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Potential of Bio Hydrogen Production from Dark Fermentation of Sewage Waste Water – A Review

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Nowadays, energy is the basic need for social life. Bioenergy is the powerful renewable substitution of fossil fuels. Hydrogen is a clean fluid that can be easily provided with the help of certain biomass. Hydrogen production can be produced by various techniques such as electrolysis, steam-methane reforming process, and biological process. The majority of the countries produced biohydrogen as bioenergy because it is carbon-free fuel and it is eco-friendly and it does not contribute to ozone depletion or greenhouse emissions. Waste (sewage water, industrial waste, domestic waste) can be used as an alternative source. Hydrogen production from the various biological process, such as dark fermentation some of the bacteria (Enterobacter and clostridium) involved in hydrogen production are yield very high energy. In this article, we can discuss the advanced techniques, parameters affecting biohydrogen production during the fermentation process, limitations, and importance.

Keywords: Bioenergy; Bacteria; Dark Fermentation; Hydrogen Production; Renewable energy.

In recent days bioenergy is the main source of energy because it can be renewable and eco-friendly. Nearly 95% of world demand on fossil fuel[35.] Biohydrogen as biogas is very effective than other gases. Hydrogen is one of the most abundant on earth, it doesn't cause any pollutants and it is a non-poisonous substance [1]. Hydrogen is considered a renewable and sustainable source of energy, waste minimization and it also overcomes the negative effects of fossil fuel while the combustion of fossil fuel releases CO2[3,4.] Bio hydrogen does not cause any greenhouse gas effect, ozone depletion, and global warming. Hydrogen gas is safe to use than other natural gas. For hydrogen gas production biomass and water can be used as renewable resources, biohydrogen production from renewable resources is also known as "Green technology"[4.] This hydrogen production can also be done through dark fermentation which efficient method to produce biohydrogen [17, 20.] There are several ways to produce hydrogen with the help of domestic waste, agriculture waste, food waste, industrial wastewater, and sewage water. Sewage water, industrial wastewater is cheap and effective methods for biohydrogen production and wastewater treatment.

Sewage water Wastewater or Industrial wastewater can be widely used for hydrogen production because it includes various substance that enhances the hydrogen yield. Sewage water

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contains several organic and inorganic substances which help to enhance bacterial growth with involved in biohydrogen production.

Biohydrogen production from wastewater with the help of the dark fermentation method is an efficient method. It also involved in the treatment of Wastewater Mass spectrometry was used to evaluate wastewater [17.] Some of the studies show that wastewater substrate concentration yields hydrogen production[34]. Now we discuss the biohydrogen production process involved during dark fermentation (refer Figure.1) [44]

Dark fermentation

Dark fermentation is the effective method for obtaining bio hydrogen because of its efficiency, mode of operation is simple [23.] In this production total net ratio is equal to 1.9 whereas, in steam, methane reforming the ratio is equal to 0.64 [22.] It is a process that is carried out in a dark room, under anaerobic conditions without oxygen. This dark fermentation produces hydrogen continuously day and night. It is a complex process that is similar to anaerobic conversion anaerobic digestion. In this method, various organic materials act as a substrate. The organic compounds are the only carbon source to provide metabolic for the dark fermentation [9.] In this fermentation mostly, pure culture is used because its metabolic activity is easier to control under mesophilic to thermophilic temperature ranges and when we are using wastewater as substrate, we can use this mixed culture [6, 9.] The Pretreated organic is fed into the bioreactor which contains some microorganisms such as acidogenic, acetogenic, homoacetoagenic, hydrolytic, and methanogenic bacteria, these are produced methane and carbon-di-oxide as the final product [6.] This fermentation takes place with the help of microorganisms it will convert hydrocarbons molecule from complex to simple finally hydrogen and acid as intermediate products to carbon-di-oxide and methane [25.] Theoretically, once the completion of glucose oxidation the conversion yield is 12 molecules of H_2 with the single substrate [6.] Dark fermentation includes both facultative anaerobe and obligate anaerobe. Facultative anaerobe can survive in the presence and absence of oxygen (for example *Enterobacter* and *Citrobacter*). Obligate anaerobe can grow only in the absence of oxygen (for example *Clostridium*). In this method, the residual biomass can be used and transformed into value-added products [21.] Hydrogen is the key substrate for anaerobic metabolism. To obtain effective bio hydrogen temperature, pH, substrate, nutrition feed should be maintained. pH is one of the important factors which affects the hydrogenase enzyme activity [3.] The microorganisms involved in dark fermentation uses energy-rich hydrogen molecules (if it is available) and it utilizes the electron from hydrogen oxidation to produce energy. In this fermentation hydrogenase is the enzyme which plays a vital role in hydrogen metabolism activity. In the absence of an external acceptor, during the metabolic processes organism generated an excess of electrons at the end of the result reduction protons yielding hydrogen molecule[22.] During this fermentation four molecules of hydrogen have been produced. Some of them think that energy yield is very low in dark fermentation, by using appropriate substrate concentration and mixed cultures we can yield more amount of hydrogen in the equation no.1 [6]

Equation 1: $C_6 H_{12} O_6 + 12 H_{2O}$ ——— CH_3 $COOH + 4H_2$.

In this fermentation, it also produces volatile fatty acids (acetic acid, butyric acid, propionic acid. Hydrogen production mostly occurred through acetate (67% of H_2 yield) and butyrate (50% of H_2 yield) pathways in equation no.2 [22, 31]. The overall dark fermentation was explained in the Figure.2[44]

Equation 2: $C_6 H_{12} O_6 + 2 H_{2O}$ ——— $2 CH_3$ $COOH + 2 CO_2 + 4H_2$ (acetate)

$C_6 H_{12} O_6$ ————- $CH_3 CH_2CH_2 COOH + 2 CO_2 + 2H_2$ (butyrate)

Hydrogenase

An enzyme that plays a key role in hydrogen production, it involves reversible oxidation

$2H+ + 2e — H_2$ [22.]

Hydrogenase is classified into three types [Fe] hydrogenase, [NiFe] hydrogenase, an Fe hydrogenase. FeFe hydrogenase is the most efficient in terms of fermentative hydrogen production, the activity of this FeFe hydrogenase is 10-100 times higher than another hydrogenase. Clostridia species contain a large number of hydrogenases with a different modular structure. During hydrogen production, the activity of hydrogenase can be improved by adding appropriate Fe^{2+} concentration [23].

Hydrogen producing microorganisms

There are several types of microorganisms such as cyanobacteria, algae, photosynthetic bacteria, and fermentative bacteria that are used for hydrogen production. Among that *Enterobacter* and *Clostridium* species are very effective in dark fermentation for hydrogen production. Anaerobic bacteria are involved in hydrogen production due to their high production rate and it is used a wide range of carbohydrates from wastewater.

Clostridium

This microorganism belongs to the group of gram-positive bacteria, which is rod-shaped (Figure 3. *Clostridium* Species [41]) It can be found easily in soil and water. It comes under obligate anaerobe bacteria. It is an anaerobic spore former. This type of bacteria produces acid intermediates and hydrogen, and it also includes acetate production from hydrogen and carbon-di-oxide by acetogenins and homoacetogens [25.] It is responsible for adverse conditions like oxygen, heat, low (or) high pH, alcohol, and toxic compounds. It has greatly reduced metabolic activity due to its changeover from vegetative cells to endospores. This endospore formation can achieve the genus clostridium can be isolated from mixed culture by heating the culture at 70°C for 10 minutes to kill the vegetative clostridium cells and non-spore-forming organism. Carbohydrates are the common substrate for the genus of clostridium. During the exponential growth phase, it yields very good hydrogen production [1.] *C. saccharolytic*, helps to catalyze carbohydrates, simple sugar, disaccharides, oligosaccharides, and cellulose. *C. Proteolytic*, helps to catalyze proteins and ferment amino acids. These organisms play a crucial role in hydrogen production during acidogenesis because it helps to break down the complex molecules into simple molecules. In this method, glucose is converted into pyruvate by the process of glycolysis .[2]

Enterobacter

This microorganism belongs to gram-negative bacteria, non-spore formation, which is rod-shaped (Figure 4. *Enterobacter* Species [42]). It comes under the family Enterobacteriaceae. *Enterobacter aerogens* (facultative anaerobe) can grow under aerobic and anaerobic conditions. It can be found in the soil, water, sewage, plants, etc., This *Enterobacteriaceae* can have several beneficial properties for hydrogen production. To obtain a high yield of hydrogen, the utilization of a carbon source is required. In this hydrogen production, Enterobacter does not inhibit by high hydrogen pressures.

MATERIAL AND METHODS

Pretreatment of sludge

The complex which is present in the sewage is difficult to hydrolyze because of its high resistance. Pretreatment is undertaken before

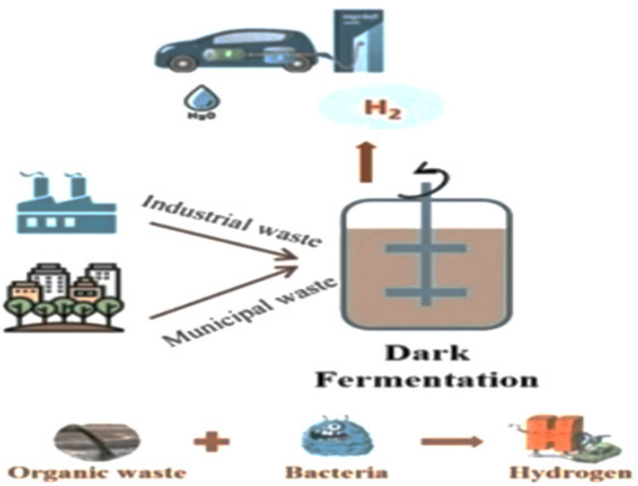

Fig. 1. Outline for hydrogen production

hydrogen production activity. The raw sludge is collected from a municipal wastewater treatment plant through an aeration tank and the sludge is treated for pH, chemical oxygen demand (COD), dry solid (DS), volatile solid (VS), and stored at 4°C for 2 - 3 days before used [24,28]. There are four Pretreatment methods to treat sludge. These pretreatment steps involved inactivating methanogens and promoting the endospore-forming hydrogen producers [7.] The purpose of the pre-treatment is to enrich the spore-forming bacteria and reduce the hydrogen-consuming bacteria [17.]

Acid pretreatment

It helps to remove hemicellulose components which present in the sludge with the help of nitric, sulfuric, and hydrochloric acids. pH was adjusted to 2.0 [28.]

Alkaline pretreatment

With the help of alkaline solutions such as calcium hydroxide ($Ca(OH)_2$), sodium hydroxide (NaOH), or ammonium to remove the lignin. pH was adjusted to 12.0 [28.]

Thermal pretreatment

It increases the bioavailability of organic compounds and also improves biodegradation (i.e., accessible to the microbes for their degradation). The sample was autoclaved at 121°C [28.]

Ultrasonic pretreatment

Ultrasonic Pretreatment helps to disrupt the physical, chemical, and biological processes of the sludge. It is a very effective mechanical method to enhance the biodegradability of the sludge.

Reactor for hydrogen production

There are several kinds of bioreactors used for hydrogen production. There are different bioreactors for different substrates as we used. Continuous Stirred Tank reactors (CSTR) and Up-flow anaerobic Sludge Blanket (UASB) reactors are preferable for industries due to their high yield and application of a wide range of wastewater. Anaerobic Fluidized Bed Reactor

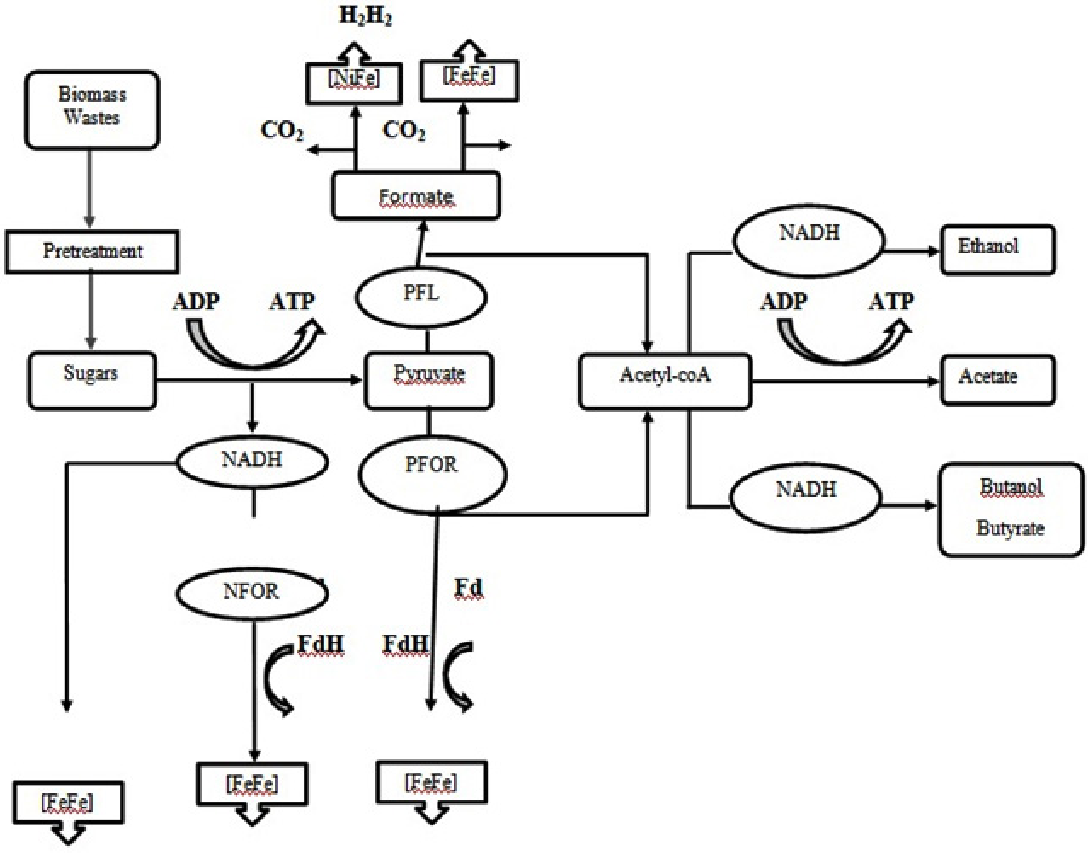

Fig. 2. Dark Fermentation Pathways for Hydrogen Production

(AnFBR) produces 4.26 ± 0.04 mol H_2. This AnFBR is suitable for sewage sludge substrate. This reactor maintains temperature stability and excellent mass transfer and reaction rates. The contact between substrate and biomass has been improved by mixing completely. In AnFBR the direction is always vertical-up flow and the flow of velocity is equal or greater than maximum velocity which drags the force exerted by the particles in the reactor [31.] The AnFBR should be 2.5 cm in diameter and 230 cm in height and the distributor have 32 holes and each hole has 2 mm in diameter.

Factors affecting hydrogen production

Substrate composition

The substrate is depending on the source we choose. In most of the studies, simple sugar like glucose and Sucrose are taken. Only a few studies included agriculture waste, industrial waste, domestic waste, and sewage sludge. These are mainly involved in fermentative hydrogen production and produce sustainable hydrogen production.

Temperature

Temperature is one of the important parameters involved in bacterial activity and hydrogen production rate[9.] There are four different temperatures for dark fermentative, mesophilic (25-40°C), thermophilic (40-65°C), extreme thermophilic (65-80°C) or hyperthermophilic (>80°C), most of the dark fermentative process conducted under 35-55° C and the mesophilic condition is favorable for hydrogen-producing bacteria such as *Clostridia* and *Enterobacter* [9, 22, 31]. The selection of optimum temperature is based on the bacterial culture, kind of substrate, and enzyme activity. Hydrogen production is much in extreme thermophilic compared with mesophilic and thermophilic. 4 molecules of hydrogen mole per glucose are obtained under extreme thermophilic. At this extreme thermophilic bacterium shows better tolerance to hydrogen partial pressure and it generates metabolic shift to non-hydrogen producing Pathway. When the temperature rises it provides energy to the dark fermentative process [9]. At psychrophilic conditions (4°C to 20°C) of dark fermentative have been studied [22.]

pH

pH is the main factor that affects the enzyme activity, redirected metabolic pathway, and microbial activity since the enzyme is active only in particular pH [9, 22, 31.] The initial pH for the feeding solution is 4-9.3. The reactors are fitted with a pH probe to control pH during fermentation process [31.] The optimum pH for dark fermentation is 5.5. It will also affect the enzyme hydrogenase (FeFe) which is mainly responsible for hydrogen production. Under unoptimal pH, the fermentation will lead to solvent production and it also changes its pathway from acetate to butyrate pathway, decreasing in hydrogen production [9.]

Hydraulic retention time

Hydraulic retention time (HRT) is an important parameter for the dark fermentation process, it requires the utilization of continuous and semi-continuous processes. HRT is the measure of the average length of time that the substrate remains in the reactor. In CSTR, short regular interval hydraulic retention time is washed out the methanogenic bacteria which grow slowly and selected for acid-producing bacteria [9, 22.] It is allowed to use a small reactor. Optimal HRT for

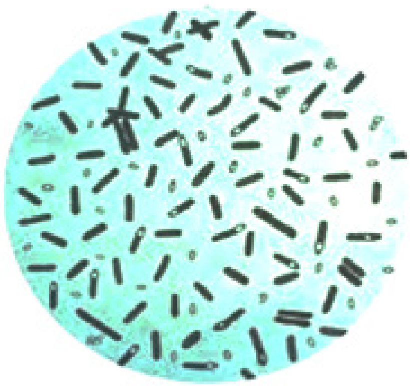

Fig. 3. *Clostridium* Species

Fig. 4. *Enterobacter* Species

simple carbohydrates 2 hours, 4 hours, or 12 hours. To find the best optimal HRT for dark fermentation is, by conducting semi-continuous on solid wastes by varying the range of HRT, it took 6 hours to 10 days. The optimal HRT for sewage biosolid is 24 h, after 3 days stable biohydrogen is produced [22.] In anaerobic digestion pH and HRT are combined, it can easily demonstrate the hydrogen-producing bacteria and hydrogen consuming bacteria under mesophilic and thermophilic conditions [9.]

Volatile fatty acids

Volatile fatty acids (VFA) are the important factor that affects the anaerobic digestion process. There are two important roles in VFA, decomposition of organic substrate and generating gases, when it occurs continuously oxygen demand decreases. Most of the VFA is produced in the acidogenic phase by the hydrolysis process. During this anaerobic process, a sudden drop of pH can cause accumulation of VFA or generate excessive carbon-di-oxide or both. The type of metabolic pathway and microbial activity can easily understand through VFA during the process.

Hydrogen partial pressure

Hydrogen partial pressure (HPP) is a parameter affecting the fermentative hydrogen pathway. It is an important parameter especially in the continuous synthesis of hydrogen. During the production of the process, the enzyme hydrogenase is oxidized and reduces ferredoxin in a reversible process. A suitable stirring speed range from 20rpm to 100rpm was to be found to improve the hydrogen production rate from 35 to 214 (cm^3 H_2 /g) [22.] When the concentration of hydrogen increases, decreases of H_2 synthesis and the metabolic pathway shifts to the production of reduced substrates. Continuous H_2 synthesis requires hydrogen partial pressure of 50 kPa at 60 degrees Celsius, 20kPa at 70 degrees Celsius, 2kPa at 98 degrees Celsius [9.] An increase in partial pressure of hydrogen is favorable for the reduction in ferredoxin while further inhibition causes the conversion of substrate to hydrogen [22.] The effect of HPP is reduced by sparging of inert nitrogen gas in the reactor.

Substrate inhibition

With the help of initial substrate concentration to activate germination process, prevention of re-sporulation and it also carry out the energy-efficient operation. Initial substrate concentration with optimum ranges enhances hydrogen production. The concentration of volatile fatty acids, variations in pH, hydrogen partial pressure are the factors affected by high substrate concentration. Most of the studies focused on carbohydrate sources and few are used organic material. Optimal microbial is significant for reducing the substrate inhibition such bacteria like *Bacillus sp*, *Enterobacter aerogens* can stimulate the microbial activity in batch mode the initial concentration of 1-50g(COD)/L, later the concentration beyond 20g(COD)/L may decrease the hydrogen production via substrate inhibition. It is not consistent in dark fermentation, because various factors are responsible for inhibition.

Trends and perspective

Biohydrogen is a clean H_2 provide with simple technology Dark fermentation hydrogen production seems good for future commercial production. To overcome substrate degradation, lower yield hydrogen, and VFA are the major challenges and possibilities to enhance fermentative production. Asian countries mainly focused on dark fermentation. Industrial biohydrogen production is a potential for future uses [11.] By using wastewater as substrate, it is rich in the organic source which helps to grow to microorganisms in dark fermentation, and with the help of biological tools hydrogen is produced [11, 29.] By removal of a microbial population which is responsible for lower yield production, enhanced hydrogen productivity. By enhancing hydrogenase enzyme is also responsible for hydrogen activity [29.] Recently, hydrogen is produced by using microbial culture immobilization, modification of bioreactor, modifying optimum conditions (HRT, VFA, temperature, pH,) [11.]

Sustainable biohydrogen and its challenges

Sustainable biohydrogen production can be derived from its purity and production rate at affordable costs. In recent years biological methods show significance to hydrogen production due to their mild conditions and lower energy demand. Criteria for sustainability to achieve economic, environmental, and social party. The energy ratio and GHG emission are favorable for hydrogen production. Biohydrogen production consideration as the worth of planning and developing H_2 economy from an energy and environmental perspective. Many researchers are still working on transportation, industrial production, and storage processes to reach a satisfactory level [11]. The replacement of natural gas with the help of biohydrogen can reduce greenhouse gas emissions and thus prevent environmental factors [40].

Some of the challenges for sustainable use of biohydrogen are:

• Thermodynamic barrier.
• Low photochemical efficiency.
• Suitability of low-cost substrate.
• Kinetics suitable design of reactors.
• Cost material for hydrogen storage.

Outlook and future directions

Industrial wastewater is the Potential feedstock for biohydrogen production[39]. The concentration of recent techniques in hydrogen production by dark fermentation has been substantial recent improvements in both the production yield and volumetric production rates of hydrogen fermentation. The major question is raised "can practical approaches for hydrogen production be possible from the H_2 from the substrate (12 H2/glucose)?". This question is mainly focused and challenging for future use. Using ametabolic engineered microbes is an important approach to improve fermentative hydrogen production. Hydrogenase is complex and its maturation proteins are due to the fact of 14% of the *E.coli* genome remains completely unexplored [36]. Hydrogen production improves more biological techniques which are significant for the improvement of rapid separation of gas removal and separation, hybrid system, reverse micelles, and metabolic engineering [36,37]. Nowadays hydrogen production has become the most challenging area of biotechnology. The future of bio-hydrogen production also depends on the genetically engineered microbes and the development of bioreactors is important for economic considerations (i.e) depends on the cost of fossil fuel. Now, the number of targets is going for renewable energy including hydrogen [38,39,40]

Advantages

• High energy density.
• It can be renewable and eco-friendly.
• More powerful and energy-efficient.
• Zero-emission gas.
• Biohydrogen can be stored for a longer period.

Limitations

• The biomass feedstock was costly and developed low-cost methods for growing, harvesting, transporting, and pretreating biomass.
• Difficult to maintain continuous H_2 rate in a long time.
• Microorganisms should be metabolically engineered to produce more yield.
• Low commercial application.
• The yield is comparatively low.
• Highly flammable.

CONCLUSION

In this review article, we have discussed the dark fermentative biohydrogen production by using *clostridia* and *Enterobacter sp*. with the help of wastewater as substrate. Using wastewater is an efficient method to produce hydrogen. With the help of waste as a substrate, we can derive many fossils fuels. There are different methods for hydrogen production and different sources have high, low yields, with more advantages and disadvantages. Researchers are still working on dark fermentative production to yield high hydrogen production on industrial bases. Most of them are searching for renewable energy sources because their availability is more. This method is challenging for future technology. It should be a key solution for our global needs.

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Competing interest statement

The authors declare no conflict of interest.

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