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Biohydrogen Production from Wastewaters

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Abstract

Biohydrogen production technology is an emerging field for the advanced wastewater treatment with cogeneration of energy. Besides, hydrogen is an excellent candidate with high energy value (122 kJ/g) than other known carbon-based fuels with no adverse effects to the environment as it releases only water vapor as the by-products during the combustion. Biohydrogen production technology can be assisted through two major pathways: (a) light-dependent reaction (biophotolysis and photofermentation) and (b) light-independent reaction (dark fermentation and microbial electrohydrogenesis cells). The light-dependent reaction can be catalyzed by photosynthetic bacteria, whereas the dark fermentation catalyzed by the heterotrophic bacterial group of facultative and obligate anaerobes. The wastewaters are a rich source of organic nutrients which supports the growth of hydrogen producers along with the disposal of waste and energy recovery. In the present chapter, the recent advancements on biohydrogen production technology from wastewaters with respect to the (a) inoculum development, (b) process optimization, (c) scale-up and (d) the challenges and perspectives toward the improvement of this emerging technology for the wastewater treatment.

Keywords: biohydrogen, dark fermentation, wastewater

1. An overview of biohydrogen production

The growing demand of the energy for daily life purposes urged us to seek an alternative and renewable energy carrier with less emission of the pollutants. Hydrogen is an essential and promising candidate for replacing the fossil fuels depletion and greenhouse gas emission

reduction. When burning, it releases only water vapor as a by-product with no adverse harmful gases such as NO_x and SiO_2 , and hence, it is considered as clean and carbon-free energy carrier. The energy content of hydrogen is 122 kJ/g, which is 2.75-fold greater than the existing hydrocarbon fuels makes an ideal energy carrier for various industrial, transportation and power generations.

Different types of hydrogen production are available such as fossil fuel by hydrocarbon reforming, coal gasification and partial oxidation which requires high temperature and pressure. The biologically adopted hydrogen production methods can be classified as (i) biophotolysis of water using algae/cyanobacteria, (ii) photodecomposition of organic compounds using photosynthetic bacteria, (iii) dark fermentative hydrogen production using strict anaerobic or facultative bacteria and (iv) microbial fuel cells (MFC). Each biological production method had distinct advantages and limitations. For example, the green algae/cyanobacteria decomposes the water into gas (H_2) and liquid (H_2O) in the presence of sunlight by photosynthesis pathway, whereas the slow growth of the algal cells and an inhibition of hydrogenase enzyme with the presence of traces of oxygen limit their application in large scale extent. The photosynthetic bacteria and dark fermentation bacteria share a similar metabolism for the breakdown of organic compounds for their energy and the liberation of energy [1, 2]. The photosynthetic bacteria use organic acids as a substrate and prone to the ammonium and oxygen toxicity, making it as unsuitable for commercial hydrogen production. In contrast, the dark fermentation degrades wide range of organic waste from complex lignocellulose, food waste and industrial wastewater to simpler monomers (sucrose, glucose). However, the chemical oxygen demand (COD) removal efficiency of the dark fermentation is relatively lower 33%, as it requires further treatment before discharge into the system. Moreover, the biomass growth rate and hydrogen production rate of the dark fermentation are comparatively higher than the other hydrogen production methods and make it as attractive candidate for industrial and commercial biohydrogen production [3]. Recently, the auxiliary methods for the hydrogen production from hydrogen effluent have been emerged through microbial fuel cell (MFC) or bioelectrochemical systems (BES) technology.

2. Hydrogen-producing microorganisms

Table 1 displayed the microbial strains helpful for biohydrogen production through dark fermentation [4]. Hydrogen production during fermentation involves either facultative anaerobic bacteria or strict anaerobic bacteria. Facultative anaerobes are capable of growing in the absence of oxygen. The most common hydrogen-producing facultative anaerobes are *Klebsiella pneumoniae* [5], *Escherichia coli* [6], *Enterobacter aerogenes* [7], *Rhodospirillum rubrum*, *Methanobacterium formicicum* [4]. Chookaew et al. [5] reported that *Klebsiella* sp. TR17 is able to produce biohydrogen from crude glycerol in an up-flow anaerobic sludge blanket (UASB) reactor with highest HPR of 242.15 mmol H_2 /L/d and HY of 44.27 mmol H_2 /g glycerol. Besides, the *Klebsiella pneumoniae* produce valuable by-products such as 1,3-propanediol and 2,3-butanediol [8]. Reungsang et al. [7] reported that the immobilized *E. aerogenes* ATCC

Wastewater type	Inoculum source	Hydrogen yield (HY) (mol/mol hexose added)	References
Distillery effluent	<i>Enterobacter cloacae</i>	165.3 mL/g COD	[32]
Cassava WW	<i>Clostridium acetobutylicum</i>	2.41 mol/mol glu	[33]
Rice mill WW	<i>Enterobacter aerogens</i>	1.74 mol/mol sugar	[34]
Rice mill WW	<i>Citrobacter ferundii</i>	1.40 mol/mol sugar	[34]
Rice mill WW	<i>Enterobacter aerogens</i> RM08	1.97 mol/mol	[35]
CMS	<i>Clostridium tyrobutyricum</i>	0.7 mmol H ₂ /g COD	[36]
CMS	<i>Clostridium pasteurianum</i>	1.1 mmol H ₂ /g COD	[36]
CMS	<i>Clostridium sporosphaeroides</i>	0.9 mmol H ₂ /g COD	[36]

Table 1. Hydrogen production using pure cultures WW, wastewater; CMS, condensed molasses soluble.

13048 produced major soluble metabolite products (SMPs), such as ethanol, 1,3-propanediol (1,3-PD), formic acid and acetic acid.

2.1. Facultative anaerobes

Facultative anaerobes play important roles in H₂ production by biological routes, as it can grow in the presence of oxygen, higher biomass growth rate and utilization of wide range of organic wastes. The widely studied facultative anaerobic model for hydrogen production is *E. coli* and *E. aerogenes*. Facultative anaerobes convert pyruvate to acetyl-coA and formate with the catalysis of pyruvate formate-lyase complex and then release H₂ with formate hydrogen lyase. The maximum theoretical hydrogen yield is 2 mol of H₂ per mole of glucose. The glucose metabolic pathway yields succinate, lactate, acetate, ethanol and formate, as fermentation end-products. *Enterobacter* sp. have been widely used in various reactor configuration from batch to continuous mode operation. Several attempts like coculture of the facultative anaerobes with strict anaerobes have been assessed to improve the biohydrogen production. The coculture has advantages over pure culture due to the less maintenance, technical feasibility and faster substrate utilization rate. Sivagurunathan et al. [9] demonstrated that the addition of enriched mixed culture with *Enterobacter cloacae* enhanced the hydrogen production rate of 2.25 L/L-d from beverage wastewater. In another report [6], immobilization of *E. coli* cells using sodium alginate increased the hydrogen production efficiency from fructose (1.17 mol/mol hexose) and beverage wastewater (1.65 mol/mol hexose), respectively.

2.2. Mixed consortia

The mixed consortia can be derived from a variety of different natural sources, such as sewage sludge, anaerobically digested sludge, compost, animal manure and contaminated soil (Table 2). Mixed culture contains different types of bacteria; it also contains methanogens or hydrogen-consuming bacteria. Mixing also determines the local shear stress that the flow applies to microorganisms. Mixed culture can be obtained from aerobic or anaerobic sludge in wastewater treatment plants or compost piles or any other source of bacteria. Currently,

Wastewater type	Inoculum source	Hydrogen yield (HY) (mol/mol hexose added)	References
BWW	EMC-sewage sludge + pig slurry	1.95 mol/mol glu	[37]
BWW	EMC + <i>E. coli</i> XL1 blue	260 mL/g COD	[11]
Sugar beet juice	Anaerobic sludge	2.0 mol/mol glu	[38]
Distillery WW	Anaerobic sludge	10.95 mmol/g COD	[39]
Dairy WW	Anaerobic sludge	15.33 mmol/g COD	[40]
Cheese processing WW	Mixed cultures	10.2 mM/g COD	[41]
Organic WW	Soil	2.32 mol/mol	[42]
Herbal WW	Slaughter house sludge	165 mL/g COD	[43]
CMS	Anaerobic sludge	1.5 mol/mol	[44]
Brewery WW	Anaerobic sludge	1.21 mol/mol	[45]
GWW	Anaerobic sludge	0.75 mol/mol	[46]

WW, wastewater; BWW, beverage wastewater; CMS, condensed soluble molasses; GWW, glycerine wastewater.

Table 2. Hydrogen production using mixed consortia.

researchers mainly focused two routes for microbial fermentative hydrogen production: one utilizes pure microbial strains and the other employs a mixed microbial consortium. Generally, the hydrogen-producing efficiency and hydrogen yield of pure bacteria are lower than mixed consortia. Several investigators have focused on hydrogen production by microbial fermentation using a mixed microbial consortium, because of low-cost organic substrates, high hydrogen yields and operated in non-sterile conditions.

3. Process optimization for scale-up

Biohydrogen production is an emerging research area in the sustainable biofuel production via anaerobic fermentation technology. Though the hydrogen production from biological routes seems attractive over other commercial process, the operational conditions are essential to optimize in order to attain the maximum achievable hydrogen production rates and yields. A few important parameters on these aspects are as follows:

- (a) Inoculum pretreatment
- (b) pH
- (c) Nutrient availability
- (d) Hydraulic retention time

Biohydrogen production through mixed consortia is a complex bioprocess where the inoculum source, substrate type, environmental factors (pH, temperature and substrate concentration), nutrient availability and HRT can influence the metabolic reactions of hydrogen

producers. Optimizing these factors is a paramount importance for enhancing the hydrogen production efficiency from organic wastes.

3.1. Inoculum pretreatment

The active acidogenic hydrogen-producing biocatalyst role is crucial, notably in a complex mixed culture microenvironment. In general, the hampering hydrogen yield from mixed consortia was observed due to (i) the competition of hydrogen-consuming microbes and (ii) diversion of the metabolic flux toward non-favorable hydrogen by-products. The hydrogen consumers, such as lactic acid bacteria, methanogenes and sulfur-reducing bacteria, not only act as a competitor for the hydrogen producers but also synthesize various by-products, which affect the growth of hydrogen producers. For instance, the release of proteinaceous toxin (bacteriocins) by lactate-producing bacteria acts as a suppressing factor for hydrogen production and microbial growth [10]. Thus, when the mixed culture is used as an inoculum source, pretreatment step acts as an important role in determining the efficiency of the hydrogen production from mixed consortia. **Table 3** showed the various pretreatment methods for enriching the hydrogen producers. The pretreatment step promotes the selective enrichment of hydrogen producers with a suppression of the hydrogenotrophic methanogenes and other hydrogen consumers. The suppression of the hydrogen consumers by pretreatment process allows the mixed consortia to produce the hydrogen as a major product. The fundamental basics relied with the pretreatment method are the physiological difference of the micro-organisms. The spore-forming hydrogen producers survive under the harsh pretreatment conditions, whereas the vegetative cells ruptured/killed during the pretreatment. Various pretreatment methods, such as heat shock, acid shock, alkali shock, chemical agents, load shock and oxygen shock, have been assessed for enriching the hydrogen producers from mixed consortia. Each pretreatment step has a significant impact on the suppression of the microbial populations and also the distribution of the microbial metabolism.

Among the various pretreatment methods, the heat-shock [11] pretreatment has been widely accepted as a suitable method for preparing the hydrogen-producing seed inocula, due to the relatively simple method for the suppression of the hydrogen consumers and selective enrich-

Substrate	Inoculum source	Pretreatment method	Hydrogen yield (HY)	References
Deoiled jatropha waste	Anaerobic digester sludge	Heat shock	20 mL H ₂ /g VS	[11]
Glucose	Anaerobic sludge	Acid shock	0.80 mol/mol	[12]
Sucrose	Anaerobic digester sludge	Base shock	3.06 mol/mol	[13]
Glucose	Anaerobic granular sludge	Chloroform	1.55 mol/mol	[14]
Desugared molasses	Digested manure	Load shock	237 mL H ₂ /g- sugar	[16]
Glucose	Anaerobic sludge	Repeated aeration	1.96 mol/mol	[17]
Glucose	Anaerobic sludge	Gamma irradiation	2.15 mol/mol	[47]

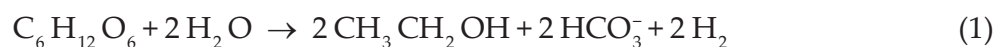
Table 3. Inoculum pretreatment method for enriching hydrogen production mixed consortia.

ment of the sporulating hydrogen-producing bacteria such as *Clostridium* sp. The acid-shock [12] and base-shock [13] pretreatments suppress the methanogenic activity by the narrow selective growth pH range of the methanogenes (6–7.5), whereas the *Clostridium* populations survive in the harsh condition due to the spore-forming capability. The chemical shock methods such as chloroform [14] and 2-bromoethanesulfonic acid (BESA) [15] have a complex structure, analog to the methanogenic coenzyme, and it acts as a inhibitor for the methanogenes. This method facilitated the suppression of the methanogenes, whereas the other non-spore-forming hydrogen producers such as *Enterobacter* sp. can also survive with the presence of *Clostridium* sp., thus enhancing the substrate utilization and hydrogen yield. The load-shock [16] treatment is directed by the exposure of the inoculum to a higher substrate concentration, and it leads to the surge in the pH with an accumulation of organic acids and inhibits the methanogenic populations.

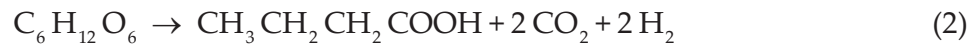
Ren et al. [17] demonstrated that application of various pretreatment methods, such as acid, alkaline, heat-shock and repeated aeration, can greatly affect the metabolic pathway and the microbial community distribution pattern. The dominant butyric acid-mediated hydrogen metabolism was observed with heat-shock and alkaline treatment, and mixed-type fermentation pathway was observed with the acid pretreatment, whereas the ethanol-type pathway was observed with repeated aeration treatment with a maximum hydrogen yield of 1.96 mol/mol glucose. The microbial community characterized by denaturing gradient gel electrophoresis (DGGE) revealed that the changes in the composition of the microbial dynamics affect the hydrogen yield. The strain *Ethanoligenens harbinensis* was detected under repeated aeration condition with an ethanol-mediated pathway, and the hydrogen-consuming propionic acid bacterium *Propionibacterium propionicus* was detected in acid treatment with low hydrogen productivity. The heat-shock-mediated mixed culture was dominated with *Clostridium* sp. which represents the butyric-acid-type metabolic pathway. Based on the evidence, the appropriate pretreatment method is essential for enriching the hydrogen-producing bacterial populations and enhanced hydrogen production.

3.2. pH

pH is the key driven parameter affecting the cellular metabolism of hydrogen-producing bacterial populations, since the prevalent end products of the bacterial metabolism vary with the changes in the medium pH. Based on the pH and the major end products formation, three metabolic pathways have been proposed (a) ethanol type (EtOH) (Eq. 1), (b) butyric type (HBu) (Eq. 2) and (c) propionic type (HPr) (Eq. 3). The former, HBu type, involved in the hydrogen-generating reactions, whereas the latter, HPr type, involved in the hydrogen-scavenging reactions. Hence, the elimination of the propionate formation is an essential step for the enhancement of hydrogen production.



$$\Delta G^0 = -235.0 \text{ kJ/mol}$$



$$\Delta G'_O = -254.0 \text{ kJ/mol}$$



$$\Delta G'_O = -279.4 \text{ kJ/mol}$$

pH affects the physiological conditions of the bacterial growth, metabolism and ions transport. Optimizing the pH is considering a key factor influenced the redox environment and the direction of electron flow toward the hydrogen formation. The experimental reports demonstrated that the optimal pH for the bacterial growth does not result in the elevated hydrogen production performances [3]. For the dark fermentative hydrogen fermentation, the optimal pH for efficient hydrogen production lied between 5.5 and 6.5 for various wastewaters and pure substrates [18]. In addition, the acidic pH induces the pyruvate transformation to volatile fatty acids (VFA) with concomitant hydrogen production, whereas the neutral pH facilitated the methanogenic pathway. Maintaining the acidogenic (5.5–6.5) pH is essential for controlling the methanogenic populations and efficient hydrogen production.

3.3. Nutrients

The inorganic nutrient supplements, such as nitrogen (N), phosphorus (P) and iron (Fe), along with carbon (C) source, are important for microbial growth and improvement in the hydrogen production. The nutrient at proper concentration is beneficial for hydrogen production. For instance, Lin and Lay [19] explained that at a carbon/nitrogen (C/N) ratio of 47, the hydrogen yield from sucrose was 1.9 times higher than the control with a value of 4.8 mol/mol substrate. In a pure culture thermotolerant *Kelbsiella* sp., the maximum hydrogen yield of 0.28 mol/mol glycerol was observed with 11.21 g/L glycerol, 2.84 g/L KH_2PO_4 and 5.66 g/L NH_4Cl , respectively [20]. Wang et al. [21] mentioned that the hydrogen production efficiency of glucose (313.3 mL/g glucose) was improved with low supplementation of nitrate 0.1 g/L; however, increased concentration of nitrate over 0.1 g/L significantly affected the hydrogen yield and the substrate consumption rate. The drop in hydrogen production is attributed by the inhibition of nitrogenase activity by surplus ammonium ions [22, 23]. The iron (Fe) is an important element essential for the hydrogenase activity, which directs the metabolic pathway by stimulating the active site for the ferredoxin (Fd). The addition of iron supplement was shown to improve the hydrogen production. Gadhe et al. [24] demonstrated the effects of nano-sized iron and nickel oxide nanoparticles by using dairy wastewater as a substrate, and it showed that an enhancement in hydrogen yield of 17.2 mmol/g COD is due to the enhanced activity of the ferredoxin oxidoreductase, ferredoxin and hydrogenase enzymes. Moreover, the optimal value for the Fe^{2+} concentration is varied with the type of substrates used. For instance, the optimal concentration reported by Liu and Shen [25] was 10 mg/L from starch, whereas palm oil mill effluent showed an optimal value of 257 mg/L [26].

3.4. Hydraulic retention times

The hydraulic retention time (HRT) is one of the key process control parameters influencing the continuous hydrogen production. HRT enables the better process control of the microorganisms that can regulate the metabolic pathway favorable for efficient hydrogen production. The long HRT permits the growth of hydrogen consumers mainly archaea, which is unsuitable for hydrogen production, whereas too low HRT leads to the washout of active biomass and deterioration of the reactor performances. The optimization of HRT is a paramount importance for the scale-up, long-term and sustainable hydrogen production. HRT controls the organic loading rate (OLR), substrate degradation and reaction kinetics. The organic wastes required long HRT, whereas the simple organics required short HRT [2]. The reported optimum HRT value for the wastewater ranges from 0.5 to 24 h. For example, the short HRT (0.5 h) provided the maximum hydrogen production rate of 14 L/L-d from condensed soluble wastewater [27], whereas the long HRT (24 h) is required for efficient conversion of olive mill wastewater with a HPR of 7.0 L/L-d [28]. The process parameters discussed above significantly influenced the hydrogen production; hence, careful assessment of each individual factor is important for stable hydrogen production.

4. Bioreactor design considerations for continuous hydrogen production

Bioreactor configuration is a notable factor in dark fermentative hydrogen production, as it influences the contact between the organic waste and hydrogen producers, substrate utilization, biomass dilution rate, etc. According to the feeding regime, the biohydrogen production can be conducted in batch, semi-continuous and continuous mode (**Table 4**). The batch mode operation is relatively simple and easier to control. Hence, the batch mode hydrogen reactors have been widely used to determine the feasibility of the organic waste feedstock and to optimize the environmental parameters such as pH, temperature, substrate concentration. In semi-continuous mode operation, the organic substrate was operated in a sequencing batch which includes feeding, reaction, settle and decant stages [29]. The sequencing batch operation is recommended for a viscous substrate like a POME and solid organic biomass like food waste and lignocellulosic biomass, where the physical contact between the substrate and microorganisms is limited, and this reactor mode operation enables the better hydrolysis rate, avoids clogging in the pipes and retains the effective biomass concentration. In continuous mode operation, the continuous supply of nutrients and the removal of the pollutants occur simultaneously with the aid of peristaltic pumps.

Although various reactor models assessed, the continuous mode operation is preferred for bench-scale and commercial-scale applications. The widely investigated model for continuous mode operation is the CSTR type, wherein the substrates and feedstocks are well mixed inside the reactor with the aid of the mechanical rotor; however, the biomass washout usually occurred at lower HRT [27, 30]. In some cases, the biofilm formed inside the CSTR is resistance to the biomass washout and thereby enhancing the hydrogen production performances. Chu et al. [27] investigated the CSTR reactor model by using condensed soluble molasses as a substrate with suspended and immobilized cells as inoculum source. The hydrogen production

Substrate	Inoculum source	Reactor mode	HPR (L/L/d)	References
Palm oil mill effluent	Anaerobic digester sludge	ASBR	6.7	[29]
Condensed molasses	Anaerobic sludge	CSTR	14.04	[27]
Beverage WW	Enriched mixed cultures	CSTR	37.56	[30]
Tofu processing WW	Anaerobic digester sludge	MBR	19.86	[48]
Desugared molasses	Anaerobic sludge	UASB	5.6	[49]
Olive mill WW	Anaerobic sludge	PBR	7.0	[28]
Beverage WW	Enriched mixed cultures	ICBR	55.4	[31]
Beverage WW	Anaerobic digester sludge	PBR	88.7	[50]

WW, wastewater; ASBR, anaerobic sequencing batch reactor; CSTR, continuously stirred tank reactor; MBR, membrane bioreactor; PBR, packed bed reactor; ICBR, immobilized cell bioreactor.

Table 4. Bioreactor types used in hydrogen production.

from immobilized cell was relatively lower with a maximum HPR of 7.6 L/L/d; however, the suspended cells operation provided the maxim HPR of 14.04 L/L/d, respectively. The observed variation is attributed by the washout of the active biomass in immobilized cells system (9.8 g volatile suspended solids (VSS)/L), poor mass transfer between the microbes and substrates and the increased lactic acid formation. On the other hand, the suspended cell system formed a hydrogen-producing granule (HPG) inside the reactor, and thus, it retains the active biomass (12.30 g VSS/L) and less formation of the lactic acid. Sivagurunathan et al. [30] demonstrated that the hydrogen production from ICBR [31] was higher (55 L/L/d) than the suspended cells CSTR (37.56 L/L/d) operation. The superior performance of the ICBR is due to the formation of granular biomass at short HRT of 3 h with the presence of *Selenomonas* sp. and further maturation of granules with the presence of active hydrogen-producing *Clostridium* Sp. The *Selenomonas* sp. act as a bio-glue for the development of granules. Moreover, the energy content analysis of the beverage wastewater with immobilized cells system analysis showed that it has the capability of reducing the CO₂ reduction efficiency of 2832 ton CO₂ equivalent/year.

5. Conclusion

Biohydrogen production from industrial wastewaters seems to be appropriate and environmental benign option for future sustainable hydrogen economy with simultaneous energy recovery and waste disposal. Various studies revealed the hydrogen production potential of wastewaters. Among them, sugar-rich wastewaters are the promising substrate for high-efficient hydrogen production rates and yields, due to their easier degradation rate and higher substrate concentration. Other key challenges that rely on dark fermentative hydrogen production from organic wastes are the low substrate conversion efficiency, moderate-to-low

hydrogen yield and residual organics in the effluents. In general, biohydrogen production is a primary step for wastewater treatment, in which a maximum 4 mol/mol glucose representing 33% of COD removal efficiency; nearly 70–80% of the residual organics remain untreated with the hydrogen-producing effluent, thus seeks further disposal of the effluent in the wastewater streams. The post-residual effluent has to be integrated with various two-step processes, such as methane production, photofermentation, microbial electrolysis cells, bioplastics production and microalgae cultivation, for maximizing the energy recovery.

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