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## Production of Bio-Hydrogen Gas from Wastewater by Anaerobic Fermentation Process: A Review

**Ravi Kumar Parihar, Kanjan Upadhyay**

### Abstract

Hydrogen is viewed as a very clean energy source, since its combustion releases mainly dihydrogen monoxide as a reaction product. Additionally, it has the advantage of having the highest energy density when compared to any other fuel. Biological production of hydrogen gas has consequential advantages over chemical methods. The major biological process utilized for hydrogen gas production is photo-fermentation, dark fermentation of organic material, conventionally carbohydrates by bacteria and bio-photolysis. This review article summarizes the fermentative bio-hydrogen production from wastewater.

**Keywords:** Wastewater; Bio-hydrogen; Fermentation; Dark-Fermentation.

### 1. Introduction

One of the great challenges in the coming decade is how to get new renewable energy sources that are environmentally friendly and to replace high dependency on fossil fuels. Until recently, almost all of the energy needed is derived from the conversion of fossil energy sources, such as for power generation, industrial and transportation equipment that uses fossil fuels as a source of energy. Fossil fuels are a source of non-renewable energy and also have seriously negative impacts on the environment (Wahab *et al.*, 2014) [43]. Hydrogen gas is a clean energy source with a high energy content of 122 KJg<sup>-1</sup>. Unlike fossil fuels, hydrogen does not cause any CO<sub>2</sub>, CO, SO<sub>x</sub> and NO<sub>x</sub> emissions, producing water as its only by-product when it burns, reducing greenhouse effects considerably. Hydrogen is considered to be a major energy carrier of the future and can directly be used in fuel cells for electricity generation (Gonzalez *et al.*, 2011).

Nowadays, global energy requirements are mostly dependent on fossil fuels, which eventually lead to foreseeable depletion due to limited fossil energy resources. In recent times, a great deal of attention is being paid to the usage of hydrogen as an alternative and eco-friendly fuel throughout the world (Mohan *et al.*, 2007) [24]. Recent reviews on hydrogen indicated that the worldwide need on hydrogen is increasing with a growth rate of nearly 12% per year for the time being, and the contribution of hydrogen to the total energy market will be 8-10% by 2025 (Pandur and Joseph, 2012) [34]. Biological production of H<sub>2</sub> is one of the alternative methods where processes can be operated at ambient temperatures and pressures, and are less energy intensive and more environmentally friendly (Mohan *et al.*, 2007) [24].

Biological hydrogen production from renewable resources using microorganisms appears to be the most attractive method compared to other hydrogen production processes because it has fewer environmental concerns. Biological methods mainly include photosynthetic hydrogen production (photo-fermentation) and fermentative hydrogen production (dark fermentation) (González *et al.*, 2011) [10]. Broadly, biological H<sub>2</sub> production processes can be classified as biophotolysis of water using algae and cyanobacteria, photodecomposition of organic compounds by photosynthetic bacteria, and fermentative H<sub>2</sub> production from organic compounds. So far, H<sub>2</sub> production by photosynthetic microorganisms was extensively studied while H<sub>2</sub> evolution by fermentation was treated with little attention. The fermentative evolution is more advantageous than photochemical evolution for mass production of H<sub>2</sub> by microorganisms, where various wastewaters can be used as substrates. Of late, H<sub>2</sub> production through anaerobic fermentation using wastewater as a substrate has been attracting considerable attention (Mohan *et al.*, 2007) [24].

Dark fermentation, traditionally known as anaerobic digestion, is considered as a feasible process because it generates biohydrogen from carbohydrate substrates including biomass and organic waste materials. However, the yield of bio-H<sub>2</sub> is relatively low, since H<sub>2</sub> is produced as an intermediate and can be further reduced to methane, acetate and propionate by hydrogen-consuming bacteria (HCB) during dark fermentation. To increase the production rate of biohydrogen, more attention needs to be given to developing methods that inhibit the activity of HCB and exclusively enrich hydrogen-producing bacteria. Critical factors in biological H<sub>2</sub> production are pH, temperature, feed concentration, bacterial population, retention period, etc. Recently, experiments have been carried out to study the possibility of hydrogen production using organic wastes from various industries in combination with the wastewater treatment strategy (González *et al.*, 2011) [10].

Starch manufacturing factories discharge huge amount of wastewater which is rich in biodegradable organic matter. This wastewater has to be treated prior discharging into sewer network. Generally, the chemical oxygen demand levels of starch wastewater range from 6 to 10 g/l and it can impose heavy loads on the environment or be expensive in terms of sewer disposal (Nasr *et al.*, 2012) [32]. Rice Bran De-oiled Wastewater is the carbohydrate rich and easily hydrolysable wastewater. Wastewater has a high chemical oxygen demand value and is therefore suitable for anaerobic treatment process (Sivaramakrishna *et al.*, 2010) [41]. Dairy wastewater contains complex organics such as polysaccharides, proteins and lipids, which on hydrolysis form sugars, amino acids and fatty acids. In subsequent acidogenic reaction, these intermediate products are converted to volatile fatty acids, which are further degraded by acetogens, forming acetate, CO<sub>2</sub>, and H<sub>2</sub>. Lastly, both acetate and H<sub>2</sub>/CO<sub>2</sub>, are converted by methanogens to CH<sub>4</sub>. To harness H<sub>2</sub> as end product from anaerobic process instead of CH<sub>4</sub>, inhibition of methanogenic reaction and enhancement for acidogenic reaction are important prerequisites. Also optimized operating conditions can result in good H<sub>2</sub> yield. In this direction, we have made an attempt to harvest H<sub>2</sub> from dairy wastewater treatment through anaerobic fermentation in suspended growth bioreactor using anaerobic mixed consortia, by restricting the methanogenic activity and manipulating operating conditions of the reactor (Mohan *et al.*, 2007) [24].

## 2. Types of Wastewater and Waste Materials

The major criteria for the selection of waste materials to be used in biohydrogen production are the availability, cost, carbohydrate content and biodegradability. Simple sugars such as *D*-glucose, sucrose and lactose are readily biodegradable and preferred substrates for hydrogen production. However, pure carbohydrate sources are expensive raw materials for hydrogen production. Major waste materials which can be used for hydrogen gas production may be summarized as follows (Kargi *et al.*, 2006) [16].

### 2.1. Carbohydrate rich industrial wastewaters

Some biodegradable carbohydrate containing and non-toxic industrial effluents such as dairy industry, olive mill, baker's yeast and brewery wastewaters can be used as raw material for bio-hydrogen production. Those wastewaters may require pretreatment to remove undesirable components and for nutritional balancing. Carbohydrate rich food industry effluents may be further processed to convert the carbohydrate content to organic acids and then to hydrogen gas by using proper bio-processing technologies (Kargi *et al.*, 2006) [16]. Lactose-rich wastewater can be found in the cheese and dairy

industry wastewater. Cheese whey contains about 5% lactose, which can be a substrate for fermentation purposes. Even though several technologies have been applied to convert lactose-rich wastewater to other products, utilization and disposal of wastewater are still one of the problems in dairy industries. Hassana *et al.*, (2009) [11], conducted experiment to study the possibility of hydrogen production from crude cheese whey by *Clostridium saccharoperbutylacetonicum*. Hydrogen production rate was affected by pH with the optimum at mild acidic range. The highest hydrogen production potential and yield of hydrogen were achieved at 1432 ml and 2.7 molH<sub>2</sub>/mol lactose, respectively at pH 6. An increase of yield of hydrogen production was achieved at 3 molH<sub>2</sub>/mol lactose *Clostridium thermolacticum* at continuous mode had evaluated the potential of hydrogen production from dairy wastewater coupled with wastewater treatment. Hydrogen gas production was found highest with the organic loading rate of 3.5 kg COD/m<sup>3</sup> day, with yield of hydrogen production at 1.105 mmolH<sub>2</sub>/ m<sup>3</sup>-min and 64.7% COD removal.

### 2.2. Waste sludge from wastewater treatment plants

The waste sludge generated in wastewater treatment plants contains large quantities of carbohydrate and proteins which can be used for energy production such as methane or hydrogen gas. Anaerobic digestion of excess sludge can be realized in two steps. Organic matter will be converted to organic acids in the acidogenic phase and the organic acids will be used for hydrogen gas production by using photo-heterotrophic bacteria.

### 2.3. Starch and cellulose containing agricultural or food industry wastes

Many agricultural and food industry wastes contain starch and/or cellulose which are rich in terms of carbohydrate contents. Complex nature of these wastes may adversely affect the biodegradability. Starch containing solid wastes is easier to process for carbohydrate and hydrogen gas formation (Kargi *et al.*, 2006) [16]. Starch wastewater has high organic content and suspended solids and nutrients (Tawfik *et al.*, 2013) [42]. Starch can be hydrolyzed to glucose and maltose by acid or enzymatic hydrolysis followed by conversion of carbohydrates to organic acids and then to hydrogen gas. Cellulose containing agricultural wastes requires further pre-treatment. Agricultural wastes should be ground and then delignified by mechanical or chemical means before fermentation. Cellulose and hemicelluloses content of such wastes can be hydrolyzed to carbohydrates which are further processed for organic acid and hydrogen gas production. It was reported that there is an inverse relationship between lignin content and the efficiency of enzymatic hydrolysis of agricultural wastes (Kargi *et al.*, 2006) [16].

## 3. Different Technologies for Production of Hydrogen Gas from Effluent Wastewater.

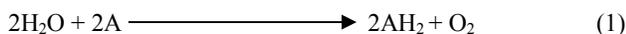
There are some common processes for biologically hydrogen production from waste water

### 3.1 Bio-photolysis

#### 3.1.1. Bio-photolysis-Direct process

The action of light on a biological system that results in the dissociation of a substrate, usually water, to produce hydrogen is referred to as biophotolysis. A direct biophotolysis of H<sub>2</sub> production is a biological process which utilizes solar energy and photosynthetic systems of algae to convert water into chemical energy.

The concept of the bio photolysis of wastewater with the formation of oxygen and hydrogen is the bringing together of two biological fields of scientific endeavour. The two areas of progress referred to are: (1) a greater understanding of the molecular events which occur in photosynthesis, and (2) a greater understanding of molecular events in microbial metabolism. The photosynthetic system which consists of two photo-systems operating in series can, by capturing two quanta of radiant energy, place an electron from the water-oxygen couple (0.8 volts pH 7.0) to a negative value as much as -0.7 volt which is 0.3 volts more negative than the hydrogen electrode. A minimum of eight quanta of radiant energy are required for the following photosynthetic equation:



Where A is an electron acceptor. For the purpose of employing these photosynthetic electrons for the reduction of protons to hydrogen by the action of a bacterial hydrogenase, the acceptor must have an oxidation-reduction potential near the potential of the hydrogen electrode and in its reduced state serve as a substrate for the hydrogenase. In this reaction oxygen produced by the photosynthesis strongly inhibits the hydrogen production. Inhibition is not due to the oxygen inactivation of hydrogenase, mainly due to the reaction of oxygen with the photo system.

### 3.1.2. Bio-photolysis-Indirect process

The most believable processes for future applied research and development are those which couple separate stages of microalgal photosynthesis and fermentations. These involve obsession of  $\text{CO}_2$  into storage carbohydrates (e.g. starch in green algae, glycogen in cyanobacteria) followed by their conversion to  $\text{H}_2$  by the reversible hydrogenase, both in dark and possibly light-driven anaerobic metabolic processes. In indirect biophotolysis, the problem of sensitivity of the  $\text{H}_2$  evolving process to  $\text{O}_2$  is usually circumvented by separating

$\text{O}_2$  and  $\text{H}_2$ . In a typical indirect biophotolysis hydrogen is produced as follows:



Based on a preliminary engineering and economic analysis, biophotolysis processes must achieve close to an overall 10% solar energy conversion efficiency to be competitive with alternatives sources of renewable  $\text{H}_2$ , such as photovoltaic electrolysis processes. Such high solar conversion efficiencies in photosynthetic  $\text{CO}_2$  fixation could be reached by genetically reducing the number of light harvesting chlorophylls and other pigments in microalgae. Similarly, greatly increased yields of  $\text{H}_2$  from dark fermentation by microalgae could be obtained through application of the techniques of metabolic engineering. Solar energy driven microalgae processes for biohydrogen production are potentially large-scale, but also involve long-term and economically high-risk (Wahab *et al.*, 2014) [43].

### 3.2 Dark Fermentation

#### Anaerobic suspended growth treatment processes

In the post ten years a number of different anaerobic processes have been developed for the treatment of sludges and high-strength organic wastes. Some commonly use anaerobic suspended growth treatment processes are given as follows: A) Anaerobic digestion, B) Anaerobic contact process, C) Up-Flow Anaerobic Sludge-Blanket Process (Mulik *et al.*, 2013) [26].

#### Anaerobic Biotreatment Process:

Dairy waste water was anaerobically treated by batch and repeated batch processes with the application of certain growth supporting material individually in glass aspirator.

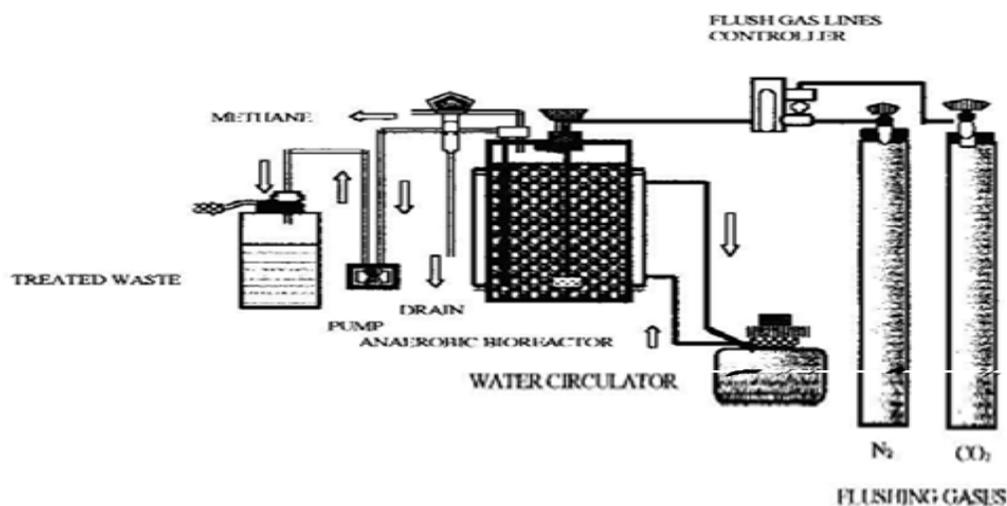


Fig. 1: Schematic diagram of Anaerobic Bio-treatment System. Support materials used for immobilization of bacterial population are bamboo rings PVC rings Foam cubes and Firebricks Gravels. Each reactor material was sealed with rubber stopper facilitated with thermometer, gas measuring system, gas sampling port and Hg manometer to measure gas pressure over the surface of the liquid and bioreactor temperature was maintained at  $35 \pm 0.5$  °C. Each bioreactor was also covered with black plastic sheet to protect it from the

bacterial photosynthesis and algal growth during cultivation operation. A mixture of  $\text{N}_2$  and  $\text{CO}_2$  gases (3:1) was sparged in the culture vessel to create anaerobic conditions prior to inoculation with enriched culture of mixed population of methanogenic bacteria. The samples of the treated effluent were taken from the bottom of the reactor through drain line. In the repeated batch process, 80% v/v treated waste was replaced with fresh medium based on initial concentration of volatile suspended solids (Qazi *et al.*, 2011).

### Up-Flow Anaerobic Sludge-Blanket Process

In the up flow anaerobic sludge-blanket process, the wastewater flows upward through a sludge blanket composed of biologically formed granules or particles. Treatment produced under anaerobic conditions (principally Methane and carbon dioxide) cause internal circulation, which helps in the formation and maintenance of the biological granules. Some of the gas produced within the sludge blanket becomes attached to the biological granules. The free gas and the particles with the attached gas rise to the top of the reactor.

### Factors Affecting Anaerobic Digestion

A. Environmental factors: 1) PH & Alkalinity 2) Volatile acid concentration 3) Temperature 4) Nutrient Availability 5) Toxic materials B. Basic Factors: 1) Bacteria 2) Food 3) Contact 4) Time (Mulik *et al.*, 2013) [26].

### 3.3 Photo-fermentation

Photo-fermentation differs from dark fermentation because it only proceeds in the presence of light. Hydrogen production by purple non sulfur bacteria was mainly due to the presence of nitrogenases under oxygen-deficient conditions using light energy and reduced compounds (organic acids). Photosynthetic bacteria undergo anoxygenic photosynthesis with organic compounds or reduced sulfur compounds as electron donors. Some non-sulfur photosynthetic bacteria were potent hydrogen producers, utilizing organic acids such as lactic, succinic and butyric acids, or alcohols as electron donors. Since light energy was not required for water oxidation, the efficiency of light energy conversion to hydrogen gas by photosynthetic bacteria was in principle much higher than that by cyanobacteria. Hydrogen production by photosynthetic bacteria was mediated by nitrogenase activity, although hydrogenases might be active for both hydrogen production and hydrogen uptake under some conditions. Photosynthetic bacteria had long been studied for their capacity to produce significant amounts of H<sub>2</sub>. The advantage of their use was in the versatile metabolic capabilities of these organisms and the lack of Photo system, which automatically eliminates the difficulties associated with O<sub>2</sub> inhibition of H<sub>2</sub> production. These photo-heterotrophic bacteria have been found suitable to convert light energy into H<sub>2</sub> using organic wastes as substrate in batch processes, continuous cultures or immobilized whole cell system using different solid matrices like agar gel and polyurethane foam. The overall reaction of hydrogen production is as follows:



Major drawbacks of the process involved low photochemical efficiencies (3-10%). This might be overcome by using co-cultures having different light utilization characteristics (Pandu *et al.*, 2012) [34].

### 4. Relevant Literature

Wastewater and waste materials used as economic source for the production of biohydrogen gas at different parameters such as organic loading rate, pH value, chemical oxygen demand, different temperature range etc.

In the year (2015) Lingfeng *et al.*, [21] studied the biohydrogen gas production from Beer wastewater in an internal circulation reactor. It was concluded when the condition of organic loading rate was at 30-42 kg COD/(m<sup>3</sup>.d), Hydrogen production rate was about 7.0-7.8 m<sup>3</sup>/(m<sup>3</sup>.d) and hydrogen content was 42-46%; COD removal rate was up to 20-30%, maximum hydrogen production rate was 7.83 m<sup>3</sup>/(m<sup>3</sup>.d) with

corresponding OLR of 36 kg COD/(m<sup>3</sup>.d). Wimonsong and Nitisoravut (2015) [44], investigated on the comparison of different catalysts for fermentative hydrogen production. That activity of different catalysts namely, Mg-Al hydrotalcite, Au/Zn-Mg-Al hydrotalcite and nanoporous activated carbon were tested in batch fermentative production of hydrogen using sucrose-fed anaerobic mixed culture at 37 °C. It was found the Au/Zn-Mg-Al hydrotalcite the maximum hydrogen yield of 2.74 ± 0.14 mol H<sub>2</sub>/mol sucrose at applied catalyst dosage of 167 mg/L at 37 °C. High initial activity of nanoporous activated carbon for hydrogen production accompanied with a high concentration of acetic acid. Jadhav *et al.*, (2015), reported that the production of biohydrogen was carried out by using dairy and rice mill effluent as a substrate by using *Clostridium acetobutylicum* NCIM 2877 in immobilized state. It was found that maximum production was 71.67 ± 0.88 ml with rice mill effluent and 52.0 ± 0.57 ml with dairy effluent at temperature 35 °C and 33 ± 1.2 ml and 64.33 ± 0.67 ml was obtained for rice mill effluent and dairy effluent respectively at pH 5.

In the year (2014) Romao *et al.*, [40] Studied the fermentative biohydrogen production from biomass waste product, in the batch operation with a reaction volume of 75 mL using lactose (20g/L) as substrate. It was found that the maximum hydrogen gas conversion of 4.84 mol H<sub>2</sub>/mol lactose at MgSO<sub>4</sub> concentration of 1.2 to 1.6 g/L and temperature range of 30 to 35°C. This clearly showed that increases in the temperature had an effect on cell growth and then in the synthesis of the target-product. Mullai and Sridevi (2014) [27], Studied that substrate utilization kinetics of biohydrogen production using Michaelis–Menten model. The kinetics substrate degradation rate v<sub>m</sub> was the maximum specific substrate degradation rate k<sub>m</sub> were estimated at 0.106 g COD h<sup>-1</sup> and 32.78 g COD h<sup>-1</sup>, respectively. The regression line had a correlation coefficient of 0.974, suggesting the applicability of the model for study of biohydrogen production. Chen *et al.*, (2014), investigated that the enhanced bio-hydrogen production from protein wastewater by altering protein structure and amino acids acidification type via pH control. It was obtained that the hydrogen production reached 205.2 mL/g-protein when protein wastewater pre-treated at pH 12 and then fermented at pH 10. Himabindu *et al.*, (2014) [12], studied on brewery effluent as the promising substrate source for hydrogen production using various mixed microbial consortia. In that anaerobic digestion process using mixed microbial consortia, hydrogen generated is consumed by the hydrogen utilizing methanogens. It was concluded maximum hydrogen production of 293ml/liter of brewery effluent was obtained with sewage mixed consortia as the inoculum at 55 °C and pH 6.5. Reginatto *et al.*, (2014) [37], reported that the methods for enrichment of an anaerobic sludge with H<sub>2</sub>-producing bacteria have been compared by using cassava processing wastewater as substrate. The sludge was submitted to three different pre-treatment: 1) heat pretreatment by boiling at 98 °C for 15 min., 2) heat pretreatment followed by sludge washout in CSTR operated at a dilution rate of 0.021 h<sup>-1</sup>, and 3) sludge washout as the sole enrichment method. It was concluded that the use of sludge washout as the sole sludge pretreatment method the most effective in terms of H<sub>2</sub> production, as compared to the heat and to the combined heat and washout pretreatments. Nasr *et al.*, (2014) [31], investigated biological treatment of dairy wastewater by using two units, conventional sequencing batch reactor and hybrid sequencing batch reactor. Reactor was operated at organic loading rates of 1.75, 2.33, and 3.50 g COD L<sup>-1</sup> d<sup>-1</sup>. Results obtained in the two reactors, the chemical oxygen demand and total Kjeldahl nitrogen removal

efficiencies decreased with increasing OLRs. It was obtained (hybrid system) higher specific substrate utilization rates (0.096-0.13 h<sup>-1</sup>) and biomass growth yield coefficient (0.25-0.67 g VSS g<sup>-1</sup> COD), as compared to the conventional sequencing batch reactor. Amorim *et al.*, (2014) [44], studied the bio hydrogen production from cassava wastewater in an anaerobic fluidized bed reactor. The effluent pH was approximately 5.0, while the influent chemical oxygen demand measured 4000 mg COD/L. The hydrogen yield production increased from 0.13 to 1.91 molH<sub>2</sub>/mol glucose as the HRT decreased from 8 to 2 h. The hydrogen production rate significantly increased from 0.20 to 2.04 L/h/L when the HRT decreased from 8 to 1 h. The main soluble metabolites were ethanol (1.87-100%), acetic acid (0.00-84.80%), butyric acid (0.00-66.78%) and propionic acid (0.00-50.14%). Overall concluded that the best hydrogen yield production was obtained at an HRT of 2 h. Nivedhan *et al.*, (2014) [33], investigated of hydrogen production from glycerol using microbial electrolysis cell. Cationic exchange membrane was used for the exchange of hydrogen ions from anode chamber (graphite plate) to cathode chamber (stainless steel) at external voltage supplied of 0.8 V. *Pseudomonas* bacteria were added along with the substrate. The hydrogen gas evolve was collected by the downward displacement of water, the concentration of hydrogen in the gas was found to be 50.3%. In the year (2013) Krishna *et al.*, [18] Studied the biohydrogen gas production from pharmaceutical wastewater treatment by a suspended growth reactor using environmental anaerobic technology. The optimization process included the selection of ideal co-substrate (sucrose) and nitrogen source (DAP) to examine the feasibility of hydrogen production from industrial effluent in a 50%-50% mixture of the complex feed and the industrial effluent. That study demonstrated the process of H<sub>2</sub> generation was found to be dependent on the Organic loading-rate applied and the selected reactor operating conditions pH-6 were found to be optimum for effective H<sub>2</sub> yield. Mullai *et al.*, (2013) [28, 29], reported the biohydrogen production through anaerobic dark fermentation and kinetic modeling using sediment microorganisms of pichavaram mangroves, India. The influence of process parameters studied such as effect of initial glucose concentration, initial medium pH, and trace metal (Fe<sup>2+</sup>) concentration. A maximum hydrogen yield of 2.34, 2.3, and 2.6 mol H<sub>2</sub> mol<sup>-1</sup> glucose, respectively, was obtained under the following set of optimal conditions: initial substrate concentration-10,000 mg L<sup>-1</sup>, initial pH-6, and ferrous sulphate concentration-100mg L<sup>-1</sup>, respectively. Kim *et al.*, (2013) [17], studied on the simultaneous organic solids disintegration and fermentative hydrogen production by pretreated sludge generated from wastewater treatment plant. Pretreated (30 min) sludge presented an optimal condition, resulting in maximum hydrogen yield (25.1 ml H<sub>2</sub>/g-VS) and the highest hydrogen content (60.0%). Maeda T. *et al.*, (2013) [23], It was studied biohydrogen production from oil palm frond juice and sewage sludge as substrates. The juice with sewage sludge provided an optimum carbon/nitrogen ratio since the yield of biohydrogen increased to 1.5 from 1.3 molH<sub>2</sub>/mol glucose. The results demonstrate the feasibility of our engineered strain for utilizing industrial biomass with an outstanding improvement towards hydrogen production when compared to the unmodified host. Mullai *et al.*, (2013) [28, 29], reported that the artificial neural network modelling for hydrogen production in a continuous anaerobic sludge blanket filter (ASBF). It was found that the maximum hydrogen yield at an initial glucose concentration of 11g/L and HRT of 24 h were substrate concentration (2.53-13 g glucose/L) and hydraulic retention time (6-30 h HRT) at mesophilic

temperature (32 ± 2 °C) and pH 5.5. It was obtained the performance function determination coefficient (R<sup>2</sup> = 0.9981) value between experimental and simulated hydrogen production rate revealed that ANN model could reliably be used as a simulation model in ASBF. Raj *et al.* (2013), investigated on the exploitation of Sago effluent as a source for hydrogen production. The pH was varied from 4.0-6.5, temperature ranges from 30 °C - 70 °C and substrate concentration (Glucose and Nitrogen) varied from 0.25 g/l - 1.25 g/l and their interaction on hydrogen gas production were studied. The raw cow dung was used as inoculum for hydrogen gas production. The maximum hydrogen production was occurred in acidic condition and at 1.25 g/l of substrate concentration under 55 °C.

In the year (2012) Nasr *et al.*, [32] Conducted experimental work to investigate the efficiency of up-flow anaerobic staged reactor (UASR) for hydrogen production from starch wastewater industry. An influenced pH value from 6.86 ±0.5 to 5.65 ±0.76 during the UASR was associated by increase in VFAs from 46 ±27 to 160±58 mg/l respectively. The UASR observed an average hydrogen production yield of 6 l/d, corresponds to 0.35 mol H<sub>2</sub>/mol glucose. Ibrahim *et al.* (2012) [13], Used five pretreatment methods namely acid, chemical, heat-shock, freezing and thawing, and base, for producing bacteria in anaerobic granulated sludge and its subsequent enhancement of bio hydrogen production. It was investigated the granulated sludge pre-treated by heat-shock showed maximum accumulated H<sub>2</sub> (19.48mLg<sup>-1</sup>COD), COD removal efficiency (62%), and biomass concentration (22.5 gL<sup>-1</sup>). Cavinate *et al.*, (2012) [6], reported that optimization of two-phase thermophilic anaerobic digestion of bio waste for bio-hydrogen production through reject water recirculation. It was noted that the hydrogen production at low hydraulic retention time (3d), both with and without reject water recirculation and at two organic loading rate (16 and 21 kgTVS/m<sup>3</sup>d). The better yields were obtained with recirculation where the pH reached an optimal value (5.5). The specific gas production was 51 l/kgVSfed and H<sub>2</sub> content in biogas 37%. Aravind *et al.*, (2012) [5], studied that the production of biohydrogen from dairy waste using mixed culture of enterobacter cloacae and clostridium pasteurinum in a bioreactor. Anaerobic continuous stirred tank reactor was used with working volume of 12.8L constructed and operated for 20 days and at 37 °C. It was noted that the process of hydrogen generation to be dependent on the OLR applied. The selected reactor operating conditions (acidophilic pH 6) were found to be optimum for effective hydrogen yield.

In the year (2011), Gonzalez *et al.*, Studied the fermentative hydrogen production in packed bed batch reactors to assess the influence of environmental factors over yield hydrogen production from dairy wastewater. Dried stems of *Opuntia imbricata* were used as substratum adding a pretreated mixed culture for biofilm formation under an acidic regime contributed to achieve a complete inhibit of methanogenic activity. It was observed that High-yield hydrogen production achieved (11.05 mM H<sub>2</sub>/g CODc) at pH 4 and 55 °C. Rohit *et al.*, (2011) [39], studied that large amounts of livestock manure, which come from cattle feedlots, poultry, and swine buildings, are causing a major environmental issue. It was well known that anaerobic digestion had successfully been used for the disposal of manures to produce methane in the last two decades. An alternative strategy has been developed to convert dairy manures to bio-hydrogen as a high value-added clean energy source instead of methane. The focus of this study was investigating the performance and optimal operating conditions of bio-hydrogen production from dairy manure.

Ismail *et al.*, (2011) <sup>[14]</sup>, investigated on production of bio hydrogen from palm oil mill effluent (POME) experiments were conducted in a continuous stirred tank reactor seeded with polydimethyl-siloxane (PDMS) immobilised mixed cultures. It was charged with hydrogen producing bacteria from an anaerobic digester used for treating POME were acclimatised and immobilised in PDMS. The soluble carbohydrate degradation efficiency was highest at 81.2% during HRT 4 days at 55 °C.

In the year (2010), Sivaramakrishna *et al.*, <sup>[41]</sup> Investigated biohydrogen production in an anaerobic batch reactor operated at 57 °C and pH6 with rice bran de-oiled wastewater as substrate. The hydrogen generating mixed micro flora was enriched from slaughter house sludge through acid treatment (pH 3-4, for 24h) coupled with heat treatment (1h at 100 °C) to eliminate nonspore forming bacteria and to inhibit the growth of methanogenic bacteria prior to inoculation in the reactor. It was observed that the hydrogen production rate was maximum at 57 °C (1861±14ml/L-WW/d) compared to 37 °C (651±30ml/L-www/d). Krishna *et al.*, (2010) <sup>[19]</sup>, studied that the influence of various wastewaters on bio hydrogen production by anaerobic fermentation using periodic discontinuous suspended growth reactor. Hydrogen production through anaerobic fermentation of synthetic feed consists of specific concentrations of several nutrients required for anaerobic fermentation. The process aimed at studying the variations of hydrogen production with nutrient addition. It was concluded that the substrate conversion efficiencies of the complex feed is higher than synthetic feed. Lin *et al.*, (2010) <sup>[20]</sup>, studied the effects of pH (4.0-8.0), substrate concentration (1.24-6.20 g COD L<sup>-1</sup>) and nutrient addition on the fermentative hydrogen production from preserved fruits soaking solution in batch assays using response surface methodology. The peak H<sub>2</sub> yield of 2.64 mol H<sub>2</sub> mol<sup>-1</sup> hexose was obtained at a preserved fruits soaking solution concentration of 3.72 g COD L<sup>-1</sup>, initial pH cultivation of 6 and without nutrient addition.

In the year (2009) Nakhla *et al.*, <sup>[30]</sup> reported biological hydrogen production from corn-syrup waste using a novel system. It was found that hydrogen production rate was a function of the organic loading rate; it increased from 10 to 34 L H<sub>2</sub>/L·d with the increase of OLR from 26 to 81 gCOD/L·d. It was observed that the highest hydrogen yield 3.2 molH<sub>2</sub>/mol hexose corresponds to 430mLH<sub>2</sub>/gCOD. Alves *et al.*, (2008) <sup>[3]</sup>, studied the effect of methanogenic inhibitors, inoculums type, and temperature on biohydrogen production using food components. It was found the bromoethanesulfonate-inhibited sludge produced more hydrogen with higher rates and smaller lag times than heat treated suspended sludge and granular sludge inoculums.

In the year (2007) Mohan *et al.*, <sup>[24]</sup> Studied the anaerobic biohydrogen production from dairy wastewater treatment in sequencing batch reactor (AnSBR). Bioreactor was operated at under acidophilic conditions (pH 6.0) mesophilic temperature (28±2 °C) with a total cycle period of 24 hrs phases at three different organic loading rates of 2.4, 3.5, and 4.7 Kg COD/m<sup>3</sup>-day, respectively. It was found that H<sub>2</sub> evolution rate dependent on the OLR applied at pH 6. Logan *et al.*, (2007) <sup>[22]</sup>, reported that the Hydrogen production from domestic wastewater using a bio-electrochemically assisted microbial reactor. When graphite granules were added to the anode chamber current density was increased when the domestic wastewater had a high initial chemical oxygen demand (COD>360 mg/L). It was concluded the final BOD of the treated wastewater was reduced to less than 7.0 ± 0.2mg/L and maximum hydrogen production 0.0125 mg-H<sub>2</sub>/mg-COD with an energy requirement equivalent to 0.0116 mg-H<sub>2</sub>/mg-COD,

producing an 8% net yield of H<sub>2</sub>. Yang *et al.*, (2007) <sup>[45]</sup>, reported that the hydrogen production from cheese processing wastewater by anaerobic fermentation. Anaerobic batch fermentation was using mixed microbial communities under mesophilic conditions. It was concluded fermentation experiments H<sub>2</sub> yields of 8 and 10 mM/gCOD at food-to-microorganism ratios of 1.0 and 1.5, respectively. In the continuous H<sub>2</sub> fermentation experiments were also performed using a completely mixed reactor at pH maintain in a range of 4.0–5.0. It was found that maximum H<sub>2</sub> yields 1.8 and 2.3 mM/gCOD fed for the loading rates tested with a hydraulic retention time of 24 h.

In the year (2006) Reungsang *et al.*, <sup>[38]</sup> Investigated on batch production of biohydrogen from cassava wastewater using, anaerobic seed sludge, a mixed culture of anaerobic seed sludge and *Rhodospirillum rubrum*, and a two-step batch culture of anaerobic seed sludge and *R. rubrum*. It was observed that the maximum specific hydrogen production of 429 mL H<sub>2</sub> g<sup>-1</sup> -VSS and a hydrogen yield of 71.3 mLg<sup>-1</sup> – COD at 55 °C and pH 5.0. Mohan *et al.*, (2006) <sup>[25]</sup>, reported on effect of various pretreatment methods on anaerobic mixed microflora to enhance biohydrogen production utilizing dairy wastewater as substrate. It was found the chemical pretreatment (2-bromoethane sulphonic acid sodium salt (0.2 g/l); 24 h) procedure enabled higher H<sub>2</sub> yield along with concurrent substrate removal efficiency. On the contrary, heat-shock pretreatment (100 °C; 1 h) procedure resulted in relatively low H<sub>2</sub> yield. In the case of combination integration of pH (pH 3; adjusted with ortho-phosphoric acid) and chemical pretreatment evidenced higher H<sub>2</sub> production. Hansen *et al.*, (2006) <sup>[8]</sup>, reported that the effect for selectively enriching hydrogen-producing microbial species present in biological sludge produced during animal wastewater treatment. It was noted that the maximum hydrogen composition of biogas at 32% to 45% was achieved within 50 h of batch culture, with no indication of methane throughout the study. Maximum specific hydrogen production achieved over the entire culture time was 223 mLH<sub>2</sub>/gcell and 379 mLH<sub>2</sub>/g cell in culture at pH 7. Agrawal *et al.*, (2006) <sup>[1]</sup>, investigated biological hydrogen production at different biomass by using phototrophic bacteria *Rhodobacter sphaeroides*, anaerobic dark fermentative bacteria's *Clostridium pasteurinum*, *Bacillus licheniformis* and *Enterobacter cloacae* with different substrates. It was found that the *Rhodobacter sphaeroides* took relatively longer duration (48 hrs) for hydrogen production. The optimum temperature and pH for maximum production of hydrogen (35%) where as in case of *Rhodobacter sphaeroides* it was found to be 32 °C and 7.5 respectively, hydrogen (21%) 32 °C and 7 for *Clostridium pasteurinum* respectively, hydrogen (8%) 30 °C and 6.8 respectively for *Bacillus licheniformis* and *Enterobacter cloacae*.

In the year (2005), Youn and Shin <sup>[46]</sup>, Studied the conversion of food waste into hydrogen by thermophilic acidogenesis. Hydrogen-producing microorganism by acclimation of raw seed sludge to the food waste without heat pretreatment and the growth of methanogenesis could be prevented at the thermophilic and acidogenic operational condition. It was found that the optimum operational condition for continuous hydrogen production from the food waste at 8 gVSI<sup>-1</sup> d<sup>-1</sup>, 5 days HRT and pH 5.5 ± 0.1 where the hydrogen production rate, content, yield and the efficiency of carbohydrate decomposition were 1.0 l H<sub>2</sub>/l-days, 60.5% (v/v), 2.2 mol-H<sub>2</sub>/mol-hexose consumed and 90%, respectively. Akiyama *et al.*, (2005) <sup>[2]</sup>, reported that the hydrogen production from waste Aluminum sources, such as dross, using an aqueous

solution of sodium hydroxide in a beaker and an autoclave. The result obtained from an XRD analysis showed that the white product that precipitated during the experiments contained aluminum hydroxide, the rate of hydrogen generation significantly increased with the concentration of sodium hydroxide and temperature of the aqueous solution, and the activation energy was  $68.4 \text{ kJ mol}^{-1}$ .

In the year (2002), Fang and Liu<sup>[9]</sup>, reported hydrogen production from wastewater by acidogenic granular sludge. The sludge was granulated in a hydrogen-producing acidogenic reactor when operated at  $26 \text{ }^\circ\text{C}$ , pH 5.5 treating a sucrose-rich wastewater. The influence of hydraulic retention time (HRT) and sucrose concentration on hydrogen production by the acidogenic granular sludge at a constant loading rate of  $25 \text{ g-sucrose/(l.day)}$ . The hydrogen accounted for 57% to 68% of the biogas at HRT ranging 4.6–28.6 h and sucrose concentration ranging 4,800–29,800 mg/l. It was investigated the hydrogen yield was more dependent on HRT and sucrose concentration. It ranged from 0.19 to 0.27 l/g-sucrose with the maximum yield occurring at HRT 13.7 h and sucrose concentration 14,300 mg/l in the wastewater. The acidified effluent was composed of volatile fatty acids and alcohols.

### Conclusion

This review article show anaerobic system of digestion can be used in treatment of various industries wastewater. Transformation of wastewater to reusable items are increasing much significance in today's reality. Elective strategies for getting biohydrogen from sewage wastewater are likewise broadly being explored. Point by point characterization of pH, COD, lipid and sugar division, carbon to nitrogen ratio, organic loading etc. of various sewage samples are to be done to get better understanding of wastewater to biohydrogen conversion.

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