

State of the Art of Bio-Hydrogen Production from Biomass

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INTRODUCTION

As a clean and renewable energy resource, hydrogen is regarded as the fuel of the future. Biotechnology of hydrogen production has aroused a broad attention around the world as an environmentally friendly process [1–4]. Hydrogen production by microorganisms is divided into two main categories:

- 1) Production by algae or phototrophic bacteria and
- 2) Production by anaerobic fermentation bacteria.

Currently, more research focuses on the utilisation of algae and phototrophic bacteria [5–10]. However, the efficiency of hydrogen production by phototrophic microorganism is low. The hydrogen evolution rate of *Rhodobacter sphaeroides* RV was only 1.4–1.6 L/L reactor/day [6] and 1.3 mL/mL porous glass media/h, and it cannot be continuously operated in the absence of light. In contrast, anaerobic hydrogen fermenting bacteria can produce hydrogen continuously without the need for photo-energy [11, 12]. Another attraction of anaerobic hydrogen bioproduction is that highly concentrated organic wastewater and biomass, such as municipal solid wastes, sewage sludge, can be used as raw material, which can solve pollution as well as generate hydrogen [4]. Currently, anaerobic hydrogen production has been mainly studied on bench scale using pure cultures such as *Clostridium* sp. [13], *Enterobacter aerogenes* [14–19], and *Ectothiorhodospira vacuolata* [20]. Although some studies have been conducted on mixed cultures of anaerobic bacteria [11, 21–28], the optimal condition for hydrogen production has not been fully understood.

Bacterial fermentation requires substrates such as glucose and/or sucrose to obtain energy for growth and maintenance, and produces several intermediate by-products such as

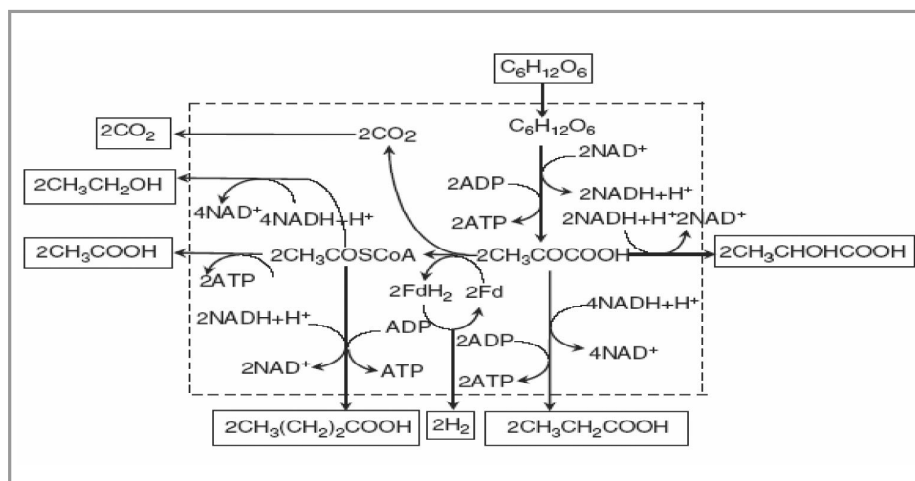


Figure 1: Representative pathways of fermentative hydrogen evolution and some other by-products (Fd, oxidised ferredoxin; FdH₂, reduced ferredoxin).

organic acids, alcohols and hydrogen during the metabolic pathways, as shown in Figure 1 [29]. Gibbs free energy values of these fermentation reactions are all positive, indicating these reactions are not spontaneous. The accumulation of intermediate products in systems can inhibit fermentation. The amount of hydrogen produced from glucose is affected by fermentation pathways and liquid end-products [28]. A maximum of 4 mol hydrogen is theoretically produced from 1 mol of glucose with acetic acid as the end-product, while a maximum of 2 mol hydrogen is theoretically produced from 1 mol of glucose with butyrate as the end-product. There is no hydrogen produced when the end-product is propionic acid. In practical operation, high hydrogen yields are associated with a mixture of acetate and butyrate as fermentation products. *Clostridium pasteurianum*, *Clostridium butyricum* and *Clostridium beijerinckii* are high hydrogen producers while *Clostridium propionicum* is a poor hydrogen producer [30, 31]. *Enterobacter aerogenes* strain E.82005 is another high hydrogen producer with 1.58 mol hydrogen/mol glucose [32]. However, little information is available for anaerobic hydrogen production at pilot scale with mixed

microbial cultures, although pilot-scale study is critical to testify the productivity before a new biotechnology is put into full scale operation, and using mixed microbial cultures is a more cost-effective and promising approach to achieve hydrogen bioproduction in large scale. In addition, as a by-product through fermentation pathways, hydrogen production is affected by fermentation end-products, including, acetic acid, propionic acid, butyric acid, and lactic acid (as shown Figure 1). But there is very limited information of the correlation between fermentation pathways and hydrogen production ability.

Our previous study [11] had confirmed that the acclimatised anaerobic activated sludge had a high hydrogen producing ability (as high as 10.4m³ H₂/m³reactor/d) in a continuous reactor with an available volume of 9.6 L. It has been found that a high cell density (higher than 5 g/L) in bioreactor is required to keep high hydrogen yield, since low cell intensity cannot efficiently convert organic substrates to hydrogen, especially at short hydraulic retention time (HRT<4h). To achieve this purpose, a variety of reactors with several microbial growth carriers, such as foam, plastic media, have been

Table 1: Micro-organism used for hydrogen generation

Classification	Name of microorganism	References
Green algae	<i>Scenedsmus obliquus</i>	Schnackenberg <i>et al.</i> , 1993
	<i>Chlamydomonas reinhardtii</i>	Greenbaum, 1990
Cyanobacteria Heterocystous	<i>C. moewusii</i>	Greenbaum, 1990
	<i>Anabaena azollae</i>	Banerjee <i>et al.</i> , 1989
	<i>Anabaena CA</i>	Kumar and Kumar, 1991
	<i>A. variabilis</i>	Tsygankov <i>et al.</i> , 1997
	<i>A. cylindrica</i>	Smith <i>et al.</i> , 1992
	<i>Notoc muscorum</i>	Spiller <i>et al.</i> , 1978
	<i>N. sponiaeforme</i>	Vyas and Kumar, 1995
Photosynthetic Bacteria	<i>Westiellopsis prolifica</i>	Vyas and Kumar, 1995
	<i>Rhodobater sphaeroides</i>	Fascetti <i>et al.</i> , 1998
	<i>R. capsulatus</i>	Krahn <i>et al.</i> , 1996
	<i>R. sulidophilus</i>	Singh and Srevstava, 1991
Fermentative Bacteria	<i>Enerobacter aerogens</i>	Tanisho <i>et al.</i> , 1999
	<i>E. cloacae</i>	Bothe and Kentemicj, 1990
	<i>Clostridium butyricum</i>	Tanisho <i>et al.</i> , 1987

developed [19, 15]. However, all matrices of growth carriers have a limitation on the microbial cell density due to their spatial occupation in bioreactors. High concentration of anaerobic biomass can overcome this limitation, since it possesses a strong ability of flocculation even in continuous bioreactors without growth carriers. Thereby, a high cell density is expected to be attained by employing the floccular anaerobic activated sludge other than by immobilising cells in a growth carrier.

BIOLOGICAL HYDROGEN PRODUCTION PROCESSES

Biological processes of hydrogen production are fundamentally dependent upon the presence of a hydrogen-producing enzyme. Hydrogen producing enzymes catalyse the chemical reaction $2H^+ + 2e^- \rightarrow H_2$ [33]. Biological production of hydrogen, using microorganisms (Table 1), is an exciting new area of biotechnology development that offers the potential production of usable hydrogen from a variety of renewable resources.

Biological hydrogen production processes, which are favorable in terms of their operational cost, can be classified to photo and dark-fermentation processes. Photo-fermentation processes by photosynthetic microorganisms, such as algae and cyanobacteria, produce the hydrogen from organic acids and water utilising sunlight [34, 35]. This may be considered the most economic process, but can only be operated during the daytime. Also, the production of oxygen

from the process may decrease the hydrogen production efficiency. Conversely, dark-fermentation, by anaerobic microorganisms, produces hydrogen from a general anaerobic metabolism.

The anaerobic bio-hydrogen production process is not only stable, but also fast in the production of H_2 compared to the photo-fermentation process. However, research on the anaerobic bio-hydrogen production has been relatively deficient, as most of studies have focused on the role of hydrogen to the thermodynamic conversion of volatile fatty acids (VFAs) for the production of methane only [36,37].

It has often been reported that the rate of hydrogen evolution from an anaerobic fermentation was dependent on the pH, loading rate, biogas circulation and hydraulic retention time (HRT) for the acidogenic phase [38, 39]. These parameters are used mostly to control the operation by blocking the methanogenesis of the anaerobic pathways. The methanogenesis is the critical stage for the anaerobic bio-hydrogen production

process due to the rapid H_2 consumption rate of hydrogen utilising methanogens. Many researchers have reported that the blocking of the methanogenesis was possible by adjusting the pH to one that was weakly acidic and by shortening the shorter solids retention time (SRT) even further than the minimum for the growth of methanogens. However, information on the detailed operational parameters was not available as the operation of a continuous reactor involves many variables, and due to the lack of understanding of the mechanism of hydrogen production.

FERMENTATIVE HYDROGEN PRODUCTION

Hydrogen production by fermentative bacteria is technically simple than by photosynthetic bacteria [51]. Hydrogen can be produced by anaerobic bacteria, which grow in the dark on carbohydrate rich substrates. Fermentation reactions can be operated at mesophilic (25-40°C), thermophilic (40-65°C), extreme thermophilic (65-80°C), or hyperthermophilic (> 80°C) temperatures. While indirect and direct photolysis systems produce pure hydrogen. Dark fermentation processes produce a mixed biogas containing hydrogen, carbon dioxide, and lesser amounts of methane (CH_4), CO, and/or H_2S [52]. Carbohydrates are the preferred substrates for fermentative hydrogen producing bacteria; hydrogen was hardly produced from protein and lipids (Table 2).

Glucose, isomers of hexoses or polymers in the form of starch or cellulose yield different amounts of hydrogen per mole of glucose, depending on the fermentation pathway and end products (Table 2).

Table 2: Effect of carbon sources on hydrogen production and cell growth of *Rb. Sphaeroides* KD131 wild-type strain

			(ml H_2 /ml-broth)	hydrolysis (%)
Glucose	6.26-6.3	3.4	0.363	49.1
Glyccrol	7.89-8.19	1.35	0	ND
Sucrose	7.47-7.61	1.83	0.140	ND
Starch	7.44-7.53	0.81	0	<1.8
Malate	7.51-7.75	3.10-3.36	1.590	>95
Lactate	7.19-7.48	2.77	0.800	60.9
Acetate	9.22-9.43	3.10	0.235	55.2

Fermentative Hydrogen Production by Using Mixed Culture

Experiments were conducted to investigate H₂ production from glucose by mixed anaerobic cultures (anaerobic microflora) at various temperatures in the mesophilic range [53]. Results showed that glucose degradation rate and efficiency, H₂ yield, and growth rate of H₂-producing bacteria all increased as the temperature increased from 33 to 41 °C. However, the specific H₂ production rate increased with increasing temperature from 33 to 39 °C, and then decreased as the temperature was further increased to 41 °C. The distribution of aqueous products was also greatly influenced by temperature variation. H₂ yield and growth rate of H₂-producing cultures had a linear relationship with temperature. A modified Gompertz model was able to adequately describe the H₂ production and microbial growth in the mesophilic range. The activation energies for H₂ production and microbial growth were estimated as 107.66 and 204.77 kJ/mol, respectively.

Further more, examination and comparison were carried out on the biological fermentative production of hydrogen from glucose in a continuous stirred tank type bioreactor (CSTR) and an upflow anaerobic sludge blanket bioreactor (UASB) at various hydraulic retention times (2–12 h HRT) under mesophilic conditions (35 °C) [54]. Also the biohydrogen production from glucose in the CSTR at mesophilic and thermophilic (55 °C) temperature range was studied and compared. From the CSTR experiments it was found that thermophilic conditions combine high hydrogen production rate with low production of microbial mass, thus giving a specific hydrogen production rate as high as 104 mmole H₂/h/l/g VSS at 6 h retention time compared to a specific hydrogen production rate of 12 mmole H₂/h/l/g VSS under mesophilic conditions. On the other hand, the UASB reactor configuration is more stable than the CSTR regarding hydrogen production, pH, glucose consumption and microbial by-products (e.g. volatile fatty acids, alcohols etc.) at the HRTs tested. Moreover, the hydrogen production rate in the UASB reactor was

significantly higher compared to that of the CSTR at low retention times (19.05 and 8.42 mmole H₂/h/l, respectively at 2 h HRT) while hydrogen yield (mmole H₂/mmole glucose consumed) was higher in the CSTR reactor at all HRT tested. This implies that there is a trade-off between technical efficiency (based on hydrogen yield) and economic efficiency (based on hydrogen production rate) when the attached (UASB) and suspended (CSTR) growth configurations are compared.

Another study was conducted to evaluate the capability of microflora to produce hydrogen from Palm oil mill effluent (POME) sludge, sludge compost from Malaysia and CREST compost from Philippines. The capability of this microflora to produce hydrogen was examined with 500 ml artificial wastewater containing 1% glucose, 0.2% yeast extract and 0.018% magnesium chloride hexahydrate under anaerobic fermentation in a batch culture.

The microflora in POME sludge, sludge compost and CREST compost were found to produce significant amounts of hydrogen. The maximum production yield of hydrogen per decomposed glucose was 2.1 mol/mol-glucose at a conversion rate of 0.137 L/(L-med h) at 50 °C obtained by sludge compost. All fermentations were carried out without pH control. It was also found that the addition of nitrogen source in the medium caused a change in hydrogen produced. There was no methane gas in the evolved gas [55].

Fermentative Hydrogen Production by Using Pure Culture

Currently, anaerobic hydrogen production has been mainly studied on bench scale using pure cultures such as *Clostridium* sp. [44], *Enterobacter aerogenes* [45, 46], and *Ectothiorhodospira vacuolata* [47]. *Rhodospseudomonas capsulata* can use volatile fatty acids as electron donors for continuous H₂ production at the expense of light energy. In this study a mixture of acetate, propionate and butyrate was used as the substrate for continuous H₂ production from a photo-bioreactor seeded with *R. capsulata*. For a mixture of acetate 1.8 g/l, propionate of 0.2 g/l and butyrate of 1.0 g/l, a maximum H₂

production rate of 37.8 ml/g dry weight (dwt)/h, a light conversion efficiency of 3.69% and a substrate conversion efficiency of 45% were achieved. Hydrogen was also produced with the effluent from an acidogenic H₂-producing reactor at a maximum production rate of 24.9 ml/gdwt/h by *R. capsulata*. The H₂ yield increased by three-fold through combining H₂ production of anaerobic fermentative bacteria and *R. capsulata*, compared with the dark fermentation only [56].

In the production of acetate by *Clostridium thermolacticum* growing on lactose, considerable amounts of hydrogen were generated. Lactose available in large amounts from milk permeate, a wastestream of the dairy industry, appears to be a valuable substrate for cheap production of biohydrogen. In this study, continuous cultivation of *C. thermolacticum* was carried out in a bioreactor, under anaerobic thermophilic conditions, on minimal medium containing 10 g l⁻¹ lactose. *C. thermolacticum* growing on lactose produced acetate, ethanol and lactate in the liquid phase. For all conditions tested, hydrogen was the main product in the gas phase. Hydrogen specific production higher than 5 mmol H₂ (g cell)⁻¹h⁻¹ was obtained. By operating this fermentation at high-dilution rate and alkaline pH, the hydrogen content in the gas phase was maximised [56]. ■

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