COMPARISONS AND LIMITATIONS OF BIOHYDROGEN PRODUCTION PROCESSES: A REVIEW

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ABSTRACT

Hydrogen gas can be produced by conventional methods such as thermo-chemical gasification, pyrolysis, solar gasification and supercritical conversion. Hydrogen productions through biological methods are an attractive and alternate method for the replacement of fossil fuel in future. In this review, the major biological processes discussed for hydrogen production are bio-photolysis of water by algae, dark fermentation, photo–fermentation of organic materials and the sequential dark and photo-fermentation processes. Major constraints in dark and photo-fermentative hydrogen production include the raw material cost, lower hydrogen yield and rate of hydrogen production. To overcome those constraints, intensive research works are strongly recommended to be carried out on the advancement of these processes. The review showed effective utilization of low cost substrate such as agricultural, food industry wastes and effluents such as diary industry wastewater for hydrogen production with inexpensive energy generation and simultaneous wastewater treatment. It reveals that the hydrogen yield could be even achieved greater with the effective pretreatment methods of inoculum and substrates. This review explores the recent status and developments that have been made to improve the hydrogen production particularly with pretreatment methods and gave an outline about the unit cost of various hydrogen production processes.

KEYWORDS: *Biohydrogen, Bio-Photolysis, Dark-Fermentation, Photo-Fermentation and Pretreatment Methods.*

I. INTRODUCTION

Hydrogen becomes a significant and alternate energy carrier to fossil fuels because of its property of clean, renewable, high energy content and does not contribute to environmental problems such as greenhouse effect that leads to global warming. 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 contribution of hydrogen to total energy market will be 8-10% by 2025 [1]. Conventional hydrogen gas production methods are energy intensive processes requiring high temperatures (>840^{o}C). Electrolysis of water may be the cleanest technology for hydrogen gas production. However, electrolysis should be used in areas where electricity is inexpensive since electricity costs account for 80% of the operating cost of H_2 production. At present the total annual world hydrogen production is around 368 trillion cubic meters (2008). Of this amount, about 40% is used in the chemical industry, 40% in refineries and the other 20% in a large variety of processes [2] including its use as energy carrier. In 2005, 48% of the global demand for hydrogen was produced from steam reforming of natural gas, about 30 % from oil /naphtha reforming from refinery/chemical industrial off- gases, 18% from coal gasification, 3.9% from water electrolysis and 0.1% from other sources [3]. Due to increasing trend of hydrogen demand, development of cost effective and efficient hydrogen production technologies has gained significant attention. Biohydrogen production technology can

utilize the renewable energy sources like biomass for the generation of hydrogen, a cleanest energy carrier for the use of mankind. The hydrogen is considered as a dream fuel by virtue of the fact that it has high energy content per unit mass of any known fuel (142MJ/kg), is easily converted to electricity by fuel cells and on combustion it gives water as the only byproduct. Presently, 40 % H_2 is produced from natural gas, 30 % from heavy oils and naphtha, 18 % from coal, and 4 % from electrolysis and about 1 % is produced from biomass.

The advantages and disadvantages of various hydrogen production processes are outlined in Table 1[4]. Among various hydrogen production processes, biological method is known to be less energy intensive, for it can be carried out at ambient temperature and pressure [5]. Biological method mainly includes photosynthetic hydrogen production and fermentative hydrogen production.

Table 1: Comparison of various hydrogen production processes

Process	Advantages	Disadvantages
Solar gasification	Good hydrogen yield	Effective solar collector plates are required
Thermo-chemical gasification	Higher conversion can be achieved	Gas conditioning and tar removal is to be done
Pyrolysis	Gives carbonaceous material with oil, chemicals and minerals	Catalyst deactivation will occur
Supercritical conversion	Sewage sludge can be used easily, difficult by gasification	Selection of supercritical medium
Direct bio-photolysis	H_2 can be produced directly from water and sunlight	Requires high intensity of light, low photochemical efficiency and O_2 is inhibitory.
Indirect bio-photolysis	Blue green algae can produce hydrogen from water. It has the ability to fix N_2 from atmosphere	Uptake hydrogenates are to be removed.
Photo-fermentation	A wide spectral energy can be used by photosynthetic bacteria.	O_2 is inhibitory on nitrogenase enzyme and light conversion efficiency is low.
Dark fermentation	It can produce H_2 without light. No oxygen limitations and can produce several metabolites as by-products. Various substrates can be used in this anaerobic process.	Relatively lower H_2 yield. At higher H_2 yield, process becomes thermodynamically unfavorable.
Two-stage fermentation	Can produce relatively higher H_2 yield. By-products (metabolites) can be efficiently converted to H_2.	Requires continuous light source which is difficult for large scale processes.

Even though photosynthetic hydrogen production is a theoretically perfect process with transforming solar energy into hydrogen by photosynthetic bacteria, applying it to practice is difficult due to the low utilization efficiency of light and difficulties in designing the reactors for hydrogen production [6]. However, fermentative hydrogen production has the advantages of rapid hydrogen production rate and simple operation. Moreover, it can use various organic wastes as substrate for fermentative hydrogen production. Thus, compared with the photosynthetic hydrogen production, fermentative hydrogen production is more feasible and thus widely used. In addition, it is of great significance to produce hydrogen from organic wastes by fermentative hydrogen production, because it can not only treat organic wastes, but also produce very clean energy. Therefore fermentative hydrogen production has been received increasing attention in recent years. For cost effective, we need to go for the utilization of agricultural and some food industry effluents such as cheese whey, olive mill and baker's yeast industry wastewaters for hydrogen production. It also provides inexpensive energy generation with simultaneous wastewater treatment.

II. BIOHYDROGEN PRODUCTION METHODS

2.1. Bio-photolysis

2.1.1. 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_2 production is a biological process which utilizes solar energy and photosynthetic systems of algae to convert water into chemical energy.

The concept of the biophotolysis of water with the formation of oxygen and hydrogen is the bringing together of two biological fields of scientific endeavor, each of which has made phenomenal progress during the last two decades. 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, although thermodynamically feasible, heretofore not much thought has been given to the possibility of bio-photolysis. 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:

$$2H_2O + 2A \longrightarrow 2AH_2 + O_2 \quad (1)$$

where A is an electron acceptor. A variety of compounds may serve as Hill reagents. 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 [7]. Inhibition is not due to the oxygen inactivation of hydrogenase, mainly due to the reaction of oxygen with the photo system (ferredoxin or hydrogenase). Development by biotechnology of an oxygen stable Hydrogenase reaction is not plausible on thermodynamic and other backgrounds. To overcome oxygen inhibition, photosynthesis process needs to produce oxygen absorbers (eg; glucose-oxidase). Biological hydrogen production is often conducted in two stages under different atmospheric conditions, the first stage for cell growth followed by the second stage for hydrogen evolution. Nitrogen starvation is often at the end of the growth stage as an efficient metabolic stress to induce the nitrogenase activity. It was clear that a necessary technology breakthrough need to be attained for hydrogen productivity by nitrogen fixing cyanobacteria. However, direct biophotolysis, though limited by its relatively low hydrogen productivity, provides a working model for hydrogen production from water and sunlight energy. Therefore it is necessary to develop knowledge and technical innovations in hydrogen enzymes, electron carriers that would lead to a bio-mimetic water photolysis system avoids intrinsic incompatibility of simultaneous hydrogen and oxygen evolution and splits water into separated gas streams.

2.1.2. Bio-photolysis-Indirect process

The most credible processes for future applied research and development are those which couple separate stages of microalgal photosynthesis and fermentations (`indirect biophotolysis'). These involve fixation of CO_2 into storage carbohydrates (e.g. starch in green algae, glycogen in cyanobacteria) followed by their conversion to H_2 by the reversible hydrogenase, both in dark and possibly light-driven anaerobic metabolic processes [8]. In indirect biophotolysis, the problem of sensitivity of the H_2 evolving process to O_2 is usually circumvented by separating O_2 and H_2 [9]. In a typical indirect biophotolysis hydrogen is produced as follows:

$$12H_2O + 6CO_2 \longrightarrow C_6H_{12}O_6 + 6O_2 \quad (2)$$

$$C_6H_{12}O_6 + 12H_2O \longrightarrow 12H_2 + 6CO_2 \quad (3)$$

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 H_2, such as photovoltaic electrolysis processes. Such high solar conversion efficiencies in photosynthetic 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 H_2 from dark fermentation by microalgae could be obtained through application of the techniques of metabolic engineering. Another challenge is to scale-up biohydrogen processes with economically viable bioreactors. Solar energy driven microalgae processes for biohydrogen production are

potentially large-scale, but also involve long-term and economically high-risk. In the nearer-term, it may be possible to combine microalgal H_2 production with wastewater treatment.

2.2. Dark Fermentation

Dark fermentation is the fermentative conversion of organic substrate to biohydrogen. It is a complex process manifested by diverse group of bacteria by a series of biochemical reactions. Fermentative/hydrolytic microorganisms hydrolyze complex organic polymers to monomers which further converted to a mixture of lower molecular weight organic acids and alcohols by necessary H_2 producing acidogenic bacteria. Utilization of wastewater as a potential substrate for biohydrogen production has been drawing considerable interest in recent years especially in dark fermentation process. Industrial wastewater as fermentative substrate for H_2 production addresses most of the criteria required for substrate selection viz., availability, cost and biodegradability [10]. Chemical wastewater [11], cattle wastewater [12] and starch hydrolysate wastewater [13] have been reported to produce biohydrogen apart from wastewater treatment from dark fermentation process using selectively enriched mixed culture under acidophilic conditions. Various wastewaters viz., paper mill wastewater [14], food processing wastewater [15], rice winery wastewater [16], distillery and molasses based wastewater [17]; wheat straw wastes [18] and palm oil mill wastewater were also studied as fermentable substrates for H_2 production along with wastewater treatment. Utilizing mixed culture is extremely important and well-suited to the non-sterile, ever-changing, complex environment of wastewater treatment. Some anaerobic mixed cultures cannot produce H_2 as it is rapidly consumed by the methane-producing bacteria. Successful biological H_2 production requires inhibition of H_2 consuming microorganisms, such as methanogens and pre-treatment of parent culture is one of the strategies used for selecting the requisite microflora. The physiological differences between H_2 producing bacteria and H_2 consuming bacteria (methanogenic bacteria) form the fundamental basis behind the development of various methods used for the preparation of H_2 producing seeds. When the inoculum was exposed to extreme environments such as high temperature, extreme acidity and alkalinity, spore forming H_2 producing bacteria such as Clostridium survived, but methanogens had no such capability. Also, hydrogen production from different agricultural wastes has been reviewed by Guo et al [19] given the present state of knowledge; further experimentation is required to better understand the impact on biohydrogen production performances of the compositions and the characteristics of different organic substrates. Pretreatment processes of agricultural waste also require specific investigation since the origins and compositions of the agricultural wastes determine which specific pretreatment is the most suitable.

In the Table 2, it was shown that higher hydrogen yield was achieved by utilizing mixed consortia rather than pure culture. Also mutant strain or genetically engineered microorganism will yield higher than the others. Though possessed significant advantages, the main challenge observed with fermentative H_2 production process was relatively low energy conversion efficiency from the organic source. Typical H_2 yields range from 1 to 2 mol of H_2/mol of glucose, which resulted in 80-90% of the initial COD remaining in the wastewater in the form of various volatile organic acids (VFAs) and solvents [38]. Even under optimal conditions about 60-70% of the original organic matter remains in solution [39]. Usage of unutilized carbon sources present in acidogenic process for additional biogas production sustains the practical applicability of the process. One way to utilize the remaining organic matter in a usable form is to produce additional H_2 by terminal integration of photo-fermentative process for H_2 production and methane by integrating acidogenic process to terminal methanogenic process [40]. Baghchehsaree et al [21] reported the H_2 yield of 2.18mol/mol glucose by using *Thermotoga neapolitana*, whereas Cakir et al [25], reported 2.4mol/mol glucose from wheat straw wastes using anaerobic sludge. Though the earlier have used pure culture and substrate (glucose), it yields lesser than the later using anaerobic sludge. The possible reason is that the waste must be subjected to appropriate pretreatment before deploying it for fermentative hydrogen production to enrich the hydrogen producing bacteria, repressed the methanogenic activity. This enhances the higher yield, in dark fermentation, thereby paves the way for simultaneous waste treatment and inexpensive energy generation from the low cost substrate.

Perera et al [41] evaluated the net energy gain for dark fermentation processes using various substrates. Evaluation reported that the improvement in hydrogen yield at higher temperature is not

justified as the net energy gain not only declined with increase of temperature, but also was mostly negative for above the ambient temperature.

Table 2: Comparison of various processes on biohydrogen production by dark fermentation

System	Culture	Substrate	Volumetric H_2 production rate,(mL^{-1} h^{-1})	H_2 Yield, ($molH_2$ mol $glucose^{-1}$)	Reference
Batch	*Thermotoga neapolitana*	Glucose	51	3.85	[20]
Batch	Anaerobic digester sludge	Glucose	-	2.18	[21]
Continuous	Mixed culture	Glucose	5100	2.1	[22]
Batch	Rice Rhizosphere microflora	Apple pomace wastes	-	2.3	[23]
Continuous	*C.acetobutylicum* ATCC 824	Glucose	1270	-	[24]
Batch	Heat treated anaerobic sludge	Acid-Hydrolyzed ground wheat	7.42	2.4	[25]
Batch	Defined consortium	Sewage sludge	-	35.54mL g $sludge^{-1}$	[26]
Batch	*Bacillus coagulans*	Glucose	-	2.28	[27]
Batch	*Klebsielle oxytoca* HP1	Glucose	350	3.6	[28]
Batch	Cow dung compost	Wheat straw wastes	10.14mL g TVS^{-1} h^{-1}	68.114mL g TVS^{-1}	[18]
Batch	*Clostridia sp*	Glucose	1496	1.7	[29]
Batch	*E. cloacae* IIT-BT 08	Sucrose	660	6.0	[30]
Batch	*Thermoanaerobacterium*	Starch in wastewater	1.9 ml/h	92	[31]
Batch	*C. pasteurianum*	Starch	4.2mlh^{-1}	106[b]	[32]
Batch	Mixed culture	Glucose	-	2.1	[33]
Continuous	Anaerobic mixed consortia	Diary wastewater	-	0.122[a]	[34]
Batch	Mixed culture	Sewage bio-solids	-	700[a]	[35]
Continuous	Sludge from WTP	Rice winery wastewater	-	2.14	[16]
Batch	Sludge from WTP	Potato processing wastewater	-	6.0[a]	[16]
Batch	*Thermoanaerobacterium*	Starch wastewater	-	92[b]	[31]
Continuous	Activated sludge and digested sludge	Glucose	2360	1.16	[36]
Continuous	*T.kodakaraensis* KOD1	Starch	146[c]	-	[37]

a - mmol H_2 $gCOD^{-1}$
b - mL H_2 g^{-1} starch
c - mmol H_2 gdw^{-1} h^{-1}

It was concluded to operate the dark fermentation at the near-ambient temperatures to maximize the net energy gain [41]. And this should be done by direct electricity production from the dark fermentation via microbial fuel cells. But this was contradictory to the earlier study by Wu et al [42], has achieved positive net energy gain of 0.8kJ/g COD in spite of high fermentation temperatures of

40^{o} C. Reason for this higher achievement of net energy gain is that they utilized high sucrose concentration of 30g/L and high hydrogen producing bacteria [42].

2.2.1. Effect of Pretreatment Methods

Pre-treatment helps to accelerate the hydrolysis step, thus, reducing the impact of rate limiting step and augment the anaerobic digestion to enhance the H_2 generation [43]. Several pre-treatment procedures viz., heat-shock, chemical, acid, alkaline, oxygen-shock, load-shock, infrared, freezing, etc., were employed on a variety of mixed cultures [44, 45] for selective enrichment of acidogenic H_2 producing inoculum. pH also played a critical role in governing the metabolic pathways of the organism where the activity of acidogenic group of bacteria was considered to be crucial. Optimum pH range for the methanogenic bacteria was reported to be between 6.0 and 7.5, while acidogenic bacteria functioned well below the 6 pH [46]. The pH range of 5.5-6.0 was considered to be ideal to avoid both methanogenesis and solventogenesis which was the key for effective H_2 generation. Effect of various pretreatment methods for the production of biohydrogen was outlined in the Table 3. The pretreatment methods used for selective enrichment of hydrogen producing anaerobic consortia considerably influenced the H_2 yield and acid forming pathways of glucose.

Table 3: Comparison of biohydrogen yield by different pretreatment methods

Inoculum	Substrate	Pre-treatment method employed	Max.H_2 yield($molH_2$ mol glucose^{-1})	Optimal pre-treatment method	References
Anaerobi sludge	Sea water culture medium	Acid, alkali, heat-shock, KNO_3 and control	1.2[c]	Heat-shock	[47]
Dairy manure wastes	Dairy manure	Acid, NaOH, infrared radiation	31.5 [b]	Acid pretreatment	[48]
Sludge of WTP	Corn Stover	Steam explosion process-neutral and acid	3.0	Steam explosion process with acid	[49]
Digested sludge	Glucose	Acid, base, heat-shock, aeration and chloroform	1.8	Heat-shock	[50]
Cattle manure sludge	Glucose	Freezing and thawing, acid, heat-shock, and sodium 2-bromoethane sulfonate	1.0	Acid	[51]
Cow dung compost	Corn stalk wastes	Acidification	149.69 [b]	Acid	[36]
Methanogenic granules	Glucose	Acid, heat-shock and chloroform	1.2	Chloroform	[52]
Digested wastewater sludge	Sucrose	Heat-shock, aeration, acid, base, 2-bromoethanesulfonic acid and iodopropane	6.12[c]	Base	[53]
Anaerobic sludge	Dairy wastewater	Sodium 2-bromoethane sulfonate, acid, heat-shock and their combinations	0.0317 [a]	Sodium 2-bromoethanes ulfonate	[34]
Clostridium bifermentans	Wastewater sludge	Freezing and thawing, ultrasonication, acidification, sterilization and methanogenic inhibitor	2.1[a]	Freezing and thawing	[35]
Clostridium bifermentans	Wastewater sludge	Freezing and thawing, sonication, Acidification and	4.1 g kg DS^{-1}	Freezing and thawing	[54]

		sterilization			
Pseudomonas sp. GZ1	Waste sludge	Sterilization, microwave and ultrasonication	15.02ml g $TCOD^{-1}$	Sterilization	[55]
Anaerobic mixed microflora	Glucose	Heat, alkaline and acidification	1405mL	Heat treatment	[56]
Anaerobic mixed culture	Sugar-beet pulp	Alkaline, thermal, microwave, thermal-alkaline and microwave-alkaline	5.15[a]	Alkaline	[57]
Anaerobic sludge	Corn stover	Microwave assisted acid treatment and thermal acid treatment	1.53	Microwave assisted acid treatment	[58]

a - mmol H_2 g COD^{-1}
b - mlH_2 g TVS^{-1}
c - molH_2 mol $sucrose^{-1}$

Different pretreatment methods resulted in variations in the fermentation pathways of glucose. Along with an increase in the temperature or use of alkaline or acid pretreatment, the fermentation pathway of glucose converted from ethanol and butyric types to propionate and butyric types, resulting in a decrease in H_2 yield [56]. From the Table 3, after thorough examination, it was strongly recommended that the chemical pretreatment method was most preferable, though few results showed heat shock and base treatment method optimum. Addition of chemicals altered the physiological conditions and environment and enhanced the production and yield. This showed that some inoculum or substrates need suitable treatment based on their waste characteristics. Also there exists some disagreement in the optimum pretreatment method, for example, in the recent published work; Ozkan et al [57] reported alkaline pretreatment on beet pulp is optimum, whereas Liu and Cheng [58] reported microwave-assisted acid pretreatment on corn stover is optimal. Based on these results, it was understood that the possible reason for the disagreement is the difference in their studies with the type of substrates (sugar-beet pulp and corn stover) used for pretreatments, since it has different composition and characteristics in its origin.

2.2.2. Effect of pH

pH is another important factor that influences the activities of hydrogen producing bacteria and the fermentative hydrogen production, because it may affect the metabolism pathway. It has been examined that in an appropriate range, increasing pH could increase the ability of hydrogen producing bacteria to produce hydrogen. Since some studies on fermentative hydrogen production were conducted in batch mode with pH control, while some others were conducted in continuous mode, in these cases, the effect of pH on fermentative hydrogen production was investigated; there exists certain disagreement on the optimal pH for fermentative hydrogen production. For example, the optimal pH for fermentative hydrogen production reported by Mu et al [59] was 4.2, while that reported by Zhao and Yu [60] was 7.0. The possible reason for this disagreement was the difference among these studies in terms of inoculum, substrate and pH range studied. Similarly optimal H_2 production appears to take place with a pH of 5.0 to 6.0 for food wastes [61, 62]; whereas a neutral pH is recommended for crop residues and animal manure [63]. Two different types of experimentation have been performed to determine the optimal pH: one involved adjusting different initial pH in a series of batch tests while the other maintained the same pH in continuous reactors during the fermentation process [63, 64]. In addition, sucrose was the most widely used substrate during the investigation of the effect of pH on fermentative hydrogen production. Thus, investigating the effect of pH on fermentative hydrogen production using organic wastes as substrate is recommended. Ren et al reported the batch experiments with maximum hydrogen yield of 1.77mmol/mmol of glucose were achieved at pH 6.0. Low hydrogen yields observed at pH 4.0 are due likely to inhibitory effects on the microbial growth, although a low pH can be thermodynamically favorable for hydrogen production. Lower yields not only attributed to thermodynamically unfavorable, but also metabolically unfavorable for hydrogen production [65].

2.3. 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 nitrogenase 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_2. The advantage of their use was in the versatile metabolic capabilities of these organisms and the lack of Photo system II, which automatically eliminates the difficulties associated with O_2 inhibition of H_2 production. These photo-heterotrophic bacteria have been found suitable to convert light energy into H_2 using organic wastes as substrate in batch processes, continuous cultures [66] or immobilized whole cell system using different solid matrices like agar gel and polyurethane foam. The overall reaction of hydrogen production is as follows:

$$C_6H_{12}O_6 + 6H_2O + hv \longrightarrow 12H_2 + 6CO_2 (\Delta G_o = +3.2 \text{ kJ}) \quad (4)$$

Major drawbacks of the process involved low photochemical efficiencies (3-10 %). This might be overcome by using co-cultures having different light utilization characteristics. Production of biohydrogen by photo fermentation using different substrates and inoculum were outlined in Table 4. It was clear from the Table 4 that, Koku et al. [69] reported the hydrogen production rate of 5.0mLH_2/L h, whereas Basak and Das [70] have reported the maximum production rate of 6.55mLH_2/L h from the same substrate Maleic acid. The possible reason for the deviation in their production rate is on the characteristics of microorganism. It was clearly evident that, production rate and yield could be improved by studying the characteristics and engineering the hydrogenase enzyme to enhance its activity.

Table 4: Comparison of Biohydrogen production rate by various photo-fermentation processes

System	Organism	Substrate	H_2 production rate,(mlL^{-1} h^{-1})	References
Batch	*Rhodopseudomonas*	Starch	25.0	[67]
Batch	*Rhodobacter capsulata*	Starch	0.88	[68]
Batch	*Rhodopseudomonas*	Acetate	25.2	[67]
Batch	*Rhodobacter capsulata*	Acetate	0.88	[68]
Batch	*Rhodobacter sphaeroides* RV	Lactate	62.5	[66]
Batch	Rhodobacter sphaeroides	Malate	5.0	[69]
Continuous	*Rhodobacter sphaeroides* O.U 001	Malic acid	6.55	[70]
Continuous	Rhodobacter sphaeroides GL-1	Lactate	2100	[71]
Batch	*Anabaena variabilis*	Water	2.0	[72]

The fermentation process for hydrogen production has been investigated so far, but yet there is lack of details regarding the application of kinetic studies in the photo-fermentation. This review has shown the kinetic studies on biohydrogen production by using the modified Gompertz equation for fitting the

experimental data of cumulative hydrogen production [68, 46, 59, 73]. The modified Gompertz equation is:

$$H(t) = Hexp\left\{-exp\left[\frac{R_{max} \times e}{H}(\lambda - t) + 1\right]\right\} \quad (5)$$

H(t) is cumulative hydrogen production(g/l) during the fermentation time t(h), H is the maximum gas production potential (g/l), R_{max} is the maximum production rate(g/lh), λ is lag time to exponential product formed(h) and e is 2.7183. The values of H, R_{max} and λ are normally determined by best fitting the experimental data using the software curve Expert 1.3[74]

2.4. Two-Stage Fermentation

Combined dark and photo fermentation was a rather new approach in biological hydrogen gas production. It has certain advantages over single stage dark-fermentation or photo-fermentation processes. A three step process scheme consisting of pre-treatment-hydrolysis, dark fermentation and photo-fermentation could be used for this purpose. The first step of pre-treatment includes grinding, acid hydrolysis, neutralization and nutrient balancing to produce carbohydrate solution from the biomass. Fermentable sugars were converted to organic acids, CO_2 and hydrogen in the dark fermentation phase. Light-fermentation was used for production of hydrogen from organic acids under anaerobic conditions in the presence of light. The effluent of dark fermentation in hydrogen production provides sufficient amount of organic acids for the photo-fermentation. Therefore, the limitation by the organic acid availability would be eliminated. Using dark and photo fermentative bioreactors hybrid fermentation technology might be one of the promising routes for the enhancement of H_2 production yields. The synergy of the process lie in the maximum conversion of the substrate which otherwise fail to achieve a complete conversion due to thermodynamic and other limitations [75]. Thus, in this system the light independent bacteria and light dependent bacteria provide an integrated system for maximizing the H_2 yield. Further utilization of organic acids by photo-fermentative bacteria could provide better effluent quality in terms of COD. However, the system should be well controlled to provide optimum media composition and environmental conditions for the two microbial components of the process. In such a system, the anaerobic fermentation of carbohydrate (or organic wastes) produces intermediates, such as low molecular weight organic acids, which are then converted into H_2 by the photosynthetic bacteria in the second step in a photo-bioreactor. Higher hydrogen production yields could be obtained when two systems are combined. In the Table 5, it was observed that maximum yield of 14.2mol/mol sucrose was achieved from sucrose by utilizing *Caldicellulosiruptor* and photosynthetic bacteria of *Rhodopseudomonas capsulatus*.

Table 5: Comparison of Biohydrogen production and yield by two stage dark and photo-fermentation processes

Substrate	Inoculum	H_2 yield (molmol glucose^{-1})	Rate of H_2 production (mlL^{-1}h^{-1})	References
Beet molasses	*Caldicellulosiruptor* and *Rhodopseudomonas capsulatus*	4.2	7.1	[76]
Sucrose	*Clostridium pasteurianum* and *Rhodopseudomonas palustris* WP3-5	14.2mol H_2mol sucrose^{-1}		[77]
Starch manufac turing waste	*C. butyricum* and *Enterobacter* HO-39 and *Rhodobacter sp.* M-19	7.2	-	[78]
Cassava starch	*Clostridium sp* and *Rhodopseudomonas palustris*	6.07mol H_2 mol sucrose^{-1}	334.8	[79]
Glucose	*Lactobacillus delbrueckii* and *Rhodobacter sphaeroides* RV	7.1		[80]
Olive mill wastewater	Activated sludge and *Rhodobacter sphaeroides* O.U.001	-	11	[81]
Glucose	*E.coli* HD701and *Rhodobacter sphaeroides* O.U.001	-	5.2	[82]
Water hyacinth	Mixed bacteria and immobilized *Rhodopseudomonas palustris*	596ml H_2gTVS$^{-}_{1}$	-	[83]
Food waste	Anaerobic mesophilic and thermophilic	1.8	-	[84]

	acidogenesis			
Glucose	*Enterobacter clocae* DM11 and *Rhodobacter sphaeroides* O.U.001	5.3	-	[85]
Makkoli wastewater	*Clostridium butyricum* NCIB 9576 and *Rhodobacter sphaeroides* E15-1	-	16	[86]
Glucose	*C. butyricum* and *Rhodopseudomonas palustris*	5.48	100	[87]

The major challenge in biohydrogen production by dark and light fermentation was to improve the rate and the yield of hydrogen production for an economic process. Biological and engineering studies must be concentrated on these issues. Raw material cost was another concern in biohydrogen fermentations. Therefore, waste materials and renewable resources such as biomass were strongly recommended to utilize as a substrate. Also in the case of two stage fermentation, there exists certain disagreement that Su et al [87] reported a maximum hydrogen yield of 5.48mol/mol glucose, whereas Cheng et al [79] reported a maximum yield of 6.07mol/mol hexose. The possible reason for the disagreement is the difference in their utilization of substrate as glucose and starch. The later was inoculated with immobilized cells and hence its yield was increased.

III. COST COMPARISON OF HYDROGEN PRODUCTION PROCESSES

The cost of H_2 generated from biological processes and other available conventional processes were tabulated in Table 6. Biological hydrogen was comparatively high than that of hydrogen from pyrolysis. Strategies should therefore be developed to effectively lower the cost of H_2 production for commercialization. From the Table 6, it was proven, hydrogen production by conventional methods are not affordable except in the case pyrolysis. Hence, it was shown that despite of its high energy content; the cost of biological hydrogen production was still not a cost effective when compared to the existing pyrolysis method (conventional hydrogen production). It was strongly recommended therefore for the future research in the biological method to overcome or to replace efficiently, the available conventional processes with the cost effective method for biological hydrogen production.

Table 6: Comparison of unit cost of hydrogen production processes with conventional processes

Name of the processes	Raw materials	Energy content of the fuel (MJ kg^{-1})	Unit cost of energy content of the fuel US \$/ $MBTU^{-1}$
Photo-biological hydrogen	H_2O, organic acids	142	10
Fermentative hydrogen	Molasses	-	10
Pyrolysis for hydrogen production	Coal, biomass	-	4
H_2 from advanced electrolysis	H_2O	-	11
H_2 from Nuclear Energy	Electrolysis and water splitting	-	12-19
H_2 by biomass gasification	Biomass	-	44-82
H_2 from Wind Energy	Wind mill	-	34
H_2 from Photovoltaic power station	Solar energy	-	42
H_2 from thermal decomposition of steam	H_2O	-	13
H_2 from photochemical	Organic acids	-	21
Gasoline	Crude petroleum	43.1	6
Fermentative ethanol	Molasses	26.9	31.5
Biodiesel	Jatropha seeds	37	0.4
Natural gas	Raw natural gas	33-50	10

Among the alternative energies, at present, biomass and bio-fuel are the ones closer to the parity in conventional and distributed systems, respectively. Increased efforts in the development of advanced

technologies to improve the technical feasibility and scalability of the hydrogen production based on renewable energy, higher carbon emission, large investment growth in renewable energies, etc., could make cost parities to be reached in the near future. Also, as the hydrogen production based in renewable technologies avoid fuel prices in uncertainties, mainly produced in the natural gas market, massive investment in hydrogen production with renewable technologies could be produced before the parities are reached [88].

IV. LIMITATIONS IN BIOLOGICAL HYDROGEN PRODUCTION

Few limitations are somehow discussed here for the smooth transition from the fossil fuel based economy to the hydrogen energy based economy as follows:

- Direct bio-photolysis processes, though essentially attractive, seems to suffer from the intractable barriers of oxygen sensitivity, intrinsic limitations in light conversion efficiencies, and very tedious economics.
- In indirect bio-photolysis, the use of nitrogenase enzyme with its inherent high energy demand and the low solar energy conversion efficiencies are the insurmountable factors.
- Although biological processes for the production of gaseous hydrogen have been well demonstrated with cultured microalgal biomass, these processes must still be integrated into a system capable of meeting basic requirements for overall efficiency of converting solar energy into fuels.
- Processing of some biomass feed stock was too costly and therefore need to develop low cost methods for growing, harvesting, transporting and pretreating energy crops and/or biomass waste products.
- There was no clear contender for a robust, industrially capable microorganism that can be metabolically engineered to produce more hydrogen.
- Several engineering issues need to be addressed which include the appropriate bioreactor design, difficult to sustain steady continuous H_2 production rate in the long term, scale-up, preventing interspecies H_2 transfer in non sterile conditions and separation/purification of H_2.
- Sensitivity of hydrogenase enzyme to O_2 and H_2 partial pressure severely decreases the efficiency of the processes.
- Insufficient knowledge on the metabolism of H_2 producing bacteria and the levels of H_2 concentration tolerance of these bacteria.
- A lack of understanding on the improvement of economics of the process by combination of H_2 production with other processes.
- The productivity and yield of H_2 from any of the processes explained above was low for commercial application.

To overcome these constraints a numerous improvements need to be done in the future research. The advancements in the scientific research such as development of bioreactor design, engineering of hydrogenase enzyme and genetic modification of microorganism are therefore strongly recommended to improve the yield and rates of production. Many studies are currently carrying out in these technical and scientific advancements for better output as a futuristic goal.

V. CONCLUSIONS

Biohydrogen production was the most challenging area with respect to environmental problems. Due to the energy potential of hydrogen, new processes were to be developed for sustainable hydrogen production in biological methods. This review emphasized the raw material cost as major limitations for bio-hydrogen production showing that the utilization of some carbohydrate rich or starch containing solid wastes or some industry wastewaters are an attractive approach for bio-hydrogen production. Many of the results have proved in better hydrogen yield from different substrates and inoculum followed by their effective and appropriate pretreatment technique. Also it was examined that the sequential or combined bioprocesses of dark and photo-fermentations seem to be the most attractive approach as compared to other methods used for bio-hydrogen production from carbohydrate rich wastes. Two major aspects need indispensable optimization, viz., a suitable substrate and ideal microbial culture that can convert the substrate efficiently to hydrogen. A

comparative study on available processes indicated that biohydrogen production requires greater improvement on the process mainly with respect to hydrogen yield from the cheaper raw materials. Hydrogen production can be improved with the scientific advancements such as genetic modification of organism, engineering of hydrogenase enzyme, by using improved bioreactor and also by hybrid process. The future of biological H_2 production mainly not only depends on research advances, i.e. improvement in efficiency through genetically engineering microorganisms and/or the development of bioreactors, but also on economic considerations (the cost of fossil fuels), social acceptance, and the development of hydrogen energy systems.

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