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Review on Bio-hydrogen Production Methods

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Abstract: Hydrogen is a promising replacement for fossil fuels as a long-term energy source. It is a clean, recyclable, high efficient nature and environmentally friendly fuel. Hydrogen is now produced mostly using water electrolysis and natural gas steam reformation. However, biological hydrogen production has substantial advantages over thermochemical and electrochemical. Hydrogen can be produced biologically by bio-photolysis (direct and indirect), photo fermentation, dark fermentation. The methods for producing biological hydrogen were studied in this study.
Keywords: Biological hydrogen, steam reformation, bio-photolysis, photo-fermentation, dark fermentation

I. INTRODUCTION

In recent years, the provision of high energy demands, as well as serious environmental challenges like greenhouse gas emissions and global warming, need the use of clean, renewable, and long-term energy sources [1]. Among various alternative clean fuels, hydrogen is a promising green fuel that is accepted as an environmentally safe, renewable, and high energy yield (122 kJ g^{-1}) [2]. Hydrogen is one of the foremost abundant elements within the universe in its ionic form. It is an odorless, colorless, tasteless, and non-poisonous gas. When hydrogen is employed as a fuel, it generates no pollutants but produces water [3]. It also contents the highest energy per unit weight of any known fuel [4]. Hydrogen has the calorific value (higher heating value) of 141 MJ kg^{-1}, which is the highest of all the known commercial fuels. But the lower heating value (120 MJ kg^{-1}) is considered, 1 kg of hydrogen is equivalent to about 2.75 kg of gasoline [5]. Hydrogen is expected to have about 11% of the total renewable energy share of 36% by 2025 and up to 34% of the total renewable energy share of 69% by 2050 [6]. Hydrogen can be generated by thermochemical, electrochemical, and biological processes [2]. Both thermochemical and electrochemical processes are non-renewable in nature and depend on fossil fuels [7]. These methods not only consume energy but pollute the environment, so the depletion of fossil fuels has led the researcher to find renewable and non-polluting energy resources [8].

Biological hydrogen production is a promising alternative approach for the production of fuel from low cost, renewable, environmentally friendly resources, and non-polluting in nature [9]. Globally, the demand for hydrogen is increasing so the use of organic wastes as a substrate it is a renewable way for the production [10]. As cost of the substrate is a major factor in the economics of bio-hydrogen production, it is necessary to use less expensive and abundant feedstocks to keep the process affordable. Organic waste from industry and agriculture is not only used to generate green energy, but it also aids in bioremediation [11]. Lignocellulosic biomass, which makes up a major portion of municipal, industrial, and agricultural waste, is both renewable and inexpensive, making it ideal for biofuel generation [12]. The utilization of organic waste as a substrate to generate hydrogen provides a simultaneous solution of waste treatment along with clean energy generation [13-14]. Currently, biological hydrogen can be produced through different metabolic pathways, including direct
water bio-photolysis by green algae, indirect water bio-photolysis by cyanobacteria, photo-fermentation by sulfur-free purple photosynthetic bacteria, water gas exchange reaction to synthesize hydrogen by microalgae, and heterotrophic anaerobic bacteria by dark fermentation [15-16].

At present, there are numerous studies on several aspects of biological hydrogen production. Tami et al. [17] reviewed bio-hydrogen production by dark fermentative bacteria using starch-containing waste as a substrate. Vinod et al. [18] published a review on bio-hydrogen production from waste materials. Prakash et al. [19] reviewed bio-hydrogen production through dark fermentation.

However, a review article on bio-hydrogen production methods is limited in the literature. Based on these facts, the objective of this paper is to present an overview of biological hydrogen production methods by using organic waste biomass.

II. BIOLOGICAL HYDROGEN PRODUCTION METHODS

Bio-hydrogen production methods can be classified as

A. Direct bio-photolysis

This method is analogous to the processes found in plants and algal photosynthesis. In this process, solar energy is directly converted to H_2 via photosynthetic reactions.

$2H_2O$ + light energy → $2H_2 + O_2$

1) *Organisms:* Cyanobacteria and green algae are two types of organisms that aid in the generation of hydrogen through direct bio-photolysis [20]. Algae use photosynthesis to split water molecules into hydrogen ions and oxygen. The generated H^+ is converted into hydrogen gas by the hydrogenase enzyme. *Chlamydomonas reinhardtii* is one of the best-known hydrogen-producing algae [15].
2) *Advantage:* The advantage of this method is that the primary feed is water, which is cheap and almost universally available (Table 1).
3) *Disadvantages:* Three characteristics that restrict hydrogen production by direct photolysis using green algae: (i) Solar conversion efficiency of photosynthetic system; (ii) H_2 synthesis processes (i.e. the necessity to separate the processes of H_2O oxidation and H_2 synthesis); and (iii) bioreactor design and cost [21].

The simultaneous synthesis of hydrogen and molecular oxygen in direct bio-photolysis is a disadvantage that has resulted in the inhibition of hydrogen-related processes ranging from gene expression to hydrogenase catalytic activity. While constant purging of an inert gas (argon) to remove the produced oxygen could be a viable option, it is not cost-effective for long-term large-scale hydrogen generation. Several methods for improving H_2 generation by green algae are currently under investigation.

B. Indirect Bio-photolysis

In indirect bio-photolysis, involves the separation of H_2 and O_2 evolution reactions into different stages, which are then linked via CO_2 fixation and evolution. Cyanobacteria have unique characteristics in that they use CO_2 in the air as a carbon source and solar energy. CO_2 is first taken by the cells to form cellular components, which are then used to produce hydrogen. The reactions below describe the general mechanism of hydrogen generation in cyanobacteria:

$12H_2O + 6CO_2$+ light energy → $C_6H_{12}O_6 + 6O_2$

$C_6H_{12}O_6 + 12H_2O$ + light energy → $12H_2 + 6CO_2$

Key enzymes (nitrogenase and hydrogenase) in cyanobacteria carry out metabolic functions to generate hydrogen [22].

1) *Advantage:* One of the benefits of indirect bio-photolysis is that it requires minimal nutrients. However, adding trace metals like Ni^{2+} to the culture medium can help nitrogen-fixing cyanobacteria like *Nostoc muscorum* and *Anabaena cylindrical* grow faster and fix more CO_2 [23]. Anabaena species and strains have been studied extensively due to their greater rates of H_2 generation [21].

C. Fermentation

Fermentation is the process of generating energy by utilizing an endogenous electron acceptor derived from the oxidation of organic waste products by a variety of microorganisms. The fermentation process can be either aerobic or anaerobic in nature [24]. Organic waste materials can be fermented with microbes under anaerobic conditions to yield H_2 and various organic alcohols or acids as by-products. Bio-fermentation can be classified into two categories, (a) Photo fermentation and (b) Dark fermentation [25].

1) *Photo Fermentation:* Photo-fermentation produces hydrogen by decomposing organic acids with the help of light-dependent, sulphur, and non-sulphur purple bacteria. Purple sulphur bacteria refers to a group of bacteria that can perform photosynthesis. Photobacteria are purple non-sulphur bacteria, which produces H_2 mainly due to the presence of nitrogenase under nitrogen-deficient conditions which uses light energy and reduced compounds [4]. Photo fermentative bacteria produce H_2 and CO_2 by oxidizing organic acids such as acetic acid, propionic acid, butyric acid, lactic acid, and malic acid. As a result, the two-stage dark fermentation method is frequently followed by photo fermentation to obtain a larger H_2 yield [26]. The generation of ATP by photophosphorylation provides the energy required for microbial development [27].

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

$2CH_3COOH + 4H_2O \rightarrow 8H_2+ 4CO_2$

a) *Effect of Substrate:* Carbon source is one of the most crucial aspects of producing sustainable biofuels. The benefits of such a sustainable process with high efficiency, cost-effective, and minimal pollution are highly dependent on selecting an appropriate feedstock [28].
b) *Effect of Illumination:* The most significant factor in the photo-fermentation process is light; specifically, the intensity, sources, and distribution of light. Because the generation of ATP in photosynthetic organisms is a light-dependent process, making

efficient use of light is critical. In the nitrogen fixation process and subsequent hydrogen synthesis, a large amount of ATP is required to encourage electron transit [29].

c) *Effect of Trace Metals and Minerals:* Nitrogenase is an important enzyme in the metabolism of PNS bacteria. It is a binary enzyme and made up of two proteins: (i) a molybdenum and iron-containing protein (MoFe protein or dinitrogenase) and (ii) an iron-containing protein (Fe protein or dinitrogenase reductase). In the presence of both proteins, the nitrogenase complex is active [20]. The electron transfer chain (ETC) uses Fe ions as electron carriers, such as Fd and cytochromes. As a result, the availability of an adequate amount of molybdenum and iron in the medium is critical for photo-fermentation hydrogen production, particularly when wastewater is employed as a feedstock [30].

2) *Dark Fermentation:* Dark fermentation is one of the most well-known bio-hydrogen generation systems. H_2 can be produced by anaerobic bacteria grown in the dark on carbohydrate-rich substrates [31]. *Enterobacter, Bacillus,* and *Clostridium* species are found to generate hydrogen [21]. Carbohydrates, specifically glucose, are the preferred carbon sources for fermentation processes that produce acetic and butyric acids as well as hydrogen gas [32]. The theoretical output of hydrogen can be determined based on the microorganisms metabolic pathway and the initial sugar concentration in the fermentation medium. Hexose to acetic acid, hexose to butyric acid, and acetate to ethanol are three thermodynamically favorable dark fermentation metabolic routes for the conversion of organic substrates to bio-hydrogen [33-34]. Depending on the microorganisms and substrate, a mixed gas containing H_2 and CO_2 is produced, along with additional trace gases such as CH_4, CO, and H_2S [35-38]. Bacteria can convert glucose to pyruvic acid via glycolytic pathways by simultaneously generating ATP from ADP and NADH. With the help of pyruvate ferredoxin- oxidoreductase and hydrogenase, pyruvic acid is further converted to CO_2 and H_2. The level of bio-hydrogen generation can be determined by the conversion of pyruvate to acetyl-CoA and then to acetate, butyrate, and ethanol. The availability of hydrogen from glucose is determined by the ratio of butyrate to acetate. H_2 can be produced from acetic acid and butyric acid in Eq. (a) and Eq. (b) [39-40]. H_2 production from glucose is also represented in Eq. (c) and Eq. (d).

$$C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CH_3COOH + 2CO_2 \quad \text{(a)}$$
$$7C_6H_{12}O_6 + 6H_2O \rightarrow 24H_2 + 6CH_3CH_2CH_2COOH + 18CO_2 \quad \text{(b)}$$
$$C_6H_{12}O_6 + 4H_2O \rightarrow 2CH_3COO^- + 2HCO_3^- + 4H^+ + 4H_2 \quad \text{(c)}$$
$$C_6H_{12}O_6 + 12H_2O \rightarrow 6HCO_3^- + 12H_2 + 6H^+ \quad \text{(d)}$$

a) *Organisms:* Some of the bacterial communities which generate H_2 include anaerobes, such as Clostridia, methanogenic bacteria, and archaea, facultative anaerobes, such as *Enterobacter, Escherichia coli,* and *Citrobacter*, and some aerobes, such as *Alcaligenes* and *Bacillus*. Some other bacterial species that aid in hydrogen production belong to the group of *Bacillaceae*, Gram-positive cocci, such as *Micrococcaceae* and *Peptococcaceae.*

III. FACTORS INFLUENCING DARK FERMENTATION

Factors that influence the dark fermentation are temperature, pH, hydraulic retention time, volatile fatty acids, and partial pressure of H_2 and inorganic content.

A. *Effect of temperature*

The process temperature has a direct impact on bacterial growth and metabolic activity, as well as the rate of hydrogen production. Bacteria that produce hydrogen by dark fermentation include those that are mesophilic (25–40°C), thermophilic (40–65°C), extreme thermophilic (65–80°C), and hyper-thermophilic (>80°C). The temperature range of 35–55°C is ideal for dark fermentation processes. When bacterial species are exposed to high temperatures, they produce more bio-hydrogen than when they are exposed to low temperatures [41].

B. *Effect of pH*

The pH level has the potential effect to improve biohydrogen generation. Microorganisms enzymatic activity during the bioconversion process works best at a certain pH range. According to most experts, the pH level of 5.5 is ideal for biohydrogen generation [42-44].

C. *Effect of partial pressure*

The aspect that influences hydrogen generation is the partial pressure of hydrogen inside the bioreactor. When the partial pressure inside the bioreactor drops, the amount of hydrogen transferred from the liquid to the gas phase increases [45-46]. The activity of hydrogenase is influenced by the reversible oxidation-reduction of ferredoxin. As a result of the higher hydrogen content in the liquid phase, ferredoxin oxidation becomes unfavorable, lowering ferredoxin [47], and clearing the way for bio-hydrogen synthesis.

D. Effect of Hydraulic retention time

The hydraulic retention time (HRT) is another crucial factor in biohydrogen synthesis by dark fermentation. Short HRTs are used to clean up the methanogens in a typical continuous stirred-tank reactor (CSTR) system by choosing acid-producing bacteria [48]. Kim et al. suggest that [49], in a CSTR system, a short HRT of fewer than 3 days could enhance hydrogen synthesis. For hydrogen generation, simultaneous effects of pH and HRT are occasionally reported. In general, a short HRT can result in a low pH level during anaerobic activity [50]. The dilution rate, on the other hand, has an impact on the hydrolysis of organic wastes [51].

Table1. Advantages and disadvantages of different hydrogen production processes

Process	Advantages	Disadvantages
Direct bio- photolysis	H_2 may be produced directly from sunlight and water. In comparison to trees and crops, solar conversion energy rose ten folds.	A high-intensity light is required. The system can be harmed by O_2. Photochemical efficiency is reduced.
Indirect bio-photolysis	H_2 can be produced by cyanobacteria using water as a source.	To prevent H_2 degradation, uptake hydrogenase enzymes are to be removed. In a gas mixture around 30% O_2 is present
Photo-fermentation	In this, bacteria can utilize a broad spectrum of light energy. Various organic wastes can be used.	Nitrogenase is inhibited by the presence of oxygen. The effectiveness of light conversion is quite low, only 1–5%.
Dark fermentation	It can produce H_2 all day long without light. As substrates, a wide range of carbon sources can be employed. It produces useful metabolites such as butyric, lactic, and acetic acids as by-products. There is no O_2 is required, as it is an anaerobic process.	Hydrogenase is strongly inhibited by O_2. H_2 yields are feasible at a lower rate. H_2 fermentation becomes thermodynamically unfavorable as yields rise. CO_2 is present in the product gas combination, and it must be separated.

IV. CONCLUSIONS

For the future of the zero-carbon economy, hydrogen production through biological processes plays a significant role as it can utilize sustainable energy sources, and it will also contribute to the world's economy long-term viability by ensuring a steady supply of energy and reducing future greenhouse gases. This review study provides an overview of biological hydrogen production methods by using organic waste biomass. Among the various methods, In the photo fermentation process use of waste resources such as wastewater which is a suitable carbon source for PNS bacteria. This bacteria allows for long-term hydrogen synthesis from low-cost substrates and abundant solar energy. This will minimize the issues concerning with fossil fuel and ultimately benefits the environment from pollution.

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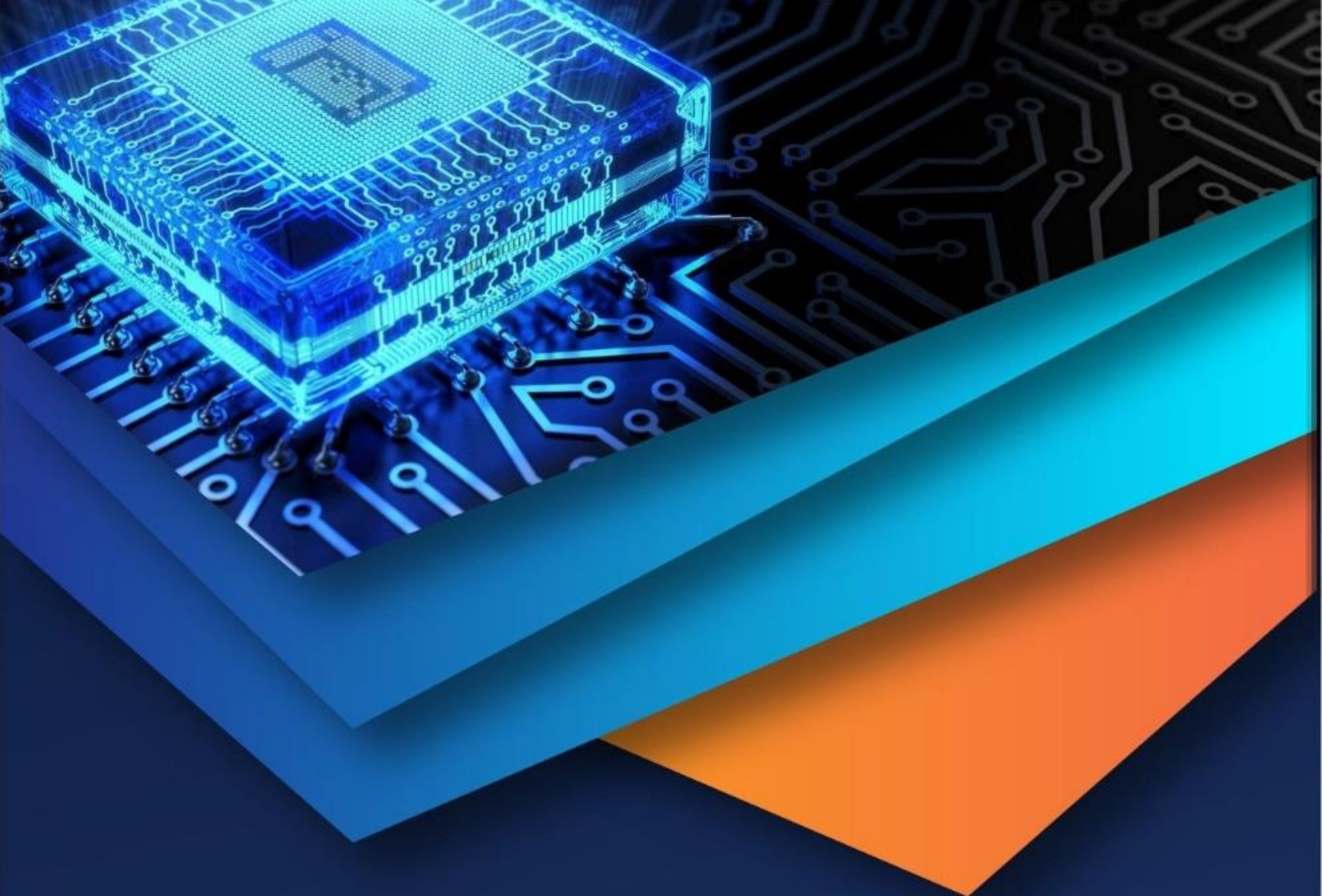

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