A–C). Under the optimal conditions of photo-fermentation, the maximum cumulative hydrogen content of corn stalk could reach 688.8 ml at an optimum nano-TiO\textsubscript{2} concentration of 300 mg L\textsuperscript{-1}. During the process of PFHP, the photoinduced electrons generated by nano-TiO\textsubscript{2} under the excitation of light energy, can be effectively transferred to the enzyme system of photosynthetic bacteria to promote the reduction reaction between electrons and hydrogen ions provided by organic compounds, thus accelerating hydrogen production. And the addition of nano-TiO\textsubscript{2} significantly promoted photosynthetic microorganisms to metabolize butyric acid and acetic acid in the process of
PFHP, which could notably avoid the adverse impact of acidified environment on hydrogen production due to the excessive accumulation of intermediate by-products.\textsuperscript{113} Another similar study showed that nano-TiO\textsubscript{2} significantly enhanced the photolysis of proteins and polysaccharides to small molecular compounds except for CO\textsubscript{2}, and was more easily utilized by photo-fermentation bacteria.\textsuperscript{117}

To screen for more effective semiconductor nanoparticles, Liu et al. investigated hydrogen production by photo-fermentative bacteria with the addition of TiO\textsubscript{2}, ZnO,
and SiC NPs in batch culture. The result demonstrated that these three NPs could promote the hydrogen production performance of *Rhodopseudomonas sp.* nov. strain A7 under respective optimal conditions. Among them, SiC NPs exhibited the greatest potential to enhance photo-hydrogen. Furthermore, the preparation temperature of nanomaterials was also the key factor in determining their properties. When the synthesis temperature was 1500°C, SiC NPs had excellent properties and could significantly promote the growth of bacterial cells. Compared with alone strain A7, the hydrogen production effect increased by 18.6% with the addition of SiC NPs.

Graphitic carbonitrides have attracted extensive attention in the fields of solar energy conversion and environmental remediation on account of their attractive electronic band structures, high physicochemical stability, and “earth-abundant” properties, thus becoming a new research hotspot. Attia et al. used laser photoactivated g-C₃N₄ NPs and Ni NPs in rich light media around photosynthetic hydrogen-producing bacteria to promote bacterial photo-fmentation to produce hydrogen, which stimulated bacterial activity during anaerobic digestion of substrates (food waste), thereby enhancing the tolerance of bacteria to unstable light irradiation. Compared with micronutrients, photactivated Ni NPs are co-factors and can be more efficiently absorbed by hydrogen production bacteria, while g-C₃N₄ nanosheets have a large specific surface area (158 m² g⁻¹) and a narrow bandgap (2.7 eV).

These characteristics enable them to absorb a large amount of light, resulting in a rich light-medium around the photo-fermentation bacteria. Ultimately, this explains the positive effect of g-C₃N₄ nanosheets over Ni NPs in stimulating photo-fermentation bacteria (Figure 10D,E). And H₂ production increased rate by up to 5.4 times (3.3 ml/l/h) and 3.4 times (2.1 ml/l/h) compared with the blank control group (0.61 ml/l/h), respectively.

Ji et al. studied the effect of silica-core gold-shell NPs on hydrogen production of organic acid photo-fermentation over enhanced near-infrared illumination. The purpose of adding NPs was to capture light in the culture medium by scattering, and to activate local surface plasmons to enhance the near field of electromagnetic field with photons of resonant wavelengths, thus providing more light energy for biological hydrogen production of CGA009 cells. A 115% increase in hydrogen production was obtained in this work at suitable concentrations (10 mg L⁻¹). Defect engineering on photocatalytic materials has proved to be a promising and attractive method to remarkably improve the performance in energy-related applications. This technology has also been applied to photo-fermentation hydrogen production. Nadeem et al. studied the controlled defect engineering in SnO₂ NPs. In the reduction environment, the maximum redshift of the material energy band is 0.56 eV, which is more conducive to the capture of visible light. Meanwhile, the change of oxidation-reduction potential (ORP) value is...
related to the generation of more surface defects of oxygen vacancies (OVs), which increases the concentration of free electrons in the fermentation medium, and the activity of ferredoxin-oxidoreductase is also increased, thus increasing the total hydrogen yield. Eventually, accumulative \( H_2 \) of 345 ml and a hydrogen production rate of 147 ml h\(^{-1}\) were obtained for NPs annealed in reducing gas at a concentration of 200 mg⋅L\(^{-1}\), which is 23.66% and 25.64% increase as a comparison to the control group.

The application of these novel materials can improve the effect of photo-fermentation on hydrogen production, but they are still at the experimental stage. In order to produce biohydrogen stably and continuously, its operation in large reactors deserves further study.

4 | SUMMARY AND OUTLOOK

The applications of nanomaterials and the bioelectrochemical systems have a significant improvement on hydrogen production by fermentation. Compared with the traditional industrial hydrogen production methods, fermentative hydrogen production is safer and can reduce costs by using organic waste. These summaries of this review will be beneficial to beginners in this field, and we also hope that this field will receive further development and attention to realize practical applications.

Finally, in order to achieve breakthroughs in the utilization of new energy, the current scientific barriers to biological hydrogen production need to be solved. Here are our suggestions for future research:

1. Carbon-based nanomaterials have attracted interest because of their low cost, high stability and good biocompatibility. A few cases show that they can significantly improve the hydrogen production performance. Therefore, the development of heteroatom-doped carbon-based materials may simultaneously enhance hydrogenase activity and electron transfer rate in the reaction process, and increase the hydrogen production rate.

2. At present, geometric configuration needs attention in the research of nanomaterials in fermentative hydrogen production and the same nanomaterials with different geometric structures, such as cubes, spheres, and rods, are also worth studying in biological hydrogen production.

3. The actual hydrogen production is often lower than the theoretical value due to the demand for microbial growth. Therefore, some methods can help increase hydrogen production rates, such as single-bacteria surface modification. The nanocatalytic materials with good biocompatibility like graphitic carbon nitride, could be uniformly coated on the surface of bacteria to limit their proliferation, allowing them to focus more on hydrogen production rather than microbial growth.

4. Up to now, the theoretical simulation of fermentative hydrogen production has not been implemented. However, it is instructive for future catalysts design and worth exploring.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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