Review Article

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Nanotechnology approaches for Enhancement in Biohydrogen Production

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ABSTRACT

The rapid surge of renewable energy sources has been influenced by the high rate of energy consumption and the low sustainability of traditional energy sources. Being an excellent energy source, hydrogen does not leave any negative carbon footprint as it only produces water during the combustion process. It is carbon neutral which can be produced from a variety of waste feed stocks or biomass, making it the most efficient and environmentally friendly form of energy amid all biofuels. To meet the future hydrogen demand, biological processes like bacterial fermentation, are considered to be environmentally favourable option. Since biomass is abundant, cheap, and biodegradable, it is considered profitable for biohydrogen production. Though photo-biological and dark fermentation methods are regarded as successful in generating biohydrogen, their lower yields pose significant challenges for its commercial production. Studies are being conducted to improve efficiency, and here is where nanomaterials come into play by influencing biological processes at the cellular level. They can act as catalysts speeding up the reactions that create hydrogen and making the process more sustainable. Owing to their distinct properties such as stability, crystalline nature, high ratio of surface to volume, adsorption ability, and increased electroconductivity significantly enhance hydrogen generation. In this paper, the applications of nanomaterials such as metals, metal alloys, metal oxides, nanocomposites, and inorganic nanoparticles to improve biohydrogen production have been reviewed.

Keywords: Biohydrogen; Production; Nanotechnology; Nanoparticles; Fermentation.

1. INTRODUCTION

The goal of the recommended net-zero targets for climate change is to find low-cost, carbon-free alternative energy sources that can power a country's energy security and autonomy without posing any pollution (Karthikeyan *et al.* 2024). Since fossil fuels produce pollution that contributes towards the exhaustion of fossil fuel reserves, depending exclusively on fossil fuels to power modern economies is unsustainable and detrimental to the environment. Renewable energy is a possible solution to numerous issues associated with fossil fuels. Akia *et al.* (2014) and Sekoai *et al.* (2019) cited biofuels as economical, simple to produce, eco-friendly, emission-free, effective, and ecologically beneficial renewable energy sources. According to Ladole *et al.* (2017), some examples of liquid and gaseous biofuels are bioethanol, bio-butanol, bio-diesel, bio-oil, biogas, bio-methane, bioethane, bio-butane and biohydrogen. Because of its several advantages, biohydrogen has drawn great interest over other biofuels. These include the production of water vapour without pollution, having a high energy content of 120 kJ/g, utilizing diverse feedstock and bacteria from varied natural environments, and ease of production on a large scale at ambient temperature and pressure.

An attractive alternative to fossil fuels is hydrogen, which has a high energy density and produces no greenhouse gases during combustion. When it comes to power balance, fermentation-based hydrogen synthesis from cellulosic materials is an inexpensive, ecologically safe method that offers a reliable path for exploiting vast quantities of underutilized biomass. It is a clean fuel because, when it burns, the only byproduct it creates is water, unlike fossil fuels. Only the utilization of engines and energy that use hydrogen as a fuel, which significantly reduces fuel gas emissions, can really "greenify" automobiles. The formation of hydrogen through biological channels is carbon neutral and may have advantages over other processes such as thermochemical and electrochemical ones, as it may use a wide range of biologically flexible renewables to function at ambient pressure and temperature while requiring less energy input (Karthikeyan *et al.* 2024).

Agriculture, water purification, drug administration, food, cancer therapy, and energy are among the rapidly developing fields that use nanotechnology. In particular, iron and nickel nanomaterials play an important role as cofactors in

enzymes that produce biohydrogen. Stability, adsorption capacity, high ratio of surface to volume, catalytic properties, enhanced electroconductivity, and crystalline nature are among the distinctive characteristics of nanomaterials. Furthermore, adding nanomaterials to the lignocellulosic biomass during the pre-treatment stage could improve lignin removal, which would enhance the sugar yield and accelerate the overall process. The enzyme cellulase catalyzes the process of enzyme hydrolysis, or the transformation of cellulose into glucose (sugar). Its pH and thermal stability are influenced by nanomaterials, which further creates an anaerobic environment that allows the hydrogenase enzyme to work, increasing the amount of hydrogen produced (Salame *et al.* 2018)

Beyond the benefits of raising biohydrogen yield and enzymatic activity, their expensive and risky synthesis procedures imply that the complete process is not yet viable from an environmental and pilot-scale production perspective (Srivastava *et al.* 2020). One possible and sustainable option is a low-cost "Green synthesis" which would use organic waste to create nanomaterials economically. A yield of 3428 mL/L was observed for 410 hours in the biohydrogen synthesis carried out by Srivastava *et al.* (2020) utilizing crude enzyme and sugarcane bagasse treatment applied to magnetite particles. Using cobalt ferrite nanoparticles doped with copper and aluminium, Li *et al.* (2022) achieved a biohydrogen yield of 213 mL/g glucose. Green synthesized magnetic iron nanoparticles were used to boost biohydrogen synthesis synergistically with a yield of 0.72 mol H_2/mol of glucose (Aziz *et al*. 2022). In an experiment conducted by Yildirim *et al.* (2024) green engineered nanoparticles were used to produce 213 mL H_2/g glucose of biohydrogen. The research for biohydrogen production using nanoparticles as a catalyst has intensively increased, and with the use of nanomaterials with particular physiochemical and structural characteristics nanoscience and technology play a significant role in enhancing the production of biohydrogen.

2. BIOHYDROGEN

One potential alternative energy source derived from sustainable and renewable sources is hydrogen (Plangklang *et al.* 2012). It is an environmentally friendly fuel that produces both electricity and water, mitigating the adverse effects caused by fossil fuel consumption (Poleto *et al.* 2016). Hydrogen is the purest fuel among all commonly used fuels, including coal and oil and has the highest calorific value because bacterial fermentation produces it as a byproduct (Liu *et al.* 2020). The term "biohydrogen" refers to the dihydrogen gas (H_2) produced by various microorganisms like bacteria, algae, and archaea. Several biological processes, such as microbial electrolysis, direct and indirect bio-photolysis, dark and photo-fermentation, can yield biohydrogen (Srivastava *et al.* 2017). Different biological methods for generating biohydrogen require different organic substrates and micro-organisms. These methods, which make use of biowastes, are pollution-free and less expensive than other energy-producing techniques. In addition to being a viable sustainable energy source, biohydrogen provides a good substitute for carbon-based fuels. Due to its renewable nature, high cellulose concentration, and abundant availability, the most flexible organic substrate for large-scale biological biohydrogen production is lignocellulosic biomass (Wang *et al.* 2017). Even though the most sustainable method for producing biofuels from biomass is biomass into biohydrogen, there are still several issues that must be resolved before it can be widely used in industry (Nikolaidis *et al.* 2017). In this context, Fani *et al.* (2018) discussed the role of nanotechnology in many biochemical areas. According to Pugazhendhi *et al.* (2019), nanomaterials possess unique features that can significantly improve the method of transforming biomass into biohydrogen.

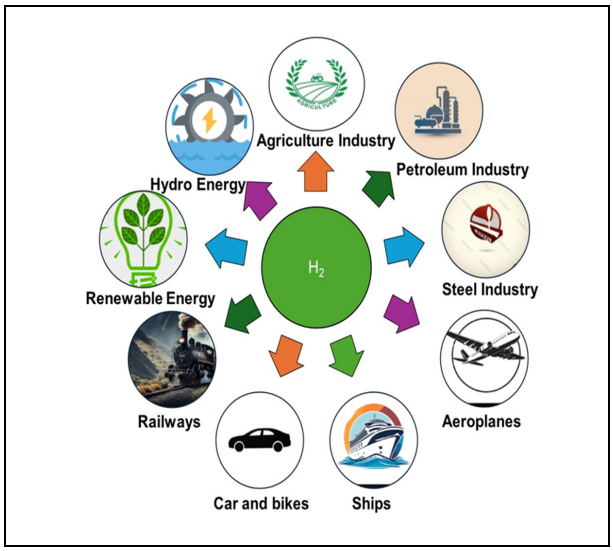


Fig. 1: Various applications of biohydrogen

According to Zilouei and Taherdanak (2015), many nanomaterials, including iron, nickel, gold, titanium, and silver have been shown to improve the production of biohydrogen through several biological processes, such as dark and photo-fermentation, bio-photolysis, and others. Nanomaterials are present in large concentrations near the active sites of hydrogenase and nitrogenase enzymes, and they function as co-factors by enhancing the synthesis of biohydrogen. They can scavenge oxygen, and reduce the possibility of oxidation-reduction by removing excess oxygen during fermentation. As a result, an ideal anaerobic environment for the hydrogenase enzyme's activity can be created, improving the yield of biohydrogen (Lin *et al.* 2016). Biohydrogen finds applications over a wide range from chemical industries, transportation to the generation of electricity which are summarized in Fig. 1.

3. PRODUCTION OF BIOHYDROGEN

Being an essential gas, hydrogen is utilized as a source of sustainable energy and as fuel in some industries. As a result, there has been a rapid growth in the need for hydrogen generation. While auto-thermal technologies, water electrolysis, and hydrocarbon steam reforming are popular methods for generating hydrogen, their large power consumption needs render them impractical (Zhao *et al.* 2013). The current state of biohydrogen production involves four main processes: dark fermentation, photo-fermentation, microbial electrolytic cell, and bio-photolysis. All of these technologies are environment-friendly, sustainable, and renewable energy-producing as they use organic waste and water as substrates to generate biohydrogen (Feng *et al.* 2023). Hydrogen is produced by biological processes that are photosensitive or photo-independent. While bacteria are employed in dark fermentation, microalgal processes similar to photo-fermentation and bio-photolysis depend on light to take place. Hydrogenase and nitrogenase are two vital enzymes for biohydrogen production (Das, 2001).

According to Kim *et al.* (2011), the bidirectional oxygenation of hydrogen into protons and electrons is carried out by hydrogenase. Nitrogenase, which is mostly found in bacteria produces hydrogen following nitrification in an anoxic environment. However, compared to hydrogenase, it is less successful at producing biohydrogen (Nagarajan *et al.* 2017). Cyanobacteria are highly relevant and profitable as potential hydrogen generators because they make hydrogen by converting solar energy from water. The complete classification of biohydrogen production is depicted in Fig. 2.

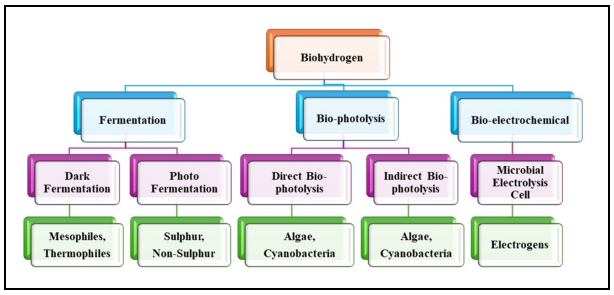


Fig.2: Biohydrogen production process

3.1 Dark Fermentation Process

Dark fermentation is reported as the most popular technique for producing biohydrogen (Sambusiti *et al.* 2015). It is an efficient and effective method for the generation of hydrogen (Fig. 3). This is because of its high rate of hydrogen production, versatility in using different substrates (especially lignocellulosic biomass), and less energy consumption while operating at ambient conditions. Under dark fermentation, a variety of adaptable fermentative microbes, including *Citrobacter, Rhodopseudomonas, Enterobacter, Lactobacillus, and Clostridium*, can be employed to generate biohydrogen. Numerous effective research on the microbial decomposition of organic waste for biohydrogen production has been reported by Khamtib *et al.* (2012). The dark fermenting bacteria thrive on the sugar-rich organic substrate to produce pyruvate by glycolysis. This pyruvate by ferredoxin reduction is then oxidized to acetyl CoA, which yields ATP and acetate. The enzyme hydrogenase then oxidizes the reduced ferredoxin, resulting in the generation of biohydrogen (Ramprakash *et al.* 2018).

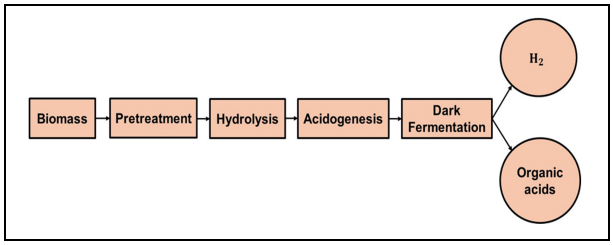


Fig. 3: Representation of dark fermentation process

The acetate process can theoretically produce four moles of hydrogen per mole of glucose, whereas the butyrate pathway can produce two moles of hydrogen per mole of glucose. The representation equation for dark fermentation is given below as:

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

A study on the synthesis of biohydrogen using dark fermentation was published by Khamtib *et al.* (2012). Under optimal conditions of temperature 60 °C, C:N ratio of 20:1, pH6.5, and a xylose concentration of 10 g/L. Because the hydrogenase enzyme is not inhibited by light or oxygen, this technique can produce hydrogen at a greater rate continuously and at a continuous supply (Khamtib *et al.* 2012). Also, this method yields many metabolic intermediates, particularly organic acids with additive qualities, which can be utilized to synthesize additional commercial goods (Das, 2001).

Since hydrogen is produced at a higher rate *via* dark fermentation and requires less time for microbial growth than biophotolysis and photo fermentation, it is more economical in laboratory settings. The hydrogen produced by dark fermentation is easy to use, reliable, and has the highest yield relative to several alternative non-fermentative techniques (Senthil Rathi *et al.* 2024). Additionally, a variety of bio-waste can be utilized as dark fermentation feedstock for hydrogen production, adding even more benefits to recycling and reuse. Incremental enrichment is a process enhancement approach that has gained attention recently due to its ability to improve process performance along with the benefits of simpler operations and lower energy utilization (Yang *et al.* 2018). Several benefits come with

dark fermentation, which include the capacity to use a range of feedstock sources, illumination independence, and a comfortable atmosphere. Anaerobic micro-organisms can also be introduced as mixed or single cultures. Dark fermentation is preferable since photo-fermentation needs light energy (Saravanan *et al.* 2022).

However, the dark fermentation process is constrained by the limited hydrogen yield. The main drawback of dark fermentation is the formation of toxic compounds. It is also affected by different factors such as temperature, pH, hydraulic retention time, and inoculum percentage. Furthermore, the accumulation or high concentration of acid metabolites and ethanol has a negative impact generation of hydrogen. A substantial amount of feedstock comprising micro and macronutrients together with metal ions is needed for effective dark fermentation.

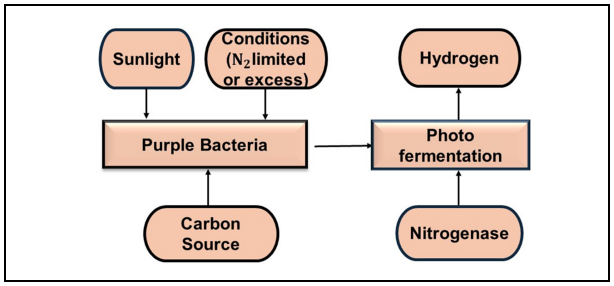


Fig. 4: Process of photo-fermentation

3.2 Photo-fermentation Process

The rapid breakdown of organic materials and moderate fermentation environment make photo-fermentation a popular method for producing biohydrogen. Photo-fermentative H_2 generation becomes feasible when free solar energy is combined with waste disposal regulation and renewable energy delivery (Policastro *et al.* 2021). In the presence of the nitrogenase enzyme, it is the process by which photo-fermentative bacteria use volatile fatty acids and organic acid created by the reaction of dark fermentation as a natural substrate. This process produces biohydrogen using light energy when nitrogen is deficient (Das, 2001; da Silva Veras *et al.* 2017). *Rhopaenasps, Rhodobactersps, Rhodovulumsps, Dunaliellasps, Anabaena sps* and *Chlorella* are a few common species of photo-fermentative bacteria (Corneli *et al.* 2016). The primary raw material used to produce photo-fermentative biohydrogen at the moment is straw feedstock. It can be treated using enzymolysis, shattering, and other procedures. Microalgae are unicellular organisms having a high-power density they act on carbohydrates which can be utilized in biochemical processes. Therefore, it is crucial to ascertain whether it is feasible to produce biohydrogen from microalgae (Jiang *et al.* 2021; Jing *et al.* 2022). Through photo-fermentation, purple non-sulfur bacteria is used in non-oxygenic hydrogen synthesis. They utilize sunlight for heterotrophic growth, during which hydrogen is generated. Nitrogenase facilitates the hydrogen generation from electrons generated during substrate oxidation. Fig. 4 depicts a photo-fermentation mechanism.

The main enzymes responsible for producing hydrogen are nitrogenase and hydrogenase. A regulated environment is necessary for photo-fermentation (Lin *et al.* 2021). In particular, pure and impure glycerol fractions can be effectively converted to hydrogen through the photosynthetic bacteria *Rhodopseudomonas palustris* through photo-fermentation which also has the potential to produce energy in the future. Under anaerobic conditions, nitrogenase enzymes use ATP as an energy source to decompose organic wastes into simpler chemicals like CO_2 and H_2. This process is carried out by the photo-fermentative bacteria and can be represented by the following equations.

$$6CO_2 + 12\ H_2 \rightarrow C_6H_{12}O_6 + 6H_2O$$

This process is amongst the most promising methods for creating biohydrogen due to its high hydrogen production and high theoretical efficiency of 8 mol of H_2/mol of acetic acid (Das, 2001; Liu *et al.* 2023). Additionally, these authors investigated the effects of several substrates (carbon sources), such as fructose, glucose, sucrose, and acetate, and determined the highest quantity of hydrogen produced (Liu *et al.* 2021). Photo-fermentation can work on variety of biowaste. Because solar energy is used efficiently, green microalgae and photosynthetic bacteria together yield good results in photo-fermentation. Targeting the disruptive qualities of gene products may be the focus of future studies, including genetic manipulation. Biohydrogen production efficiency can also be increased by lowering the costs associated with manufacture, supply, storage, transformation, and practical applications. The production of biohydrogen involves many factors that require several optimization processes to get a practical and economical approach. The production of biohydrogen through photo-fermentation is highly influenced by several parameters, such as light intensity, pH, stirring mode, pretreatment conditions, and substrate concentration. Compared to dark fermentation, photo-fermentation is significantly more difficult since it needs continuous light intensity, careful atmospheric monitoring, and specific nutrition.

3.3 Biophotolysis Process

In photoautotrophic organisms like cyanobacteria and microalgae, a process known as biophotolysis uses light as its primary energy source to split down molecules of water into hydrogen and oxygen (Dincer *et al.* 2015). It is widely considered to be an environmentally beneficial method as it produces hydrogen using just light energy and the photosystems of algae. Different algae engaged in biophotolysis include

Cholrella, Scenedesmus, Chlamydomonas reinhardtii, and *Tetraselmis* (Rashid *et al.* 2011; Oey *et al.* 2016). The two types of biophotolysis that differ in the way hydrogen is produced are direct biophotolysis (DBP) and indirect biophotolysis (IBP). Using nitrogenase and/or hydrogenase enzymes, both microalgae and cyanobacteria, which are heterocystous and non-heterocystous strains, can perform direct photolysis. The process of direct bio-photolysis involves the splitting of molecules of water into hydrogen ions (H^+) and oxygen (O_2) through the absorption of light energy by photosystems (PS II and PS I) present as photosensors in the cells of green algae and cyanobacteria. Energy absorption causes electrons in PS II to be excited, and these electrons are then linearly transported to ferredoxin via PS I (Das, 2001). Then, enzyme hydrogenase helps in the generation of hydrogen. Fig. 5 represents the biophotolysis process for hydrogen production.

Giannelli *et al.* (2012) reported that pigments including chlorophyll, phycobiliproteins, and carotenoids found in cyanobacteria tend to absorb sunlight and mediate the multiple phases of IBP. According to Nikolaidis *et al.* (2017), these organisms undergo photosynthesis, when water and light energy are present, which transforms the absorbed light energy into chemical energy and produces biomass. Nitrogenase or hydrogenase hydrolyses this biomass, in the presence of water and light energy producing hydrogen in the process (Das, 2001). Indirect biophotolysis techniques based on reversible hydrogenase have many advantages over nitrogenase-based systems: Reversible hydrogenases have unique hydrogen evolution activities that are approximately a thousand times more powerful than those of nitrogenase and do not require adenosine triphosphate (ATP). Certainly, there are a few drawbacks. Reversible hydrogenases are frequently expressed at low degree activity in microalgal species, which results in lower H_2 generation rates than any found using nitrogenase-based methods. The oxygen sensitivity of the process, which acts as the main inhibitor of the hydrogenase enzyme that produces H_2, is one of the difficult challenges to biophotolysis-based hydrogen synthesis. Using oxygen-reducing scavengers, antioxidant additions, mineral deprivation, and co-culturing bacteria on microalgae are some methods to control molecular oxygen (Javed *et al.* 2022).

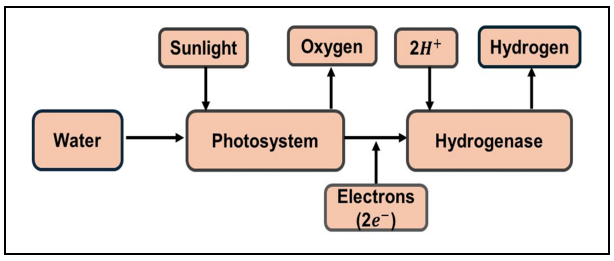


Fig: 5: Process of biophotolysis

3.4 Microbial Electrolysis Cell Process

Microbial Electrolysis Cell stands as another significant technique for biohydrogen generation similar to the microbial fuel cell (MFC). This process of applying an external potential to convert organic materials found in the garbage into biohydrogen (Sarangi *et al.* 2020). This system, which converts waste into biohydrogen, typically includes cathode and anode chambers with matching electrodes (Fig. 6). The electrodes can be positioned individually in different chambers or a single chamber. Proton exchange membranes are often employed to separate the two specialized chambers in a two-chamber MEC; however, other membranes, such as charged mosaic, bipolar, and cation-anion exchange membranes, can also be used in the applicable process (Varanasi *et al.* 2019). Anoxic or anaerobic conditions are typically enabled throughout the two-chamber MEC by the presence of microorganisms and organic waste water in the anode chamber. However, other solutions, especially phosphate buffer solution and/or salt solution, are typically filled in the cathode chamber throughout the operation. To produce electrons and protons, the microorganisms in the anodic chamber must oxidize the organic matter this is the fundamental premise of MEC. A proton exchange membrane diffuses protons while combining electrons with oxygen at the cathode, where they are transferred. Hydrogen gas is produced when protons and electrons combine. Using MEC to produce hydrogen has many benefits, the most significant of which is that 1.23 V is the theoretical voltage needed for the hydrolysis of water to produce hydrogen. As a result, this bioconversion process needs a low potential (Keçebaş *et al.* 2019). Water electrolysis needs an electrical energy of 7.5–50.6 times more than MEC to produce biohydrogen (Kadier *et al.* 2018). *Clostridium beijerinckii* and *Clostridium butyricum* have reportedly been to exhibit high hydrogen yields in the context of biohydrogen synthesis from agro-industrial residues, with 235 ml H_2/g and 310 ml H_2/g removal of COD, respectively (Martinez-Burgos *et al.* 2022). Furthermore, enzymatic saccharification can increase the yield and rate of hydrogen production. On an average, 0.015 L/day of hydrogen generation from household water in the 13–21 °C temperature range is reported by the MEC study (Heidrich *et al.* 2013). The efficiency of MEC in producing hydrogen is approximately 90%, and its performance is entirely dependent on the concentration and composition of the substrate, temperature, the type of MEC, pH, electrode material, operating voltage, microbe type, and membrane type (Heidrich *et al.* 2013).

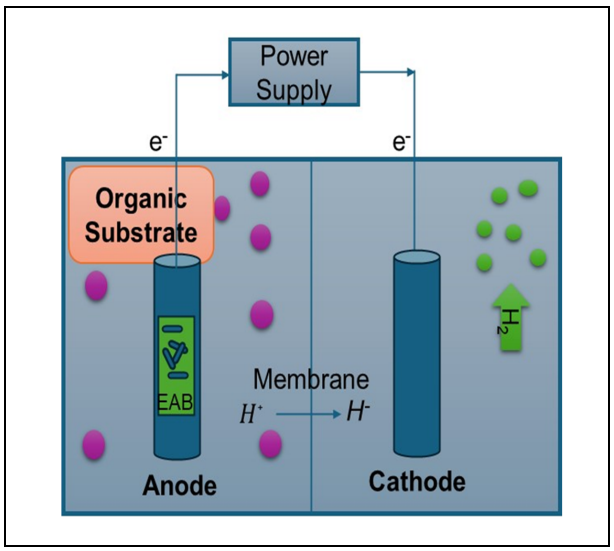


Fig. 6: Microbial electrolytic cell

3.5 Comparison of Production Process

Though the above discussed methods are useful for the production of biohydrogen, fermentation has better features as compared to other methods. Table 1 depicts the advantages and disadvantages of different production methods of biohydrogen.

4. ROLE OF NANOTECHNOLOGY

The use of nanomaterials in the production of biohydrogen results in increased productivity and yield, as well as improved enzyme reusability, the ability to retain activity after repeated cycles and to complete reactions more quickly. Further, nanotechnology may be responsible for making the overall process more sustainable and cost-effective. The role of nanotechnology at various processes in production of biohydrogen is summarized in Fig. 7.

Table 1. Advantages and challenges of biohydrogen methods of production

Process	Conditions	Advantages	Challenges	Reference
Biophotolysis	Light	Increased hydrogen generation rate Cheaper due to simple design of reactor Potential substrates include glycerol and wastes high in carbohydrates	Enzymes hydrogenase and nitrogenase are inhibited by oxygen created during the process Low light energy conversion efficiency A stream of mixed oxygen and hydrogen are generated	Ahmed *et al.* (2021)
Microbial electrolysis cell	External voltage, anaerobic condition	Energy is obtained from light rather than glucose The substrates used are volatile fatty acids and organic acids	Costly procedure Constraints on scalability Requirement of external voltage supply	Singh *et al.* (2022)
Photo fermentation	nitrogen-deficient conditions, anaerobic, light	Using photosynthetic microalgae and cyanobacteria wastewater can be converted to hydrogen by photolysis Operated with std temperature and pressure settings	Less advantageous economically since photobioreactors and processing are expensive Poor light energy conversion efficiency	Bolatkhan *et al.* (2019)
Dark Fermentation Anaerobic	Without light	Yields valuable byproducts such as butyric, lactic and butyric acids No oxygen limitation problem	Lower yield of H_2 Product gas mixture contains CO_2	Akhlaghi et al. (2020)

Due to their special physicochemical characteristics, nanomaterials are becoming more and more recognized as ways to improve the process of producing biohydrogen (Beckers *et al.* 2013). According to Patel *et al.* (2018), the metabolic activity of microorganisms is significantly impacted by nanomaterials which promote the efficient transfer of electrons to acceptors, which increases biohydrogen output and productivity. The synthesis of biohydrogen is catalyzed by its small size and vast surface area providing more reaction sites for the interaction of the enzyme with the substrate (Vaghari *et al.* 2016). Furthermore, the small size and substantial surface area of the nanomaterials significantly boost the rate of electron transfer between the enzyme and nanomaterial, which in turn promotes the synthesis of hydrogen (Patel *et al.* 2018). The two enzymes nitrogenase and hydrogenase are involved in all biological processes that result in the synthesis of biohydrogen. These enzymes require nickel and iron as metallic co-factors at their reactive sites to maintain both their structural integrity and functionality (Engliman *et al.* 2017). Nanoparticles with pore volumes of 1–100 nm are known to have a higher number of active (functional) sites, a greater contact surface area, and good selectivity (Gadhe *et al.* 2015).

In addition, a great deal of nanomaterials possesses improved catalytic characteristics, crystalline structures, greater absorption potential, and good chemical stability. Nanoparticles improve anaerobic microbial activity, reduce inhibitor emergence, and increase electron transfer and chemical catalytic activities in the context of biofuels (Elreedy *et al.* 2019). The dimensionality of nanostructured materials is typically used to categorize them into one of four categories: zero, one, two, or three. Nanoparticles (NPs) differ greatly from their macro counterparts in terms of their physical and chemical properties, which are broadly

used in the production of bioenergy. In addition, it has been noted that additional NP qualities including crystallinity, stability, efficient storage and adsorption capacity are much sought for in the biohydrogen generation industry to enhance the cellulase enzyme's hydrolysis, productivity, and stability (Hamawand *et al.* 2020). In general, NPs fall into one of two categories: organic or inorganic. Organic NPs include liposomes, polymersomes, polymer constructions, and micelles. These materials have been extensively used in drug and gene delivery, imaging, and other related fields. Inorganic NPs such as carbon nanotubes, quantum dots, gold, and magnetic nanoparticles have gained a lot of interest from researchers due to their optical and magnetic properties as well as their chemical properties (stability, inertness and ease of usage). The characteristics of both parent NPs are displayed by hybrid NPs, which blend inorganic and organic nanoparticles. In some circumstances, hybrid NPs have outperformed the parent NPs in terms of catalyst recovery and selectivity. Fig. 8 shows the different nanomaterials used in generation of biohydrogen.

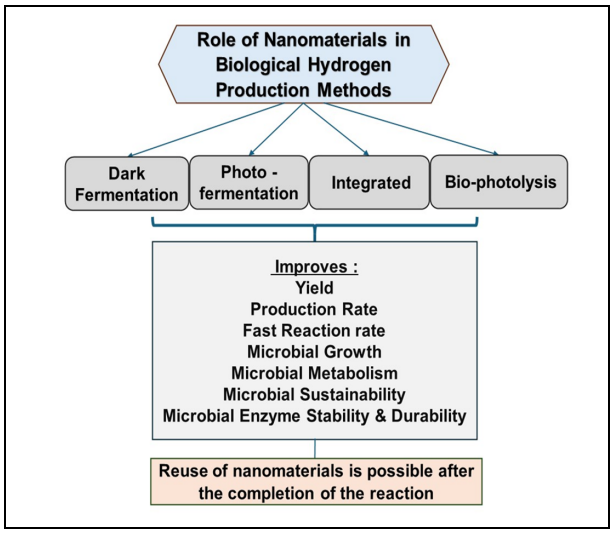


Fig. 7: Role and advantages of nanomaterials to enhance hydrogen production

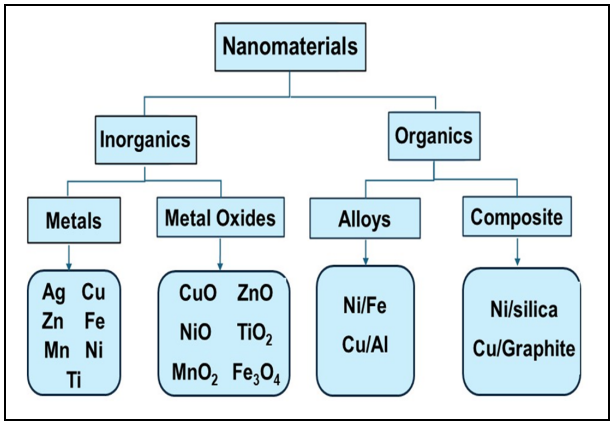


Fig. 8: Various nanomaterials used in biohydrogen production

4.1 Prospective Nanomaterials for the Production of Biohydrogen

4.1.1 Metal based Nanomaterials

Metal oxides (Al_2O_3/Fe_2O_3/ZnO/CaO/KOH/MoO_3/NiO) and metallic nanoparticles (Al/Ni/Fe/Au/Co) are examples of metal-based nanomaterials (NMs). Other metal oxide-based nanomaterials include mixed metal oxides, transition metal oxides, and alkali earth metal oxides. The preparation technique, as well as the shape, dispersion in a reaction and size, all affect the activity of these metal-based nanomaterials (Arya *et al.* 2021). Numerous chemical, physical, and biological processes are capable of producing them. Metal-based NMs are frequently employed to increase the generation of high-value products such as bioethanol, biodiesel, biogas, and biohydrogen (Sanusi *et al.* 2021). This is accomplished by using metal-based NMs for lignocellulosic biomass catalytic conversion, enzyme and biocatalyst immobilization, digestion and agro and biowaste treatment, and as additives at nanoscale to microalgal cultures to boost biomass.

(i) Gold and Silver NPs

By plasmonic phenomenon, gold, a colourful transition metal can absorb visible quantum electromagnetic radiation and produce photogenerated electrons and biohydrogen. Gold nanoparticles (AuNPs) improve catalytic properties in chemical reactions (oxidation and hydrogenation) furthermore supporting activity of enzyme and their immobilization in biological applications (Zhang *et al.* 2007). AuNPs were shown to play a role in improving fermentative biohydrogen production. The anaerobic culture yield was 62.3% higher when the source was synthetic wastewater containing sucrose (Zhang *et al.* 2007). Crucially, the amount of AuNPs that influenced the intermediate concentration during the hydrogen generating process was directly correlated with the increase in hydrogen generation.

Significant volume to surface area ratio of 5 nm AuNPs provided a stimulating effect for the production of biohydrogen, improving biohydrogen yield and substrate utilization by 46% and 56%, respectively (Zhang *et al.* 2007). They have also been shown in other studies to increase the enzyme activity for biohydrogen production, such as ferredoxins and the [Ni-Fe]-[Fe-Fe] hydrogenases which bring out the transfer of electrons (Zhao *et al.* 2013)

(ii) Palladium Nanoparticles

Palladium Nanoparticles (PdNPs) might have a major impact on organisms that produce biohydrogen as well. The effect of palladium nanoparticles synthesized via a photogenic process using *Coriandrum sativum* leaf extract on hydrogen production by glucose-fed

E. cloacae strains 811 and 101 was investigated by Mohanraj *et al.* (2016). In this investigation, Pd^{2+} ions significantly reduced glucose conversion efficiency under the same conditions by inhibiting production of H_2 more than PdNPs. Compared to mixed cultures, pure *E. cloacae* cultures produced more biohydrogen. Interestingly, PdNPs up to 20.0 mg/L addition did not affect the biological characteristics of mixed culture or E. cloacae. Moreover, a rise in the synthesis of propionate, an intermediate metabolite, demonstrated its detrimental effect on the production of Hydrogen.

In MECs, Pd nanoparticles are used as cathode catalysts instead of Ni nanoparticles. Using a bio-electrochemical deposition technique, Pd nanoparticles were produced on carbon fabric to serve as a cathode electrode. The results showed that MECs work in bio-hydrogen production had significantly improved. By using nanoparticles in the MEC, the anode reaction efficiency, the cathode enzymatic activity, and the proton exchange membrane kinetics improved.

(iii) Silica, Iron, and Nickel NPs

Proteins and microbes have long been recognized to benefit from silica's greater biodegradability (Pandey *et al.* 2015). Using mixed consortia from wastewater of conventional effluent treatment plants, Venkata Mohan *et al.* (2008) demonstrated the effective use of mesoporous SiO_2 particles in H_2 production. Because of insufficient degradation or inefficient feed use, excessive feed loading often had a detrimental effect on H_2 generation by mixed consortia (Venkata Mohan *et al.* 2008). The immobilization of bacteria on silica particles (120 mg/L) under fermentation conditions surprisingly stimulated a much larger H_2 synthesis of 7.02 mol/kg COD/day compared to the high feed loading control group (2.55 kg COD/day) (Mishra *et al.* 2018). According to Beckers *et al.* (2013), the metabolite intermediate type and the yield of H_2 production was unaffected by SiO_2 particles with high permeability (5.1 mg/L).

In their 2015 study, Zilouei and Taherdanak (2015) investigated the effects of Fe NPs versus Fe^{2+} ions on the fermentative hydrogen generation from glucose by the anaerobic sludge at concentrations ranging from 0 to 50 mg/L. Fe^{2+} ions and Fe NPs both enhanced hydrogen yield by 15 and 37% at doses of 10 and 25 mg/L, respectively, in contrast to controls. Iron (Fe) and titanium (TiO_2) NPs exhibited increased hydrogenase activity in various photo-fermentation processes, thus this characteristic property can be used for bio photolytic hydrogen production (Pandey *et al.* 2015). $SiCeFe_3O_4$ reported 3.02 mol H_2/mol acetate, PdNi/CdS (54 mmol/g.h), and graphite C_3N_4 (64.2 mol H_2/mol sugar) are a few examples of nanocatalysts in photo-fermentative biohydrogen generation (Wimonsong *et al.* 2015). In additional research, dark fermentation of grass was carried out using zero-valent iron (FeO) nanoparticles, it was demonstrated that FeO nanoparticles increased the activity of a hydrogenase enzyme to provide a greater biohydrogen production and highest hydrogen yield 73% higher than control studies (Cheng *et al.* 2020). Fe nanoparticles and Fe^{2+} ions were found to be able to significantly reduce propionate formation by up to 75%, which would aid in the creation of more hydrogen. Additionally, they showed increased hydrogen yields of 15% and 37%, respectively, in comparison to the control. Because of their small size, broad surface area, and biocompatibility, nanomaterials are important catalysts for biohydrogen production *via* dark fermentation reaction (Mohanraj *et al.* 2016; Srivastava *et al.* 2017; Zilouei and Taherdanak, 2015). Fe nanoparticles from *Syzygiumcumini* that were "greenly" synthesized affected the production of biohydrogen using *E. cloacae* DH-89 was shown by (Bao *et al.* 2013). The addition of Fe NPs at a concentration of 100 mg/L was found to significantly boost glucose utilization by two times, resulting in the generation of 1.9 mol H_2/mol glucose a much higher amount than that of the control. Fe^{2+} ions involvement in the hydrogenase and ferredoxin enzymes may be connected to this notable improvement. Lin *et al.* (2016) goal was to increase *Enterobacter aerogenes* ATCC13408's biohydrogen production in a starch medium by adding Fe_2O_3 as a nano addition. There was an upward trend in the hydrogen yield, rising from 164.5 mL H_2/g to 192.4 mL H_2/g. Using a chemical deposition method, Jayabalan (2020) produced a cathode catalyst from nickel molybdate ($NiMoO_4$), nickel oxide, and cobalt oxide (Co_3O_4) nanoparticles which improved the yield of hydrogen in the microbial electrolytic cell process.

(iv) Titanium Oxide, Copper Nanoparticles

The effect of TiO_2 NPs on the yield of hydrogen generation and nitrogenase intake by *R. palustris* was studied by Zhao *et al.* (2011). The nitrogenase activity was significantly improved. There was a 46.1% increase in the generation of hydrogen with a yield of 1.02 mol/kg. When utilizing Cu-NPs instead of copper sulfate ($CuSO_4$), some bacteria, such as *Enterobacter cloacae* and *Clostridium acetobutylicum*, can produce more biohydrogen. While Cu-NPs can be beneficial at low concentrations, high concentrations can have an inhibitory effect on hydrogen production by bacteria (Liu *et al.* 2021).

Dudek *et al.* (2018) detected biohydrogen generation of 138.45 mL by *Platymonas subcordiformis* cultivated on medium consisting of the addition of 10 g/L of glucose after 11 days of biomass development. According to Jafari *et al.* (2016), the biomass treated with titanium oxide (TiO_2) produced more biohydrogen (44.2 kg) than the control group, but TiO_2 also decreased the activity of hydrogenase uptake. By significantly boosting

the hydrogenases' activities, NPs enhance the biohydrogen generation's contribution to biophotolysis.

Nanomaterials, especially photocatalytic nanomaterials like ZnO, and TiO_2, improve the photoconversion efficiency of photo-fermentative bacteria and provide the energy needed to produce biohydrogen (Chen *et al.* 2014). These nanomaterials give increased reactive surface area, more active sites, and a quantum size effect, which improves surface adsorption and photocatalysis (Zhu et al. 2020). Additionally, these nanomaterials speed up the transfer of photo-induced electrons to the enzyme system, increasing biohydrogen productivity and yield (Liu *et al.* 2023).

(v) Magnetic Nanoparticles

A growing number of bioenergy research are focusing on magnetic nanoparticles (MNPs), such as Fe_3O_4, $CoFe_2O_4$, $MnFe_2O_4$, and Fe_2O_4, as they are highly effective enzyme nanocarriers because of their high surface area, quantum size, non-toxic nature, strong thermal stability and biocatalytic activity (Ghosh *et al.* 2024). Furthermore, MNPs possess other qualities like superior compatibility, high enzyme loading capacity, and above allow processing costs, which enable their commercial application (Markandan *et al.* 2022). Strong magnetism and high porosity are two of MNPs key advantages over equivalents. They have been used in a variety of biohydrogen production contexts, such as the increase in methanogens activity for biohydrogen production and enzyme immobilization in hydrolysis of lignocellulosic biomass or H_2 production (Yazdani *et al.* 2019; Cheng *et al.* 2020). Magnetic nanoparticles like iron and nickel can be added to a structure to provide the necessary availability to preserve and enhance structural integrity in photo-fermentation process.

4.1.2 Carbon Nanotubes

Carbon Nanotubes (CNTs) are cylindrical carbon structures made of rolled-up graphite sheets with a nanometric diameter. They possess exceptional mechanical, electrical, and thermal properties, making them attractive for various applications. CNTs offer outstanding mechanical, electrical, thermal properties and biocompatibility for various applications. Furthermore, the big surface area of CNTs allows for low diffusion resistance and a high enzyme loading capacity (Markandan *et al.* 2022). Using fermentative mixed culture made from starch waste as feed, along with activated carbons in granulated (10 g/L) and powdered (5 g/L) forms, significantly increased the production of hydrogen gas by 94.5 and 44%, respectively (Mohan *et al.* 2008).

Using anaerobic muck as the inoculum, the Carbon nanotubes (CNTs) using a UASB reactor demonstrated a competent generation of hydrogen of roughly 2.45 mol H_2/mol glucose at hydraulic retention time of (Hallenbeck *et al.* 2005). Under similar conditions anaerobic process in the absence of CNTs was not feasible with 15 days activity in the reactor. The UASB reactor based on CNTs produced a hydrogen output that was 1.7 times higher than that of granular activated carbon. Similarly, using the granular activated carbon and its powder, acidogenic culture blends of the starch waste as a feed, separately, showed a significant shift in the hydrogen generation, i.e., 94.5 and 44.0 % (Basak *et al.* 2007). To successfully improve hydrogen generation, concentrations of activated carbons at 33.0 mg/L and 33.3 mg/L were found to yield 62.5% and 73.0% under both batch and UASB mode from sucrose-based anaerobic sludge as inoculum, respectively (Mohan *et al.* 2008). The hydrogenase protein source in ferredoxin is formed by the hydrogen fermentation process.

4.1.3 Metal Organic Frameworks

According to Ahmed *et al.* (2021), metal organic frameworks (MOFs) are a unique class of three-dimensional architectures put together by metal ion/inorganic clusters and carbon-based ligands that are bonded together by strong covalent and/or coordination interactions. The chemical functions, topological structure and porosity of MOF can all be developed or modulated by carefully selecting which organic and inorganic components to use (Yang *et al.* 2018). Because of their increased catalytic activity, vast surface area, porosity and variety of chemical groups MOFs have attracted a lot of attention (Basumatary *et al.* 2022). Due to these remarkable and adaptable qualities, MOF is now a hot topic for research, particularly when it comes to catalysts. Creatively engineered catalysts are rapidly replacing the traditional homogeneous and heterogeneous catalysts for application in bioenergy research. There are two main ways that MOF nanoparticles can be used for hydrogen production: 1) Electrocatalytic water splitting: In this process, electricity is used to split water molecules into hydrogen and oxygen gas. Metal organic framework nanoparticles can be used as electrocatalysts to improve the efficiency of this process 2) Photocatalytic water splitting: In this process, sunlight is used to split water molecules into hydrogen and oxygen gas. Metal organic framework nanoparticles can be designed to absorb light and generate the energy needed to split water. Nickel-based MOF and palladium-based MOFs have shown to be effective electrocatalysts for hydrogen evolution reaction (Xiang *et al.* 2017; Chung *et al.* 2023).

4.1.4 Combination of Inorganic and Organic Nanoparticles

(i) Fe_2O_3/C Nanoparticles (FOC NPs)

Fe_2O_3/C nanoparticles help the development and concentration of the microorganisms that produce hydrogen, and they may also enhance hydrogenase

activity and electron transfer processes in the dark hydrogen fermentation pathway. The addition of 200 mg/L FOC nanoparticles increased the production of hydrogen by 33.7%. Furthermore, few results showed that the hydrogen production was reduced by the uneven concentration of these nanoparticles. The yield that was seen was 218.63 mL H_2/g glucose, which is 33.7% more than the 163 mL H2/g glucose of the control group that did not include Fe_2O_3/C nanoparticles (Zhang *et al.* 2018).

(ii) Ni-Ni Graphene Nanocomposite (Ni-NiGr NC)

The maximum hydrogen yields for nickel nanoparticles (NPs) and nickel-graphene nanocomposite (Ni-NiGr NC) using industrial wastewater and mono-ethylene glycol (MEG) through anaerobic digestion was roughly 24.73 ± 1.12 and 41.28 ± 1.69 mL/g COD for Ni nanoparticles and NiGr nanocomposites, respectively. Using batch reactors, the yield was measured at different doses of Ni NPs and NiGr NC, ranging from 0 to 100 mg/L (Han *et al.* 2015). When 60 mg/L of these were present, it was clear that the hydrogen generation greatly improved by around 23% and 65%, respectively, compared to the absence of nanomaterials. However, adding more of these nanomaterials lowers the hydrogen yield to an initial 20.80 ± 1.12 mL/g (Han *et al.* 2015). In another investigation, it was found that the use of Ni-graphene-based NPs improved the synthesis of dark fermentative biohydrogen, with a maximum yield of 41.3 H_2/g COD and a 65% increase in H_2 yield (Elreedy *et al.* 2019)

(iii) AlOA Nanoparticles

Aluminum-oleic acid core-shell nanoparticles or AlOA core shell nanoparticles are stable (towards non-polar solvent and air) due to sonochemical production of their shell. Bunker *et al.* (2011) produced >95% yield of hydrogen from tap water at ordinary room temperature. This was made possible by the thermal breakdown of aluminum hydride (alane) in the presence of a catalyst, titanium (IV) isopropoxide, and was meant to be used as power in a basic fuel cell (Ergal *et al.* 2018). Through the mutual action of organic acid-coated aluminum nanoparticles, such a potentiality is produced. Due to the reactions simplicity that generates hydrogen whenever and wherever it is needed, there is no need for large scale direct storage of hydrogen. The remarkable stability of these new nanoparticles and the high-level water-aluminum reactivity energy density are the determining factors (Ergal *et al.* 2018). Table 2 summarises the effect of different nanoparticles on the yield of hydrogen production.

Table 2. Summary of various nanoparticles in the biohydrogen production process

Nanoparticles	Feedstock/ Substrate	H_2 yield rate Nanosci (%) Increase	References
Ag	Glucose	Improved hydrogen yield (2.48 mol/mol glucose) with increase in 61.7 % yield	(Zhao *et al.* 2013)
Cu	Glucose	2.5 mg/L enhanced hydrogen generation	(Mohanraj *et al.* 2016)
Fe	Glucose	2.5 mg/L enhanced hydrogen production with an increase in 55% yield	(Zilouei and Taherdanak, 2015)
Fe	Glucose	A maximum H_2 yield 1.9 mol/l glucose	(Mubarak *et al.* 2014)
FeO	Glucose	2.07 mol H_2/mol glucose with 7.9 % more yield	(Mohanraj *et al.* 2016)
Fe_2O_3	Glucose	192.4 ml H_2/g casava starch with 17%	(Engliman *et al.* 2017)
CNT's	Glucose	2.45 mol/mol substrate	(Esmaeili *et al.* 2021)
Au	Acetate	Maximum production rate 1052 mL/L per day with 56 % yield	(Khan *et al.* 2013)
Fe	water hyacinth	57 mL/g of the plant biomass with a 68.4 % yield	(Mubarak *et al.* 2014)
Au	Artificial wastewater	4.48 mol/mol sucrose with a 67.5% increase in yield	(Khan *et al.* 2013)
FeO	Glucose	2.07 mol H_2/mol glucose with a 7.9% increase in yield	(Mohanraj *et al.* 2016)
Fe_2O_3	Glucose	192.4 ml H_2/g cassava starch with a 17% increase in yield	(Engliman *et al.* 2017)
Fe_3O_4	Wastewater	44.28 ml H_2/g COD with a 72.5 % increase in yield	(Malik *et al.* 2014)
TiO_2	Malate	The rate of production increased 1.54 times with a 69.6% increase in yield	(Reddy, 2017)
SiO_2	Air: CO_2	3121 H_2/l/h with a 45.2% yield increase	(Wang *et al.* 2023)
Biochar	Municipal solid waste	96.3 ml/g	(Pattarkine *et al.* 2012)
Ni+Graphene	Mixed culture	41.3 ml/g COD	(Cheah *et al.* 2020)

4.2 Nanoparticle Synthesis

Many techniques, including "top-down (physical procedures) and bottom-up (chemical methods)" synthesis, have been used to create the nanomaterial (Wang *et al.* 2004; Iqbal *et al.* 2012). Top-down synthesis is a destructive technique wherein larger molecules are divided into smaller components, which are then converted into nanoparticles (NPs) with the appropriate size and shape (Iravani *et al.* 2011).

Polishing, tearing, slicing, spraying, electroplating, machining, chemical precipitation, sputtering, pulsed laser deposition and vapor deposition are some of the methods used to break large components down into smaller ones. Bello *et al*. (2015) effectively produced coconut shell nanoparticles using granules from the milling process. A nanoscale change in the material's characteristics was demonstrated by their observations that the NPs grew smaller and lost their brown color with time. By using processes like sedimentation and reduction, bottom-up approaches generate the smallest NPs from progressively fundamental elements (Salame *et al.* 2018). Nanoparticles synthesised from simpler building elements are a distinguishing feature of bottom-up approaches. Instead of using mechanical methods like spinning, most work is completed by the use of biological and chemical processes. The following procedures are examples of bottom-up techniques: biological synthesis, sol-gel, spinning, chemical vapor deposition, hydrothermal, laser evaporation, and supercritical fluid creation (Iqbal *et al.* 2012). Top-down and bottom methods can be used to manufacture Fe, Ag, Cu, and Bi nanoparticles, according to several studies (Salame *et al.* 2018). Green synthesis techniques use a variety of plants, fungus, bacteria, and microalgae as biosynthesis agents. Nanoparticle synthesis is one of the many biotechnological processes that use microalgae because they are non-pathogenic and can use sunlight, carbon dioxide, and ammonium salts as sources of energy and nitrogen (Yildirim *et al.* 2024). For the production of nanoparticles, the most significant benefit of using plant extracts on microalgae is their ease of availability. This method works well for large-scale production because of its abundance in nature (Kamath *et al.* 2020). The antioxidant activity of the selected plant extract, which acts as a reducing agent, is a crucial component in the green pathway synthesis of nanoparticles.

4.3 Nanotechnology Devices for Biohydrogen Production

The next step from macro to micro scale is developing nanotechnology for miniature devices and systems, as well as synthesizing nanoparticles for biohydrogen production. Microfluidic systems are a major technical advancement because of their uniform flow, quick and effective mass and heat transfer, high proton transfer, large surface-to-volume ratio, uniform flow, and ability to create sturdy, compact fuel processors (Karthikeyan *et al.* 2024). Focused on producing necessary microfluidic components, developing microfabrication methods, synthesizing nanomaterials, and integrating parts into intricate microfluidic systems are the areas of study for small-scale devices. In the case of MEC, MFC or microbial electrochemical cells (MXC), microfluidics could be downsized. Producing biohydrogen requires several microfluidics characteristics, including mass and flow transfer, a high surface-to-volume ratio, cell immobilization, cell culture, downstream processing, and a quick reaction time. Microfluidics provides various benefits through data collection on light irradiation, cell behaviour, and other parameters. These benefits can be used to maximize yield on a macroscale or for effective $BioH_2$ generation at the microscale. According to Fadakar *et al.* (2020), a non-photogenic strain of *Escherichia coli* was used as the biocatalyst to generate hydrogen at cumulative rates of 46 and 28 parts per million per hour using substrates based on glucose and urea. Steps were taken to enhance biohydrogen production by increasing the volume-to-surface area ratio, electrical conductivity, and biocompatibility. These measures include the use of Fe_3O_4 nanospheres and reduced graphene oxide on the surface, nickel nanostructure incorporation, and the competitive advantage of metal-based electrodes over carbon-based electrodes (Tang *et al.* 2008; Mousavi *et al.* 2019). Due to their capacity to accurately control, observe, and manipulate samples at the nano- to pico-liter levels, microfluidic lab-on-a-chip systems combine multiple processes within a particular biological assay.

4.4 Outlooks

A clean, renewable energy source, biohydrogen still falls short of large-scale industrial production owing to its expensive and restricted efficiency at the moment of production. Even if several non-metallic and metallic nanoparticles have shown a great capacity to support dark fermentation in microbes for the synthesis of biohydrogen or have shown enormous potential for light-aided biohydrogen generation, they are far from sufficient. The following problems, in our opinion, must be resolved to overcome the technological barrier of enhanced biohydrogen generation using nanomaterials:

- Provide more details about the deeper molecular mechanism by which metal nanoparticles affect bacteria that produce hydrogen by use of material science, spectroscopy, proteomics, and other biological methods.

- There are now just two types of metal nanoparticles being studied by the biohydrogen production system: iron and nickel. More research should be done on materials that have exceptional physical and chemical properties, are less poisonous, and are environmentally beneficial. This must extend beyond single–metal nanoparticles to other nanomaterials with superior physical-chemical properties, such as inorganic non-metallic, organic, and alloy nanoparticles that are bimetallic or even polymetallic, whose impact on the production of biohydrogen and related mechanisms need to be investigated, should also be checked. Nanomaterials' dimensions, shape, size, and

distribution on or within cells must also be taken into consideration.

- It's essential to investigate the ideal dose of nanomaterials in various strains of metal nanomaterials-preferring bacteria and the impact of NPs on biohydrogen production since NP concentration and bacterial species always influence biohydrogen production. To decrease the harmful effects of nanomaterials on hydrogen-producing as much as feasible, it's also necessary to investigate the optimal compatibility between metal nanoparticles and hydrogen-producing bacteria, along with the process of cell poisoning.

- Examine the way that nanomaterials in the system of mixed bacteria produce hydrogen by screening them. The mechanism underlying this action is yet unknown, while some research has indicated that specific nanoparticles, like TiO_2 nanoparticles and iron nanoparticles (Fe NPs), can change the population levels of biohydrogen-producing microbes within a mixed bacterial system. More investigation is also required to find and screen biohydrogen-producing microbial populations that exhibit superior activity for producing biohydrogen, strong environmental resistance, and ecological friendliness, as well as to develop novel techniques for effectively using nanomaterials for microorganism screening.

- Assess the environmental effects of waste liquid and residue from biohydrogen production that contain nanomaterials. Additionally, to examine the toxicity of nanoparticles made of metals in these materials to the environment and the technology involved in recovering and reusing nanomaterials

- Utilize metal nanoparticles to create high-value byproducts through the integration of synthesis of biohydrogen and other bioproduction methods, such as methane synthesis and biological fixation of nitrogen, to increase the input economy of nanomaterials.

- Provide a large-scale photobiohybrid system design for a professional photoreactor to produce biohydrogen. Because photo-bioreaction is unique in producing biohydrogen, photo-bioreactor design must account for tight anaerobicity, fast biohydrogen discharge, and high-efficiency light energy absorption.

4.5 Future Prospects

Since utilizing renewable energy sources is expected to lessen present reliance on conventional fossil fuels, it is vital to take action to address energy security, a significant concern facing modern society. Utilizing co-culture or mixed-culture microorganisms, creating thermophilic or thermotolerant genetically engineered microbes, utilizing various substrates, and implementing effective enzyme systems can be used for long-term practical applications. One of the biggest obstacles to the application of nanomaterials-based biohydrogen generation technologies may be the expense associated with their preparation. Reuse of the nanomaterials, which are used in every stage of the process, is another problem with this method. According to this reference, when nanomaterials are produced using greener methods as opposed to chemical methods, the cost of the synthesis process may be further reduced. Recycling of nanomaterials may be possible through the use of sophisticated filtration techniques. Nonetheless, it is important to take into account the constraints that both chemical and green synthesis approaches have. To fully comprehend how nanomaterials affect the biochemical and molecular processes involved in the generation of cellulosic biohydrogen, more research is still required.

5. CONCLUSIONS

This review explains how various biohydrogen generation processes, including dark fermentation and photo-fermentation, can be improved by nanoparticles. Since iron and nickel serve as hydrogenase's active centers, a great deal of research has been done on the use of metal nanoparticles, specifically those of iron and nickel and their oxides, to improve the production of biohydrogen through dark fermentation. There has been a variation in the amount of biohydrogen produced, ranging from 5.47 to 23%. Additionally, biohydrogen synthesis during dark fermentation can be enhanced by gold, silver, and palladium (biohydrogen production varies from 5.4 to 67.1 %). Furthermore, the nano device utilized in biohydrogen research has been examined. The application of nanoparticles in various biohydrogen system modes has been assessed through the literature, although this field is very new. According to the existing data available, nanoparticles can significantly enhance this process in a sustainable manner to obtain a high yield of biohydrogen on a practical scale. More study is required to close the current gap in the practical applications and long-term viability of this field, as it is still in its early phases.

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CONFLICTS OF INTEREST

The authors declare that they have known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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REFERENCES

Ahmed, S. F., Rafa, N., Mofijur, M., Badruddin, I. A., Inayat, A., Ali, M. S., Farrok, O. and Yunus Khan, T. M., Biohydrogen Production From Biomass Sources: Metabolic Pathways and Economic Analysis, *Front Energy Res.,* (2021). https://doi.org/10.3389/fenrg.2021.753878

Akia, M., Yazdani, F., Motaee, E., Han, D. and Arandiyan, H., A review on conversion of biomass to biofuel by nanocatalysts, *Biofuel Res. J.,* 01(01), 16–25 (2014). https://doi.org/10.18331/BRJ2015.1.1.5

Arya, I., Poona, A., Dikshit, P. K., Pandit, S., Kumar, J., Singh, H. N., Jha, N. K., Rudayni, H. A., Chaudhary, A. A. and Kumar, S., Current Trends and Future Prospects of Nanotechnology in Biofuel Production, *Catalysts,* 11(11), 1308 (2021). https://doi.org/10.3390/catal11111308

Bao, M. D., Su, H. J. and Tan, T. W., Dark fermentative bio-hydrogen production: Effects of substrate pre-treatment and addition of metal ions or L-cysteine, *Fuel,* 112, 38–44 (2013). https://doi.org/10.1016/j.fuel.2013.04.063

Basak, N. and Das, D., The Prospect of Purple Non-Sulfur (PNS) Photosynthetic Bacteria for Hydrogen Production: The Present State of the Art, *World J. Microbiol. Biotechnol.,* 23(1), 31–42 (2007). https://doi.org/10.1007/s11274-006-9190-9

Basumatary, B., Basumatary, R., Ramchiary, A. and Konwar, D., Evaluation of Ag@TiO2/WO3 heterojunction photocatalyst for enhanced photocatalytic activity towards methylene blue degradation, *Chemosphere,* 286, 131848 (2022). https://doi.org/10.1016/j.chemosphere.2021.131848

Beckers, L., Hiligsmann, S., Lambert, S. D., Heinrichs, B. and Thonart, P., Improving effect of metal and oxide nanoparticles encapsulated in porous silica on fermentative biohydrogen production by Clostridium butyricum, *Bioresour. Technol.,* 133, 109–117 (2013). https://doi.org/10.1016/j.biortech.2012.12.168

Bolatkhan, K., Kossalbayev, B. D., Zayadan, B. K., Tomo, T., Veziroglu, T. N. and Allakhverdiev, S. I., Hydrogen production from phototrophic microorganisms: Reality and perspectives, *Int. J. Hydrogen Energy,* 44(12), 5799–5811 (2019). https://doi.org/10.1016/j.ijhydene.2019.01.092

Bunker, C. E. and Smith, M. J., Nanoparticles for hydrogen generation, *J. Mater. Chem.,* 21(33), 12173 (2011). https://doi.org/10.1039/c1jm10856e

Chen, J., Li, K., Chen, L., Liu, R., Huang, X. and Ye, D., Conversion of fructose into 5-hydroxymethylfurfural catalyzed by recyclable sulfonic acid-functionalized metal–organic frameworks, *Green Chem.,* 16(5), 2490–2499 (2014). https://doi.org/10.1039/C3GC42414F

Cheng, J., Li, H., Ding, L., Zhou, J., Song, W., Li, Y.-Y. and Lin, R., Improving hydrogen and methane co-generation in cascading dark fermentation and anaerobic digestion: The effect of magnetite nanoparticles on microbial electron transfer and syntrophism, *Chem. Eng. J.,* 397, 125394 (2020). https://doi.org/10.1016/j.cej.2020.125394

Chung, W.-T., Mekhemer, I. M. A., Mohamed, M. G., Elewa, A. M., EL-Mahdy, A. F. M., Chou, H.-H., Kuo, S.-W. and Wu, K. C.-W., Recent advances in metal/covalent organic frameworks based materials: Their synthesis, structure design and potential applications for hydrogen production, *Coord. Chem. Rev.,* 483, 215066 (2023). https://doi.org/10.1016/j.ccr.2023.215066

Corneli, E., Dragoni, F., Adessi, A., De Philippis, R., Bonari, E. and Ragaglini, G., Energy conversion of biomass crops and agroindustrial residues by combined biohydrogen/biomethane system and anaerobic digestion, *Bioresour. Technol.,* 211, 509–518 (2016). https://doi.org/10.1016/j.biortech.2016.03.134

da Silva Veras, T., Mozer, T. S., da Costa Rubim Messeder dos Santos, D. and da Silva César, A., Hydrogen: Trends, production and characterization of the main process worldwide, *Int. J. Hydrogen Energy,* 42(4), 2018–2033 (2017). https://doi.org/10.1016/j.ijhydene.2016.08.219

Das, D., Hydrogen production by biological processes: a survey of literature, *Int. J. Hydrogen Energy,* 26(1), 13–28 (2001). https://doi.org/10.1016/S0360-3199(00)00058-6

Dincer, I. and Acar, C., Review and evaluation of hydrogen production methods for better sustainability, *Int. J. Hydrogen Energy,* 40(34), 11094–11111 (2015). https://doi.org/10.1016/j.ijhydene.2014.12.035

Dudek, M., Dębowski, M., Zieliński, M., Nowicka, A. and Rusanowska, P., Water from the Vistula Lagoon as a medium in mixotrophic growth and hydrogen production by Platymonas subcordiformis, *Int. J. Hydrogen Energy,* 43(20), 9529–9534 (2018). https://doi.org/10.1016/j.ijhydene.2018.04.039

Elreedy, A., Fujii, M., Koyama, M., Nakasaki, K. and Tawfik, A., Enhanced fermentative hydrogen production from industrial wastewater using mixed culture bacteria incorporated with iron, nickel, and zinc-based nanoparticles, *Water Res.,* 151, 349–361 (2019). https://doi.org/10.1016/j.watres.2018.12.043

Engliman, N. S., Abdul, P. M., Wu, S.-Y. and Jahim, J. M., Influence of iron (II) oxide nanoparticle on biohydrogen production in thermophilic mixed fermentation, *Int. J. Hydrogen Energy,* 42(45), 27482–27493 (2017). https://doi.org/10.1016/j.ijhydene.2017.05.224

Ergal, İ., Fuchs, W., Hasibar, B., Thallinger, B., Bochmann, G. and Rittmann, S. K.-M. R., The physiology and biotechnology of dark fermentative biohydrogen production, *Biotechnol. Adv.,* 36(8), 2165–2186 (2018). https://doi.org/10.1016/j.biotechadv.2018.10.005

Fadakar, A., Mardanpour, M. M. and Yaghmaei, S., The coupled microfluidic microbial electrochemical cell as a self-powered biohydrogen generator, *J. Power Sources,* 451, 227817 (2020). https://doi.org/10.1016/j.jpowsour.2020.227817

Fani, M., Haddadzadeh Niri, M. and Joda, F., A Simplified Dynamic Thermokinetic-Based Model of Wood Gasification Process, *Process Integr. Optim. Sustain.,* 2(3), 269–279 (2018). https://doi.org/10.1007/s41660-018-0042-5

Feng, S., Hao Ngo, H., Guo, W., Woong Chang, S., Duc Nguyen, D., Thanh Bui, X., Zhang, X., Ma, X. Y. and Ngoc Hoang, B., Biohydrogen production, storage, and delivery: A comprehensive overview of current strategies and limitations, *Chem. Eng. J.,* 471, 144669 (2023). https://doi.org/10.1016/j.cej.2023.144669

Gadhe, A., Sonawane, S. S. and Varma, M. N., Enhancement effect of hematite and nickel nanoparticles on biohydrogen production from dairy wastewater, *Int. J. Hydrogen Energy,* 40(13), 4502–4511 (2015). https://doi.org/10.1016/j.ijhydene.2015.02.046

Ghosh, P., Deepshikha, K., Kumar, R. R., Chaturvedi, V. and Verma, P., Recent advances of nanotechnology in ameliorating bioenergy production: A comprehensive review, *Sustain. Chem. Pharm.,* 37, 101392 (2024). https://doi.org/10.1016/j.scp.2023.101392

Giannelli, L. and Torzillo, G., Hydrogen production with the microalga Chlamydomonas reinhardtii grown in a compact tubular photobioreactor immersed in a scattering light nanoparticle suspension, *Int. J. Hydrogen Energy,* 37(22), 16951–16961 (2012). https://doi.org/10.1016/j.ijhydene.2012.08.103

Hallenbeck, P. C., Fundamentals of the fermentative production of hydrogen, *Water Sci. Technol.,* 52(1–2), 21–29 (2005). https://doi.org/10.2166/wst.2005.0494

Hamawand, I., Seneweera, S., Kumarasinghe, P. and Bundschuh, J., Nanoparticle technology for separation of cellulose, hemicellulose and lignin nanoparticles from lignocellulose biomass: A short review, *Nano-Struct. Nano-Obj.,* 24, 100601 (2020). https://doi.org/10.1016/j.nanoso.2020.100601

Han, W., Ye, M., Zhu, A. J. and Zhao, H. T., Li, Y. F., Batch dark fermentation from enzymatic hydrolyzed food waste for hydrogen production, *Bioresour. Technol.,* 191, 24–29 (2015). https://doi.org/10.1016/j.biortech.2015.04.120

Heidrich, E. S., Dolfing, J., Scott, K., Edwards, S. R., Jones, C. and Curtis, T. P., Production of hydrogen from domestic wastewater in a pilot-scale microbial electrolysis cell, *Appl. Microbiol. Biotechnol.,* 97(15), 6979–6989 (2013). https://doi.org/10.1007/s00253-012-4456-7

Iqbal, P., Preece, J. A. and Mendes, P. M., Nanotechnology: The "Top-Down" and "Bottom-Up" Approaches, Supramol Chem., https://doi.org/10.1002/9780470661345.smc195

Iravani, S., Green synthesis of metal nanoparticles using plants, *Green Chem.,* 13(10), 2638 (2011). https://doi.org/10.1039/c1gc15386b

Jafari, O. and Zilouei, H., Enhanced biohydrogen and subsequent biomethane production from sugarcane bagasse using nano-titanium dioxide pretreatment, *Bioresour. Technol.,* 214, 670–678 (2016). https://doi.org/10.1016/j.biortech.2016.05.007

Javed, M. A., Zafar, A. M., Aly Hassan, A., Zaidi, A. A., Farooq, M., El Badawy, A., Lundquist, T., Mohamed, M. M. A. and Al-Zuhair, S., The role of oxygen regulation and algal growth parameters in hydrogen production via biophotolysis, *J. Environ. Chem. Eng.,* 10(1), 107003 (2022). https://doi.org/10.1016/j.jece.2021.107003

Jiang, D., Zhang, X., Ge, X., Yue, T., Zhang, T., Zhang, Y., Zhang, Z., He, C., Lu, C. and Zhang, Q., Insights into correlation between hydrogen yield improvement and glycerol addition in photo-fermentation of Arundo donax L., *Bioresour. Technol.,* 321, 124467 (2021). https://doi.org/10.1016/j.biortech.2020.124467

Jing, Y., Li, F., Li, Y., Jiang, D., Lu, C., Zhang, Z. and Zhang, Q., Biohydrogen production by deep eutectic solvent delignification-driven enzymatic hydrolysis and photo-fermentation: Effect of liquid–solid ratio, *Bioresour. Technol.,* 349, 126867 (2022). https://doi.org/10.1016/j.biortech.2022.126867

Kadier, A., Kalil, M. S., Chandrasekhar, K., Mohanakrishna, G., Saratale, G. D., Saratale, R. G., Kumar, G., Pugazhendhi, A. and Sivagurunathan, P., Surpassing the current limitations of high purity H2 production in microbial electrolysis cell (MECs): Strategies for inhibiting growth of methanogens, *Bioelectrochemistry,* 119, 211–219 (2018). https://doi.org/10.1016/j.bioelechem.2017.09.014

Kamath, V., Chandra, P. and Jeppu, G. P., Comparative study of using five different leaf extracts in the green synthesis of iron oxide nanoparticles for removal of arsenic from water, *Int. J. Phytoremediation,* 22(12), 1278–1294 (2020). https://doi.org/10.1080/15226514.2020.1765139

Karthikeyan, B. and Velvizhi, G., A state-of-the-art on the application of nanotechnology for enhanced biohydrogen production, *Int. J. Hydrogen Energy,* 52, 536–554 (2024). https://doi.org/10.1016/j.ijhydene.2023.04.237

Keçebaş, A., Kayfeci, M. and Bayat, M., Electrochemical hydrogen generation, *Sol. Hydrog. Prod.*, 299–317 (2019). https://doi.org/10.1016/B978-0-12-814853-2.00009-6

Khamtib, S. and Reungsang, A., Biohydrogen production from xylose by Thermoanaerobacterium thermosaccharolyticum KKU19 isolated from hot spring sediment, *Int. J. Hydrogen Energy,* 37(17), 12219–12228 (2012). https://doi.org/10.1016/j.ijhydene.2012.06.038

Kim, D.-H. and Kim, M.-S., Hydrogenases for biological hydrogen production, *Bioresour. Technol.* 102(18), 8423–8431 (2011). https://doi.org/10.1016/j.biortech.2011.02.113

Ladole, M. R., Mevada, J. S. and Pandit, A. B., Ultrasonic hyperactivation of cellulase immobilized on magnetic nanoparticles, *Bioresour. Technol.,* 239, 117–126 (2017). https://doi.org/10.1016/j.biortech.2017.04.096

Li, W., Zhang, J., Yang, J., Zhang, J., Li, Z., Yang, Y. and Zang, L., Comparison of copper and aluminum doped cobalt ferrate nanoparticles for improving biohydrogen production, *Bioresour. Technol.,* 343, 126078 (2022). https://doi.org/10.1016/j.biortech.2021.126078

Lin, H.-N., Hu, B.-B. and Zhu, M.-J., Enhanced hydrogen production and sugar accumulation from spent mushroom compost by Clostridium thermocellum supplemented with PEG8000 and JFC-E, *Int. J. Hydrogen Energy,* 41(4), 2383–2390 (2016). https://doi.org/10.1016/j.ijhydene.2015.11.078

Lin, L., Han, X., Han, B. and Yang, S., Emerging heterogeneous catalysts for biomass conversion: studies of the reaction mechanism, *Chem. Soc. Rev.,* 50(20), 11270–11292 (2021). https://doi.org/10.1039/D1CS00039J

Liu, L., Fang, H., Wei, Q. and Ren, X., Extraction performance evaluation of amide-based deep eutectic solvents for carboxylic acid: Molecular dynamics simulations and a mini-pilot study, *Sep. Purif. Technol.,* 304, 122360 (2023). https://doi.org/10.1016/j.seppur.2022.122360

Liu, X., He, D., Wu, Y., Xu, Q., Wang, D., Yang, Q., Liu, Y., Ni, B.-J., Wang, Q. and Li, X., Freezing in the presence of nitrite pretreatment enhances hydrogen production from dark fermentation of waste activated sludge, *J. Clean. Prod.,* 248, 119305 (2020). https://doi.org/10.1016/j.jclepro.2019.119305

Liu, Y., Liu, J., He, H., Yang, S., Wang, Y., Hu, J., Jin, H., Cui, T., Yang, G. and Sun, Y., A Review of Enhancement of Biohydrogen Productions by Chemical Addition Using a Supervised Machine Learning Method, *Energies,* 14(18), 5916 (2021). https://doi.org/10.3390/en14185916

Markandan, K. and Chai, W. S., Perspectives on Nanomaterials and Nanotechnology for Sustainable Bioenergy Generation, *Materials (Basel),* 15(21), 7769 (2022). https://doi.org/10.3390/ma15217769

Martinez-Burgos, W. J., do Nascimento Junior, J. R., Medeiros, A. B. P., Herrmann, L. W., Sydney, E. B. and Soccol, C. R., Biohydrogen Production from Agro-industrial Wastes Using Clostridium beijerinckii and Isolated Bacteria as Inoculum, *BioEnergy Res.,* 15(2), 987–997 (2022). https://doi.org/10.1007/s12155-021-10358-1

Mishra, P., Thakur, S., Mahapatra, D. M., Wahid, Z. A., Liu, H. and Singh, L., Impacts of nano-metal oxides on hydrogen production in anaerobic digestion of palm oil mill effluent – A novel approach, *Int. J. Hydrogen Energy,* 43(5), 2666–2676 (2018). https://doi.org/10.1016/j.ijhydene.2017.12.108

Mohan, S., Mohankrishna, G., Reddy, S., Raju, B., Rao, K. and Sarma, P., Self-immobilization of acidogenic mixed consortia on mesoporous material (SBA-15) and activated carbon to enhance fermentative hydrogen production, *Int. J. Hydrogen Energy,* 33(21), 6133–6142 (2008). https://doi.org/10.1016/j.ijhydene.2008.07.096

Mohanraj, S., Anbalagan, K., Rajaguru, P. and Pugalenthi, V., Effects of phytogenic copper nanoparticles on fermentative hydrogen production by Enterobacter cloacae and Clostridium acetobutylicum, *Int. J. Hydrogen Energy,* 41(25), 10639–10645 (2016). https://doi.org/10.1016/j.ijhydene.2016.04.197

Mousavi, M. R., Ghasemi, S., Sanaee, Z., Nejad, Z. G., Mardanpour, M. M., Yaghmaei, S. and Ghorbanzadeh, M., Improvement of the microfluidic microbial fuel cell using a nickel nanostructured electrode and microchannel modifications, *J. Power Sources,* 437, 226891 (2019). https://doi.org/10.1016/j.jpowsour.2019.226891

Nagarajan, D., Lee, D.-J., Kondo, A. and Chang, J.-S., Recent insights into biohydrogen production by microalgae – From biophotolysis to dark fermentation, *Bioresour. Technol.,* 227, 373–387 (2017). https://doi.org/10.1016/j.biortech.2016.12.104

Nikolaidis, P. and Poullikkas, A., A comparative overview of hydrogen production processes, *Renew. Sustain. Energy Rev.,* 67, 597–611 (2017). https://doi.org/10.1016/j.rser.2016.09.044

Oey, M., Sawyer, A. L., Ross, I. L. and Hankamer, B., Challenges and opportunities for hydrogen production from microalgae, *Plant Biotechnol. J.,* 14(7), 1487–1499 (2016). https://doi.org/10.1111/pbi.12516

Pandey, A., Gupta, K. and Pandey, A., Effect of nanosized TiO2 on photo-fermentation by Rhodobacter sphaeroides NMBL-02, *Biomass and Bioenergy,* 72, 273–279 (2015). https://doi.org/10.1016/j.biombioe.2014.10.021

Patel, S. K. S., Lee, J.-K. and Kalia, V. C., Nanoparticles in Biological Hydrogen Production: An Overview, *Indian J. Microbiol.,* 58(1), 8–18 (2018). https://doi.org/10.1007/s12088-017-0678-9

Plangklang, P., Reungsang, A. and Pattra, S., Enhanced bio-hydrogen production from sugarcane juice by immobilized Clostridium butyricum on sugarcane bagasse, *Int. J. Hydrogen Energy,* 37(20), 15525–15532 (2012). https://doi.org/10.1016/j.ijhydene.2012.02.186

Poleto, L., Souza, P., Magrini, F. E., Beal, L. L., Rodrigues Torres, A. P., Paula de Sousa, M., Laurino, J. P. and Paesi, S., Selection and identification of microorganisms present in the treatment of wastewater and activated sludge to produce biohydrogen from glycerol, *Int. J. Hydrogen Energy,* 41(7), 4374–4381 (2016). https://doi.org/10.1016/j.ijhydene.2015.06.051

Policastro, G., Luongo, V., Frunzo, L. and Fabbricino, M., A comprehensive review of mathematical models of photo fermentation, *Crit. Rev. Biotechnol.,* 41(4), 628–648 (2021). https://doi.org/10.1080/07388551.2021.1873241

Pugazhendhi, A., Shobana, S., Nguyen, D. D., Banu, J. R., Sivagurunathan, P., Chang, S. W., Ponnusamy, V. K. and Kumar, G., Application of nanotechnology (nanoparticles) in dark fermentative hydrogen production, *Int. J. Hydrogen Energy,* 44(3), 1431–1440 (2019). https://doi.org/10.1016/j.ijhydene.2018.11.114

Ramprakash, B. and Muthukumar, K., Influence of sulfuric acid concentration on biohydrogen production from rice mill wastewater using pure and coculture of Enterobacter aerogenes and Citrobacter freundii, *Int. J. Hydrogen Energy,* 43(19), 9254–9258 (2018). https://doi.org/10.1016/j.ijhydene.2018.03.198

Rashid, N., Lee, K. and Mahmood, Q., Bio-hydrogen production by Chlorella vulgaris under diverse photoperiods, *Bioresour. Technol.,* 102(2), 2101–2104 (2011). https://doi.org/10.1016/j.biortech.2010.08.032

Salame, P. H., Pawade, V. B. and Bhanvase, B. A., Characterization Tools and Techniques for Nanomaterials, *Nanomater. Green Energy*, 83–111 (2018). https://doi.org/10.1016/B978-0-12-813731-4.00003-5

Sambusiti, C., Bellucci, M., Zabaniotou, A., Beneduce, L. and Monlau, F., Algae as promising feedstocks for fermentative biohydrogen production according to a biorefinery approach: A comprehensive review, *Renew. Sustain. Energy Rev.,* 44, 20–36 (2015). https://doi.org/10.1016/j.rser.2014.12.013

Sanusi, I. A., Suinyuy, T. N. and Kana, G. E. B., Impact of nanoparticle inclusion on bioethanol production process kinetic and inhibitor profile, *Biotechnol. Reports,* 29, e00585 (2021). https://doi.org/10.1016/j.btre.2021.e00585

Sarangi, P. K. and Nanda, S., Biohydrogen Production Through Dark Fermentation, *Chem. Eng. Technol.,* 43(4), 601–612 (2020). https://doi.org/10.1002/ceat.201900452

Saravanan, A., Kumar, P. S., Mat Aron, N. S., Jeevanantham, S., Karishma, S., Yaashikaa, P. R., Chew, K. W. and Show, P. L., A review on bioconversion processes for hydrogen production from agro-industrial residues, *Int. J. Hydrogen Energy,* 47(88), 37302–37320 (2022). https://doi.org/10.1016/j.ijhydene.2021.08.055

Sekoai, P. T., Ouma, C. N. M., du Preez, S. P., Modisha, P., Engelbrecht, N., Bessarabov, D. G. and Ghimire, A., Application of nanoparticles in biofuels: An overview, *Fuel,* 237, 380–397 (2019). https://doi.org/10.1016/j.fuel.2018.10.030

Senthil Rathi, B., Senthil Kumar, P., Rangasamy, G. and Rajendran, S., A critical review on Biohydrogen generation from biomass, *Int. J. Hydrogen Energy,* 52, 115–138 (2024). https://doi.org/10.1016/j.ijhydene.2022.10.182

Singh, N. and Sarma, S., Biological routes of hydrogen production: a critical assessment, *Handb. Biofuels*, 419–434 (2022). https://doi.org/10.1016/B978-0-12-822810-4.00021-X

Srivastava, N., Srivastava, M., Manikanta, A., Singh, P., Ramteke, P. W. and Mishra, P. K., Nanomaterials for biofuel production using lignocellulosic waste, *Environ. Chem. Lett.,* 15(2), 179–184 (2017). https://doi.org/10.1007/s10311-017-0622-6

Srivastava, R. K., Shetti, N. P., Reddy, K. R. and Aminabhavi, T. M., Biofuels, biodiesel and biohydrogen production using bioprocesses. A review, *Environ. Chem. Lett.,* 18(4), 1049–1072 (2020). https://doi.org/10.1007/s10311-020-00999-7

Tang, G.-L., Huang, J., Sun, Z.-J., Tang, Q.-Q., Yan, C.-H. and Liu, G.-Q., Biohydrogen production from cattle wastewater by enriched anaerobic mixed consortia: Influence of fermentation temperature and pH, *J. Biosci. Bioeng.,* 106(1), 80–87 (2008). https://doi.org/10.1263/jbb.106.80

Vaghari, H., Jafarizadeh-Malmiri, H., Mohammadlou, M., Berenjian, A., Anarjan, N., Jafari, N. and Nasiri, S., Application of magnetic nanoparticles in smart enzyme immobilization, *Biotechnol. Lett.,* 38(2), 223–233 (2016). https://doi.org/10.1007/s10529-015-1977-z

Varanasi, J. L., Veerubhotla, R., Pandit, S. and Das, D., Biohydrogen Production Using Microbial Electrolysis Cell, In: Microbial Electrochemical Technology. Elsevier, 843–869 https://doi.org/10.1016/B978-0-444-64052-9.00035-2

Venkata Mohan, S., Lalit Babu, V., Srikanth, S. and Sarma, P. N., Bio-electrochemical evaluation of fermentative hydrogen production process with the function of feeding pH, *Int. J. Hydrogen Energy,* 33(17), 4533–4546 (2008). https://doi.org/10.1016/j.ijhydene.2008.05.073

Wang, S., Ma, Z., Zhang, T., Bao, M. and Su, H., Optimization and modeling of biohydrogen production by mixed bacterial cultures from raw cassava starch, *Front. Chem. Sci. Eng.,* 11(1), 100–106 (2017). https://doi.org/10.1007/s11705-017-1617-3

Wang, Y. and Xia, Y., Bottom-Up and Top-Down Approaches to the Synthesis of Monodispersed Spherical Colloids of Low Melting-Point Metals, *Nano Lett.,* 4(10), 2047–2050 (2004). https://doi.org/10.1021/nl048689j

Wimonsong, P. and Nitisoravut, R., Comparison of Different Catalysts for Fermentative Hydrogen Production, *J. Clean Energy Technol.* 3(2), 128–131 (2015). https://doi.org/10.7763/JOCET.2015.V3.181

Xiang, W., Zhang, Y., Lin, H. and Liu, C., Nanoparticle/Metal–Organic Framework Composites for Catalytic Applications: Current Status and Perspective, *Mol.,* 22(12), 2103 (2017). https://doi.org/10.3390/molecules22122103

Yang, G. and Wang, J., Improving mechanisms of biohydrogen production from grass using zero-valent iron nanoparticles, *Bioresour. Technol.,* 266, 413–420 (2018). https://doi.org/10.1016/j.biortech.2018.07.004

Yazdani, M., Ebrahimi-Nik, M., Heidari, A. and Abbaspour-Fard, M. H., Improvement of biogas production from slaughterhouse wastewater using biosynthesized iron nanoparticles from water treatment sludge, *Renew. Energy,* 135, 496–501 (2019). https://doi.org/10.1016/j.renene.2018.12.019

Yildirim, O. and Ozkaya, B., Enhancing fermentation yield for biohydrogen production using eco-friendly nickel and cobalt ferrite nanoparticles, Biomass Convers Biorefinery. https://doi.org/10.1007/s13399-024-05354-2

Zhang, J., Fan, C., Zhang, H., Wang, Z., Zhang, J. and Song, M., Ferric oxide/carbon nanoparticles enhanced bio-hydrogen production from glucose, *Int. J. Hydrogen Energy,* 43(18), 8729–8738 (2018). https://doi.org/10.1016/j.ijhydene.2018.03.143

Zhang, Y. and Shen, J., Enhancement effect of gold nanoparticles on biohydrogen production from artificial wastewater, *Int. J. Hydrogen Energy,* 32(1), 17–23 (2007). https://doi.org/10.1016/j.ijhydene.2006.06.004

Zhao, W., Zhang, Y., Du, B., Wei, D., Wei, Q. and Zhao, Y., Enhancement effect of silver nanoparticles on fermentative biohydrogen production using mixed bacteria, *Bioresour. Technol.,* 142, 240–245 (2013). https://doi.org/10.1016/j.biortech.2013.05.042

Zhao, Y. and Chen, Y., Nano-TiO 2 Enhanced Photofermentative Hydrogen Produced from the Dark Fermentation Liquid of Waste Activated Sludge, *Environ. Sci. Technol.,* 45(19), 8589–8595 (2011). https://doi.org/10.1021/es2016186