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Fermentative Hydrogen Production From Wastewater and Solid Wastes by Mixed Cultures

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Over 160 publications related to fermentative hydrogen production from wastewater and solid wastes by mixed cultures are compiled and analyzed. Of the 98 reported cases, 57 used single substrates (mainly carbohydrates), 8 used actual wastewater, and 33 used solid wastes for hydrogen conversion. The key information is compiled in four tables: (1) pretreatment conditions for screening hydrogen-producing bacteria from anaerobic sludge or soil, and the process and performance parameters for (2) single substrates in synthetic wastewaters, (3) actual wastewaters, and (4) solid wastes. Process parameters discussed include pH, temperature, hydraulic retention time, seed sludge, nutrients, inhibitors, reactor design, and the means used for lowering hydrogen partial pressure. Performance parameters discussed include hydrogen yield, maximum volumetric production rate, maximum specific production rate, and conversion efficiency. The outlook for this new technology is discussed at the end.

KEY WORDS: fermentation, hydrogen, mixed cultures, reactor, waste, wastewater

I. INTRODUCTION

Energy supply and environment protection are two crucial issues for the sustainable development of global prosperity. Over 80% of the energy consumed today in the world is derived from fossil fuels (Das and Veziroğlu, 2001), which will eventually become depleted in the not too distant future. In addition, burning of fossil fuels contributes severely to the climate change,

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environmental deterioration, and the threatening of public health (Bockris, 1972; Levin et al., 2004).

For over two decades, environmental engineers have successfully commercialized anaerobic technology for the treatment of wastewater (Fang and Liu, 2001) and solid wastes (Mata-Alvarez et al., 2000). In these processes, organic pollutants and wastes are converted into methane through a series of chain reactions by distinct groups of anaerobic microorganisms. Complex organics are first hydrolyzed and fermented into fatty acids, which are then further converted into acetate and hydrogen, both of which are lastly converted into methane. As compared to the aerobic waste/wastewater treatment processes, the methanogenic process offers several intrinsic advantages: (a) saving the energy that is otherwise needed for aeration, (b) lowering sludge yield, and (c) producing a readily useable fuel—methane. Over 2000 full-scale methanogenic wastewater treatment systems have since been installed worldwide (Fang and Liu, 2001; Gallert et al., 2003).

Recently a new anaerobic process has been developed to convert organic pollutants into hydrogen, instead of methane. Hydrogen is favored over methane for two reasons. First, hydrogen has a wider range of industrial applications as compared to methane. It can be used for the syntheses of ammonia, alcohols, and aldehydes, as well as for the hydrogenation of edible oil, petroleum, coal, and shale oil (Hart, 1997), whereas methane is mostly used as fuel. Second, hydrogen is an ideal fuel, producing only water upon combustion. It can be used directly in the internal combustion engines, or used to produce electricity through fuel cells (Hart, 1997; Dincer, 2002; Dunn, 2002; Iwasaki, 2003). Many energy experts believe that hydrogen will replace fossil fuels as the next generation of energy (Hoffmann, 2001; Rocha et al., 2001). Some even predict that a new economy empowered by hydrogen will fundamentally change the nature of our market and political and social institutions, just as coal did for the 19th century and petroleum for the 20th century (Rifkin, 2002; Winter, 2004). Furthermore, methane is a greenhouse gas with 21 times the heat-trapping effect of carbon dioxide (IPCC, 1996; US EPA, 2005). Although it constitutes only 0.00017% of the atmosphere, methane accounts for 0.47 W/m^2 of radiative forcing, which is about 19% of the total global greenhouse gas forcings today (Masters, 1998).

Hydrogen is commercially produced by either electrolytic or thermochemical process, both of which are energy intensive (Rajeshwar et al., 1994). Yet, in nature, hydrogen may be produced biologically by autotrophs as well as heterotrophs (Nandi and Sengupta, 1998; Das and Veziroğlu, 2001; Hallenbeck and Benemann, 2002). Autotrophs, such as algae, use carbon dioxide as a carbon source, whereas heterotrophs use organic matter as a carbon source. From an environmental engineering point of view, heterotrophs are of more concern because they can be used to degrade organic pollutants and thus clean up the environment. Heterotrophs produce hydrogen by either phototrophic or nonphototrophic (often called "dark") fermentation of

organic matter, depending on whether light is the energy source. Although heterotrophic hydrogen production has been studied since 1960s (May et al., 1964; Gray and Gest, 1965; Zajic et al., 1978; Kondratieva, 1983; Nandi and Sengupta, 1998), most studies have been related to nonphototrophic fermentation. The scarcity of information related to phototrophic fermentation is due to two reasons: (a) It is difficult to control light penetration and its uniform distribution, and (b) the process is likely not cost-effective unless the free sunlight can be used as the light source.

For those papers of nonphototrophic fermentative hydrogen production, most, especially the early ones, were for pure cultures by microbiologists. However, environmental engineers are more interested in using mixed cultures for wastewater/waste treatment for practical reasons. A mixed culture system would be cheaper to operate, easier to control, and would have a broader choice of feedstock (Valdez-Vazquez et al., 2005). In this article, only publications related to nonphototrophic hydrogen production by mixed cultures are reviewed; those related to phototrophic fermentation are excluded.

II. HYDROGEN-PRODUCING BACTERIA AND REACTIONS

Many fermentative bacteria produce hydrogen, which serves as an ideal intermediate energy carrier and storage medium in cells. Hydrogen production is a specific mechanism to dispose of excess electrons through the activity of hydrogenase in bacteria. Bacteria that possess such capability include strict anaerobes (*Clostridia*, methylotrophs, rumen bacteria, methanogenic bacteria, archaea), facultative anaerobes (*Escherichia coli*, *Enterobacter*, *Citrobacter*), and even aerobes (*Alcaligenes*, *Bacillus*). Nandi and Sengupta (1998) published a comprehensive review on the hydrogen-producing characteristics of these bacteria.

Among the hydrogen-producing bacteria, *Clostridium* sp. and *Enterobacter* are the most widely studied. Species of genus *Clostridium* are gram-positive, rod-shaped, strict anaerobes and endospore formers (Holt et al., 1994; Nandi and Sengupta, 1998), whereas *Enterobacter* are gram-negative, rod-shaped, and facultative anaerobes (Holt et al., 1994). The hydrogen-producing characteristics of these two genera and three thermophilic species were recently reviewed by de Vrije and Claassen (2003). Species of *Clostridium* and *Enterobacter* that have been studied for hydrogen production include *Clostridium* sp. no. 2 (Taguchi et al., 1992, 1993, 1994, 1995), *C. parapatrificum* M-21 (Evvyernie et al., 2000, 2001), *C. butyricum* LMG 1213t1 (Heyndrickx et al., 1986, 1990), *E. aerogenes* E.82005 (Tanisho et al., 1983, 1987, 1989; Tanisho and Ishiwata, 1994), *E. aerogenes* HU-101 (strains wt and m AY-2) (Rachman et al., 1998), and *E. cloacae* IIT-BT 08 (strains wt and m DM_{11}) (Kumar and Das, 2000, 2001; Kumar et al., 2000). The thermophiles include *Thermotoga maritime* (Schröder et al., 1994), *Thermotoga*

elfii (de Vrije et al., 2002), and *Caldicellulosiruptor saccharolyticus* (van Niel et al., 2002).

Studies of hydrogen production were mostly conducted at pH 5.8–6.0 and 37 ± 1°C for *Clostridium* and *Enterobacter*, and pH 7.0–7.4 and 65–80°C for thermophiles (de Vrije and Claassen, 2003). The substrates used in all the studies were carbohydrates (mostly glucose, plus xylose, sucrose, and molasses), with only one exception using *N*-acetyl-D-glucosamine (Evvyernie et al., 2000, 2001). Results showed that species of *Clostridium* had a hydrogen yield of 190–340 ml H_2/g hexose and a maximal rate of 4.2–18.2 L H_2/L/d. The corresponding values were 82–460 ml H_2/g hexose and 11.8–34.1 L H_2/L/d for *Enterobacter* and 450–540 ml H_2/g hexose and 1.6–5.9 L H_2/L/d for thermophiles (de Vrije and Claassen, 2003).

Many pathways have been proposed for hydrogen production. Figure 1 illustrates the pathway of dark fermentation using glucose as the model substrate (Yan et al., 1988; Ren and Wang, 1994; Tanisho, 2001; Liu, 2002; Prescott et al., 2002). It shows that glucose is first converted to pyruvate, producing adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and the reduced form of nicotinamide adenine dinucleotide (NADH) via the glycolytic pathway. Pyruvate is then further converted to acetylcoenzyme A

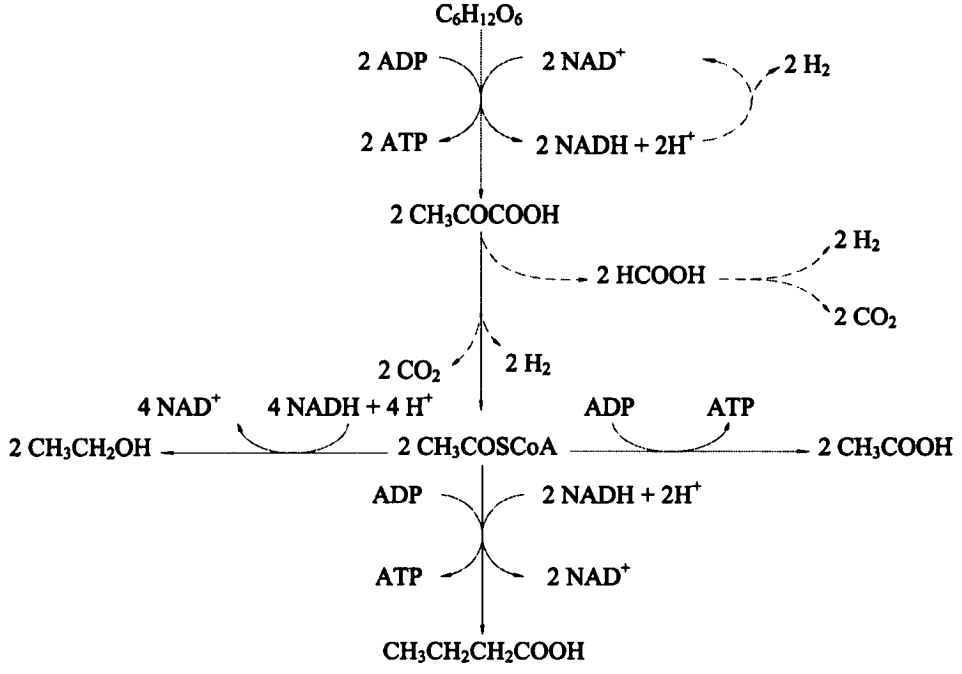

FIGURE 1. Pathway of hydrogen production from fermentation of glucose (Yan et al., 1988; Ren and Wang, 1994; Tanisho, 2001; Liu, 2002; Prescott et al., 2002).

(acetyl-CoA), carbon dioxide, and hydrogen by pyruvate–ferredoxin oxidoreductase and hydrogenase. Pyruvate may also be converted to acetyl-CoA and formate, which may be readily converted to hydrogen and carbon dioxide by bacteria such as *Escherichia coli*. Acetyl-CoA is finally converted into acetate, butyrate, and ethanol, depending on the microorganisms and the environmental conditions. NADH is used in the formation of butyrate and ethanol and the residual NADH may be oxidized, producing hydrogen and NAD^+. ATP is generated in the formation of butyrate and acetate from acetyl-CoA.

The most common products in the fermentation of carbohydrate are acetate and butyrate. This acidification process may be expressed by the two following reactions, using glucose as the model (Nandi and Sengupta, 1998):

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (1)$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad (2)$$

Thus, the stoichiometric yields are 4 moles of hydrogen for each mole of glucose (i.e., 544 ml H_2/g hexose at 25°C) in the production of acetic acid, according to reaction (1), and 2 moles of hydrogen (i.e., 272 ml H_2/g hexose at 25°C) in the production of butyric acid, according to reaction (2). In addition to these acids, ethanol may also be produced, as shown in the following reaction (Gaudy and Gaudy, 1980; Hwang et al., 2003, 2004):

$$C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2OH + CH_3COOH + 2H_2 + 2CO_2 \quad (3)$$

The corresponding stoichiometric yield is 2 moles of hydrogen for each mole of glucose.

However, the actual hydrogen yield may be substantially lower than these stoichiometric values for at least four reasons. First, glucose may be degraded through other pathways without producing hydrogen. Second, a fraction of glucose is consumed, instead, for biomass production. Third, a stoichiometric yield is achievable only under near equilibrium condition, which implies a slow production rate and a low hydrogen partial pressure (Woodward et al., 2000; Hallenbeck and Benemann, 2002). Lastly, some hydrogen produced may be consumed for the production of other by-products, such as propionate (Vavilin et al., 1995), as shown in the following reaction:

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \quad (4)$$

About 40 hydrogenase genes have been sequenced so far. All of them contain Fe, and some contain Ni and Se as well (Voordouw, 1992). Those hydrogenases containing Ni and Se facilitate the uptake of hydrogen, whereas those containing Fe alone (Fe hydrogenases) catalyze the production of hydrogen (Cammack, 1999). Several hydrogenases have been sequenced and characterized from *Clostridium* species, including *C. pasteurianum* (Meyer

and Gagnon, 1991), *C. acetobutylicum* (Santangelo et al., 1995; Gorwa et al., 1996), *C. perfringens* (Kaji et al., 1999), and *C. paraputrificum* (Morimoto et al., 2005). However, there is no information so far on Fe hydrogenase in the mixed hydrogen-producing sludge.

III. SLUDGE PRETREATMENT

Engineers would rather use mixed cultures, instead of pure cultures, for wastewater/waste treatment. Processes using mixed culture are more practical, because they are simpler to operate and easier to control, and may have a broader choice of feedstock (Valdez-Vazquez et al., 2005). Yet in a mixed culture system under anaerobic condition the hydrogen produced by bacteria, such as *Clostridium* or *Enterobacter*, is often readily consumed by other hydrogen-consuming bacteria. Thus, in order to harness hydrogen from a mixed culture system, the seed sludge needs a pretreatment process to suppress as much hydrogen-consuming bacterial activity as possible while still preserving the activity of the hydrogen-producing bacteria. The pretreatment is achieved mostly by relying on the spore-forming characteristics of the hydrogen-producing *Clostridium*, which is ubiquitous in anaerobic sludge and sediment (Brock et al., 1994). Treating an anaerobic sludge under harsh conditions, *Clostridium* would have a better chance to survive than the non-spore-forming bacteria, many of which hydrogen consumers (Lay, 2001; Oh et al., 2003a). Effective pretreatment processes include heating, acidic or basic treatment, aeration, chemicals, electric current, and so on.

Table 1 summarizes the key information in 41 cases related to the seed sludge pretreatment for the enrichment of hydrogen-producing bacteria. Overall speaking, the most common pretreatment process, heating at 100°C for 15 min, appears to be simple and effective.

III.A. Heat Treatment

Heat treatment of seed sludge has been most common for the screening of hydrogen-producing bacteria (Lay et al., 1999). Of the cases reported in Table 1, 26 employed heat treatment that may be easily and inexpensively achieved in practice by using steam. The temperature varied from 75°C (Chang et al., 2002) to 121°C (Wang et al., 2003), and the duration from 15 min (Lay et al., 1999) to 2 h (Fan et al., 2004). No study has been conducted so far to determine the optimal heating temperature and duration for the heat treatment. The most common condition (in 9 cases) was to heat the sludge by boiling (100°C) for 15 min. Table 1 also lists a variety of seed sludge sources, the most common of which (in 17 cases) was the anaerobic digester

TABLE 1. Pretreatment Condition of Seed Sludge

Treatment	Description	Sludge source	Reference
Heat	100°C 15 min	ADS	Lay et al., 1999
Heat	100°C 15 min	ADS	Okamoto et al., 2000
Heat	100°C 15 min	ADS	Lay, 2000
Heat	100°C 15 min	SBM	Noike and Mizuno, 2000
Heat	100°C 15 min	SBM	Mizuno et al., 2000a
Heat	100°C 15 min	SBM	Mizuno et al., 2000b
Heat	100°C 15 min	ADS	Lay, 2001
Heat	104°C 2 h	Compost and soil	Ginkel et al., 2001
Heat	104°C 2 h	Soil	Logan et al., 2002
Heat	75°C 1 h	SS	Chang et al., 2002
Heat	80°C 10–60 min	ADS	Noike, 2002
Heat	100°C 2 h	Compost	Lay et al., 2003
Heat	104°C 2 h	ADS	Oh et al., 2003a
Heat	121°C 30 min	Waste biosolids	Wang et al., 2003
Heat	90°C 10 min	ADS	Cheng et at., 2003
Heat	80°C 20 min	SS	Noike et al., 2003
Heat	100°C 15 min	ADS	Han and Shin, 2003
Heat	90°C 10 min	Compost	Chien et al., 2004
Heat	100–105°C 2 h	Compost	Fan et al.,2004
Heat	100°C 15 min	ADS	Han and Shin, 2004a
Heat	100°C 45 min	SS	Chang and Lin, 2004
Heat	105°C 2 h	Compost	Khanal et al., 2004
Heat	100°C 45min	AS	Lin and Lay, 2004a
Heat	100°C 45min	AS	Lin and Lay, 2004b
Heat	100°C 45 min	AS	Lin and Lay, 2005
Heat	100°C 30 min	ADS	Fang et al., 2006
Acid/base	pH 3, 10 for 24 h	SS	Chen et al., 2002
Acid/base	pH 3–4 for 24 h	SS	Chang et al., 2002
Acid/base	—	ADS	Cheng et al., 2003
Aeration	—	Compost	Ueno et al., 1995
Aeration	—	Compost	Ueno et al., 1996
Aeration	—	Compost	Ueno et al., 200la
Aeration	—	Compost	Ueno et al., 2001b
Aeration	Incubated under air for 24 h	ADS	Sparling et al., 1997
Aeration	60°C 3 days	Compost	Morimoto et al., 2004
Methanogenic inhibitors	BES 25 m*M*	ADS	Sparling et al., 1997
Methanogenic inhibitors	BESA 100 m*M*	Waste biosolids	Wang et al., 2003
Methanogenic inhibitors	Acetylene 1%	ADS	Sparling et al., 1997
Methanogenic inhibitors	Chloroform 28 mg/L	ADS	Cheng et al., 2003
Methanogenic inhibitors	Chloroform	ADS	Liang et al., 2002
Electric current	Low voltage of 3.0–4.5 V	ADS	Roychowdhury, 2000

Note. AS, acclimated sludge; SS, sewage sludge; ADS, anaerobic digested sludge; SBM, soybean meal.

sludge. Others included fermented soybean meal, soil, sewage sludge, and compost residues.

On the other hand, some reported that heat treatment could not inhibit the activity of all hydrogen-consuming bacteria (Oh et al., 2003a). Oh

et al. (2003a) found that some homoacetogenic bacteria may survive the heat treatment, and consume hydrogen for the production of acetate, resulting in the decrease of the overall hydrogen production.

III.B. Acid/Base Treatment

In the conventional methanogenic process treating wastewater or solid wastes, the pH is controlled at near pH 7. Methane production rate would drop sharply at pH below 6.3 or above 7.8 (van Haandel and Lettinga, 1994; Chen et al., 2002). Thus, adjusting the pH of anaerobic sludge substantially away from pH 7 would effectively inhibit the bioactivity of methanogens, many of which are hydrogen consumers. This, on the other hand, would not affect the bioactivity of the endospore-forming *Clostridia* (Brock et al., 1994). For example, Chen et al. (2002) found that hydrogen yield of a sewage sludge was increased 333 times after treating the sludge at pH 3 for 24 h, and 200 times at pH 10 for 24 h. Similar results were reported by Chang et al. (2002).

III.C. Methanogen Inhibitors

Methanogens are strict anaerobic and very sensitive to many chemicals. Thus, activity of hydrogen-consuming methanogens can be inhibited by simple aeration or by the addition of toxic chemicals. Ueno et al. (1995, 1996) reported that a compost sludge after forced aeration was able to produce 330–340 ml H_2/g hexose from a cellulose-containing wastewater without producing methane.

2-Bromoethanesulfonate (BES), acetylene, and chloroform are commonly used methanogen inhibitors. BES is an analog of the coenzyme M in methanogens (DiMarco et al., 1990), and is thus very specific against methanogens (Sparling and Daniels, 1987; Sparling et al., 1997). Use of BES at concentrations up to 25 m*M* (Sparling et al., 1997) or 100 m*M* (Wang et al., 2003) has been reported effective for hydrogen production. However, treating sludge at these levels of concentration would be too costly for large-scale operations.

Acetylene at a partial pressure of 500 Pa was able to inhibit methanogenic activities of pure cultures and environmental samples (Sprott et al., 1982). Sparling et al. (1997) found that the presence of acetylene in headspace was as effective as the addition of BES for the hydrogen conversion from paper waste by anaerobic digested sludge. Their results also showed that acetylene had no effect on the hydrogen yield and production rate of *C. thermocellum*.

Similarly, use of chloroform may also inhibit the hydrogen-consuming activity and thus enhance the hydrogen yield from peptone (Cheng et al., 2003) and glucose (Liang et al., 2002).

III.D. Electric Current

Roychowdhury (2000) found that hydrogen-producing bacteria could be screened from an anaerobic sludge by electric current. After a treatment of low-voltage (3.0–4.5 V) electric current, a cellulosic landfill sludge and a sewage sludge were able to produce hydrogen without methane.

IV. FEEDSTOCK

Table 2 lists the key process and performance parameters on fermentative hydrogen production in 57 cases using synthetic wastewaters, most of which containing a single carbohydrate substrate. The process parameters include pH, temperature, hydraulic retention time (HRT), reactor type, and seed sludge. Although the performance parameters include hydrogen content in biogas (%), conversion efficiency (%), yield (ml H_2/g hexose), maximum volumetric production rate (L H_2/L/d), and maximum specific rate (L H_2/g VSS/d). In this article, volatile solids (VS) represents the organic content of a solid waste, whereas volatile suspended solids (VSS) represents the biomass content in reactor. The hydrogen content may affect the cost of recovering hydrogen as a final product. Conversion efficiency was calculated assuming a theoretic yield of 544 ml H_2/g hexose, according to reaction 1. Hydrogen yield was calculated for each gram of degraded substrate, which may be expressed as g hexose (in most cases), g VS, or g COD. Two kinds of daily production rates were reported: one based on 1 L of reactor volume and the other based on 1 g of biomass (expressed as VSS).

Table 3 is the compilation of the same parameters for the fermentative hydrogen production in 8 cases treating actual wastewaters, whereas Table 4 is in 33 cases treating solid wastes.

IV.A. Synthetic Wastewaters

Most studies were conducted using synthetic wastewaters comprising a single substrate. Table 2 shows that carbohydrates were used in nearly all single-substrate fermentations. Of the 57 cases compiled, glucose and sucrose were used as individual substrates in 21 and 22 cases, respectively, and cellulose and starch were used in 6 cases each. The two exceptions used lactate (Logan et al., 2002) and a mixture of peptone (40%) and glucose (60%) (Cheng et al., 2003). In both cases, the hydrogen yields were substantially lower than those using carbohydrates as substrate.

TABLE 2. Process and Performance Parameters for Synthetic Wastewaters

Feedstock	pH Range	pH Opt	Temperature (°C)	HRT Range (h)	HRT Opt (h)	Reactor type	Seed sludge	Hydrogen in biogas (%)	Yield (ml H_2/g hexose)	Maximum volumetric rate (L H_2/L/d)	Maximum specific rate (L H_2/g VSS/d)	Conversion (%)	Reference
Glucose	5.7, 6.4	5.7	35	6–48	6	CSTR	SS	43	231[a]	17.4[a]	11.2[a]	42	Lin and Chang, 1999
Glucose	6.0	—	36	8.5	—	CSTR-gs	SBM	53	195[a]	4.8[a]	4.5[a]	36	Mizuno et al., 2000a
Glucose	5.0–7.0	7.0	35	—	—	Batch	SS	—	114[a]	—	0.04[a]	21	Chen et al., 2002
Glucose	4.0–7.0	5.5	36	6	—	CSTR	AS	64	286[a]	—	4.6	53	Fang and Liu, 2002
Glucose	5.5	—	36	6.6	—	CSTR	AS	64	260	—	4.6	48	Fang et al., 2002b
Glucose	5.0–8.0	6.0	37	3.0–12.5	5.4	CSTR	SS	48	—	4.1[a]	—	—	Horiuchi et al., 2002
Glucose	—	—	35	—	—	MBR	ADS	72	126[a]	—	1.8[a]	23	Liang et al., 2002
Glucose	6.0	—	26	—	—	Batch	Soil	64	125[a]	—	—	23	Logan et al, 2002
Glucose	4.5	—	35	216	—	SCR	AS	50	110	—	—	20	Hwang et al., 2003
Glucose	5.0	—	37	20–50	20	PBR	SS	50	—	3.6[a]	—	—	Kim et al., 2003
Glucose	(6.2, 7.5) i	(6.2) i	25	—	—	Batch	ADS	72	132[a]	—	—	24	Oh et al., 2003a
Glucose	5.5	—	36	6.6	—	CSTR	SS	64	260	—	4.6	48	Fang et al., 2004
Glucose	5.0	—	35	72	—	SCR	SS	40	200	—	—	37	Hwang et al., 2004
Glucose	5.5	—	30, 37	10,30	—	CSTR	Soil	57	245	5.6[a]	5.8[a]	45	Iyer et al., 2004
Glucose	5.5	—	35	9	—	MBR	ADS	53	117[a]	5.9	0.4[a]	22	Lee et al., 2004
Glucose	5.9–6.3	6.2	15–34	6–60	6	CSTR	SS	43	193[a]	8.0[a]	4.8[a]	36	Lin and Chang, 2004
Glucose	—	—	50, 60	—	—	Batch	Compost	—	286[a]	3.3[a]	—	52	Morimoto et al., 2004

Feedstock	pH Range	pH Opt	Temperature (°C)	HRT Range (h)	HRT Opt (h)	Reactor type	Seed sludge	Hydrogen in biogas (%)	Yield (ml H_2/g hexose)	Maximum volumetric rate (L H_2/L/d)	Maximum specific rate (L H_2/g VSS/d)	Conversion (%)	Reference
Glucose	5.5	—	—	3.3–10	5	MBR	Soil	60	201[a]	9.2[a]	1.0[a]	37	Oh et al., 2004a
Glucose	5.0–60	5.5	55–64	4–12	4	TBR	AS	53	151[a]	25.8[a]	—	28	Oh et al., 2004b
Glucose	5.5	—	23	4.5	—	CSTR	Soil	63	140	10.3[a]	2.2[a]	26	Zhang et al., 2004
Glucose	5.0	—	37	20–50	20	PBR	SS	50	—	3.6[a]	—	—	Kim et al., 2005
Sucrose	6.7	—	35	6–13.3	8	CSTR	SS	—	233[a]	—	—	43	Chen et al., 2001
Sucrose	(6.0) i	—	37	—	—	Batch	SBM	—	125[a]	—	0.6[a]	23	Lee et al., 2001
Sucrose	(4.5–7.5) i N	(5.5) i	37	—	—	Batch	Compost and soil	40	333	1.8[a]	—	61	Van Ginkel et al., 2001
Sucrose	(6.7) i	—	35	0.5–5	1	PBR	SS	35	64[a]	29.0[a]	2.0[a]	12[a]	Chang et al., 2002
Sucrose	5.5	—	26	6 h	—	CSTR	SS	63	266[a]	13.0	0.7	49	Fang et al., 2002a
Sucrose	(3.0–12.0)i	(9.0) i	37	—	—	Batch	SBM	—	120[a]	—	0.9[a]	22	Lee et al., 2002
Sucrose	6	—	26	—	—	Batch	Soil	—	123[a]	—	—	22	Logan et al., 2002
Sucrose	(6.1) i	—	35	—	—	Batch	SS	50	269[a]	—	146[a]	49	Wu et al., 2002
Sucrose	6.47–7.0	6.9	35	2–13.3	3	CSTR	SS	47	308[a]	26.9	18.8[a]	56	Chen and Lin, 2003
Sucrose	6.7–6.9	6.8	35	4–12	8	SCR	AS	35	177[a]	11.5[a]	2.2[a]	32	Lin and Jo, 2003
Sucrose	5.8–6.9	—	3.5	1–6	2	FBR	AS	38	182	22.3[a]	—	33	Wu et al., 2003
Sucrose	6.7	—	35	4–24	8	PBR	SS	42	102[a]	6.6[a]	1.3[a]	19	Chang and Lin, 2004

(Continued on next page)

TABLE 2. Process and Performance Parameters for Synthetic Wastewaters *(Continued)*

Feedstock	pH Range	pH Opt.	Temperature (°C)	HRT Range (h)	HRT Opt. (h)	Reactor type	Seed sludge	Hydrogen in biogas (%)	Yield (ml H_2/g hexose)	Maximum volumetric rate (L H_2/L/d)	Maximum specific rate (L H_2/g VSS/d)	Conversion (%)	Reference
Sucrose	(4.5–6.5) i	(5.5) i	37	—	—	Batch	Compost	61	139[a]	—	0.05[a]	28	Fan et al., 2004
Sucrose	5.5	—	26	6	—	CSTR	SS	63	266[a]	—	0.7	49	Fang et al., 2004
Sucrose	5.5	—	26	4.6–28.6	13.7	CSTR	Granular sludge	68	256[a]	—	—	47	Fang and Liu, 2004
Sucrose	(4.5–6.5) i	(4.5) i	37	—	—	Batch	Compost	40	228[a]	—	—	42	Khanal et al., 2004
Sucrose	(7.4–8.0) i	(7.5) i	35	—	—	Batch	AS	53	114[a]	3.2[a]	1.2[a]	21	Lin and Lay, 2004a
Sucrose	—	—	35	—	—	Batch	AS	55	327[a]	6.6[a]	2.7[a]	60	Lin and Lay, 2004b
Sucrose	(7.0) i	—	39	2–30	3	PBR	AS	57	186[a]	4.8[a]	—	34	Mu and Yu, 2004
Sucrose	5.4	—	35	12	—	CSTR	ADS	62	148[a]	7.6	3.8	27	Shin et al., 2004
Sucrose	5.2	—	32	15	—	CSTR-gs	ADS	54	259[a]	10.1[a]	—	48	Hussy et al., 2005
Sucrose	(6.6–7.0) i	(6.6) i	35	—	—	Batch	AS	51	233[a]	1.3[a]	—	43	Lin and Lay, 2005
Cellulose	(7.0) i	—	60	—	—	Batch	ADS	33	123	—	—	22	Ueno et al., 1995
Cellulose	(7.0) i	—	60	—		Batch	Compost	58	327[a]	—	—	60	Ueno et al., 1995
Cellulose	(7.0) i	—	37	—	—	Batch	ADS	50	48[a]	—	0.2[a]	9	Lay, 2001

Feedstock	pH Range	pH Opt.	Temperature (°C)	HRT Range (h)	HRT Opt. (h)	Reactor type	Seed sludge	Hydrogen in biogas (%)	Yield (ml H_2/g hexose)	Maximum volumetric rate (L H_2/L/d)	Maximum specific rate (L H_2/g VSS/d)	Conversion (%)	Reference
Cellulose	6.4	—	60	72	—	CSTR	Compost	57	272[a]	0.7[a]	—	50	Ueno et al., 2001a
Cellulose	6.0	—	26	—	—	Batch	Soil	—	0.4[a]	—	—	0.1	Logan et al., 2002
Cellulose	(5.5–8.5) i	(6.5) i	55	—	—	Batch	AS	68	92	—	0.3	18	Liu et al., 2003
Starch	4.0–6.0	5.2	37	10-30	17	CSTR	ADS	61	1240[a]	1.5	2.2	Unreasonable	Lay, 2000
Potato starch	6.0	—	26	—	—	Batch	Soil	—	81[a]	—	—	15	Logan et al., 2002
Wheat starch	4.5, 5.2	5.2	30,35	12–18	15	CSTR-gs	ADS	57	254[a]	3.0[a]	—	47	Hussy et al., 2003
Starch	(4.0–9.0)i	(6.0) i	55	—	—	Batch	AS	60	83[a]	—	0.4	17	Zhang et al., 2003
Starch	(4.5–6.5)i	(4.5)I	37	—	—	Batch	Compost	40	133[a]	—	—	24	Khanal et al., 2004
Starch	(4.0–9.0)i	(7.0–8.0)i	35	—	—	Batch	Cereals	58	175[a]	—	0.2	39	Liu and Shen, 2004
Lactate	6.0	—	26	—	—	Batch	Soil	—	2.2 ml/g lactate[a]	—	—	0.5	Logan et al., 2002
Peptone (40%)/glucose (60%)	—	6.5	55	—	—	CSTR	ADS	—	36.2 ml/g peptone[a]	—	—	— 2003	Cheng et al.,

Note. pH or HRT: i, initial; opt, optimal. Reactor: CSTR, continuous stirred tank reactor; MBR, membrane bioreactor; PBR, packed-bed reactor; SCR, sequencing continuous reactor; FBR, fluidized-bed reactor; TBR, trickling biofilter reactor; gs, gas sparging. Seed sludge: AS, acclimated sludge; SS, sewage sludge; ADS, anaerobic digested sludge; SBM, soybean meal. Conversion: assuming maximum conversion of 544 ml H_2/g hexose at 25°C.

[a]Calculated from provided data assuming temperature of 25°C.

TABLE 3. Process and Performance Parameters for Actual Wastewaters

Feedstock	pH Range	pH Opt	Temperature (°C)	HRT Range (h)	HRT Opt. (h)	Reactor type	Seed sludge	Hydrogen in biogas (%)	Yield (ml H_2/g hexose)	Yield (ml H_2/g COD)	Maximum volumetric rate (L H_2/L/d)	Maximum specific rate (L H_2/g VSS/d)	Conversion (%)	Reference
Sugar factory wastewater	6.8	—	60	12–72	12	CSTR	Compost	64	343[a]		4.8[a]	—	63	Ueno et al., 1996
Wastewater containing sugar and ethyl alcohol	6.0–6.5	—	37	8	—	PBR	ADS and *Clostridium butyricum*	60	—	—	1.8[a]	—	—	Kim, 2002
Molasses	6.0	—	26	—	—	Batch	Soil	—	109[a]	—	—	—	—	Logan et al., 2002
Noodle manufacturing wastewater	4.0–8.5	5.2	35	18	—	CSTR	ADS	—	200[a]	—	—	—	37	Noike, 2002
Rice winery wastewater	4.5–6.0	5.5	55	2–24	2	PBR	AS	61	291[a]	—	3.8	9.3	53[a]	Yu et al., 2002
Filtered leachate of waste biosolids	6.7–6.9	—	35	—	—	Batch	Waste biosolids	—	—	184[a]	—	—	—	Wang et al., 2003
Sugarbeet wastewater	5.2	—	32	15	—	CSTR-gs	ADS	57	231[a]	—	3.0[a]	—	42	Hussy et al., 2005
Food processing and domestic wastewater	(4.0–6.4)i	—	23	—	—	Batch	Soil	60	—	100	3.0[a]	—	—	Van Ginkel et al., 2005

Note. pH or HRT: i, initial; opt, optimal. Reactor: CSTR, continuous stirred tank reactor; PBR, packed-bed reactor; gs, gas sparging. Seed sludge: AS, acclimated sludge; ADS, anaerobic digested sludge. Conversion: assuming maximum conversion of 544 ml H_2/g hexosoe at 25°C.

[a]Calculated from provided data, assuming temperature of 25°C.

TABLE 4. Process and Performance Parameters for Solid Wastes

Feedstock	pH Range	pH Opt.	Temperature (°C)	HRT (h)	Reactor type	Seed sludge	Hydrogen in biogas (%)	Yield (ml H_2/g hexose)	Yield (ml H_2/g VS)	Maximum volumetric rate (L H_2/L/d)	Maximum specific rate (L H_2/g VSS/d)	Conversion (%)	Reference
Mixed waste	(5.0) i	5.6	37	—	Batch	SBM	60	—	180	—	0.9	—	Lay et al., 1999
Mixed waste	(5.0) i	5.6	37	—	Batch	ADS	60	—	140	—	1.1	—	Lay et al., 1999
Bean curd manufacturing waste	(6.0) i	—	35	—	Batch	SBM	63	346[a]	—	—	—	64	Mizuno et al., 2000b
Bean curd manufacturing waste	5.0	—	35	—	Batch	SBM	78	346[a]	—	—	—	64	Noike and Mizuno, 2000
Rice bran	5.0	—	35	—	Batch	SBM	68	176[a]	—	—	—	32	Noike and Mizuno, 2000
Wheat bran	5.0		35	—	Batch	SBM	72	235[a]	—	—	—	43	Noike and Mizuno, 2000
Rice	(7.0) i	—	37	—	Batch	ADS	46	—	96	—	—	—	Okamoto et al., 2000
Cabbage	(7.0) i	—	37	—	Batch	ADS	55	—	62	—	—	—	Okamoto et al., 2000
Carrot	(7.0) i	—	37	—	Batch	ADS	47	—	71	—	—	—	Okamoto et al., 2000
Egg	(7.0) i	—	37	—	Batch	ADS	—	—	7	—	—	—	Okamoto et al., 2000
Lean meat	(7.0) i	—	37	—	Batch	ADS	—	—	8	—	—	—	Okamoto et al., 2000
Fat	(7.0) i	—	37	—	Batch	ADS	—	—	11	—	—	—	Okamoto et al., 2000
Chicken skin	(7.0) i	—	37	—	Batch	ADS	—	—	10	—	—	—	Okamoto et al., 2000
Bean curd manufacturing waste	4.0–8.5	5.2	35	18	CSTR	ADS	—	346[a]	—	—	—	64	Noike, 2002
Wheat bran	4.0–8.5	5.2	35	18	CSTR	ADS	—	235[a]	—	—	—	43	Noike, 2002
Rice bran	4.0–8.5	5.2	35	18	CSTR	ADS	—	176[a]	—	—	—	32	Noike, 2002
Municipal waste	4.0–8.5	5.2	35	18	CSTR	ADS	—	48[a]	—	—	—	9	Noike, 2002
Food waste	(6.5) i	—	37	—	LBR	ADS	35	—	310	3.6	—	—	Han and Shin, 2003
Carbohydrate-rich high solid organic waste	(7.0) i	—	37	—	Batch	Compost	—	—	50[a]	—	—	—	Lay et al., 2003

(Continued on next page)

TABLE 4. Process and Performance Parameters for Solid Wastes *(Continued)*

Feedstock	pH Range	pH Opt.	Temperature (°C)	HRT (h)	Reactor type	Seed sludge	Hydrogen in biogas (%)	Yield (ml H_2g hexose)	Yield (ml H_2g VS)	Maximum volumetric rate (L H_2/L/d)	Maximum specific rate (L H_2/g VSS/d)	Conversion (%)	Reference
Fat-rich high solid organic waste	(7.0) i	—	37	—	Batch	Compost	—	—	3.3[a]	—	—	—	Lay et al., 2003
Protein-rich high solid organic waste	(7.0) i	—	37	—	Batch	Compost	—	—	2.5[a]	—	—	—	Lay et al., 2003
Bean curd manufacturing waste	5.5	—	35	6	CSTR	SS	58	131[a]	—	1.0	0.42	24	Noike et al., 2003
Bean curd manufacturing waste	5.5	—	35	9	MBR	SS	51	117[a]	—	2.6	0.03	22	Noike et al., 2003
Waste biosolids	6.7–6.9	—	35	—	Batch	Waste biosolids	—	15[b]	—	—	—	—	Wang et al., 2003
Dehydrated brewery mixture	—	—	37	—	Batch	Compost	—	27[b]	—	—	0.2	—	Fan and Chen. 2004
Food waste	(6.5)i	—	35	—	LBR	ADS	—	—	—	8.9[a]	—	—	Han and Shin, 2004a
Food waste	(6.5)i	—	37	—	LBR	ADS	—	—	310	3.6	—	—	Han and Shin, 2004b
Food waste	(7.0)i	—	37	—	Batch	Compost	—	207[a]	77	—	0.5	38	Lay, 2004
Food waste	5.0–6.0	5.5	55	48	CSTR	AS	55	136[a]	—	0.5	—	25	Shin and Youn, 2005
				72			55	204[a]	—	0.6	—	38	
				120			60	299[a]	—	1.0	—	55	
Mixed waste	5.5	—	55	84	SCR	ADS	58	437[a]	360	—	—	80	Valdez-Vazquez et al., 2005
Mixed waste	6.4	—	37	84	SCR	ADS	42	201[a]	165	—	—	37	Valdez-Vazquez et al., 2005
Food waste	4.0-7.0	4.5	37	—	Batch	ADS	56	346	—	—	2.1	64	Fang et al., 2006
Food waste	4.5	—	55	—	Batch	ADS	56	210	—	—	0.8	39	Fang et al., 2006

Notes. pH i: initial; opt; optimal. Reactor: CSTR; continuous stirred tank reactor; MBR; membrane bioreactor; SCR; sequencing continuous reactor; LBR; leaching-bed reactor. Seed sludge: AS; acclimated sludge; SS; sewage sludge; ADS; anaerobic digested sludge; SBM; soybean meal. Conversion: assuming maximum conversion of 544 ml H_2/g hexose at 25°C. [a]Calculated from provided data, assuming temperature of 25°C. [b]Calculated on COD basis; unit, ml/g COD; assuming temperature of 25°C.

For those cases using carbohydrates as substrates, the hydrogen yield in batch reactors varied widely from 0.4 ml H_2/g hexose using cellulose as feedstock (Logan et al., 2002) to 333 ml H_2/g hexose using sucrose (Van Ginkel et al., 2001). The corresponding conversion efficiencies were 0.1% and 61%. On the other hand, in continuous reactors the hydrogen yield and conversion efficiency ranged from 64 ml H_2/g-hexose and 12% (Chang et al., 2002) to 308 ml H_2/g hexose and 56% (Chen and Lin, 2003). Although a hydrogen yield of 1240 ml H_2/g hexose has been reported using starch as feedstock (Lay, 2000), such a yield is substantially higher than the theoretical maximum and has been considered to be erroneous (Fang et al., 2002a).

For comparison, the reported hydrogen yields from lactate and peptone were 2.2 ml H_2/g lactate (Logan et al., 2002) and 36.2 ml H_2/g peptone (Cheng et al., 2003). It is unclear, in the latter case, whether the yield included the amount of hydrogen produced by the co-substrate glucose.

IV.B. Actual Wastewaters

From pollution control and resource recovery points of view, it would be ideal if one could convert pollutants in wastewater into hydrogen (Zajic et al., 1978). However, only a few studies have been conducted for hydrogen production from actual wastewater. Table 3 shows the process condition and performance of eight cases found in literature using wastewaters from rice winery (Yu et al., 2002), noodle, sugar, and molasses manufacturing (Ueno et al., 1996; Logan et al., 2002; Hussy et al., 2005), food processing (Van Ginkel et al., 2005), and the filtered leachate of municipal solid wastes (Wang et al., 2003). All of these tests were conducted in laboratory scale at temperatures from 23°C to 60°C, and HRT from 2 to 72 h. No full-scale data are available so far.

Of all tests, the best yield was demonstrated by Ueno et al. (1996) for the treatment of sugar factory wastewater. They achieved a yield of 343 ml H_2/g hexose in a continuous stirred tank reactor (CSTR) with 63% of conversion efficiency, both of which are comparable to the best performance in treating single-substrate wastewater in a continuous reactor, that is, 308 ml H_2/g hexose and 56% (Chen and Lin, 2003). This was followed by 291 ml H_2/g hexose and 53% for the treatment of winery wastewater (Yu et al., 2002), 231 ml H_2/g hexose and 42% for sugarbeet wastewater (Hussy et al., 2005), and 200 ml H_2/g hexose and 37% for noodle manufacturing wastewater (Noike, 2002).

IV.C. Solid Wastes

Methanogenic conversion has gradually become a common solid wastes treatment process, even though it may encounter some problems associated

with collection, storage, and transport of solid wastes (Rocha et al., 2001), and the pretreatment needed for the recalcitrant lignocellulosic compounds (de Vrije and Claassen, 2002; 2003; Nath and Das, 2003). So far, in total, 33 cases have been reported for hydrogen production from solid wastes, the key process and performance parameters of which are listed in Table 4. The feedstock included those wastes from kitchen (21 cases), food processing (5), mixed wastes (5), and municipal wastes (2). The hydrogen yield varied widely from 2.5 ml H_2/g VS (Lay et al., 2003) to 360 ml H_2/g VS (Valdez-Vazquez et al., 2005), with conversion efficiency from 9% (Noike, 2002) to 80% (Valdez-Vazquez et al., 2005). Wastes rich in carbohydrate in general had higher hydrogen yield than those rich in protein and fat. For example, Lay et al. (2003) reported that the hydrogen yield from a carbohydrate-rich waste (containing rice and potato) was 50 ml H_2/g VS, which was 16 times higher than the yield from a fat-rich waste (fatty meat and chicken meat), and 20 times higher than the yield from a protein-rich waste (egg and lean meat). Similarly, Okamoto et al. (2000) reported that the yields were 62–96 ml H_2/g VS treating rice, cabbage, and carrot, but only 7–11 ml H_2/g VS for treating egg, fat, meat, and chicken skin.

The highest reported conversion efficiencies from solid wastes so far were 64% from a bean curd manufacturing waste and a rice slurry in batch process (Mizuno et al., 2000b; Fang et al., 2006) and 80% from a mixed food-and-paper waste in a semicontinuous process (Valdez-Vazquez et al., 2005). The latter also had a yield of 437 ml H_2/g hexose, the highest recorded yield from wastewaters and solid wastes so far.

V. NUTRIENTS AND INHIBITORS

Like all fermentation processes, hydrogen production requires nutrients for bacterial metabolism, growth and activity. The nutrients include nitrogen (N), phosphate (P), and some trace elements. On the other hand, hydrogen production may also be inhibited by chemicals and the presence of other bacteria. The following summarizes the effects of nutrients and inhibitors.

V.A. Nitrogen

Nitrogen is one of the most essential nitrients needed for growth. Comparisons of hydrogen yield at various N concentrations and C/N ratios were reported in four cases. Results were, however, conflicting. The optimal N concentration varied from 0.1 to 2.0 g N/L, and the C/N ratio from 3.3 to 130.

Liu and Shen (2004) investigated the effect of N concentration, using NH_4HCO_3 as the N source, on the batch production of hydrogen from starch. Seven N concentrations were studied, varying from 0.1 to 2.0 g N/L, corresponding to C/N ratios from 67 to 3.3. Results showed that the maximum

hydrogen yield (175 ml H_2/g hexose) and specific hydrogen production rate (0.2 L H_2/g VSS/d) were obtained at 1.0 g N/L or a C/N ratio of 6.7. Similar results were reported by Ueno et al. (2001a), who compared hydrogen production from powdered cellulose (10 g/L) at 2 nitrogen concentrations (0.13 and 0.9 g N/L), corresponding to C/N ratios of 34 and 5. Results showed that at 0.9 g N/L and a C/N ratio of 5 the hydrogen yield (272 ml H_2/g hexose) was about 100% higher than that (136 ml H_2/g hexose) at 0.13 g N/L and a C/N ratio of 34.

Morimoto et al. (2004) compared the hydrogen yield from glucose (10 g/L) using yeast extract as N source at 3 concentrations, 0.2, 0.4, and 0.8 g N/L, assuming an average N content of 10% in yeast extract (Sigma, 2004), corresponding to C/N ratios of 20, 10, and 5. Results showed that the highest hydrogen yield of 170 ml H_2/g hexose was obtained at 0.4 g N/L and a C/N ratio of 10. Lin and Lay (2004a) compared the hydrogen yields of sucrose at four concentrations with 0.9 g N/L of nitrogen, corresponding to C/N ratios of 130, 98, 47, and 40. The highest hydrogen yield (327 ml H_2/g hexose) was obtained at C/N ratio of 47.

V.B. Phosphate

A few studies found that phosphate was needed in hydrogen production for its nutritious value as well as buffering capacity (Oh et al., 2002, 2003b; Lin and Lay, 2004b). Hawkes et al. (2002) compared literature data for C/P ratios from 6 to 260, and concluded an optimal C/P ratio of 130. A similar optimal C/P ratio of 120 was recently reported based on a systematic study for C/P ratios from 8.7 to 800 for hydrogen production from sucrose (Lin and Lay, 2004b).

V.C. Trace Metals

Lin and Lay (2005) studied the requirement of 11 trace metals in hydrogen production using the experiment design of Taguchi orthogonal arrays. They reported that magnesium, sodium, zinc, and iron were of significance for hydrogen production; among the four, magnesium was the most crucial. At the optimal combined concentrations of 4.8 mg Mg^{2+}/L, 393 mg Na^{+}/L, 0.25 mg Zn^{2+}/L, and 1 mg Fe^{2+}/L, the maximum hydrogen yield from a sucrose-containing wastewater was 233 ml H_2/g hexose.

Nearly all the other trace-metal studies were focused on iron alone, probably because its presence is essential for hydrogenase (Junelles et al., 1988; Hawkes et al., 2002). Several studies reported that iron-limited conditions would not only lower the production of hydrogen as well as acid, but also increase the production of alcohols, such as ethanol and butanol (Junelles et al., 1988; Lee et al., 2001). However, the reported optimal iron

concentration was inconsistent, varying from 10 mg Fe^{2+}/L (Liu and Shen, 2004) to 353 mg Fe^{2+}/L (Lee et al., 2001). At the optimal concentration of 10 mg Fe^{2+}/L, Liu and Shen (2004) reported a maximum specific production rate of 0.2 L H_2/g VSS/d and a yield of 126 ml H_2 hexose.

V.D. Toxic Heavy Metals

Heavy metals, including cadmium, chromium, zinc, copper, nickel, and lead, may be present at significant concentrations in some industrial wastewater and municipal wastes. These metals are often found to be the leading cause of anaerobic reactor upset and failure. Fang (1997) compared the effect of five heavy metals on the methanogenic granular sludge, and reported that toxicity was in the following order: zinc > nickel > copper > cadmium > chromium.

For hydrogen production, similar order of inhibition was also observed. Hsieh (2003) reported that for hydrogen production from sucrose, zinc (C_{50} 4.5 mg/L) was slightly more toxic than copper (C_{50} 6.5 mg/L), which in turn was much more toxic than chromium (C_{50} 60 mg/L). In two separate studies of hydrogen production from dairy water, copper (C_{50} 65 mg/L) was reported more toxic than zinc (C_{50} 120 mg/L) (Yu and Fang, 2001a), and chromium (C_{50} 72 mg/L) was more toxic than cadmium (C_{50} 170 mg/L) (Yu and Fang, 2001b).

Zheng and Yu (2004) reported that there was a lag phase for hydrogen production from glucose in the presence of copper and zinc; however the total hydrogen yield was not adversely affected by copper at concentrations up to 400 mg/L and by zinc up to 500 mg/L.

V.E. Lactic Acid Bacteria

In continuous hydrogen production from bean curd manufacturing waste by a mixed culture, Noike et al. (2002) reported that hydrogen production by *Clostridium* ceased when two lactic acid bacteria, *Lactobacillus paracasei* and *Enterococcus durans*, were added as cocultures. They claimed that the inhibition was caused by bacteriocins secreted by the lactic acid bacteria, instead of the lowering of pH resulting from the production of acids.

VI. OPERATIONAL CONDITIONS AFFECTING HYDROGEN PRODUCTION

Operational parameters such as pH, temperature, and HRT are crucial to hydrogen production. Their effects are discussed next.

VI.A. pH

pH is one of the key process parameters on hydrogen production because it may directly affect the hydrogenase activity (Dabrock et al., 1992) as well as the metabolism pathway (Lay, 2000). In addition, it is also a crucial factor for the suppression of the hydrogen-consuming methanogenic activities (Chen et al., 2002). Fang and Liu (2002) investigated the pH effect over the range pH 4.0–7.0 (with increments of 0.5), and concluded that the optimal pH was 5.5 with a yield of 286 ml H_2/g hexose and a specific production rate of 4.6 L H_2/g VSS/d. For comparison, the hydrogen yields were only 190 ml H_2/g hexose at pH 4.0 and 41 ml H_2/g hexose at pH 7.0. Lin and Chang (1999) compared the hydrogen production from glucose at two pH, and reported that at pH 5.7 the hydrogen yield and specific production rate were 231 ml H_2/g hexose and 11.2 L H_2/g VSS/d, which were slightly higher than the 226 ml H_2/g hexose and 8.3 L H_2/g VSS/d at pH 6.4.

In total, 22 "optimal pH" values were reported in Table 2. However, many were conducted in batch reactors without pH control. In these cases, only the "initial pH" was reported, yet the actual pH in mixed liquor would have been gradually reduced due to the production of fatty acids. The degrees of pH reduction depended on many factors, such as substrate and sludge concentrations, temperature, duration, and so on. The optimal initial pH values reported for these cases were thus case specific and can only be used for references.

Of the 11 cases in Table 2 where the "actual" pH values were reported, the optimal pH was in the range of 5.2–7.0 with an average of pH 6.0 for hydrogen conversion from carbohydrates.

In total, 10 optimal pH values for hydrogen conversion were reported for actual wastewater (2 cases) and for solid wastes (8 cases). All were within the range of pH 5.2–5.6, with one exception at pH 4.5. Noike (2002) reported an optimal pH of 5.2 for a noodle manufacturing wastewater, and Yu et al. (2002) reported pH 5.5 for a rice winery wastewater. The optimal pH values for solid wastes were reported as pH 5.6 for a mixture of food waste, night soil, and sewage sludge (Lay et al., 1999), pH 5.2 for wheat bran, rice brain, municipal waste, and bean curd manufacturing waste (Noike, 2002), and pH 5.5 for a food waste (Shin and Youn, 2005). However, Fang et al. (2006) recently reported an optimal pH of 4.5 for rice slurry with a hydrogen yield of 346 mL/g carbohydrate.

The pH may also affect the metabolism pathways in hydrogen production. In most studies, butyrate and acetate were the two main products, while low pH seemed to favor butyrate production. Propionate production increased substantially at pH 7.0 and above. Fang and Liu (2002) investigated the product profiles from pH 4.0 to 7.0. Butyrate was found to be the predominant product (up to 45.6%) at pH 6.0 or below, whereas acetate became predominant (up to 34.1%) at pH 6.5 or above. Other by-products included

ethanol (4.6–10.1%), lactate (2.0–4.6%), caproate (0.5–5.8%), and propionate (0.9–15.9%). Similar observations were found in the degradation of rice slurry for pH 4.0–7.0 (Fang et al., 2006). Horiuchi et al. (2002) reported that butyrate was predominant at pH 5.0, but acetate became predominant followed by propionate at pH 8.0.

Kim et al. (2004) also reported that butyrate was the main product at pH 5.5, but butanol became predominant at pH 4.3. A butanol fermentation pathway via butyraldehyde was proposed for low pH (Gaudy and Gaudy, 1980). Hwang et al. (2004) reported that the main products were butyrate at pH 4.0–4.5, but ethanol at pH 4.5–6.0, and propionate at pH 5.0–6.0.

VI.B. Temperature

The effect of temperature on hydrogen production was studied in several studies, most of which seemed to show that hydrogen yield increased with temperature. Lin and Chang (2004) examined the effect for the range of 15–34°C and reported that the optimal temperature was 30–34°C for a maximum yield of 193 ml H_2/g hexose and a specific production rate of 4.8 L H_2/g VSS/d. Yu et al. (2002) found that hydrogen yield and specific production rate from a winery wastewater at 55°C (260 ml H_2/g hexose and 9.3 L H_2/g VSS/d) were 38% and 145% higher, respectively, than those at 20°C (188 ml H_2/g hexose and 3.8 L H_2/g VSS/d). Morimoto et al. (2004) reported that at 60°C the hydrogen yield from glucose was 60% (218 ml H_2/g hexose) higher than that at 50°C (136 ml H_2/g hexose). Valdez-Vazquez et al. (2005) compared hydrogen production at 37°C and 55°C from a mixed waste in semicontinuous reactors. They reported that the hydrogen yield at 55°C was 437 ml H_2/g hexose, substantially higher than the 201 ml H_2/g hexose at 37°C.

All the hydrogen production studies listed in Tables 2–4 were conducted in three temperature ranges: ambient (15–30°C; 15 cases), mesophilic (32–39°C; 73 cases), and thermophilic (50–64°C; 13 cases). However, due to the drastic differences in reactor, substrate, seed sludge, and other process conditions, it is difficult to compare hydrogen yield at the three temperature ranges. Among the 57 cases using carbohydrates as substrate in Table 2, the average yields were 173 ml H_2/g hexose for temperatures in the range of 15–30°C, 191 ml H_2/g hexose for 32–39°C, and 190 ml H_2/g hexose for 50–64°C. The highest reported yields were 266 ml H_2/g hexose for 15–30°C (Fang et al., 2002a, 2004), 333 ml H_2/g hexose (Van Ginkel et al., 2001) for 32–39°C, and 327 ml H_2/g hexose for 50–64°C (Ueno et al., 1995). Results suggest that hydrogen yields and production rates were comparable at mesophilic and thermophilic temperatures, but lower at the ambient temperatures.

For the 8 cases treating actual wastewater in Table 3, the average yields were 109 ml H_2/g hexose for 23–26°C, 216 ml H_2/g hexose for 32–37°C, and 317 ml H_2/g hexose for 55–60°C. The highest yield was 343 ml H_2/g hexose from treating a sugar factory wastewater at 60°C (Ueno et al., 1996). For the solid wastes in Table 4, the average yields were 224 ml H_2/g hexose for 35–37°C, and 257 ml H_2/g hexose for 55°C. The highest yield was 437 ml H_2/g hexose from treating a mixed food and paper waste at 55°C (Valdez-Vazquez et al., 2005).

Temperature may also affect the metabolic pathway, resulting in a shift of by-product compositions, which were mostly acetate and butyrate, plus some propionate and ethanol. However, results were conflicting. Valdez-Vazquez et al. (2005) reported that butyrate was the predominant by-product at 37°C and acetate at 55°C; at the latter temperature, slightly more propionate and ethanol were produced. Yet Yu et al. (2002) reported that acetate was the main by-product at 20°C, but the production of acetate decreased at 55°C with increasing production of butyrate, propionate, and ethanol. Contrary to both of these two, Zoetemeyer et al. (1982) reported that propionate and ethanol were the predominant products in the acidification of glucose at 55–65°C.

VI.C. Hydraulic Retention Time (HRT)

Some reported optimal HRT values in Table 2 are actually inconclusive because they are the lowest HRT values in the studied range (Lin and Chang, 1999, 2004; Kim et al., 2003, 2005; Oh et al., 2004a, 2004b; Ueno et al., 1996; Yu et al., 2002), meaning the actual optima could be even lower. Excluding these inconclusive findings, results in Table 2 show that most reported optimal HRT values for glucose and sucrose were in the range of 3–8 h, with the lowest being 1 h (Chang et al., 2002) and the highest 13.7 h (Fang and Liu, 2004). The two reported optimal HRT for starch, on the other hand, were much higher, that is, 15 h (Hussey et al., 2003) and 17 h (Lay, 2000). Long HRT was needed for degradation of starch due to its slow initial step of hydrolysis.

The two reported optimal HRT values for actual wastewater in Table 3 were 2 h (Yu et al., 2002) for a rice winery wastewater and 12 h (Ueno et al., 1996) for a sugar factory wastewater. They were in general in concurrence with results in Table 2 using single carbohydrate substrates. Most of the solid wastes were treated in slurry form by mixing with water. The optimal HRT of the slurry varied significantly, from 6–9 h for bean curd waste in a CSTR or a membrane bioreactor (MBR) (Noike et al., 2003) to 84 h for organic solid food waste in a semicontinuous reactor (Valdez-Vazquez et al., 2005). Shin and Youn (2005) compared the hydrogen yield at 48, 72, and 120 h for hydrogen conversion from a food waste, and reported that 120 h had the highest hydrogen yield.

VII. BIOREACTORS FOR WASTEWATER TREATMENT

Many exploratory studies were conducted in batch reactors for simple operation and efficient control. However, large-scale operations would require continuous production processes for practical engineering reasons. Reactors for continuous hydrogen production included the completely mixed, packed-bed, fluidized-bed, sequencing-continuous reactor, trickling biofilter, and membrane bioreactors.

VII.A. Continuously Stirred Tank Reactor

The continuously stirred tank reactor (CSTR) has been the most common mode of continuous hydrogen production. Complete mixing allows intimate contact between the substrate and biomass, as well as effective pH and temperature control. CSTR has been used for hydrogen conversion from synthetic wastewaters containing glucose (Lin and Chang, 1999, 2004; Mizuno et al., 2000a; Fang and Liu, 2002; Fang et al., 2002b, 2004; Horiuchi et al., 2002; Iyer et al., 2004; Zhang et al., 2004), sucrose (Fang et al., 2002a, 2004; Fang and Liu, 2004; Chen and Lin, 2003; Shin et al., 2004; Hussy et al., 2005), starch (Lay, 2000), and cellulose (Ueno et al., 2001a), as well as from actual sugar manufacturing wastewater (Ueno et al., 1996).

In a conventional CSTR, biomass is well suspended in the mixed liquor, which has the same composition as the effluent. Since biomass has the same retention time as the HRT, its concentration in the mixed liquor and, thus, the hydrogen production are limited. However, it has been recently found that hydrogen-producing biomass in CSTR could be self-granulated or flocculated under proper conditions. As a result, the biomass retention time could be decoupled from HRT, and thus its concentration could be kept at much higher levels. Fang et al. (2002a) found that the hydrogen-producing sludge could agglutinate into granules within 60 days in treating a synthetic sucrose-containing wastewater at 26°C, pH 5.5, and 6 h of HRT. The formation of granular sludge drastically increased the biomass concentration (up to 20 g/L) and consequently the hydrogen production rate (13.0 L H_2/L/d). A matured granule was 1.6 ± 0.2 mm in diameter, 1.038 g/ml in density, 11% in ash content, and over 50 m/h in settling velocity. Each gram of granule contained 179.1 mg extracellular polymers, which were composed of 61.9% carbohydrate, 14.4% of protein, 8.4% humic substance, 3.1% uronic acid, and 0.15% DNA.

Similarly, Zhang et al. (2004) reported that biomass could form dense flocs in treating a glucose-containing wastewater at 23°C, pH 5.5, and 4.5 h of HRT. The reactor contained 4.59 g/L of biomass and produced hydrogen at 10.3 L H_2/L/d with a conversion efficiency of 26%. As the loading rate increased, the floc size increased from 1.2 to 5.8 mm, the porosity from 75% to 96%, and the fractal dimension from 2.11 to 2.48.

Another approach to increase the biomass concentration in a CSTR was to immobilize biomass in biofilms or artificial granules. Such an approach was applied for hydrogen production from pure cultures (Karube et al., 1976, 1982; Kumar and Das, 2001), but more recently for mixed cultures as well. Examples included immobilization of biomass on fibers of cuprammonium rayon (Kim et al., 2002), in a polyvinyl alcohol medium (Kim et al., 2003, 2005), in alginate beads with dosages of activated carbon, polyurethane, and acrylic latex plus silicone (Wu et al., 2002), or in artificial granules containing cationic polyacrylamide and anionic silica sol (Kim et al., 2003, 2005). Wu et al. (2002) reported that immobilizing biomass improved hydrogen yield threefold and production rate fourfold. However, the improvement could be compromised in large granules where mass transfer might become a limiting factor (Wu et al., 2003).

VII.B. Packed-Bed Reactors

The common alternative to CSTR for the continuous hydrogen production is the packed-bed reactor. In such a reactor, biomass are immobilized either in granules (Chang and Lin, 2004; Mu and Yu, 2004) or in biofilms (Oh et al., 2004b), or are entrapped in packed media (Chang et al., 2002). The flow pattern within the packed-bed reactor is plug flow with little mixing. In most packed-bed reactors, wastewater enters at the bottom and exits from the top. This is commonly known as upflow packed-bed reactor. Those that use the downflow mode are also known as trickling biofilter reactors (Oh et al., 2004b).

Chang et al. (2002) entrapped hydrogen-producing biomass in three porous media: loofah sponge, expanded clay, and activated carbon. Results showed that loofah sponge was inefficient for biomass immobilization, while the other two exhibited improved yields. The reactor entrapping biomass in activated carbon had a higher production rate (29.0 L H_2/L/d) and more stability than the other two at 1 h of HRT and a sucrose concentration of 20 g/L. In two recent studies, hydrogen was produced from a sucrose-rich wastewater in packed-bed reactors with self-granulated biomass. Mu and Yu (2004) reported a maximum yield of 186 ml H_2/g hexose and a production rate of 4.8 L H_2/L/d; however, little information on the characteristics of the granules was provided. Chang and Lin (2004) reported a maximum yield of 102 ml H_2/g hexose and a hydrogen production rate of 6.6 L H_2/L/d; the diameter of granules ranged from 0.23 mm to 0.43 mm.

Oh et al. (2004b) produced hydrogen from glucose under thermophilic conditions in a trickling biofilter reactor packed with 55 mg/cm^3 of polyvinylidene dichloride fibers (0.3 mm in dia) with a high void fraction of 95%. The maximum hydrogen yield and production rate were 151 ml H_2/g hexose and 25.7 L H_2/L/d, respectively.

Wu et al. (2003) produced hydrogen from a sucrose-rich wastewater using immobilized biomass in alginate beads with acrylic latex and silicone inside a three-phase fluidized-bed reactor. Results show that the fluidized-bed reactor was flexible and stable to operate with a yield of 182 ml H_2/g hexose and a production rate of 22.3 L H_2/L/d.

Yu et al. (2002) produced hydrogen from a rice winery wastewater in an upflow sludge bed reactor, with a maximum yield of 291 ml H_2/g hexose and volumetric rate of 3.8 L/L/d. However, no granulation was observed in their study.

VII.C. Membrane Bioreactor

The membrane bioreactor (MBR) has become a mature technology in aerobic wastewater treatment (Gander et al., 2000) and has recently been applied to the anaerobic process (Vallero et al., 2005). It relies on a membrane to retain sludge in the mixed liquor by membrane separation, so that the reactor can be operated at high biomass concentrations with very low sludge yield. Attempts have been made recently to apply the MBR process for hydrogen production. However, results in three reported cases so far (Cheng et al., 2003; Lee et al., 2004; Oh et al., 2004a) have not shown many advantages. Reactors were operated at biomass concentrations of 2–13 g VSS/L with volumetric production rates of 5.9–16.5 L H_2/L/d, which were, although comparable to the rates in many CSTR, substantially lower than the highest yield of 26.9 L H_2/L/d reported by Chen and Lin (2003). The two reported hydrogen yields in MBR were 0.4 and 2.5 L H_2/g VSS/d, also similar to those in CSTR.

VIII. BIOREACTORS FOR SOLID WASTES TREATMENT

Most of the studies on hydrogen production from solid wastes were conducted in slurry form by mixing the wastes with added water. Of the 33 cases studied, 21 were conducted in batches and 12 in continuous processes, including 6 that used CSTR, 3 leaching-bed reactors, 2 sequencing continuous reactors, and 1 MBR. Using a CSTR, Noike (2002) converted hydrogen at 35°C and 18 h of HRT from bean curd manufacturing waste, wheat bran, rice bran, and municipal waste, and reported the respective hydrogen yields as 346, 235, 176, and 48 ml H_2/g hexose. For comparison, Shin and Youn (2005) reported a production rate of 1.0 L H_2/L/d and a yield of 299 ml H_2/g hexose for the conversion of a food waste at 55°C in a CSTR at a loading rate of 8 g VS/L/d and a HRT of 120 h.

In a comparative study, Noike et al. (2003) reported that the production rate of a MBR was 2.6 L H_2/L/d, as compared to 1.0 L H_2/L/d in a CSTR.

The specific production rates were respectively 0.03 L H_2/g VSS/d and 0.42 L H_2/g VSS/d, whereas the hydrogen yields were 117 and 131 ml H_2/g hexose for MBR and CSTR.

Han and Shin (2003, 2004a, 2004b) developed a process in which four leaching-bed reactors were used for hydrogen recovery from food waste. The effluent was subsequently treated for methane recovery in separate reactors. They reported that in such a process, at a loading rate of 11.9 kg VS/m^3/d, 28.2% of VS in the solid wastes were converted to hydrogen with a yield of 310 ml H_2/g VS.

Valdez-Vazquez et al. (2005) operated two reactors in sequencing continuous mode to convert a mixed waste under two conditions. They reported that at pH 5.5, each gram of waste produced 165 ml hydrogen at pH 6.4 and 37°C, and 360 ml at pH 5.5 and 55°C.

IX. REDUCTION OF HYDROGEN PARTIAL PRESSURE

Since the buildup of hydrogen partial pressure may inhibit the hydrogen production, many attempts have been made to improve hydrogen production by lowering its partial pressure in the reactor. Applying vacuum was found to have little effect on hydrogen yield (Kataoka et al., 1997). However, several other methods were reported to have substantial improvements. These include vigorous mixing to avoid supersaturation (Lay, 2000), uses of nitrogen (Mizuno et al., 2000a; Hussy et al., 2003, 2005), and hydrogen-permeable membrane to remove dissolved hydrogen from mixed liquor (Liang et al., 2002), and continuous pressure release (Logan et al., 2002).

Lamed et al. (1988) compared hydrogen yields from cellulose and cellobiose with vigorous mixing and without by three *Clostridium* cultures. Results showed that mixing enhanced the hydrogen yield by a minimum of 56%. Similarly, Lay (2000) reported that increasing mixing in an CSTR from 100 to 700 rpm enhanced the hydrogen production rate from starch by 130%, from 0.7 to 1.6 L H_2/L/d.

Mizuno et al. (2000) demonstrated that sparging inert nitrogen in a CSTR increased the hydrogen yield from 120 to 195 ml H_2/g hexose. A similar approach was reported by Nielsen et al. (2001), but without providing the details. Hussy et al. (2003) found that sparging with nitrogen not only increased the hydrogen yield from 172 to 254 ml H_2/g hexose, but also improved the process stability. Similar observations were also reported in treating sucrose and sugarbeet wastewaters that the hydrogen yields were improved by over 66% from 120–140 ml H_2/g hexose to 200–260 ml H_2/g hexose (Hussy et al., 2005). However, this method has a disadvantage, that hydrogen content in the biogas is diluted, resulting in a cost increase for its recovery.

Liang et al. (2002) submerged a hydrogen-permeable silicone rubber membrane in the mixed liquor to remove hydrogen from the reactor, resulting

in a yield increase from 110 to 130 ml H_2/g hexose and a rate increase from 1.4 to 1.6 L H_2/g VSS/d. The improvements seemed to be only marginal. Logan et al. (2002) improved the hydrogen production from glucose in batch experiments by releasing the buildup pressure continuously. As compared to the experiments in which the pressure was released intermittently, continuous release improved the hydrogen production by 43%.

X. MAXIMUM HYDROGEN YIELD AND PRODUCTION RATE

Data reviewed in this study show that hydrogen yields and production rates by mixed cultures were in general comparable to those by the pure cultures. Results of continuous hydrogen production in Tables 2–4 show that the highest reported yield was 437 ml H_2/g hexose (Valdez-Vazquez et al., 2005) from a waste mixture in a semicontinuous reactor at pH 5.5, 55°C, and 84 h of HRT. This yield by a mixed culture was substantially higher than the corresponding yield of 327 ml H_2/g hexose by a pure culture (*Clostridium* sp. no. 2) converting glucose in a CSTR at pH 6.0 and 36°C, (Taguchi et al., 1995).

The highest volumetric production rate was 29.0 L H_2/L/d treating sucrose in a packed-bed reactor at an initial pH of 6.7, 35°C, and 1 h of HRT (Chang et al., 2002). This was closely followed by the 26.9 L H_2/L/d by converting sucrose in a CSTR at pH 6.9, 35°C, and 3 h of HRT (Chen and Lin, 2003). These rates were below the corresponding rate of 34.1 L H_2/L/d from glucose at 37°C and uncontrolled pH by *Enterobacter* aerogenes HU-101 in a packed-bed reactor (Rachman et al., 1998).

The best sludge activity in continuous reactors was 18.8 L H_2/g VSS/d treating sucrose at pH 6.9, 35°C, and 3 h of HRT (Chen and Lin, 2003). No similar data for pure cultures were available for comparison.

XI. OUTLOOK

Although the technical feasibility of fermentative hydrogen production from wastewater and solid wastes has been demonstrated in hundreds of papers, the technology is still at its infancy. After reviewing over 160 related papers, it is obvious that further studies on a number of areas are crucial for the further development of this technology. First, heat treatment has been demonstrated as a simple and effective pretreatment for screening hydrogen-producing bacteria from common anaerobic sludge. However, it may favor the selection of the spore-forming *Clostridium* over many other hydrogen producers, some of which may be more effective than *Clostridium* but have been overlooked.

Second, carbohydrates have been the main substrate used so far. The technology would have a rather limited scope if other organic matters cannot be effectively used as substrates. Expanding the choice of substrate,

particularly to include fat and proteins, would drastically improve the technology's chance of successful development.

Third, most papers reported the hydrogen production characteristics, such as yield, rate, conversion efficiency, etc., under certain given conditions, such as pH, temperature, HRT, etc. Predictions, basing on these results, of reactor performance under different conditions are often unreliable. Systematic studies of the effects of key process parameters, instead of trial and error, are needed.

Fourth, CSTR has been the most commonly used reactor for its simple operation and ease of process control. However, production rate by CSTR is intrinsically limited due to its complete-mix nature. Exploration of continuous high-rate reactor configurations and the corresponding optimal operation conditions is also crucial for the commercialization of the technology.

Last but not least, fermentation alone converts, even under the most ideal condition, less than 40% of the chemical energy in wastewater or solid wastes into hydrogen. The energy residues remain in the by-products in the forms of acids and alcohols, which require additional treatment processes for further energy recovery. A sustainable strategy is to develop a technology package that includes not just fermentation, but also the downstream process for the full recovery of chemical energy in wastewater and wastes.

In a number of studies, the residual acids and alcohols were further converted to methane (Han and Shin, 2003, 2004b), which is, however, of little commercial value except for being used as fuel. Two other possibilities have been explored recently: photoconversion of residual acids for further production of hydrogen (Fang et al., 2004, 2005), and microbial fuel cells for the direct production of electricity (Liu et al., 2005). Both processes are still in their infancy stage and deserve much further studies. It has been known that phototrophic bacteria are capable of converting fatty acids, such as acetate and butyrate, into hydrogen (Barbosa et al., 2001). A two-step process is thus likely to convert organic pollutants into hydrogen by dark fermentation, followed by photofermentation to convert the residual acids into hydrogen. The technically feasibility of such a two-step process has been demonstrated recently (Fang et al., 2004, 2005). Such a process could be promising if effective photo bioreactors can be developed. Microbial fuel cells may produce electricity from by-products of dark fermentation (Liu et al., 2005; Oh and Logan, 2005). A bio-electrochemically assisted microbial system combining with dark fermentation has the potential to produce overall 8–9 moles hydrogen per glucose.

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