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Article

Biohydrogen Production from Organic Waste by Fermentation : A Review

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Abstract

Biohydrogen production from organic waste represents a promising renewable energy alternative that can contribute to reducing dependence on fossil fuels. Biohydrogen is an environmentally friendly energy carrier, as it produces no carbon emissions during utilization and possesses a high energy content. Fermentation-based processes have been extensively studied due to their ability to operate without light, under relatively mild temperature and pressure conditions, and to utilize a wide variety of organic wastes as substrates. The present review article deals with the production of biohydrogen from organic waste by different fermentation methods, emphasizing the characteristics of the feedstock and the effect of the operating parameters on the efficiency of the process. The literature review demonstrated that dark fermentation is a promising sustainable route for biohydrogen production. But the relatively low hydrogen yields and the need for strict operating conditions mean that further optimization is needed to apply this technology effectively on an industrial scale, particularly in terms of improving the yield efficiency and reducing operational expenses. This study seeks to identify and compare the operating conditions reported in the literature that correlate with higher biohydrogen yield.

Keywords: Biohydrogen, dark fermentation, organic waste, renewable energy

1. Introduction

Biohydrogen is a renewable energy source with significant potential to reduce dependence on fossil fuels due to its high energy density, environmentally friendly nature, and the absence of carbon emissions during its utilization as a fuel [1]. Based on the classification of biological pathways for biohydrogen production, as illustrated in Figure 1, biohydrogen can be generated through several major approaches, including fermentation, biophotolysis, and bioelectrochemical systems. Each pathway exhibits distinct mechanisms, operating condition requirements, and substrate utilization potentials. In fermentation-based processes, a wide range of carbohydrate-rich organic wastes can be utilized as substrates. However, the presence of lignocellulosic structures in many types of biomass often restricts microbial access to fermentable compounds. Therefore, biomass pretreatment methods, such as acid hydrolysis, alkaline treatment, or ultrasonic techniques, are required to enhance the availability of reducing sugars and improve fermentation efficiency [2]. In addition to biomass pretreatment, the selection and pretreatment of inoculum, for example, through heat-shock treatment, play a crucial role in suppressing hydrogen - consuming microorganisms and enhancing biohydrogen productivity [3].

In addition to substrate and inoculum characteristics, operational parameters such as pH, solid concentration, biomass concentration, and hydraulic retention time (HRT) play a crucial role in determining the success of the fermentation process. Recent studies have also highlighted the significant contribution of lactate-based metabolic pathways to enhanced biohydrogen production, particularly when fruit and vegetable wastes are used as substrates [4]. Overall, the integration of appropriate biohydrogen production pathways, effective pretreatment techniques, inoculum conditioning, and optimization of operating conditions enables the achievement of higher hydrogen yields. This approach not only enhances the feasibility of industrial-scale implementation but also supports the principles of a circular economy through the sustainable utilization of organic waste.

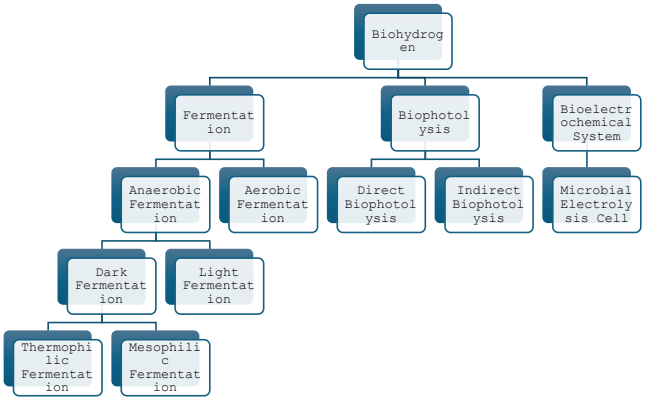

Fig. 1. Types of biohydrogen methods

2. Material and Method

2.1 Study of literature

In the present study, the approach of systematic literature review is utilized to collect and analyze the relevant scientific publications in the field of biohydrogen generation from organic waste using fermentation methods. The literature search was conducted in different academic databases such as Scopus, Google Scholar, and ScienceDirect. The search was performed by using keywords and their combinations, such as "biohydrogen production," "organic waste fermentation," "dark fermentation," "anaerobic digestion," and "hydrogen yield." The search was limited to the years 2014-2024 to include the latest advances in this field.

2.2 Data collection

To ensure quality and applicability, the literature was selected according to the following inclusion criteria : (1) Publications in peer-reviewed journals; (2) Study of the synthesis of biohydrogen from organic waste; and (3) Studies using fermentation methods, such as dark fermentation.

Exclusion criteria included: (1) work not involving hydrogen production, (2) incomplete or duplicate data, and (3) publications lacking experimental or quantitative data.

2.3 Data processing

The relevant facts systematically collected from the selected articles were the type of substrate (e.g., food waste or agricultural leftovers), the microbe used, the operating parameters (pH, temperature, and hydraulic retention time), and the hydrogen yield. The data were then classified and presented in a tabular form for easy comparison and interpretation.

2.4 Analysis and evaluation

This stage aims to critically analyze the processed data in order to obtain a comprehensive understanding, identify patterns or trends, and evaluate the relationships among the variables under investigation.

2.5 Compilation

This stage involves compiling and summarizing the results of the analysis to derive systematic and coherent conclusions in accordance with the research objectives.

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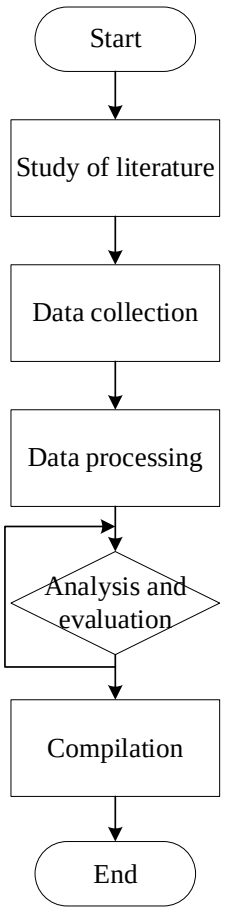

Fig. 2. Research block diagram.

3. Results and Discussion

3.1 Raw Material Preparation

Organic waste used as the feedstock generally contains major components such as carbohydrates, cellulose, hemicellulose, starch, and simple sugars, along with minor fractions of lignin and minerals. Prior to being introduced into the fermentation reactor, the feedstock undergoes a series of preparation steps, including cutting, chopping, and milling to obtain smaller and more uniform particle sizes. This size reduction stage aims to increase the surface area of the substrate, thereby enhancing the hydrolysis process and improving microbial access to organic components. Subsequently, the finely ground biomass is subjected to acid hydrolysis pretreatment, which involves treatment with dilute acid at elevated temperatures to disrupt the lignocellulosic structure and significantly increase the release of reducing sugars. The substrate preparation is then followed by a screening process to ensure that only uniformly sized particles are fed into the reactor.

In the meantime, the fermentation inoculum is subjected to heat-shock pretreatment, which entails exposure to high temperatures to render hydrogen-consuming microbes inactive without negatively impacting hydrogen-producing bacteria. One of the best methods for increasing substrate bioavailability and maximizing the activity of hydrogen-producing bacteria has been shown to be the combined use of acid hydrolysis pretreatment on the biomass and heat-shock pretreatment on the inoculum. After the pretreatment processes are completed, the substrate and inoculum are introduced into the fermentation reactor, which is maintained under anaerobic conditions [5].

3.2 Fermentation Process

The reactor feed is supplied with organic waste that has undergone size reduction and acid hydrolysis pretreatment to enhance the availability of fermentable sugars in the substrate. The reactor is then operated as a stirred anaerobic reactor, and inoculum that has been subjected to heat-shock pretreatment is added to ensure that only hydrogen-producing bacteria remain active during the fermentation process. Subsequently, nitrogen gas is introduced into the reactor to displace oxygen and maintain the anaerobic conditions required for the activity of hydrogen-producing microorganisms. Within the reactor, the organic waste is maintained at an operating temperature of approximately 35–55 °C with controlled pH, allowing the processes of hydrolysis, acidogenesis, and hydrogen formation to proceed optimally. The basic idea behind this fermentation process is that complex organic molecules are transformed by anaerobic bacteria's metabolic pathways into volatile fatty acids and hydrogen gas. The hydrogen gas produced is directed into a gas sampling bag, which is subsequently analyzed to determine the hydrogen concentration.

3.2.1 Anaerobic fermentation

Carbohydrates, proteins, and lipids are among the organic components of trash that can be valorized using an established biotechnology called anaerobic fermentation. Different microbial communities carry out the four different steps of this process, which include hydrolysis, acidogenesis, and acetogenesis [6]. Dark

fermentation and photofermentation are the two primary forms of anaerobic fermentation.

a. Dark fermentation

Dark fermentation is one of the biological routes for biohydrogen production where organic substrates are converted into hydrogen gas by anaerobic microorganisms in the absence of light [7]. This process is conducted in a closed reactor under moderate temperature conditions of approximately 30–55 °C and near-atmospheric pressure, with no presence of oxygen in the system. In addition to producing carbon dioxide and liquid byproducts like volatile fatty acids, dark fermentation focuses microbial metabolic pathways on producing hydrogen as the main product.

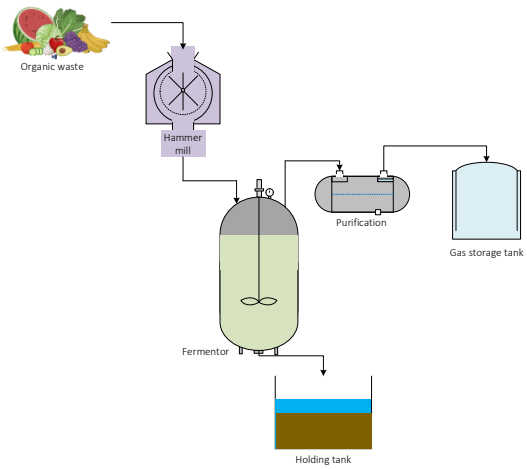

Fig. 3. A basic method of dark fermentation

According to the illustration of the dark fermentation process, biomass that has undergone size reduction and pretreatment is introduced into an anaerobic reactor. The substrate is then fermented by hydrogen-producing microorganisms under controlled temperature and pH conditions. Within the reactor, the biomass undergoes successive biodegradation stages, including hydrolysis, acidogenesis, and acetogenesis, resulting in the conversion of complex organic compounds into volatile fatty acids and hydrogen gas. The reactor effluent consists of gas, liquid, and solid phases [8]. The liquid fermentation effluent is subsequently directed to a gas–liquid separation unit to recover gas entrapped in the liquid phase. The generated gas is then conveyed to purification units to remove water vapor and other impurities, thereby obtaining hydrogen gas with higher purity. Meanwhile, the liquid and solid residues from fermentation are collected in a storage unit for substrate degradation evaluation or further utilization. The separated hydrogen gas is finally stored in gas sampling bags for compositional analysis and yield calculation, serving as the basis for evaluating the overall performance of the fermentation process.

The impact of temperature on the generation of biohydrogen shows that raising the fermentation temperature significantly increases the yield of biohydrogen. The enhanced metabolic activity of anaerobic hydrogen-producing bacteria in the mesophilic to early thermophilic temperature range is intimately linked to this phenomenon. At temperatures between 30℃ and approximately 45℃, the enzymatic activities involved in the hydrolysis and acidogenesis pathways reach near-optimal conditions, thereby promoting more efficient conversion of organic compounds into volatile fatty acids and hydrogen gas. Similar findings have been reported by [9] and [10], who identified temperature as a key parameter influencing both the rate and efficiency of biohydrogen production through dark fermentation.

b. Light fermentation

Photofermentation is another biological approach for biohydrogen production that utilizes light energy and photosynthetic bacteria to convert organic compounds into hydrogen. Photofermentation occurs under anaerobic conditions but requires illumination as the primary energy source to activate the nitrogenase enzyme, which plays a key role in hydrogen formation. In addition to substrate type, operating conditions such as light intensity and spectrum, pH, and nitrogen concentration strongly influence the performance of the photofermentation process. The work by [11] claims that photofermentation can produce more hydrogen than traditional anaerobic fermentation. Nevertheless, its industrial-scale application still faces several challenges, particularly related to light energy requirements, photobioreactor design, and long-term process stability, which hinder its economic viability and scalability in comparison to other hydrogen production methods. Therefore, advancing sustainable photofermentation technology requires the development of integrated systems, optimization of reactor design, and utilization of efficient light sources.

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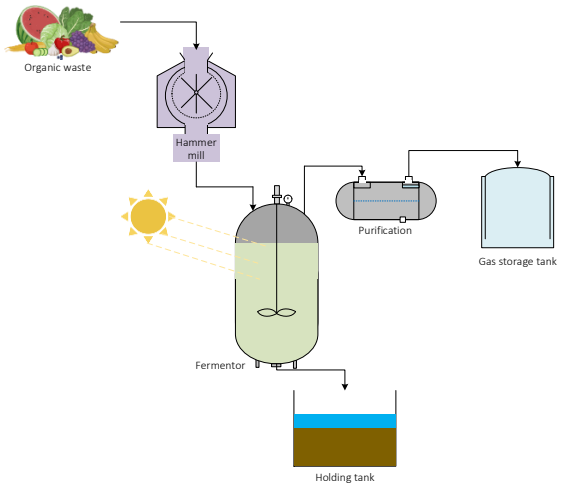

Fig. 4. A basic method of light fermentation

3.3 Aerobic fermentation

Aerobic fermentation is a biotechnological process that utilizes microbial activity under oxygenated conditions to decompose complex organic materials into simpler compounds. This process strongly depends on the availability of oxygen as the final electron acceptor, enabling faster and more stable degradation of organic matter, particularly in wastes with high organic content. According to a study by [12], aerobic fermentation of food waste dramatically modifies the biochemical properties of the substrate, as evidenced by changes in the microbial community structure and a decrease in the organic matter content. Aerobic bacteria play a crucial role in the initial stage of fermentation through the production of hydrolytic enzymes that accelerate the degradation of carbohydrates, proteins, and lipids. The dynamics of aerobic microbial communities are strongly influenced by oxygen availability and the initial composition of the substrate.

Parameters such as aeration rate, temperature, moisture content, and retention time largely govern the success of aerobic fermentation from an operational perspective [13]. Adequate aeration not only ensures sufficient oxygen supply for microbial metabolism but also assists in controlling reactor temperature due to the heat generated by microbial activity. However, excessive aeration may increase energy consumption and lead to substrate losses in the form of carbon dioxide. Overall, aerobic fermentation represents an effective approach for the treatment and stabilization of organic waste, offering advantages in terms of rapid degradation and process controllability. Although aerobic fermentation does not directly produce biohydrogen, it plays a strategic role as a preliminary waste treatment or biological pretreatment step that enhances substrate availability for subsequent processes and supports sustainable organic waste management [14].

Table 1. Type of Biohydrogen Production Method

Materials	Method	Variable	Microorganisms	H_2 produced	Reference
Pineapple and cassava peel waste	Dark fermentation	Inoculum, pH and Temperature	*Enterobacter Cancerogeous*	75,50 mL H_2/g VS	[1]
Wheat straw	Dark fermentation	Fermentation time and type of substrate	*Enterobacter Aerogenesis*	133,6 mL H_2/g TS	[2]
Grapes and coffee	Dark fermentation	pH and mixing speed	*Clostridium* and *Anaerobacter Polyendosporus*	86 mL H_2/g VS	[15]
Food waste, olive mill wastewater and rice Straw	Dark fermentation	pH and pretreatment	*Clostridium sp.*	60,6 mL H_2/g VS	[16]
Sugar cane vinasse	Dark fermentation	pH	*Clostridium sp.*	207,4 mL H_2/g VS	[17]
Cassava waste pulp	Dark fermentation	pH and HRT (hydraulic retention time)	*Anaerobic Thermophilic Bacteria*	12.39 mL H_2/g VS	[3]

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Agricultural waste	Dark fermentation	pH and Temperature (37℃)	*Clostridium sp.*	438 mL H_2/g VS	[18]
Potato	Thermophilic Fermentation	pH and temperature (25℃)	*Bacillus sp*	734.15 mL H_2/g VS	[19]
kitchen waste, food waste, vegetable waste, tea waste	Dark fermentation	Temperature (145-190℃)	*Clostridium sp. and Enterobacter sp.*	72 mL H_2 /g VS	[20]
Kitchen waste	Dark fermentation	Temperature (37℃) and mixing speed	*Clostridium sp.*	480 mL H_2/g VS	[21]
Dragon fruit waste	Anaerobic fermentation	pH	*Clostridium* and *methylothrophs*	4 mL H_2/g VS	[22]
Orange peel waste	Anaerobic fermentation	Organic Loading Rate (OLR, L/day)	*Clostridium sp.*	322,44 mL H_2/g VS	[23]
Soybean liquid waste	Dark fermentation	Temperature and liquid waste concentration	*Acetobacter sp.*	127,5 mL H_2/g VS	[24]
Melon peel waste	Dark fermentation	HRT (Hydraulic Retention Time)	*Enterobacter sp.*	50,41 mL H_2/ g VS	[25]
food waste	Dark fermentation	pH and temperature	*Clostridium sp.*	109,48 – 202,79 mL H_2/g VS	[26]
Vegetable and fruit waste	Dark fermentation	Temperature and pressure	*Cyanobacteria*	20.81 - 27.19 mL H_2/g VS	[27]
Molase	Dark fermentation and photo fermentation	pH and temperature	*Rhodobacter capsulatus*	11,207 mL H_2/L.hour	[28]
Orange peel waste	Photo fermentation	pH and substrate concentration	*Rhodobacter capsulatus*	126,5 mL H_2/g VS	[29]
agricultural waste	Dark fermentation	Inoculum	*Cellvibrio japonicus, Shewanella oneidensis, Sorangium cellulosum*	856,9 mL H_2/L	[30]
Food waste	Dark fermentation	Fermentation time	Anaerobic sludge	245,7 mL H_2/g glucosa	[31]
Agro industrial waste	Dark fermentation	Pretreatment inoculum	Anaerobic sludge	101 mL H_2/g VS	[34]
Watermelon peels	Dark fermentation	pH, yeast extrack and inoculum volume	*Clostridium butyricum*	4675,33 mL/L	[35]
Orange peels waste	Anaerobic fermentation	Fermentation time	*Enterobacter sp.*	1700 mL H_2/g VS	[36]
Banana peels	Anaerobic fermentation	pH and fermentation time	Activated sludge	15,32 mL H_2/g VS	[37]

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Fruit waste	Anaerobic fermentation	Fermentation time and type of substrate	*Clostridium sp.*	359,97 mL H_2/g VS	[38]
Fruit peels waste	Dark fermentation	Fermentation time	*Clostridium butyricum*	991 mL H_2/g VS	[39]
Vegetable waste	Dark fermentation	Kinds of vegetable and fermentation time	Anaerobic sludge	151,67 mL H_2/g VS	[40]

As seen in Table 1, biohydrogen yield varies greatly depending on the substrates, microorganisms, and operating conditions, from as low as 4 mL H_2/g VS to as high as 1700 mL H_2/g VS. Such a large difference suggests that the biohydrogen production performance is strongly influenced by several interacting factors and not only by the type of fermentation used. The yield of hydrogen primarily depends on the type and biodegradability of the substrate. Complex lignocellulosic substrates such as wheat straw and agricultural residues generally require pre-treatment to make the fermentable sugars more available. However, higher hydrogen yields are generally obtained due to the simpler carbohydrate composition of readily degradable substrates such as food waste or fruit residues. Another important factor is the type of microorganism. Mixed cultures (e.g., anaerobic sludge) may have lower yields due to competing metabolic pathways (e.g., methanogenesis or solventogenesis) but in general have higher adaptability and robustness under different conditions. However, it has been reported that controlled pure cultures such as *Clostridium sp.* and *Enterobacter sp.* were more efficient in producing hydrogen, and several reports showed yields over 200 mL H_2/g VS. However, pure cultures are more environmentally friendly. The hydrogen production is greatly influenced by the operational parameters such as temperature, pH, and hydraulic retention time (HRT). Most studies indicate that the mesophilic temperature (30-37°C) and slightly acidic conditions (pH 5–6) are the best conditions for hydrogen production. Higher yields in thermophilic systems are the result of higher metabolic rates and lower numbers of hydrogen-consuming bacteria. However, the studies reporting lower values than 50 mL H_2/g VS indicate the significant reduction in hydrogen production under unfavorable conditions. To make the comparison more objective, this review analyzes the performance of biohydrogen production using three main criteria: hydrogen yield, production conditions, and scalability potential. Dark fermentation has a broad range of performance in terms of yields but is known to reach moderate to high hydrogen yields (normally in the range of 50 to 500 mL H_2/g volatile solids) when optimized. However, processes such as photo-fermentation have a higher potential theoretical yield but are often limited by operational complexity and reliance on light. Dark fermentation offers significant benefits concerning the production rate and the simplicity of the process through the rapid reaction time, the utilization of diverse organic wastes, and the independence from light energy. Moreover, dark fermentation is considered more scalable for industrial applications due to its compatibility with existing anaerobic digestion systems and relatively simple reactor design. However, it should be noted that the statement about dark fermentation as the most promising approach, based on the data presented in Table 1, needs to be interpreted with caution. Although this method is of great potential in terms of operational feasibility and substrate versatility, the variability in the yield points out the importance of the optimization and standardization of the studies..

4. Conclusions

However, from the comparison of the reviewed studies, it can be concluded that there is not a single best method for biohydrogen production because the reported yields are completely unique (4 - 1700 mL H_2/g VS). However, dark fermentation seems very promising when considering the most important criteria, such as hydrogen yield, production efficiency, and scalability, especially when combined with appropriate pretreatment and inoculum heat shock strategies. It is claimed that the advantages of the method are shorter processing time, simplicity of operation, and flexibility in the use of different organic wastes. However, this process is highly substrate-,

microbial community-, and operational condition-dependent, and the studies provide a wide variety of hydrogen yields, such as the variations in temperature, pH, and nutrient availability, which significantly impact the results. Dark fermentation is a promising approach, but it cannot be generalized without process optimization, and further research is needed to improve yield consistency and scalability for industrial applications.

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