Cheng, K.Y., Cord-Ruwisch, R. and Ho, G. (2007) *Limitations of bio-hydrogen production by anaerobic fermentation process: An overview.* In: Jennings, P., Ho, G., Mathew, K. and Nayar, C.V. (eds), Renewable Energy for Sustainable Development in the Asia Pacific Region Conference, 4-8 February 2007, Fremantle, Western Australia. AIP Conference Proceedings Volume 941, 2007, pp 264-269

Limitations of Bio-Hydrogen Production by Anaerobic Fermentation Process: An Overview

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Abstract. Turning organic wastes into hydrogen (H2) by using anaerobic fermentative technology is an ideal concept because this can accomplish both waste treatment and energy recovery objectives. However, H2 production using anaerobic fermentative approaches faces a fundamental limitation of poor H2 production yield (i.e. < 4 mol H_2 per mol of hexose). This article gives an overview on the limitations of bio-H2 production using fermentative processes. Fundamental microbiology and thermodynamic of the processes are discussed.

1. INTRODUCTION

By decoupling H2 production from methane production, conversion of organic matter into H2 could be achieved (*1*). Many types of organic compounds, ranging from polymers to monomers such as carbohydrates, fats and amino acids are known to be the substrates for H2 production (*2*). As such, organic wastes used for the production of methane can also be the potential substrates for anaerobic fermentative production of H2 (*3*). In fact, many studies indicate that various wastes containing high organic matter have been used to produce H2 by anaerobic fermentation process (*4-10*). The major differences between the two processes are that successful biological H2 production requires inhibition of H2-consuming microorganisms and maximization of H2 production yield from the organic substrates. Most of the studies revealed that only 10-20% of stoichiometric maximum yield (i.e. 12 moles of H2 per mole hexose) of H2 could be recovered in anaerobic fermentative processes (*11-13*). Hence, some researchers even argued that fermentative H2 production should only be restricted to a pre-treatment step in a larger bioenergy production concept (*4*). The present article will discuss, from a microbiological and thermodynamic perspective, the limitations of anaerobic fermentation as H2 production process.

2. BIOCHEMICAL PATHWAYS OF FERMENTATIVE H2 PRODUCTION

Among the many species of anaerobic fermentative bacteria capable of producing H2, the H2-producing characteristics of two genera, *Clostridium* (e.g. *C. pasteurianum* (*14*); *C. beijerinckii* (*15*) and *C. butyricum* (*16*)) and *Enterobacter* (e.g. *E. aerogenes* (*17*) and *E. Cloacae (18)*), have been studied extensively. Figure 1 illustrates the pathway of anaerobic fermentation by using glucose as model substrate (*19*). The glucose monomer produced from hydrolysis is taken up by the fermentative bacteria and degraded predominantly through the Embden-Meyerhof-Parnas (glycolysis) pathways to generate ATP from ADP, leading to the formation of pyruvate ($CH_3COCOOH$). Like other bacteria or higher eukaryotic cells, the glycolytic reactions in glucose fermentative bacteria also produce electron equivalents in the form of NADH, which has to be re-oxidized in order to continue substrate degradation. Inorganic electron acceptors are usually the preferred candidate in anaerobic respiration for the bacteria to regenerate these reducing equivalents because they have higher redox potential. While in the absence of external inorganic electron acceptors, NADH is commonly reoxidized by H^+ and produce H2 and NAD^+. The pyruvate produced from glycolysis is then converted to acetyl-CoA ($CH_3COSCoA$), liberating carbon dioxide and H2. The pyruvate may also be converted into acetyl-CoA and formate (CHOOH), which may be converted to H2 and carbon dioxide by fermentative bacteria such as *Escherichia coli*. Depending on the microorganisms and the environment, the Acetyl-CoA is eventually converted into acetate (CH_3COOH), butyrate ($CH_3CH_2CH_2COOH$) or ethanol (CH_3CH_2OH).

CP941, *Renewable Energy for Sustainable Development*
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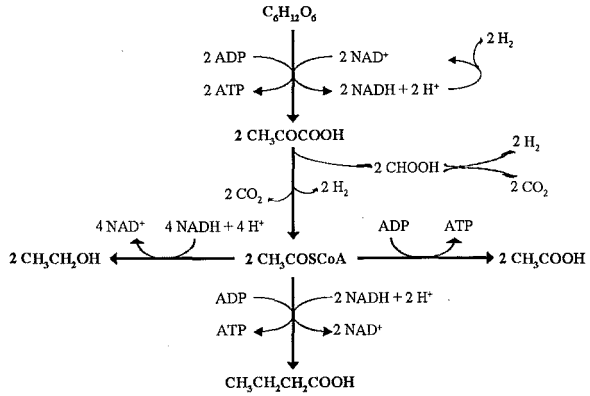

FIGURE 1. Pathways of fermentation of glucose (Adapted from (*20*)).

The formation of different end products from the pyruvate is found to be highly dependent on the H2 partial pressure of the system. For instance, since the conversion of glucose to butyrate, CO_2 and H_2 yields only 3 mol of ATP per mol of glucose, whereas the conversion of glucose to acetate, CO_2 and H_2 can yield 4 mol of ATP per mol of glucose. The production of H_2 and acetate from pyruvate is therefore usually preferred by most bacteria with hydrogenase as it allows the bacteria to conserve more energy from their substrates (*21*). In fact, from bio-H2 production stand point, formation of acetate as the end product is also preferred because it allows the greatest possible amount of H2 to be produced via fermentative pathway. However, such preferred conversion is only possible when the H2 concentration (or NADH/NAD^+ ratio) in the system is kept low. Under elevated H2 concentration environment, which may be due to accumulation of H2 because of ineffective H2 removal (e.g. inhibition of H2-consuming methanogen), H2 production from NADH can be inhibited due to thermodynamic reasons, leading to the production of propionate or butyrate from pyruvate as alternative electron sinks (Figure 2).

3. ELIMINATION OF SYNTROPHIC PARTNERS IN FERMENTATIVE H2 PRODUCTION

In methanogensis process, complex organic matter is degraded in a sequence of reactions by several distinct groups of microorganisms. Fermentative microorganisms will first breakdown the organic matter into simpler substance, such as H2, formate and acetate, which serve as the substrates for the methanogenic microorganisms. Also, a variety of other organic compounds like lactate, ethanol, propionate, butyrate, etc. are formed, and are degraded by proton-reducing acetogenic bacteria to form the methanogenic substrates, i.e. H2 and acetate. However, these organisms can only grow and keep converting the organic matter into the methanogenic substrates when the H2 concentration is maintained at low level by their syntrophic partner, i.e. methanogenic archaea (*22-24*). Such H2 (or electron + proton) transfer mechanism is an integral and vital process in the anaerobic mineralization of organic matter, and is commonly regarded as interspecies interactions between fermentative H2-producing organisms and H2-consuming methanogen (*25*). From a bioenergetic perspective, when organic electron acceptors such as pyruvate or fumurate were used, the partial oxidation of the substrates results in the formation of products that still possess high free energy. This explains why anaerobic catabolic reactions involving organic electron acceptors usually have low free energy change (ΔG), and only little energy could be conserved from the substrate metabolism by the microorganisms (*21*). Therefore, the reactions proceed very close to the dynamic equilibrium, and require constant removal of the end-products of the reactions before the reactions can proceed further (*23, 26*). Therefore, the presence of methanogenic organisms is vital because they can effectively and indirectly maintain the continuous fermentative degradation of organic matter into their own substrates, including H2. However, in fermentative H2 production process, growth of methanogens is considered as an undesirable process and should be inhibited in order to prevent any consumption of H2, allowing the H2 produced from fermentative degradation of organic matter by fermentative or acetogenic bacteria to be recovered.

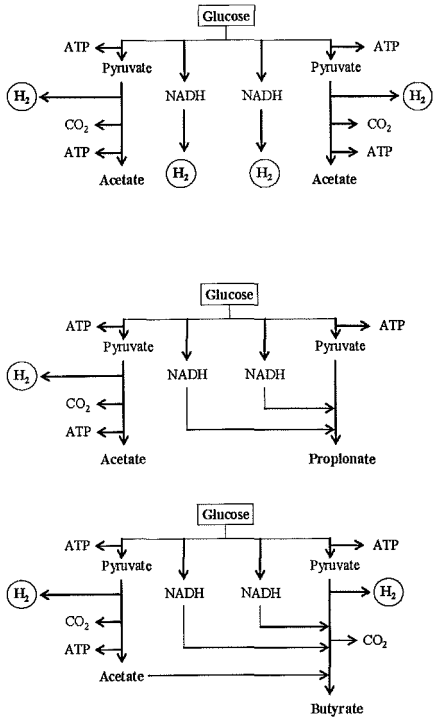

FIGURE 2. Formation of acetate, propionate or butyrate via different pathways of glucose/pyruvate degradation at elevated or low hydrogen concentrations. H_2 and acetate are produced at low H_2 concentration (< 10 Pa) whilst propionate and butyrate are produced as alternative electron sinks at elevated H_2 due to thermodynamic reasons. (Adapted from (*27*))

4. CONVERSION OF FERMENTATION END-PRODUCTS INTO H2: A THERMODYNAMIC CONSIDERATION

A variety of fermentative end-products (mainly as VFAs) are remained after the fermentative process (refer to Figure 1). However, a considerable amount of chemical energy is still remaining "unexplored" from the process. In methanogenesis, degradation of VFAs such as acetate is only possible via interspecies H_2 transfer, when H_2 is kept to a low concentration by the H_2-consuming methanogens, accumulation of H_2 to a certain level can result in the inhibition of VFA degradation reactions due to thermodynamic limitations (*21*). This explains why dissolved H_2 concentration is considered as a key controlling factor in the anaerobic processes and it can regulate the rates at which the VFAs degrade to acetate and H_2 (*28, 29*). The standard Gibbs free energy change of reaction (ΔG^o) is the amount of energy being conserved when the substrate(s) are being converted into products (s) (*21*). It can be calculated from the Gibbs free energy changes of formation from the elements (ΔG^o_f) of the substrates and products according to the following equation:

$$\Delta G^o = \sum \Delta G^o_f \text{ of products} - \sum \Delta G^o_f \text{ of substrates}$$

Since almost all reactions occur in biological systems are not under standard condition (i.e. 298 K, 1 atm pressure, 1 M concentration for all species). The Gibbs free energy change of reaction corrected for the actual reacting conditions (ΔG) can then be calculated from the ΔG^o and the concentrations of all reactants (S) and products (P) according to the following equation:

$$\Delta G = \Delta G^o + RT \log [P]/[S]$$

Where, R = universal gas constant (8.314 x 10^{-3} kJ·mol^{-1}·K^{-1}); T = absolute temperature (K). (Negative values of ΔG indicate the forward reaction is exergonic or spontaneous, and positive values of ΔG indicate the backward reaction is exergonic or spontaneous.)

According to the literature, amongst all of the end-products commonly formed during fermentative H2 production, acetate is considered as the key end-product because further conversion of acetate into H2 is unfavorable to occur in fermentative processes. Based on Gibbs free energy calculations, H2 production from acetate requires a net energy input of 104.6 kJ mol acetate^{-1} under standard conditions (*21*).

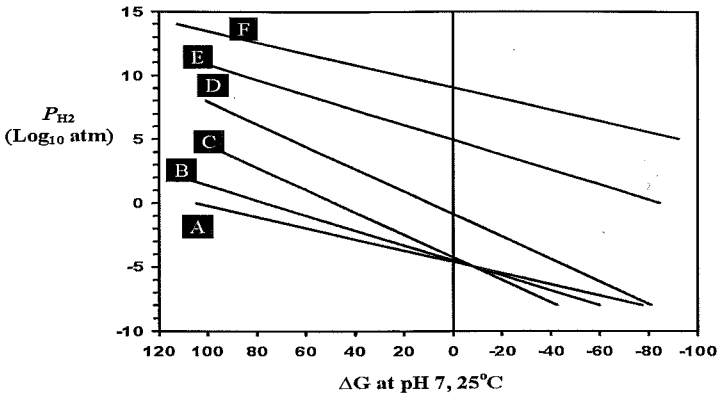

FIGURE 3. Relationship between partial pressure of hydrogen (P_{H2}) and change-in-free-energy (ΔG) for the following hydrogen-producing reactions:

	ΔG$^{o'}$ (kJ/reaction)	$P_{H2, \Delta G=0}$
(A) Acetate$^-$ + 4H_2O → 2HCO_3^- + 4H_2 + H^+	+104.6	-4.60
(B) Propionate$^-$ + 3H_2O → acetate$^-$ + 1HCO_3^- + 3H_2 + H^+	+76.5	-4.49
(C) Butyrate$^-$ + 2H_2O → acetate$^-$ + 2H_2 + H^+	+48.3	-4.25
(D) Ethanol + H_2O → acetate$^-$ + 2H_2 + H^+	+9.6	-0.85
(E) Glycerol + 2H_2O → acetate$^-$ + HCO_3^- + 3H_2 + 2H^+	-84.6	+4.96
(F) Glucose + 4H_2O → 2 acetate$^-$ + 2HCO_3^- + 4H_2 + 4H^+	-206.1	+9.06

Notes: $P_{H2, \Delta G=0}$ = hydrogen partial pressure (Log$_{10}$ atm) when ΔG is equal to zero; ΔG values (at pH 7 and 25°C) were calculated from the standard Gibbs free energy change (ΔG$^{o'}$) value in (*21*) using equation ΔG = ΔG$^{o'}$ + 2.47 x ln ([products]/[substrates]). Concentrations used for all compounds were assumed to be 1M.

Figure 3 illustrates the relationship between H2 partial pressure and change-in-free-energy for several H2 producing reactions that possibly occur in fermentative process. Under standard condition (i.e. 298 K, 1 atm pressure, 1 M concentration for all species), only the oxidation reactions of 1 mol glucose to 4 mol H_2 and 1 mol glycerol to 3 mol H_2 have negative ΔG$^{o'}$ values, whilst all the other reactions have positive ΔG$^{o'}$ values, meaning that except for glucose and glycerol, all the other reactions are energetically unfavorable to occur under standard condition. Since ΔG value of each specific reaction is in a linear function of partial pressure of the product H2, the reaction may proceed toward the product side when the partial pressure of H2 has reduced to a certain level. When ΔG is equal to zero, oxidation of 1 mol acetate to 4 mol H_2 only become energetically favorable when the partial pressure of H2 in the system is lower than -4.6 Log$_{10}$ atm (i.e. 2.55 Pa), which is extremely low that may even not allow the synthrophic action of the hydrogenotrophic methanogenic bacteria in anaerobic fermentative system to occur. According to Figure 3, glycerol should also be considered as a promising feedstock to produce H2 via fermentative processes. This is because the conversion of glycerol into H2 is energetically favorable (i.e. ΔG$^{o'}$ = -84.6 kJ/reaction), and the reaction is only susceptible to the inhibitory effect of H2 accumulation with H2 partial pressure greater than +4.96

Log_{10} atm (i.e. 9.24 x 10^9 Pa), which is very high and is not supposed to happen under biological conditions. In fact, glycerol is the major by-product of bio-diesel generation processes, and a further increase in the production of bio-diesel fuels would raise the problem of efficiently treating wastes containing glycerol (*30*). Therefore, apart from acetate, the possibility of using glycerol as the feedstock substrates in H2 production process also worthwhile to be investigated.

As aforementioned, even the fermentative process can be successfully manipulated to use acetate as the substrate for the production of H2, the produced H2 would be under very low partial pressure, i.e. concentration. This is impractical to obtain large quantity of H2 using acetate as the only substrate from fermentative process. In order for the microbes to "extract" the electrons from the substrate in a more effective manner, it is reasonable to think of using some approaches to remove the H2 that is produced from the bacteria immediately so as to maintain a low H2 partial pressure for the H_2 producing reactions to remain energetically favorable. In methanogenesis process where methane forming consortia are present, effective H2 removal is normally being achieved by the methanogenic partners, which are in syntrophic relationship at close proximity with the H_2-producing bacteria. As H2 is one of the substrates for methanogens, H2 is taken up by them immediately and effectively to produce methane, which will then be easily collected in gaseous form due to their poor aqueous solubility. In other words, the distance between the locations of H2 production and H2 consumption is so small that allowing effective mass transfer of H2 between the two locations. In fact, effective mass transfer of H2 between H2-producing and –consuming bacteria can be revealed by estimating the time required for H2 removal from H2-producing bacteria in a methanogensis environment where H2 is maintained at low level by syntrophic microbial association.

Estimation of Time required for the H2-producing bacteria to produce H2 until Inhibitory Levels are reached under Methanogenic Conditions:

Assume: Methane production rate = 1 L^{-1} CH_4 L^{-1} day^{-1} (*value based on the results obtained from an anaerobic digester in our laboratory.*)

Since: 1 mol of methane formed required 4 mol of H2, i.e. $4H_2 + CO_2 \leftrightarrow CH_4 + 2H_2O$; and molar volume of CH_4 and H_2 are 24.5 L mol^{-1} at 25 °C, 1 atm.

Then: H2 production rate = 4 L^{-1} H_2 L^{-1} day^{-1}
= 0.16 mol H_2 L^{-1} day^{-1}
= 0.16 M day^{-1}
= 1.85 x 10^{-6} M $second^{-1}$
= 1.85 μM $second^{-1}$

Assume the saturation concentration of H2 in water (25 °C, 1 atm) is 1 mmol L^{-1} (*31*)

If: H2 production was inhibited at P_{H2} of 10 Pa (*32*); i.e. 1 x 10^{-4} mmol L^{-1} = 100 nM = 0.1 μM

Then: Time Required to Produce H_2 until Inhibitory Levels are reached at this particular system would be:
= 0.1 μM/ 1.85 μM $second^{-1}$
= 54 milliseconds

This simple calculation suggests that the methanogenic partner (H_2-consuming microorganisms) could effectively take up the H2 produced by the H_2-producing bacteria within a very short time in the system (i.e. approx. <0.05 second!). However, in order to allow H2 to be collected from the process, it is an important yet difficult task to avoid H2 consumption by effectively eliminating the activities of H_2-consuming bacteria in the system while at the same time to maintain a low partial pressure of H2 to overcome the thermodynamic limitation of H2 producing processes.

5. CONCLUSION

Undoubtedly, anaerobic fermentation of organic substrates would allow energy recovery in the form of H2 gas. However, in terms of conversion efficiency the process releases only about 17% of H2 in the substrate (i.e. glucose). Most of the H2 still remain in the end products of the process and resist for further H2 conversion due to microbiological and thermodynamical limitations. Nevertheless, research efforts should be encouraged in order to generate more information and understanding on the process that can eventually allow a better technology to be developed in the future.

ACKNOWLEDGMENTS

Ka Yu Cheng would like to thank Murdoch University for the provision of full Ph.D. scholarship.

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