

Sustainable Approaches for Biohydrogen Production Technology

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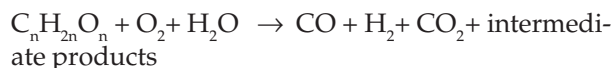
ABSTRACT

The excessive burning of fossil fuels causes environmental pollution by releasing carbon dioxide, which ultimately causes global warming. There is an urgent need for alternative sustainable fuels which give higher energy yields, are pollution-free and are renewable. The standard hydrogen production methods are the electrolysis of water and steam reformation of natural gas. However, the biological production of hydrogen proved to be safer and more renewable over thermochemical and electrochemical methods. The biological methods are a good alternative in which microorganisms play an essential role by consuming the carbon sources from the biomass resulting in the production of hydrogen in return. Biohydrogen is an alternative to fossil fuels, is clean, has high-energy content per unit of weight, and has zero greenhouse gas emissions. Hydrogen, an energy source derived from industrial and agricultural waste, can resolve sustainability issues, environmental carbon emissions, and energy security.

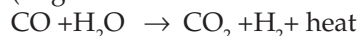
Key words: Agricultural waste, Biohydrogen, Dark fermentation, Photolysis, Sustainable production

Introduction

Biomass, like agricultural waste, forest residues, organic municipal waste, and animal waste, can produce hydrogen and other intermediate products through gasification. Gasification is a technique that converts fossil-based carbonaceous content at high temperatures in the presence of a sufficient amount of oxygen or steam into CO, H₂, and CO₂ without combustion. CO reacts with H₂O to form CO₂ and hydrogen via a water gas shift reaction.



(Organic carbonaceous source)



Biomass can be converted into liquid fuels like methanol, ethanol, biodiesel, and pyrolysis oil,

which can be further converted into hydrogen (Turner *et al.*, 2008).

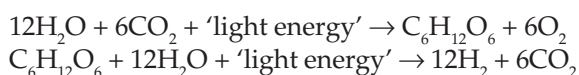
Biological Production of Hydrogen

The sustainable or environment-friendly process of producing biohydrogen can be categorized into various methods:

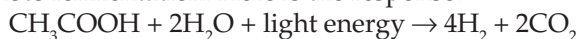
Direct and Indirect photolysis: According to Hallenbeck and Benemann (2002), this approach is founded on the idea of using sunlight as the primary source for producing hydrogen through various photosynthetic processes. It is common practice to break down water molecules into hydrogen ions and oxygen through photosynthesis, which are then transformed into hydrogen gas by the hydrogenase enzyme, using green algae like *Chlamydomonas reinhardtii*, *Scenedesmus* sp., etc.

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For optimum biohydrogen generation, several physical aspects must be tuned, including bioreactor design and cost, and chemical aspects, like hydrogenase enzyme reaction, O₂ sensitivity, solar conversion efficiency, and H₂ synthesis processes. The separation of the H₂ and O₂ evolution processes into separate stages, connected by CO₂ fixation/evolution, is necessary for indirect biophotolysis to function well (Prince and Kheshgi, 2005). Enzymes like nitrogenase and hydrogenase also enhance the hydrogen production rate. The various reactions of hydrogen production in cyanobacteria are as follows:



2. Photo and Dark fermentation: Nitrogenase in purple non-sulphur bacteria increases the rate of hydrogen synthesis utilizing light energy and organic acids in nitrogen-deficient environments. The importance of photosynthetic bacteria or algae in photo fermentation. Here is the response:



Dark fermentation: Various substrates, including carbohydrates, glucose, organic compounds, polymers (starch, cellulose), and algal biomass, etc., are utilized in this technique as an energy source without the need for light energy (Balachandar *et al.*, 2013). Various anaerobic species like *Enterobacter*, *Bacillus*, and *Clostridium* can produce hydrogen by consuming various carbon sources, especially glucose, in the dark. Role of strict or facultative anaerobic bacteria is significant in dark fermentation (Khanna and Das, 2012; Sarangi and Nanda, 2020). Firstly, the organic polymers are hydrolysed into monomers, which further face acetogenic conversion into organic acids, alcohols, and the release of hydrogen. The production of hydrogen depends on various factors like temperature, pH, hydraulic retention time (HRT), and gas partial pressure, which affect metabolic balance (Mahata and Das, 2022; Maman *et al.*, 2022). Sugars and carbohydrate-based biomass are the most efficient feedstock for biohydrogen production through dark fermentation (Ntaikouet *et al.*, 2010).

Temperature: One crucial factor for the fermentation process' optimal synthesis of biohydrogen is temperature (Saidi *et al.*, 2020). Because most of the organisms, including *Clostridium*, *Enterobacter* and *Bacillus sp.* thrive in the mesophilic range (20-42°C)

of temperature and are efficient in producing hydrogen, the majority of hydrogen is produced in the range between 20 and 45°C (Wong *et al.*, 2014).

pH: Another crucial element affecting how effectively biohydrogen fermentation works is pH. If the pH is not ideal, the microbial population will change, which eventually impacts hydrogen production (Van *et al.*, 2001). The highest amount of biohydrogen could be produced from wastewater seed sludge under optimal conditions, which included a pH of 5.5 and a temperature of 37°C.

Substrate: In comparison to protein-based substrates, carbohydrate-based substrates are more effective at producing biohydrogen (Hussy *et al.*, 2000). During fermentative hydrogen production, the substrate concentration directly correlates to the ability of hydrogen-producing bacteria to produce more hydrogen up to a point but further increases in substrate concentrations may slow down hydrogen production (Zhu and Béland, 2006; Lo *et al.*, 2008).

Carbon source: Various carbon sources like glucose, xylose, mannose, cellulose, maltose, fructose and dextran can be efficiently used to produce hydrogen. Glucose and starch are the most widely used carbon sources for fermentative hydrogen production (Liu and Shen, 2004).

Organic Wastes as a Fermentable Substrate: Waste management is the primary concern for human health and environmental sustainability as urbanization and industrialization progress (Liu *et al.*, 2020). The organic portion derived from plants or animals including industrial waste, sewage sludge, solid waste, agricultural residues, and poultry waste is readily biodegradable and can be converted to CO₂, methane, or simple organic molecules (Kapdan and Kargi, 2006; Nam *et al.*, 2016). Agricultural wastes, including rice, wheat, and maize straw; animal manure, industrial effluents like distillery wastewater, dairy waste, cheese whey effluent, palm oil mill effluent, food waste, municipal sewage waste and sewage sludge are some of the most often used organic wastes (Chong *et al.*, 2009).

Nutrients: Adding nutrients to promote bacterial growth in the presence of a carbon source is another significant factor in the rise of H₂ generation. An ideal phosphorous concentration is required since a larger concentration will encourage an excessive formation of volatile fatty acids, which will reduce hydrogen yield by diverting cellular reductants from

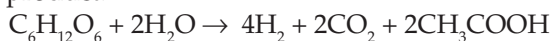
the production of H_2 . In addition, activating several enzymes and coenzymes requires the presence of appropriate metal ions, which improves microbial metabolism and cellular transport activities and promotes cell growth.

Pretreatment of substrate: Pretreatment of some substrates is essential due to their complex structures (Salem *et al.*, 2018). After being pre-treated, they can be used by hydrogen-producing bacteria for fermentative hydrogen production. Acidification of cornstalk waste resulted in higher hydrogen yield than non-treated waste. Various methods of pre-treatments, such as ultrasonication, acid/alkali/steam treatments, enzymatic hydrolysis, freezing and thawing, sterilization, and microwave, proved to be highly efficient in increasing the fermentative production of hydrogen (Wang *et al.*, 2003; Ting and Lee, 2007).

Hydraulic Retention Time (HRT): HRT is the amount of time the feedstock used to produce biohydrogen should be exposed to the inoculum in the bioreactor for a sufficient reaction and subsequent biohydrogen generation. HRT is crucial in boosting the output of biohydrogen from anaerobic fermentation. The best HRT for biohydrogen production varies depending on the type of substrate and the bioreactor being utilized, but generally speaking, the optimal HRT is 8 to 12 hours (Hawkes *et al.*, 2002).

Configuration of bioreactor: The basic aspects of a bioreactor, like dimensions, design, cost, and operational parameters, must be considered before using the bioreactor for hydrogen production (Mudhoo *et al.*, 2011). Microbial metabolism, configuration, form of energy supplied to the reactor, organic loading rates, and operational cost are the major contributors to the effective use of the reactor.

Integrated dark and photo fermentation approach: Theoretically, photo fermentation uses photons as the energy source to make 12 moles of hydrogen for every mole of glucose. However, in the dark fermentation, when there is no external energy source, the oxidation of glucose by fermentative bacteria produced additional byproducts as well, with a maximum of 4 moles of hydrogen being created per mole of glucose consumed and acetate being the only byproduct.



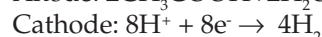
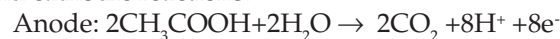
Photosynthetic bacteria can oxidize the acetate produced during the dark-fermentation stage to

produce hydrogen.



Hence, combining both dark fermentation and photo fermentation techniques will enable continuous hydrogen production at maximal yield.

Biocatalyzed electrolysis: Instead of using solar energy, another method of oxidizing the acetate to make hydrogen uses external electrical energy (Singh *et al.*, 2015). In this method, an electrolyzer cell's anodic compartment is formed by a bioreactor containing acetate, and the protons and electrons created by the bacteria are collected at the cathode, a platinum electrode that is used to catalyze the hydrogen evolution reaction. The following are anodic and cathodic reactions:



Types of waste used for sustainable hydrogen production

The accessibility, economic viability, carbohydrate content, and biodegradability of waste materials are the primary determining factors in their selection for bio-hydrogen production. According to Chong *et al.* (2009) and Mudhoo *et al.* (2011), simple sugars like glucose, sucrose, and lactose are rapidly biodegradable and the ideal substrates for hydrogen production. The following are the primary wastes that can be used to produce hydrogen gas:

Food industry waste

The wastewater of food processing industries and domestic waste contains high concentrations of carbohydrates and proteins (Ginkel *et al.*, 2005). More content of carbohydrates in waste resulted in more anaerobic hydrogen production. Starch-containing solid wastes are easier to degrade for biohydrogen production (Van *et al.*, 2005). Starch can be converted to glucose and maltose by enzymatic or acid hydrolysis, then converted to organic acids and hydrogen gas, respectively (Kapdanand Kargi, 2006). Several types of food waste, including starch waste water, waste from processing apples and potatoes, rice slurry, cheese whey, molasses, and wastewater from sugar refineries, are effective for producing hydrogen.

Agricultural and woody waste

Before being used to produce fermentative hydrogen, agricultural and woody waste containing cellu-

lose, hemicellulose, and lignin must first undergo pre-treatment. The effectiveness of enzymatic hydrolysis of agricultural wastes is inversely correlated with the amount of lignin present in the waste (Sarangi *et al.*, 2023). After pre-treatment, the hexoses and pentose sugars included in the lignocellulosic waste can be effectively used for energy production. The most prevalent type of organic waste in nature, lignocellulosic waste, is found in grasses, hardwoods, softwoods, and agricultural waste.

Cheese and Dairy waste

Lactose in large quantities in cheese whey can be fermented and used to produce hydrogen. For optimal hydrogen production, various researches suggested keeping the food-to-microbe (F/M) ratio at 1.0 to 1.5. Microbes like *Clostridium thermolacticum*, and *Lactobacillus sp* can be cultured and efficiently utilized continuously for maximum hydrogen production. The optimum pH is in the mild acidic range.

Oil waste

When animal or vegetable fats are transesterified to make biodiesel, glycerol is produced (Chong *et al.*, 2009). Diverse researchers used an immobilized bioreactor packed with polyurethane to study the anaerobic treatment of glycerol at thermophilic and mesophilic temperatures utilizing native hydrotropic bacteria for hydrogen production. Ito *et al.*, (2005) used *Enterobacter aerogenes* HU-101 to study the synthesis of hydrogen and ethanol from waste released after biodiesel production.

Brewery wastewater

After being pre-treated to eliminate any undesired components and to balance the nutrients, the effluent discharged by the dairy sector, olive mill, baker's yeast, and breweries can be used as the raw material to manufacture bio-hydrogen. Carbohydrate-rich food sector effluents can be further treated to convert the carbohydrate content to organic acids and subsequently to hydrogen gas by using two-stage anaerobic dark and photo-fermentation.

Waste sludge from effluent treatment plants

Large amounts of proteins and carbohydrates found in the sewage sludge produced in wastewater treatment plants can be used to produce energy in the form of methane or hydrogen gas (Wong *et al.* 2014). Sludge surplus can be digested anaerobically in two

phases. In the first process (the acidogenic phase), organic matter will be transformed into organic acids. In the second step, photo-heterotrophic bacteria use organic acids to produce hydrogen gas (Chang *et al.*, 2011).

Hydrogen gas production from water by algae

Through photosynthesis, algae divide water molecules into hydrogen ions and oxygen. Algal species that produce hydrogen include *Chlamydomonas reinhardtii*, *Scenedesmus obliquus*, *Chlorococcum littorale*, *Playtmonas subcordiformis*, and *Chlorella fusca* all exhibit hydrogenase activity.

Anabaena sp., *Oscillatoria sp.*, *Calothrix sp.*, and other nitrogen-fixing as well as non-nitrogen-fixing species like *Synechococcus sp.* and *Gloeobacter sp.*, are involved in the synthesis of hydrogen gas from cyanobacteria.

Conclusion

The present review detailed various biological methods, their mechanism, and also significance over other physiochemical methods for biohydrogen production. Also highlighted are the aspects including optimization of factors for an effective sustainable hydrogen production. The review paper details the primary substrates or waste products that can be economically and effectively utilized to manufacture sustainable hydrogen.

Conflict of Interest - None

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