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*Review Paper*

# Process Design for Biohydrogen Production from Waste Materials and Its Application

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***Abstract***

*Biohydrogen is regarded as an attractive future clean energy carrier due to its high energy content and environmentally friendly conversion. Biohydrogen reactor is widely used in studies concerning the anaerobic co-digestion of food waste, sewage sludge, wastewater and other organic solids. Anaerobic digestion is a series of biological processes in which microorganisms break down biodegradable material (biomass or waste feedstock) in the absence of oxygen to produce biogas, which may generate electricity and heat, or can be processed into renewable natural gas and transportation fuels. This review article explains the scientific processes of anaerobic digestion process such as hydrolysis, acidogenesis, acetogenesis and hydrogenesis as well as methods to produce biohydrogen gas such as fermentation and biophotolysis for the waste management technology and sources of renewable energy* *and concludes with solutions that may allow anaerobic digestion to become more widely adopted throughout the developing countries to control the waste management system.*

***Keywords***

*biohydrogen reactor, dark fermentation, photo fermentation, direct and indirect photolysis*

## 1. Introduction

Nowadays, waste-to-energy technologies are quite beneficial, the organic waste can be converted into a different form of energy such as activated carbon and other different adsorbents to remove the heavy metals from water, as well as composting to strong the soil fertility, methane gas, hydrogen gas which may use as a fuel purpose resulting as an environmentally friendly (Samolada & Zabaniotou, 2014; Shakoor et al., 2020; Singh et al., 2011; Srivastav & Kumar, 2020). The 'Reduce', 'Reuse' and 'Recycle' ('3 R's') referred to reduce the amount of waste to produce, considered the best ways for the green environment, many organic wastes such as banana peels, orange, sapodilla peels help to treatment of wastewater, and low-cost adsorption techniques to remove arsenic from groundwater (Baloch & Mangi, 2019; Baloch et al., 2020; Padam, Tin, Chye, Abdullah, & technology, 2014; Vu, Scarlett, & Vuong, 2018). There are so many techniques to utilized waste and convert it into different forms (Demirbas & Management, 2011; Dhanya, Mishra, Chandel, & Verma, 2020; Digman & Kim, 2008; Idumah & Nwuzor, 2019; Ramos, Monteiro, Silva, Rouboa, & Reviews, 2018) but in this study, we focus on the biological production of hydrogen gas by using sludge/wastewater and different biomass as a raw material as the process that is environmentally friendly and does not use fossil fuels. Hydrogen gas is used widely for energy purposes these days, it tends to be utilized in energy units to produce electricity, heat, and power (Kwan et al., 2020; Le, Van Dao, & Yu, 2020; Sazali, 2020). Recently, it is most commonly utilized in oil refining, production of fertilizer, and composting, while transportation and utilities are developing business sectors based on hydrogen gas (Duan et al., 2021; Esfandyari, Hafizi, & Piroozmand, 2021; Ordoñez-Frías et al., 2020). The demand for hydrogen generation has expanded significantly (Lui, Chen, Tsang, You, & Reviews, 2020; Nicita, Maggio, Andaloro, & Squadrito, 2020; Solomon & Banerjee, 2006). Hydrogen gas generating systems such as steam reforming of hydrocarbons, auto-thermal forms, and water electrolysis are well-known, but they are not cost-effective due to high energy needs. (Kapoor et al., 2020).

The hydrogen production by microorganisms can be divided into two main categories, one is by photosynthetic bacteria cultured under anaerobic light conditions, and the other is by other anaerobic bacteria. Many research has been carried out on the conversion of biomass to hydrogen by anaerobic bacteria utilizing pure cultures of various strains (Bao, Su, Tan, & fuels, 2012; Elsharnouby, Hafez, Nakhla, & El Naggar, 2013; Hallenbeck, 2009; Hiligsmann, Masset, Hamilton, Beckers, & Thonart, 2011). Natural microflora is often employed in many wastewater treatment procedures since sterilization is not required in this instance and it may be adapted to various types of wastewater components (Gray, 2004). The main form of anaerobic wastewater treatment methane fermentation.

Hydrogen produced biologically, is a possible biofuel that may be obtained from both cultivation and waste organic materials (Saba et al., 2015). The major biological processes used for hydrogen gas production are biophotolysis of water via dark-fermentation and photo-fermentation of organic materials, typically carbohydrates, via bacteria and algae; the dark and photo-fermentation process is a relatively new methodology for biohydrogen production; the only major issue with this dark and fermentative hydrogen production is that the resulting hydrogen gas is flammable (Hosseini, Abdul Wahid, Jamil, Azli, & Misbah, 2015). Although a number of procedures are available for $H_2$ generation, all of them may be grouped into two primary groups depending on raw materials utilized, namely traditional and renewable technologies.

Common anthropogenic activities include the discharge of a wide range of wate materials into the environment through everyday behaviors(Hoornweg & Bhada-Tata, 2012). Biological waste treatment methods are gaining popularity owing to their various characteristics such as technological superiority, simplicity, economy, and environmental friendliness (Jain et al., 2022; Mohan, 2016; Siwal et al., 2021). Agricultural waste, municipal trash, industrial waste, and other hazardous wastes are among the waste sources utilized in hydrogen generation (Demirbas & Management, 2011; Guo, Trably, Latrille, Carrere, & Steyer, 2010; Maji et al., 2020). Organic waste products originating from or resulting from food processing, crop leftovers, industrial, animal manures, agricultural residue, residential, and communal wastes are further classified (De Mes, Stams, Reith, Zeeman, & Bio-hydrogen, 2003; Siwal et al., 2021). Management of wastes as a potential source of $H_2$ generation has sparked considerable attention due to its sustainable nature and the possibility of opening up new avenues for the comprehensive use of eternal renewable energy sources (Dunn, 2002; Pudukudy et al., 2014). In this current review, an attempt has been made to assess the current trends, processes, and procedures in biohydrogen generation from organic waste ingredients, with a compilation of the benefits.

## 2. Process of Biohydrogen Production

### *2.1 Reactor*

Biohydrogen reactor deals with the principle of anaerobic digestion, it is an assortment of cycles by which microorganisms break down biodegradable material without oxygen (Kamaraj, Ramachandran, & Aravind, 2020). This method is utilized for domestic purposes as well as industrial to manage the waste and additionally to produce fuels. Abundant of the fermentation utilized in industrial to produce drink and food products, in addition to home fermentation, utilized anaerobic digestion (Lin et al., 2018). There are four basic key chemical and biological phases of anaerobic digestion the simplified description for the overall processes is shown below (Figure 1).

#### 2.1.1 Hydrolysis

In most cases, biomass is comprised of huge organic polymers, microbes organisms in anaerobic digesters to get to the energy capability of the material, these chains should initially be broken down into their smallest constituent parts (Kamaraj et al., 2020). These constituent parts, or monomers, for example, sugars are promptly accessible to other bacteria, thus breaking these chains and dissolving the smallest molecules into solution is called hydrolysis (Zupančič & Grilc, 2012). Hydrolysis of these high molecular weight polymeric segments is the important and initial phase in anaerobic processing and the composite organic molecules are breaken down into amino acids, unsaturated fats and sugars (Luckachan & Pillai, 2011). Hydrogen and acetate production in the primary stages can be directly utilized by methanogens (Kim, Hwang, Jang, Hyun, & Lee, 2004).

2.1.2 Acidogenesis

The organic cycle of acidogenesis brings about the additional breakdown of the excess segments by acidogenic (fermentative) microbes (Dahiya, Sarkar, Swamy, & Mohan, 2015). VFAs are made along with ammonia, hydrogen sulfide, and carbon dioxide in addition to other byproducts.

2.1.3 Acetogenesis

The third phase of anaerobic digestion is acetogenesis. In this, the molecules made through the acidogenesis stage are additionally processed by acetogens to deliver generally acetic acid, in addition to carbon dioxide and hydrogen (Kushkevych et al., 2019).

2.1.4 Hydrogenesis

The terminal phase of anaerobic digestion is the organic interaction of methanogenesis is eluded. Thus, moderate the results of the former stages and convert them into hydrogen, carbon dioxide, and methane and most of the hydrogen gas produced from the system. Thus hydrogen is delicate to both high and low pHs that is between pH 6.5 and pH 8. The remaining, indigestible material the microbes cannot use and any dead bacterial remains constitute the digestate. Microbes that consume acetic acid deliver hydrogen gas as significant products and its byproducts are carbon dioxide ($CO_2$) and methane ($CH_4$) gas.

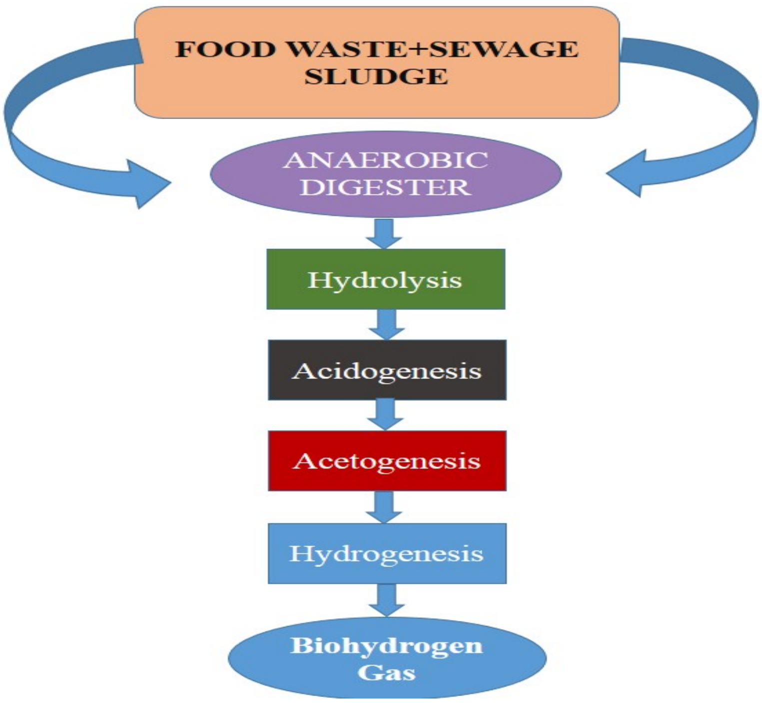

**Figure 1. Overall Processes to Produce Biohydrogen Gas**

## 3. Biohydrogen Reactor

### *3.1 Design of Biohydrogen Reactor*

Biohydrogen reactor is an anaerobic treatment technology that produces hydrogen gas, this gas is generated from biological processes takes place in a reactor to produce biohydrogen (Kapdan & Kargi, 2006). A biohydrogen reactor is a chamber or vault that facilitates the anaerobic degradation of blackwater, sludge, and/or biodegradable waste (Show, Lee, & Chang, 2011). It also facilitates the separation and collection of the hydrogen gas that is produced. Biohydrogen reactor comprises of a stainless steel double jacketed container assembled with gear motor, pressure relief valve and agitator. Other components of reactor are gas collection and measuring acrylic jar, water tank with heater and pump to circulate warm water through outer jacket of container and electric panel to control input of electricity. An electric panel is provided to control water temperature, motor rotation timing and have on/off switches. Anaerobic digestion is a technology that provides local, reliable, renewable energy through a process that disposes of the abundant trash produced by society each day.

## 4. Methods of Biohydrogen Production

Hydrogen is considered a clean energy carrier because hydrogen is constantly coupled with other elements, it is not found naturally on earth and must be synthesized (Edwards, Kuznetsov, David, & Sciences, 2007; Ji & Wang, 2021; Momirlan & Veziroglu, 2005). Fossil fuels, and biomass, are among the resources that may be utilized to produce hydrogen (Edwards et al., 2007; Ji & Wang, 2021). .It can be made in a variety of methods, depending on the hydrogen source, and how it is synthesized affects the environmental impact and energy efficiency of hydrogen generation (Demirbas, 2009; Manish & Banerjee, 2008). The most advanced hydrogen generation technologies, according to the literature, are thermo-chemical hydrogen production (e.g., natural gas reforming/gasification, reforming of renewable feedstocks), and biological hydrogen production (Iulianelli & Basile, 2014; Olabi et al., 2021; Orecchini & Bocci, 2007). Biological methods for hydrogen generation have received a lot of attention in the recent decade since the key issue is to reduce the cost of production technologies (Das & Veziroğlu, 2001; Kumar, Chakraborty, & Singh, 2017; Vijayaraghavan & Mohd Soom, 2006). Biological hydrogen generation technology may function at room temperature and pressure while consuming little energy, resulting in being environmentally friendly. Low hydrogen yields and production rates, on the other hand, are seen as key barriers to the commercialization of these technologies.

Hydrogen by fermentation is to be introduced as an industry, the fermentation process will be dependent on acids as substrate for photo-fermentation. The organic acids can be derived from any organic material source such as sewage waste waters or agricultural wastes. The most important organic acids are acetic acid (HAc), butyric acid (HBc) and propionic acid (HPc), the fermentation of hydrogen has to be a continuous fermentation process, in order sustain high production rates, since the amount of time for the fermentation to enter high production rates are in days (Kapdan & Kargi, 2006). In general, the method of biohydrogen is referred to in two main categories as shown in (Figure 2). The first category fermentation which is further divided in dark-fermentation, which does not involve light and another photo-fermentation, which requires light as the source of energy. The second is biophotolysis which is further divided in to direct biophotolysis in which the action of light on biological systems that result in dissociation of water into molecular hydrogen and oxygen and indirect biophotolysis avoids the inhabitation of hydrogenase by separating the hydrogen production process from the oxygen production. These processes are discussed in detail in the following sections.

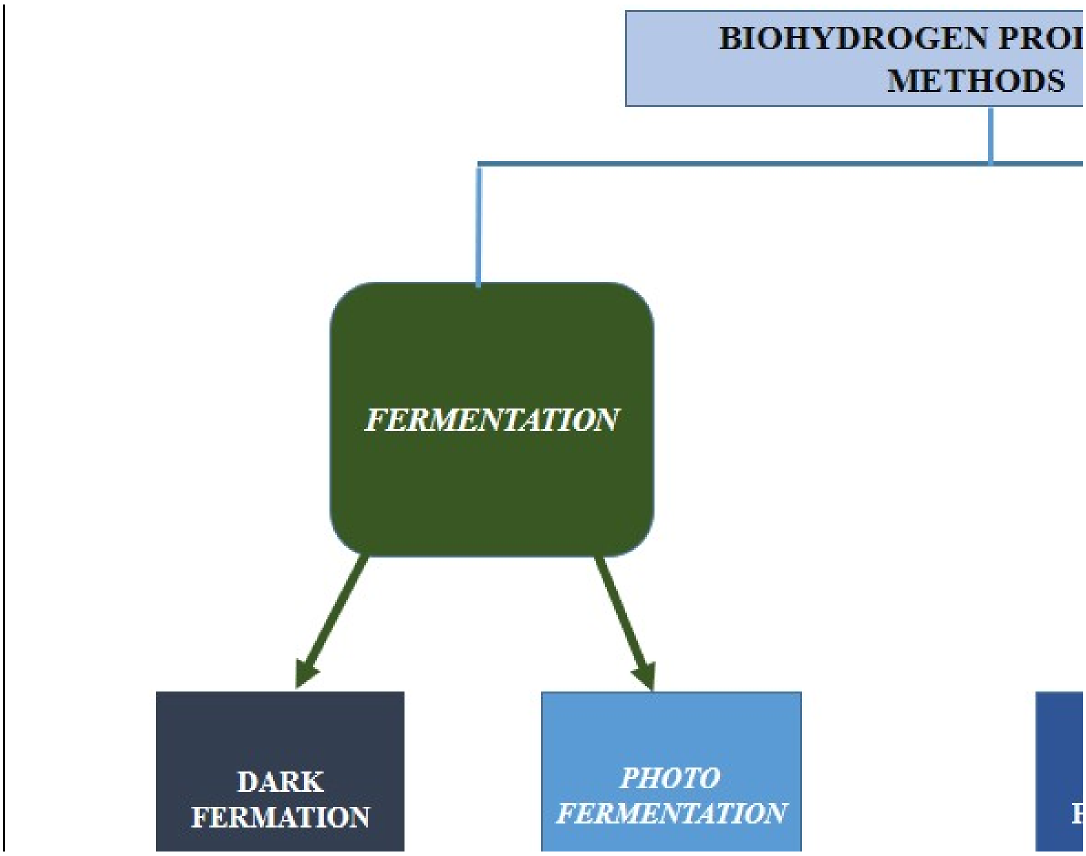


**Figure 2. Methods of Biohydrogen Production**

*4.1 Fermentation*

4.1.1 Dark Fermentation

There are various bacteria that have the ability to produce hydrogen. The Gram-positive bacterium of the *Clostridium* genus is particularly interesting since it has a naturally high hydrogen production rate (Dębowski, Korzeniewska, Filipkowska, Zieliński, & Kwiatkowski, 2014). Furthermore, it is rapidly growing and capable of generating endospores, making the bacteria easier to handle in industrial applications. *Clostridium* species can produce hydrogen in mixed cultures in mesophilic or thermophilic environments with a pH range of 5.0 to 6.5. Dark fermentation with mixed cultures appears promising because a mixed bacterial habitat within the fermenter allows diverse species to collaborate to efficiently breakdown and convert organic waste materials into hydrogen, with the creation of organic acids (Saratale, Chen, Lo, Saratale, & Chang, 2008). Clostridia produce $H_2$ through a reversible hydrogenase enzyme (2H + 2e => $H_2$); this reaction is crucial in maintaining the redox balance of fermentation (Lee, Vermaas, & Rittmann, 2010). Because $H_2$ generation raises the partial pressure of $H_2$, which might impede substrate conversion. Bacteria may respond by switching to new metabolic route to establish redox balance, energy generation, and growth through the production of solvents rather than hydrogen and organic acids (Nath & Das, 2004).

The roles of *E. coli* hydrogenases in biohydrogen generation are also of interest for enteric bacteria such as *Escherichia coli* and *Enterobacter aerogenes* (Baeyens et al., 2020b). Unlike *clostridia*, enteric bacteria create hydrogen largely (or completely in the case of *E. coli*) by formate cleavage, which helps to cleanse the medium by eliminating formate; nonetheless, cleavage is not a redox process and has no effect on the redox balance of fermentation (Baeyens et al., 2020a). This detoxification is critical for *E. coli* since it cannot defend itself by generating endospores. Because formate cleavage is an irreversible reaction, $H_2$ production is not affected by the partial pressure of hydrogen ($pH_2$) in the fermenter. However, in traditional fermentation systems, the dilution rate must be carefully controlled because it affects the concentration of bacterial cells and toxic end-products (organic acids and solvents) inside the fermenter (Owens & Basalan, 2016; Redwood, Paterson-Beedle, & Macaskie, 2009).

4.1.2 Photo fermentation

Photo-fermentation is a kind of fermentation in which light is used as an energy source; this fermentation relies on photosynthesis to sustain cellular energy levels (Hitam & Jalil, 2020). *Cyanobacteria* are commonly touted as being capable of producing hydrogen via oxygenic photosynthesis (Carrieri, Wawrousek, Eckert, Yu, & Maness, 2011). However, purple non-sulphur (PNS) bacteria (e.g., the genus *Rhodobacter*) offer great potential for hydrogen generation via oxygenic photosynthesis and photo-fermentation. *Rhodobacter sphaeroides* is very capable of producing hydrogen while feeding on organic acids, consuming 98 to 99 percent of the organic acids consumed during hydrogen synthesis (Koku, Eroğlu, Gündüz, Yücel, & Türker, 2002). Photo-fermentative bacteria, unlike algae and *cyanobacteria*, can utilize light in the wavelength range 400-1000 nm.

*4.2 Biophotolysis*

There are two types of biophotolysis. The process of direct biophotolysis is similar to that of plant and algae photosynthesis. Solar energy is directly transformed to hydrogen in this mechanism via photosynthetic processes (Eq.1).

$$2H_2O + \text{'light energy'} \rightarrow 2H_2 + O_2 \quad (1)$$

Photosynthesis allows algae to divide water molecules into hydrogen ion and oxygen. The hydrogenase enzyme converts the produced hydrogen ions into hydrogen gas. One of the most well-known hydrogen-producing algae is *Chlamydomonas reinhardtii* (Xu, Fan, & Wang, 2019). Other green algae with hydrogenase activity include *Scenedesmus obliquus, Chlorococcum littorale, Platymonas* *subcordiformis* and *Chlorella fusca* (Shaishav, Singh, & Satyendra, 2013).

4.2.1 Direct Biophotolysis

A direct biophotolysis approach must, by definition, operate at a partial pressure of around one atmosphere of $O_2$, which is a thousand times higher than the maximum likely to be tolerated (Gürtekin, 2014). Hydrogen generation by direct photolysis using green algae is currently constrained by three parameters (i) The photosynthetic apparatus's solar conversion efficiency; (ii) $H_2$ synthesis mechanisms; and (iii) Bioreactor design and cost. Several strategies are now being researched to improve $H_2$ generation by green algae, including genetically altering light-gathering antennas, optimizing light input into photobioreactors, and enhancing two-phase $H_2$ generation systems used with green algae.

4.2.2 Indirect Biophotolysis

In indirect biophotolysis, concerns of sensitivity of the hydrogen evolving process may be avoided by separating oxygen evolution and hydrogen development temporally and/or spatially (P. C. Hallenbeck & Benemann, 2002). As a result, indirect biophotolysis processes include the separation of the $H_2$ and $O_2$ evolution reactions into discrete phases that are connected by $CO_2$ fixation/evolution (Benemann, 2004). *Cyanobacteria* are unusual in that they use $CO_2$ in the atmosphere as a carbon source and sun radiation as an energy source (Eq. 2) (Gürtekin, 2014). The cells absorb $CO_2$ first to form cellular components, which are then utilized to produce hydrogen (Eq.3). The following processes illustrate the general mechanism of hydrogen generation in *cyanobacteria*.

$$12H_2O + 6CO_2 + \text{'light energy'} \rightarrow C_6H_{12}O_6 + 6O_2 \quad (2)$$

$$C_6H_{12}O_6 + 12H_2O + \text{'light energy'} \rightarrow 12H_2 + 6CO_2 \quad (3)$$

*Cyanobacteria* have key enzymes (nitrogenase and hydrogenase) that carry out metabolic activities to produce hydrogen (Tamagnini et al., 2002).

## 5. Applications and Cycle of Biohydrogen Gas

Currently, the majority of hydrogen is generated using fossil fuels, e.g., steam reforming of natural gas (Kalamaras & Efstathiou, 2013). Although prototype hydrogen cars have been built, there is presently no large infrastructure for distributing hydrogen as a transport fuel, and in-vehicle storage capacity remains a concern (Ball & Weeda, 2015). Furthermore, hydrogen fuel cells are costly to manufacture, brittle, and have a very short service life (Penner, 2006). It is a radioactive isotope used to manufacture hydrogen bombs, as well as a brilliant paint radiation source, and tritium is employed as an isotopic marker in biosciences. Hydrogen is primarily utilized to convert heavy petroleum fractions into lighter ones via the hydrocracking process, as well as for the conversion of other petroleum fractions. Hydrogen is primarily utilized to convert heavy petroleum fractions into lighter ones via the hydrocracking process, as well as for the conversion of other petroleum fractions. Solid fat substances are created with the help of nickel as a catalyst, and hydrogen is required in the petrochemical sector for the refining of crude oil. There is the biohydrogen life cycle which is based on different phases, as shown in (Figure 3). First of all, the source of the food production and consumption is generating from the houses and others residential places, which dispose of to dustbin and considered as food waste. Later this food waste carried by transportation and moved to biohydrogen processing and production plant, where these food waste converted into biohydrogen gas, this bio hydrogen gas, storage and then distributed for fuel purposes. This gas use as the fuel purposes by vehicles mostly by city buses, autos, and small transport vehicles and has several advantages, including the fact that it is fossil-free, renewable, locally generated, and reduced particle and nitrogen oxide (NOx) emissions improve air quality in general, and quieter cars benefit both drivers and passengers, as well as society as a whole.

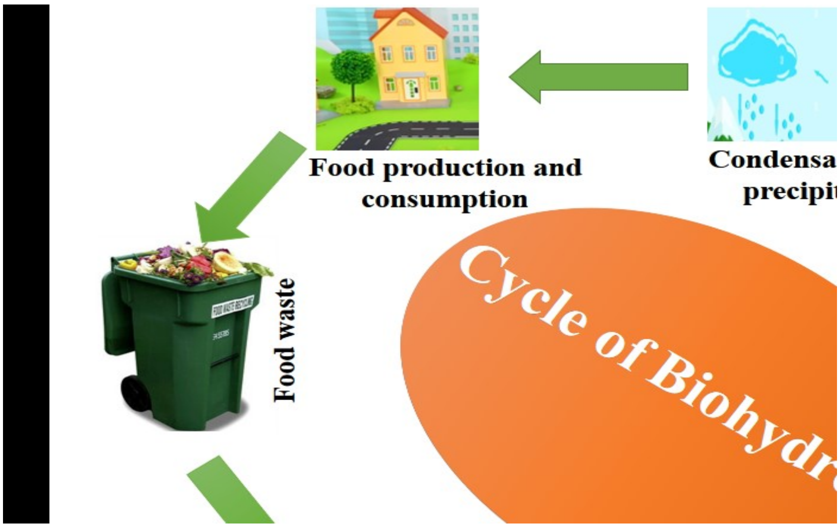


**Figure 3. Life Cycle of Biohydrogen**

## 6. Conclusion and Recommendation

Biohydrogen reactor is widely used in studies concerning the anaerobic co-digestion of food waste, sewage sludge, wastewater and other organic solids. Although it has been criticized for being more time consuming with a hydraulic retention time of 30 days or even more, but it has many advantages as well. As it is reliable, flexible and having good productivity particularly useful for evaluating the potential for co-digesting mixed wastes. This $H_2$ is an environmentally friendly it has low and easy maintenance. Biohydrogen measurement is easier than other gas measuring methods, in this system the measure gas through a transparent pipe which is ease to measure, without using any special instrument and capable producing biohydrogen gas. It is possible that in the future, the quantity of electricity required by this system will be met by a renewable energy source such as wind power or solar voltaic cells. At the commercial level, hydrogen production techniques can also save a variety of resources and money which is being wasted in conventional hydrogen production method. Conventional methods, such as gas stream reforming and electrolysis, used a considerable quantity of fossil fuel, resulting in a waste of resources and money. Anaerobic digestion of organic material causes no harm and provides greater benefits, hence hydrogen is referred to be the fuel of the future generation; it emits no pollutants and is thus environmentally friendly. Working on renewable hydrogen sources is critical for sustainable development and economic solutions. More research in the field of biohydrogen should be conducted in future to acquire a higher yield.

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