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Mini Review

The New Trends in the Production of Bio-Hydrogen

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Abstract

Photobiological hydrogen production is the most challenging research since the world's energy demand is increasing, fossil fuel resources are being reduced and the greenhouse that needs reduction of gas emissions is increasing. Hydrogen gas is a clean and efficient energy carrier that produces zero emissions and can be produced by managing renewable resources such as biomass and waste. Biological methods of hydrogen production are preferable to chemical methods because of the possibility of using sunlight. It is predicted that hydrogen gas will be effective not only in terms of being clean energy, but also in the establishment of extra- terrestrial life. However, the eco-friendly bacteria used for this purpose belongs to the non-sulfur photosynthetic bacteria such as Rhopseudomonas and *Rhodobacter* that metabolizes extraordinary carbon sources. Raw materials such as organic-based starches, cellulose-containing solid wastes obtained from different industrial wastewater or industrial wastes etc. can be used as sources, for hydrogen production as well while the waste is being treated. This work will create a process that supports the continuity of the environment in recycling and will provide significant efficiency in both a healthy environment and healthy life. This paper focuses on the importance of hydrogen production and how the recycling of waste is important for energy production by using photosynthetic bacteria.

Keywords: Bio-hydrogen; Biohydrogen production; Photobiological hydrogen production; Wastes; Dark and photo-fermentation, Photosynthetic bacteria; Purple non-sulfur bacteria

Hydrogen Production

Until recently, all of the energy needed has come from the conversion of fossil energy sources, thus the growing demand for energy has encouraged us to look for an alternative and renewable energy carrier with less emission of pollutants. In recent years, various studies have been carried out rapidly to obtain a sustainable energy source that can replace fossil fuels and has no negative impact on the environment. Wastewater treatment can be used at one time to combine the production of biohydrogen since Hydrogen is considered as an alternative fuel and promising candidate for replacing fossil fuel depletion and greenhouse gases [1-3]. Some authors suggested that these processes are suitable for integration into the existing waste water treatment plants. They have focused on multidisciplinary approach for providing integration of various technologies such as chemistry, microbiology and engineering [4]. Hydrogen gas produced by biological processes is promising because of being environmentally friendly and it can be produced at ambient temperature with minimum energy consumption in addition to this carbon-based gas emissions does not occur during this process [3,5]. The processes of biological hydrogen production can simply be classified as follows [6,7];

a. Hydrogen production by fermentation,

b. Photobiological hydrogen production,

c. Two-stage hydrogen production (integration of dark-and photo-fermentation)

The enzymes responsible for the hydrogen production processes carried out by various microorganisms are hydrogenases and nitrogenases. Therefore, hydrogen production productivities are limited by the efficiencies of these enzymes [7]. However, some scientists have expanded the scope further and analyzed biohydrogen production from wastewater by fermentation-based processes. Therefore, it should be highlighted that the nitrogen content of the substrate is also important in terms of enzyme activity. This mini-review discusses similar approaches exploring sustainable, low-energy-intensive ways to produce hydrogen from waste by analyzing hydrogen production technologies, namely dark fermentation, photobiological production, and sequential production of dark and photo-fermentation.

Hydrogen Production by Fermentation

Light-independent fermentation takes place under anaerobic conditions and in the absence of light. During the production of biological hydrogen, fermentation takes place while the biomass grows from the organic substrate. This fermentation has some advantages to other biological hydrogen production methods such as photosynthesis and photo-fermentation because hydrogen production can continue even in the absence of light, higher hydrogen production rate, low-value wastes as raw materials are used, lower net energy input and utilization. Hydrogen gas is produced from organic compounds by anaerobic microorganisms [8,9]. Considering the environmental benefit, one of the advantages of hydrogen production through the fermentation process is the use of various organic wastes as substrate, so waste reduction and energy generation can play a dual role. For this reason, hydrogen production during the fermentation process has recently attracted excessive interest from researchers and scientists. However, it should be taken into account that many parameters such as pH, temperature, organic food, feed rate and solid retention time, which are important factors for hydrogenase enzyme activity, should be checked periodically in order to obtain higher hydrogen yield during fermentation periods [10,11].

Photobiological Hydrogen Production

In biohydrogen processes (photo- fermentation and dark fermentation), two enzymes namely, nitrogenase and hydrogenase play an important role. The key enzyme nitrogenase produces one mole of H_2 per mole of N_2 fixed. While in the absence of nitrogenase, the enzyme acts as ATP-dependent hydrogenase and catalyzes the proton reduction for molecular hydrogen. Nitrogenase mediated hydrogen production using purple non-sulfur bacteria also plays a role in maintaining cellular redox status [1,12].

Photo- fermentative hydrogen production is a microbial process in which electrons and protons generated through oxidation of organic compounds such as acetates, lactate and organic acids such as butyrate. These compounds are used to produce molecular hydrogen under anaerobic, nitrogen-limited conditions by utilizing light as energy source. Some species of purple non-sulfur bacteria such as *Rhodobacter sphaeroides*, *Rhodopseudomonas palustris*, *Rhodobacter capsulatus* and *Rhodospirillum rubrum* have been used for the production of hydrogen [13]. These bacteria can produce hydrogen using nitrogenase enzyme and organic compounds such as acetate, lactate and malate also azo dye compounds under both aerobic and anaerobic conditions. Therefore, the hydrogen production process is closely linked to nitrogen metabolism where the primary function of nitrogenase here is to fix molecular nitrogen to ammonia during hydrogen formation at one time [14,15].

The purple non-sulfur bacteria could potentially redirect electrons from an inorganic substrate to hydrogen production. In these processes, photo-heterotrophs make use of small organic acids that are usually produced but not metabolized during fermentation [16]. A group of proteobacteria that have photosynthetic pigments and are capable of photosynthesis is categorized as purple sulfur bacteria. While these organisms need light to produce hydrogen, the biological pathways they use to achieve this differ

significantly. Photoheterotrophic hydrogen production occurs under light when the carbon and organic substrates are available as energy sources [17,18]. Unlike plants, algae, and cyanobacteria, do not use water as a reducing agent and therefore do not produce oxygen, instead, they use hydrogen sulfide or other reduced sulfur compounds as electron donors, which are oxidized to produce granules of elemental sulfur that become visible in cells [19]. Purple bacteria are reported to have a high potential to be used in waste treatment due to their different metabolic pathways and the ability of some to decompose aromatic ring compounds such as benzoic acid, dye, and dye derivatives [8,15,20].

Two-Stage Fermentation (Integration of Dark- and Photo-Fermentation)

The major problems encountered with biological hydrogen production in dark fermentation are the low yields and energy efficiency where in the dark fermentation for each mole of hexose, two to four moles of hydrogen are produced when acetates and butyrates are used. However, In addition to hydrogen production, products containing organic acids that causes much waste and environmental pollution could also be produced. In the photo-fermentation, the photosynthetic bacteria can be used for further processing of organic acids to hydrogen [21,22]. For decades, some researchers have shown that the best solution for obtaining a higher yield of hydrogen is to use the dark fermentation process and photo- fermentation in tandem [8, 23]. It is also found that exposure of the bacterial culture to light-dark cycles

increases the amount of hydrogen compared to continuous illumination [24]. In the two- stage fermentation, combining the two-step processes the dark and photo- fermentation will increase hydrogen production. Similarly, carbohydrate-containing substrates, organic acids are converted into CO_2 and hydrogen by mesophilic and thermophilic bacteria during the fermentation. In the second step, the organic acids such as acetic and lactic acids that are the wastes of dark fermentation, are used by the photosynthetic or purple non-sulfur bacteria for hydrogen production. Su et al. [25] have reported that glucose and starch were used to increase hydrogen yield by sequential dark and photo-fermentative processes [25]. Some attempts have been made to find the best organic acids and their optimum concentration for the production of maximum amount of hydrogen. Malic acid was found to be the most suitable for the generation of higher yield of hydrogen using *Rhodobacter sphaeroides* [24,26,27]. On the other hand, purple non-sulfur photosynthetic bacteria can decompose organic acids by using light energy and nitrogenase during a photo-fermentation process. The hybrid system is a combination of dark fermentation by fermentative bacteria followed by photo-fermentation by the purple non-sulfur photosynthetic bacteria, wherein the overall hydrogen yield can be enhanced to a great rate [12]. It is also known that aromatic compounds and xenobiotics are catabolized as carbon sources by purple non-sulfur bacteria such as *Rdopseudomonas* species [28,29]. In fact, hydrogen can be produced from wastes by these bacteria both anaerobically in light and aerobically in the dark. However, further work needs to be done in this regard.

Conclusion

Hydrogen is the fuel for the future, mainly because of its recyclability and nonpolluting nature. Biological hydrogen production processes are operated at ambient temperature and under atmospheric pressures, thus they are less energy-intensive and more environmentally friendly when compared to thermochemical and electrochemical processes. Some reports show that hydrogen is more expensive than gasoline at present. But hopefully, technological improvement will succeed in reducing the cost of biologically hydrogen production from wastes. Thus, by using suitable process parameters such as carbon and nitrogen ratio, illumination intensity, bioreactor configuration, inoculum age and by using purple non-sulfur bacteria, much higher yields of hydrogen could be generated. In the light of all this information, wastewater treatment plants can be converted into hydrogen production plants in the near future. However, there are also some issues to be considered for the production of bio-hydrogen from wastes:

A. The ratio of carbon and nitrogen source as substrate can plays important role in the hydrogen production process. The amount and structure of carbon and nitrogen sources in the waste should be determined and their ratio should be adjusted.

B. An important problem in the utilization of wastes is the impurities present in the raw materials (heavy metal content) during hydrogen production. They can negatively affect enzyme activity as well as the bacterial growth. They can also have an impact on hydrogen yield and its impurities. In general, purification of hydrogen gases generated by biological processes containing 65%-95% (v/v) hydrogen depends upon various parameters. These parameters need to be adjusted again depending on the microorganism used.

C. Various studies on the removal of wastes have reported that purple non-sulfur bacteria are very successful in the removal of wastes. It is also known that these bacteria have high efficiency in hydrogen production. Further work on the combination of hydrogen production and removal of waste at one time is needed.

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