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Review

Factors influencing fermentative hydrogen production: A review

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ABSTRACT

This review summarized several main factors influencing fermentative hydrogen production. The reviewed factors included inoculum, substrate, reactor type, nitrogen, phosphate, metal ion, temperature and pH. In this review, the effect of each factor on fermentative hydrogen production and the advance in the research of the effect were briefly introduced and discussed, followed by some suggestions for the future work of fermentative hydrogen production. This review showed that there usually existed some disagreements on the optimal condition of a given factor for fermentative hydrogen production, thus more researches in this respect are recommended. Furthermore, most of the studies on fermentative hydrogen production were conducted in batch mode using glucose and sucrose as substrate, thus more studies on fermentative hydrogen production in continuous mode using organic wastes as substrate are recommended.

1. Introduction

Fossil fuels are not renewable and will be exhausted sooner or later. In addition, the use of fossil fuels has induced very serious environmental pollution. Thus, it is necessary to find alternative energy sources that are renewable and environmentally friendly [1,2]. Hydrogen can be produced through various ways, which makes it renewable. And hydrogen produces only water, when it is combusted as a fuel or converted to electricity, which makes it very environmentally friendly [3,4]. Thus hydrogen is a very promising alternative energy source and has been received more attention all over the world in recent years. Among various hydrogen production processes, biological method is known to be less energy intensive, for it can be carried out at ambient temperature and pressure [5,6]. Biological method mainly includes photosynthetic hydrogen production and fermentative hydrogen production. Even though photosynthetic hydrogen production is a theoretically perfect process with transforming solar energy into hydrogen by photosynthetic bacteria, applying it to practice is difficult due to the low utilization efficiency of light and difficulties in designing the reactors for hydrogen production [1,7]. However, fermentative hydrogen production has the advantages of rapid hydrogen production rate and simple operation. Moreover, it can use various organic wastes as substrate for fermentative hydrogen production. Thus, compared with the photosynthetic hydrogen production, fermentative hydrogen production is more feasible and thus widely used. In addition, it is of great significance to produce hydrogen from organic wastes by fermentative hydrogen

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production, because it can not only treat organic wastes, but also produce very clean energy. Therefore fermentative hydrogen production has been received increasing attention in recent years [8].

Fermentative hydrogen production is very common under anoxic conditions. When bacteria degrade organic substrates, electrons which need to be disposed of to maintain electrical neutrality, are produced. In anoxic environments, protons can act as electron acceptor to produce molecular hydrogen [1]. Hydrogen can be produced from various substrates by hydrogen-producing bacteria. When glucose is used as the model substrate for fermentative hydrogen production, it is first converted by hydrogen-producing bacteria to pyruvate, producing the reduced form of nicotinamide adenine dinucleotide (NADH) via the glycolytic pathway. Pyruvate can then be further converted to acetylcoenzyme A (acetyl-CoA), carbon dioxide, and hydrogen by pyruvate–ferredoxin oxidoreductase and hydrogenase. Pyruvate may also be further converted to acetyl-CoA and formate, which may be readily converted to hydrogen and carbon dioxide. Acetyl-CoA is finally converted into some soluble metabolites such as acetate, butyrate, ethanol and so on [7,8].

Moreover, fermentative hydrogen production is a very complex process and influenced by many factors such as inoculum, substrate, reactor type, nitrogen, phosphate, metal ion, temperature and pH. And the effects of these factors on fermentative hydrogen production have been reported by a great number of studies throughout the world in the last few years [1–8]. This review attempts to summarize the above factors influencing fermentative hydrogen production. In this review, the effect of each factor on fermentative hydrogen production and the advance in the research of the effect were briefly introduced and discussed, followed by some suggestions for the future work of fermentative hydrogen production.

2. Inoculum

2.1. *Pure cultures*

A lot of pure cultures of bacteria have been used to produce hydrogen from various substrates. Table 1 summarizes a lot of studies using pure cultures for fermentative hydrogen production. As is shown in Table 1, *Clostridium* and *Enterobacter* were most widely used as inoculum for fermentative hydrogen production. Species of genus *Clostridium* are gram-positive, rod-shaped, strict anaerobes and endospore formers, whereas *Enterobacter* are gram-negative, rod-shaped, and facultative anaerobes [8]. Most of the studies using pure cultures of bacteria for fermentative hydrogen production were conducted in batch mode and used glucose as substrate; however, it is more desirable to produce hydrogen from organic wastes using pure cultures in continuous mode, because continuous fermentative hydrogen production from organic wastes is more feasible for industrialization to realize the goal of waste reduction and energy production. Thus more researches using pure cultures for continuous fermentative hydrogen production from organic wastes are recommended [8].

2.2. *Mixed cultures*

The bacteria capable of producing hydrogen widely exist in natural environments such as soil, wastewater sludge, compost and so on [38–41]. Thus these materials can be used as inoculum for fermentative hydrogen production. At present, the mixed cultures of bacteria from anaerobic sludge, municipal sewage sludge, compost and soil have been widely used as inoculum for fermentative hydrogen production [8]. Fermentative hydrogen production processes using mixed cultures are more practical than those using pure cultures, because the former are simpler to operate and easier to control, and may have a broader source of feedstock [8]. However, in a fermentative hydrogen production process using mixed cultures, the hydrogen produced by hydrogen-producing bacteria may be consumed by hydrogen-consuming bacteria. In addition, when mixed cultures are treated under harsh conditions, hydrogen-producing bacteria would have a better chance than some hydrogen-consuming bacteria to survive. Thus, in order to harness hydrogen from a fermentative hydrogen production process, the mixed cultures can be pretreated by certain methods to suppress as much hydrogen-consuming bacterial activity as possible while still preserving the activity of the hydrogen-producing bacteria [38]. The optimal index is highest hydrogen yield.

The pretreatment methods reported for enriching hydrogen-producing bacteria from mixed cultures mainly include heat-shock, acid, base, aeration, freezing and thawing, chloroform, sodium 2-bromoethanesulfonate or 2-bromoethanesulfonic acid and iodopropane [38]. Different pretreatment methods have different property and comparison of different pretreatment methods to obtain a better pretreatment method for a given fermentative hydrogen production process was conducted by many studies [38]. Table 2 summarizes several studies comparing various pretreatment methods for enriching hydrogen-producing bacteria from mixed cultures.

As is shown in Table 2, there exists certain disagreement on the optimal pretreatment method for enriching hydrogen-producing bacteria from mixed cultures [38–42]. The possible reason for this disagreement was the difference among these studies in the terms of inoculum, pretreatment method studied, specific condition of each pretreatment method and the kind of substrates.

Even though heat-shock was the most widely used pretreatment method for enriching hydrogen-producing bacteria from inoculum [8], it is not always effective for enriching hydrogen-producing bacteria from mixed culture inoculum compared with other pretreatment methods, for it may inhibit the activity of some hydrogen-producing bacteria [38].

In addition, in the reviewed studies, the comparisons of various pretreatment methods for enriching hydrogen-producing bacteria from mixed culture inoculum were all conducted in batch mode, and conducting these comparisons in continuous mode is recommended. Furthermore, most of the comparisons were conducted using glucose as substrate, and more comparisons conducted using organic wastes as substrate are recommended.

Table 1 – The pure bacterial cultures for fermentative hydrogen production.

Inoculum	Substrate	Reactor type	Maximum hydrogen yield	References
Clostridium acetobutylicum	Glucose	Batch	2.0 mol/mol glucose	[9]
Clostridium acetobutylicum ATCC 824	Glucose	Continuous	1.08 mol/mol glucose	[10]
Clostridium butyricum CGS5	Xylose	Batch	0.73 mol/mol xylose	[11]
Clostridium butyricum CGS2	Starch	Batch	9.95 mmol/g COD	[12]
Clostridium pasteurianum CH_4	Sucrose	Batch	2.07 mol/mol hexose	[11]
Clostridium paraputrificum M-21	Chitinous wastes	Batch	2.2 mol/mol substrate	[13]
Clostridium thermocellum 27405	Cellulosic biomass	Batch	2.3 mol/mol glucose	[14]
Clostridium thermolacticum	Lactose	Continuous	3.0 mol/mol lactose	[15]
Clostridium sp. strain no. 2	Cellulose	Continuous	0.3 mol/mol glucose	[16]
Clostridium sp. Fanp2	Glucose	Batch	0.2 mol/L medium	[17]
Enterobacter aerogenes HO-39	Glucose	Batch	1.0 mol/mol glucose	[18]
Enterobacter aerogenes NBRC 13534	Glucose	Batch	0.05 mol/L medium	[19]
Enterobacter aerogenes	Glucose	Batch	–	[20]
Enterobacter aerogenes HU-101	Glycerol	Batch	0.6 mol/mol glycerol	[21]
Enterobacter aerogenes	Starch	Batch	1.09 mol/mol starch	[22]
Enterobacter aerogenes E 82005	Molasses	Continuous	3.5 mol/mol sugar	[23]
Enterobacter cloacae IIT-BT 08	Glucose	Continuous	–	[24]
Enterobacter cloacae IIT-BT 08	Sucrose	Batch	6 mol/mol sucrose	[25]
Enterobacter cloacae IIT-BT 08	Cellobiose	Batch	5.4 mol/mol cellobiose	[25]
Escherichia coli MC13-4	Glucose	Batch	1.2 mol/mol glucose	[26]
Escherichia coli	Glucose	Batch	2.0 mol/mol glucose	[27]
Escherchia coli	Glucose	Continuous	2.0 mol/mol glucose	[28]
Pseudomonas sp. GZ1	Waste sludge	Batch	0.007 mol/g TCOD	[29]
Thermoanaerobacterium thermosaccharolyticum KU001	Glucose	Batch	2.4 mol/mol glucose	[30]
Thermococcus kodakaraensis KOD1	Starch	Continuous	–	[31]
Thermotoga elfii	Glucose	Batch	84.9 mmol/L medium	[32]
Hydrogen-producing bacterial B49	Glucose	Batch	0.1 ml/L culture	[33]
Ruminococcus albus	Glucose	Batch	2.52 mol/mol glucose	[34]
Hafnia alvei	Glucose	Batch	–	[35]
Citrobacter amalonaticus Y19	Glucose	Batch	8.7 mol/mol glucose	[36]
Ethanoligenens harbinense YUAN-3	Glucose	Continuous	1.93 mol/mol glucose	[37]

Moreover, some microbial analysis methods such as PCR-DGGE have been used to determine the community structure of mixed cultures during fermentative hydrogen production [43–45]. And they can also be used to detect the changes in the community structure of mixed cultures after certain pretreatment. For example, using PCR-DGGE technique, Kim and Shin reported that base pretreatment of mixed cultures would prevent the microbial population shift to non-H_2-producing acidogens, thus was beneficial for fermentative hydrogen production [43].

3. Substrate

A lot of substrates have been used for fermentative hydrogen production. Table 3 summarizes a lot of studies using various

Table 2 – The comparison of various pretreatment methods for enriching hydrogen-producing bacteria from mixed culture inoculum.

Inoculum	Inoculum pretreatment method studied	Substrates	Reactor type	Maximum hydrogen yield	Optimal pretreatment method	References
Digested sludge	Acid, base, heat-shock, aeration and chloroform	Glucose	Batch	1.8 mol/mol glucose	Heat-shock	[38]
Cattle manure sludge	Freezing and thawing, acid, heat-shock, and sodium 2-bromoethanesulfonate	Glucose	Batch	1.0 mol/mol glucose	Acid	[39]
Methanogenic granules	Acid, heat-shock and chloroform	Glucose	Batch	1.2 mol/mol glucose	Chloroform	[40]
Digested wastewater sludge	Heat-shock, aeration, acid, base, 2-bromoethanesulfonic acid and iodopropane	Sucrose	Batch	6.12 mol/mol sucrose	Base	[41]
Anaerobic sludge	Sodium 2-bromoethanesulfonate, acid, heat-shock and their four combinations	Dairy wastewater	Batch	0.0317 mmol/g COD	Sodium 2-bromoethanesulfonate	[42]

Table 3 – The comparison of various substrates used for fermentative hydrogen production.

Inoculum	Substrates	Reactor type	Substrate concentration (g COD/L)		Optimal index (value)	References
			Range studied	Optimal		
Clostridium butyricum CGS5	Xylose	Batch	5–40	20	Maximum hydrogen production potential (172.9 mL)	[11]
Municipal sewage sludge	Xylose	Continuous	10–100	20	Maximum hydrogen yield (2.25 mol/mol xylose)	[47]
Anaerobic sludge	Glucose	Batch	0.27–4.3	1.1	Maximum hydrogen production rate (0.13 mL/h)	[48]
Digested sludge	Glucose	Batch	1.1–320	2.1	Maximum hydrogen yield (3.1 mol/mol glucose)	[49]
Clostridium acetobutylicum ATCC 824	Glucose	Continuous	1.1–11.2	11.2	Maximum specific hydrogen production rate (1270 mL/g glucose-L reactor)	[10]
Ethanoligenens harbinense YUAN-3	Glucose	Batch	5.3–21.3	10.7	Maximum hydrogen yield (1.93 mol/mol glucose)	[37]
Thermoanaerobacterium thermosaccharolyticum PSU-2	Sucrose	Batch	5.6–56	5.6	Maximum hydrogen yield (6 mol/mol sucrose)	[50]
Mixed cultures	Sucrose	Batch	1.5–44.8	7.5 g	Maximum hydrogen yield (38.9 mL/(g COD-L culture)	[46]
Municipal sewage sludge	Sucrose	Batch	10–30	10	Maximum hydrogen yield (2.46 mol/mol sucrose)	[51]
Clostridium butyricum CGS5	Sucrose	Batch	5–30	20	Maximum hydrogen yield (2.78 mol/mol sucrose)	[52]
Anaerobic digester sludge	Sucrose	Continuous	10–60	30	Maximum hydrogen yield (1.22 mol/mol hexose)	[53]
Clostridium pasteurianum CH4	Sucrose	Batch	5–40	40	Maximum hydrogen yield (2.07 mol/mol hexose)	[11]
Cracked cereals	Starch	Batch	2.1–34.1	2.1	Maximum hydrogen yield (194 mL/g starch)	[54]
Anaerobic sludge	Starch	Batch	9.8–39.0	9.8	Maximum hydrogen yield (67 mL/g starch)	[55]
Anaerobic sludge	Starch	Batch	5–60	20	Maximum hydrogen yield (2.2 mol/mol hexose)	[56]
Municipal sewage sludge	Starch	Batch	8–32	32	Maximum hydrogen yield (11.25 mmol/g starch)	[57]
Cow dung compost	Cornstalk wastes	Batch	5.3–42.7	16	Maximum hydrogen yield (149.69 mL/TVS)	[58]
Anaerobic digester sludge	Rice slurry	Batch	2.9–23.6	5.9	Maximum hydrogen yield (346 mL/g carbohydrate)	[59]
Cow dung compost	Beer lees	Batch	5.3–53.3	21.3	Maximum hydrogen yield (68.6 mL/TVS)	[60]
Fermented soybean-meal	Bean curd manufacturing waste	Batch	1.1–6.9	4.0	Maximum hydrogen production rate (130 mL/h L culture)	[61]
Anaerobic digester sludge	Food waste	Batch	0–32.3	4.6	Maximum hydrogen yield (101 mL/g COD)	[62]
Anaerobic sludge	Food waste	Batch	3.2–10.7	6.4	Maximum hydrogen yield (1.8 mol/mol hexose)	[45]
Anaerobic digester sludge	Non-fat dry milk	Batch	0–96	4	Maximum hydrogen yield (119 mL/g COD)	[62]
Waste activated sludge	Food wastewater	Batch	10–160	40	Maximum hydrogen yield (47.1 mmol/g COD)	[63]
Municipal sewage sludge	Rice winery wastewater	Continuous	14–36	14	Maximum hydrogen yield (1.9 mol/mol hexose)	[64]

substrates for fermentative hydrogen production. As is shown in Table 3, glucose, sucrose and starch were most widely used substrate for fermentative hydrogen production. However, in recent years, a few studies have begun to use organic wastes as substrate for hydrogen production [4]. In addition, most of the studies on fermentative hydrogen production were conducted in batch mode, and more studies conducted in continuous mode are recommended.

It has been demonstrated that in an appropriate range, increasing substrate concentration could increase the ability of hydrogen-producing bacteria to produce hydrogen during fermentative hydrogen production, but substrate

concentrations at much higher levels could decrease it with increasing levels [11,46]. Furthermore, there exists certain disagreement on the optimal concentration of a given substrate for fermentative hydrogen production. For example, the optimal sucrose concentration for fermentative hydrogen production reported by van Ginkel et al. was 7.5 g COD/L [46], while that reported by Lo et al. was 40 g COD/L [11]. The possible reason for this disagreement was the difference among these studies in the terms of inoculum and substrate concentration range studied.

Some complex substrates are not ideal for fermentative hydrogen production due to their complex structures; however, after being pretreated by some methods, they can be easily used by hydrogen-producing bacteria. For example, Zhang et al. reported that the hydrogen yield from cornstalk wastes after acidification pretreatment was much larger than that from cornstalk wastes without any pretreatment [58].

Waste activated sludge from wastewater treatment plants contains high levels of organic matter and thus is a potential substrate for hydrogen production. After appropriate pretreatments such as ultrasonication, acidification, freezing and thawing, sterilization, methanogenic inhibitor and microwave, the ability of hydrogen-producing bacteria to produce hydrogen from it can be improved [65,66]. Different substrate pretreatment methods have different property and comparison of various substrate pretreatment methods was conducted by several studies. Table 4 summarizes several studies comparing various substrate pretreatment methods for fermentative hydrogen production from wastewater sludge.

As is shown in Table 4, among the substrate pretreatment methods studied, freezing and thawing and sterilization are superior pretreatment methods of wastewater sludge for fermentative hydrogen production. It is worth noting that when using *Clostridium bifermentans* as inoculum, freezing and thawing was the optimal pretreatment methods for waste activated sludge [65,66], while when *Pseudomonas* sp. GZ1 as inoculum, sterilization was the optimal pretreatment methods for waste activated sludge [29]. This demonstrates that the optimal pretreatment methods for waste activated sludge may be dependent on the inoculum used for fermentative hydrogen production.

In addition, all the reviewed comparisons of various substrate pretreatment methods for waste activated sludge were conducted in batch mode, and conducting these comparisons in continuous mode is recommended. Furthermore, all the reviewed comparisons of various substrate pretreatment methods for waste activated sludge were conducted using pure cultures as inoculum, and conducting these comparisons using mixed cultures as inoculum is recommended. Moreover, comparison of various substrate pretreatment methods for other complex organic wastes besides waste activated sludge is recommended.

4. Reactor type

As shown in Tables 1–4, most of the studies on fermentative hydrogen production were conducted in batch mode due to its simple operation and control. However, large-scale operations would require continuous production processes for practical engineering reasons. Table 5 summarizes a lot of studies using continuous reactors for fermentative hydrogen production. As is shown in Table 5, the continuous stirred tank reactor (CSTR) was widely used for continuous fermentative hydrogen production [67–75].

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, washout of biomass may occur at shorter HRT. In addition, biomass concentration in the mixed liquor and the hydrogen production is limited. Immobilized-cell reactors provide an alternative to a conventional CSTR, because they are capable of maintaining higher biomass concentrations and could operate at shorter HRT without biomass washout [8]. Biomass immobilization can be achieved through forming granules, biofilm, or gel-entrapped bioparticles [8]. For example, Zhang et al. found that the formation of granular sludge facilitated biomass concentration up to 32.2 g VSS/L and enhanced hydrogen production [67].

It has been demonstrated that in an appropriate range, increasing HRT could increase the ability of hydrogen-producing bacteria to produce hydrogen during fermentative hydrogen production, but HRT at much higher levels could decrease it with increasing levels [69]. Furthermore, there exists certain disagreement on the optimal HRT for continuous fermentative hydrogen production reactors, even for the same type reactor. For example, the optimal HRT for a CSTR reported by Zhang et al. was 0.5 h [67], while the optimal HRT for a CSTR using reported by Arooj et al. was 12 h [75]. The possible reason for this disagreement was the difference

Table 4 – The various substrate pretreatment methods for waste activated sludge.

Inoculum	Reactor type	Substrate pretreatment method	Optimal pretreatment method	Optimal index (value)	References
Clostridium bifermentans	Batch	Freezing and thawing, ultrasonication, acidification, sterilization and methanogenic inhibitor	Freezing and thawing	Maximum hydrogen yield (2.1 mmol/g COD)	[65]
Clostridium bifermentans	Batch	Freezing and thawing, sonication, acidification and sterilization	Freezing and thawing	Maximum hydrogen yield (4.1 g/Kg DS)	[66]
Pseudomonas sp. GZ1	Batch	Sterilization, microwave and ultrasonication	Sterilization	Maximum hydrogen yield (15.02 ml/g TCOD)	[29]

Table 5 – The continuous reactors used for fermentative hydrogen production.

Inoculum	Substrates	Reactor type	Hydraulic retention time (h)		Optimal index (value)	References
			Range studied	Optimal		
Municipal sewage sludge	Glucose	CSTR	0.5–2	0.5	Maximum hydrogen yield (1.81 mol/mol glucose)	[67]
Anaerobic sludge	Glucose	CSTR	2–12	4	Maximum hydrogen production rate (115.68 mmol/d)	[68]
Municipal sewage sludge	Sucrose	CSTR	2–12	4	Maximum hydrogen yield (4.70 mol/mol sucrose)	[69]
Municipal sewage sludge	Sucrose	CSTR	2–13.3	8	Maximum hydrogen yield (4.52 mol/mol sucrose)	[70]
Municipal sewage sludge	Fructose	CSTR	2–8	8	Maximum hydrogen yield (1.68 mol/mol hexose)	[71]
Anaerobic sludge	Starch	CSTR	2–12	12	Maximum hydrogen yield (1.5 mol/mol hexose)	[56]
Anaerobically digested sludge	Glucose	CSTR	6–12	10	Maximum hydrogen yield (1.95 mol/mol glucose)	[72]
Anaerobic sludge	Glucose	CSTR	4–12	10	Maximum hydrogen yield (1.63 mol/mol glucose)	[73]
Municipal sewage sludge	Xylose	CSTR	4–12	12	Maximum hydrogen yield (1.63 mol/mol xylose)	[74]
Municipal sewage sludge	Glucose	CSTR	4–12	12	Maximum hydrogen yield (1.36 mol/mol hexose)	[71]
Municipal sewage sludge	Sucrose	CSTR	2–12	12	Maximum hydrogen yield (1.60 mol/mol hexose)	[71]
Anaerobic digester sludge	Starch	CSTR	4–18	12	Maximum hydrogen yield (0.92 mol/mol glucose)	[75]
Municipal sewage sludge	Sucrose	UASB	4–24	8	Maximum hydrogen yield (1.5 mmol/mol sucrose)	[76]
Anaerobic sludge	Glucose	UASB	2–12	12	Maximum hydrogen production rate (96.0 mmol/d)	[68]
Sewage sludge	Sucrose	UASB	6–24	8	Maximum hydrogen yield (3.6 mol/mol sucrose)	[77]
Anaerobically digested sludge	Glucose	Anaerobic biofilm fluidized bed reactors	0.125–3	0.25	Maximum hydrogen yield (1.7 mol/mol glucose)	[78]
Anaerobically digested sludge	Glucose	Anaerobic granule fluidized bed reactors	0.125–3	0.25	Maximum hydrogen yield (1.6 mol/mol glucose)	[78]
Municipal sewage sludge	Sucrose	Carrier-induced granular sludge bed bioreactor	0.25–4	0.5	Maximum hydrogen yield (3.3 mol/mol sucrose)	[79]
Municipal sewage sludge	Xylose	Powder activated carbon-assisted agitated granular sludge bed reactor	2–4	4	Maximum hydrogen yield (0.7 mol/mol xylose)	[74]
Municipal sewage sludge	Sucrose	Packed-bed bioreactor	0.5–4	4	Maximum hydrogen yield (3.9 mol/mol sucrose)	[80]
Municipal sewage sludge	Glucose	Membrane bioreactor	1–4	4	Maximum hydrogen yield (1.72 mol/mol hexose)	[71]
Municipal sewage sludge	Xylose	Immobilized-cell continuously stirred anaerobic reactor	2–6	6	Maximum hydrogen yield (0.8 mol/mol xylose)	[74]

CSTR: continuous stirred tank reactor.
UASB: upflow anaerobic sludge blanket reactor.

among these studies in the terms of inoculum, substrate and HRT range studied.

As shown in Table 5, glucose and sucrose were most widely used substrate for continuous fermentative hydrogen production. Thus, more studies on continuous fermentative hydrogen production using organic wastes as substrate are recommended.

Moreover, different reactors have different property and comparison of various reactors was conducted by several studies. For example, Zhang et al. compared a biofilm-based reactor and a granule-based reactor and concluded that the granule-based reactor was better than the biofilm-based reactor for continuous fermentative hydrogen production, because the granule-based reactor has a better ability of biomass retention [78].

5. Nitrogen and phosphate

Since nitrogen is a very important component for proteins, nucleic acids and enzymes that are of great significance to the growth of hydrogen-producing bacteria, it is one of the most essential nutrients needed for the growth of

hydrogen-producing bacteria. Thus, an appropriate level of nitrogen addition is beneficial to the growth of hydrogen-producing bacteria and to fermentative hydrogen production accordingly [27]. Table 6 summarizes several studies investigating the effect of nitrogen concentration on fermentative hydrogen production.

As shown in Table 6, ammonia nitrogen was the most widely investigated nitrogen source for fermentative hydrogen production. Thus, more investigations of the effect of other nitrogen source concentration besides ammonia concentration on fermentative hydrogen production are recommended.

In addition, there exists certain disagreement on the optimal ammonia nitrogen concentration for fermentative hydrogen production. For example, the optimal ammonia nitrogen concentration for fermentative hydrogen production reported by Bisaillon et al. was 0.01 g N/L [27], while that reported by Salerno et al. was 7.0 g N/L [81]. The possible reason for this disagreement was the difference among these studies in the terms of inoculum and ammonia nitrogen concentration range studied.

As is shown in Table 6, glucose was the most widely used substrate during the investigation of the effect of nitrogen concentration on fermentative hydrogen production. Thus, more investigations of the effect of nitrogen concentration on fermentative hydrogen production using organic wastes as substrate are recommended. In addition, as is shown in Table 6, all the reviewed studies investigating the effect of nitrogen concentration on fermentative hydrogen production were conducted in batch mode, and conducting such studies in continuous mode is recommended.

Phosphate is needed for hydrogen production due to its nutritious value as well as buffering capacity. It has been demonstrated that in an appropriate range, increasing phosphate concentration could increase the ability of hydrogen-producing bacteria to produce hydrogen during fermentative hydrogen production, but phosphate concentrations at much higher levels could decrease it with increasing levels [27,82].

It had been shown that an appropriate C/N and C/P are fundamental for fermentative hydrogen production. Table 7 summarizes several studies investigating the effect of C/N and C/P on fermentative hydrogen production.

As shown in Table 7, there exists certain disagreement on the optimal C/N and C/P for fermentative hydrogen production. For example, the optimal C/N and C/P for fermentative hydrogen production reported by Argun et al. were 200 and 1000, respectively [85], while those reported by O-Thong et al. were 74 and 559, respectively [86]. The possible reason for this disagreement was the difference among these studies in the terms of substrate, C/N range and C/P range studied.

In addition, all the reviewed studies investigating the effect of C/N and C/P on fermentative hydrogen production were conducted in batch mode, and conducting such studies in continuous mode is recommended.

6. Metal ion

Even though at a higher concentration, metal ion may inhibit the activity hydrogen-producing bacteria, a trace level of metal ion is required for fermentative hydrogen production [8]. Table 8 summarizes several studies investigating the effect of metal ion concentration on fermentative hydrogen production.

As shown in Table 8, Fe^{2+} was the most widely investigated metal ion for fermentative hydrogen production, probably because its presence is essential for hydrogenase [88]. Thus, more investigations of the effect of other metal ion concentration besides Fe^{2+} concentration on fermentative hydrogen production are recommended.

In addition, there exists certain disagreement on the optimal Fe^{2+} concentration for fermentative hydrogen production. For example, the optimal Fe^{2+} concentration for fermentative hydrogen production reported by Liu and Shen was 10 mg/L [54], while that reported by Zhang et al. was 589.5 mg/L [90]. The possible reason for this disagreement was the difference among these studies in the terms of inoculum, substrate and Fe^{2+} concentration range studied.

As is shown in Table 8, glucose and sucrose were the most widely used substrate during the investigation of the effect of metal ion on fermentative hydrogen production. Thus, investigating the effect of nitrogen concentration on fermentative hydrogen production using organic wastes as substrate

Table 6 – The effect of nitrogen concentration on fermentative hydrogen production.

Inoculum	Substrates	Reactor type	Nitrogen source	Nitrogen concentration		Optimal index (value)	References
				Range studied	Optimal		
Escherichia coli	Glucose	Batch	NH_4Cl	0–0.2 g N/L	0.01 g N/L	Maximum hydrogen yield (1.7 mol/mol glucose)	[27]
Dewatered and thickened sludge	Glucose	Batch	NH_4Cl	0.5-10 g N/L	7 g N/L	Maximum hydrogen production (150 mL)	[81]
Grass compost	Food wastes	Batch	NH_4HCO_3	0–0.6 g N/L	0.4 g N/L	Maximum hydrogen yield (77 mL/g TVS)	[82]
Cracked cereals	Starch	Batch	NH_4HCO_3	0.1–2 g N/L	1 g N/L	Maximum hydrogen yield (146 mL/g starch)	[54]
Compost	Glucose	Batch	Yeast extract	2–8% yeast extract	4% yeast extract	Maximum hydrogen production (70 mmol)	[83]
Enterobacter aerogenes HO-39	Glucose	Batch	Polypepton	0–5% polypepton	2% polypepton	Maximum hydrogen production (58 mL)	[18]

Table 7 – The effect of C/N and C/P on fermentative hydrogen production.

Inoculum	Substrates	Reactor type	C/N		C/P		Optimal index (value)	References
			Range studied	Optimal	Range studied	Optimal		
Wasted activated sludge	Sucrose	Batch	40–130	47	–	–	Maximum hydrogen yield (4.8 mol/mol sucrose)	[84]
Anaerobic sludge	Wheat powder	Batch	20–200	200	50–1000	1000	Maximum hydrogen yield (281 mL/g starch)	[85]
Anaerobic sludge	Palm oil mill effluent	Batch	45–95	74	450–650	559	Maximum hydrogen yield (6.33 L/L substrate)	[86]

is recommended. In addition, as is shown in Table 8, most of the reviewed studies investigating the effect of metal ion concentration on fermentative hydrogen production were conducted in batch mode, and more studies conducted in continuous mode are recommended.

Several studies also investigated the toxicity of heavy metals to fermentative hydrogen production. For example, Li and Fang reported that the relative toxicity of six electroplating metals to fermentative hydrogen production was in the following order: Cu > Ni–Zn > Cr > Cd > Pb [94], while Lin and Shei reported that the relative toxicity of three heavy metals to fermentative hydrogen production was in the following order: Zn > Cu > Cr [95].

7. Temperature

Temperature is one of the most important factors that influence the activities of hydrogen-producing bacteria and the fermentative hydrogen production. It has been demonstrated that in an appropriate range, increasing temperature could increase the ability of hydrogen-producing bacteria to produce hydrogen during fermentative hydrogen production, but temperature at much higher levels could decrease it with increasing levels [96]. Table 9 summarizes several studies investigating the effect of temperature on fermentative hydrogen production. As shown in Table 10, even though the optimal temperature reported for fermentative hydrogen production was not always the same, it fell into the mesophilic range (around 37 °C) and thermophilic range (around 55 °C), respectively [8].

As is shown in Table 9, glucose and sucrose were the most widely used substrate during the investigation of the effect of temperature on fermentative hydrogen production. Thus, investigating the effect of temperature on fermentative hydrogen production using organic wastes as substrate is recommended. In addition, most of the reviewed studies investigating the effect of temperature on fermentative

Table 8 – The effect of metal ion concentrations on fermentative hydrogen production.

Inoculum	Substrates	Reactor type	Mental ion	Concentration (mg/L)		Optimal index (value)	References
				Range studied	Optimal		
Cracked cereals	Starch	Batch	Fe^{2+}	1.2–100	10	Maximum hydrogen yield (140 mL/g starch)	[54]
Anaerobic sludge	Starch	Batch	Fe^{2+}	0–1473.7	55.3	Maximum hydrogen yield (296.2 mL/g starch)	[87]
Grass compost	Food wastes	Batch	Fe^{2+}	0–250	132	Maximum hydrogen yield (77 mL/g TVS)	[82]
Anaerobic sludge	Palm oil mill effluent	Batch	Fe^{2+}	2–400	257	Maximum hydrogen yield (6.33 L/L substrate)	[86]
Digested sludge	Glucose	Batch	Fe^{2+}	0–1500	350	Maximum hydrogen yield (311.2 mL/g glucose)	[88]
Anaerobic sludge	Sucrose	Batch	Fe^{2+}	0–1763.8	352.8	Maximum hydrogen yield (131.9 mL/g sucrose)	[89]
Cracked cereals	Sucrose	Batch	Fe^{2+}	0–1842.1	589.5	Maximum hydrogen yield (2.73 mol/mol sucrose)	[90]
Anaerobic sludge	Glucose	Batch	Cu^{2+}	0–400	400	Maximum hydrogen yield (1.74 mol/mol glucose)	[91]
Anaerobic sludge	Glucose	Batch	Zn^{2+}	0–500	250	Maximum hydrogen yield (1.73 mol/mol glucose)	[91]
Hydrogen-producing bacterial B49	Glucose	Batch	Mg^{2+}	1.2–23.6	23.6	Maximum hydrogen yield (2360.5 mL/L culture)	[33]
Digested sludge	Glucose	Batch	Ni^{2+}	0–50	0.1	Maximum hydrogen yield (296.1 mL/g glucose)	[92]
Digested sludge	Sucrose	Continuous	Ca^{2+}	0–300	150	Maximum hydrogen yield (3.6 mol/mol sucrose)	[77]
Municipal sewage sludge	Sucrose	Continuous	Ca^{2+}	0–27.2	27.2	Maximum hydrogen yield (2.19 mol/mol sucrose)	[93]

Table 9 – The effect of temperature on fermentative hydrogen production.

Inoculum	Substrates	Reactor type	Temperature (°C)		Optimal index (value)	References
			Range studied	Optimal		
Ethanoligenens harbinense YUAN-3	Glucose	Batch	20–44	37	Maximum hydrogen yield (1.34 mol/mol glucose)	[37]
Anaerobic sludge	Glucose	Batch	25–55	40	Maximum hydrogen yield (275.1 mL/g glucose)	[96]
Anaerobic sludge	Glucose	Batch	33–41	41	Maximum hydrogen yield (1.67 mol/mol glucose)	[97]
Anaerobic sludge	Sucrose	Batch	25–45	35.1	Maximum hydrogen yield (3.7 mol/mol sucrose)	[98]
Anaerobic sludge	Sucrose	Batch	25–45	35.5	Maximum hydrogen yield (252 mL/g sucrose)	[99]
Anaerobic digester sludge	Rice slurry	Batch	37–55	37	Maximum hydrogen yield (346 mL/g carbohydrate)	[59]
Municipal sewage sludge	Sucrose	Continuous	30–45	40	Maximum hydrogen yield (3.88 mol/mol sucrose)	[100]
Thermoanaerobacterium thermosaccharolyticum PSU-2	Sucrose	Batch	40–80	60	Maximum hydrogen yield (2.53 mol/mol hexose)	[50]
Municipal sewage sludge	Starch	Batch	37–55	55	Maximum hydrogen yield (1.44 mmol/g starch)	[57]
Municipal sewage sludge	xylose	Continuous	30–55	50	Maximum hydrogen yield (1.4 mol/mol xylose)	[101]
Cow dung	Cow dung	Batch	3 7–75	60	Maximum hydrogen yield (743 mL/kg cow dung)	[102]
Cow waste slurry	Cow waste slurry	Batch	37–85	60	Maximum hydrogen yield (392 mL/L slurry)	[103]
Anaerobic digester sludge	Organic waste	Semi-continuous	37–55	55	Maximum hydrogen yield (360 mL/ g VS)	[104]

Table 10 – The effect of initial pH on fermentative hydrogen production in batch mode.

Inoculum	Substrates	initial pH		Optimal index (value)	References
		Range studied	Optimal		
Compost	Sucrose	4.5–6.5	4.5	Maximum hydrogen yield (214 mL/g COD)	[105]
Anaerobic sludge	Starch	5.0–7.0	5.0	Maximum hydrogen yield (1.1 mol/mol hexose)	[56]
Clostridium butyricum CGS5	Sucrose	5.0–6.5	5.5	Maximum hydrogen yield (2.78 mol/mol sucrose)	[52]
Waste activated sludge	Food wastewater	4.0–8.0	6.0	Maximum hydrogen yield (47.1 mmol/g COD)	[63]
Anaerobic sludge	Starch	4.0–9.0	6.0	Maximum hydrogen yield (92 mL/g starch)	[55]
Thermoanaerobacterium thermosaccharolyticum PSU-2	Sucrose	4.0–8.5	6.2	Maximum hydrogen yield (2.53 mol/mol hexose)	[50]
Municipal sewage sludge	Xylose	5.0–9.5	6.5	Maximum hydrogen yield (2.25 mol/mol xylose)	[47]
Municipal sewage sludge	Xylose	5.0–8.0	6.5	Maximum hydrogen yield (1.3 mol/mol xylose)	[106]
Cow dung compost	Cornstalk wastes	4.0–9.0	7.0	Maximum hydrogen yield (149.69 mL/TVS)	[58]
Cow dung sludge	Cellulose	5.5–9.0	7.5	Maximum hydrogen yield (2.8 mmol/g cellulose)	[107]
Municipal sewage sludge	Sucrose	5.5–8.5	7.5	Maximum hydrogen yield (2.46 mol/mol sucrose)	[51]
Anaerobic granular sludge	Glucose	3.88–8.12	7.5	Maximum hydrogen yield (1.46 mol/mol glucose)	[108]
Cracked cereals	Starch	4.0–9.0	8.0	Maximum hydrogen yield (120 mL/g starch)	[54]
Anaerobic digester sludge	Sucrose	3.0–12.0	9.0	Maximum hydrogen yield (126.9 mL/g sucrose)	[109]

Table 11 – The effect of pH on fermentative hydrogen production.

Inoculum	Substrates	Reactor type	pH		Optimal index (value)	References
			Range studied	Optimal		
Anaerobic digester sludge	Rice slurry	Batch	4.0–7.0	4.5	Maximum hydrogen yield (346 mL/g starch)	[59]
Anaerobic sludge	Sucrose	Batch	4.7–6.3	5.5	Maximum hydrogen yield (3.7 mol/mol sucrose)	[98]
Anaerobic sludge	Sucrose	Batch	4.5–6.5	5.5	Maximum hydrogen yield (252 mL/g sucrose)	[99]
Enterobacter cloacae IIT-BT 08	Sucrose	Batch	4.5–7.5	6.0	Maximum hydrogen production rate (29.63 mmol/g dry cell-h)	[25]
Mixed cultures	Sucrose	Continuous	3.4–6.3	4.2	Maximum hydrogen yield (1.61 mol/mol glucose)	[110]
Anaerobic sludge	Glucose	Continuous	4.0–7.0	5.5	Maximum hydrogen yield (2.1 mol/mol glucose)	[111]
Mixed cultures	Sucrose	Continuous	6.1–9.5	7.0	Maximum hydrogen yield (1.61 mol/mol glucose)	[112]

hydrogen production were conducted in batch mode, and more studies conducted in continuous mode are recommended.

Wang and Wan reported that the concentration of ethanol in batch tests increased with increasing temperature from 20 °C to 35 °C, but it decreased with further increasing temperature from 35 °C to 55 °C [96]. Their results also showed that the concentration of acetic acid in batch tests increased with increasing temperatures from 20 °C to 35 °C, but it trended to decrease with further increasing temperature from 35 °C to 55 °C. The changes in ethanol concentration and acetic acid concentration in the soluble metabolite of each batch test with increasing temperature may result from the metabolic pathway shift induced by the different bacteria that were dominant at each temperature. In addition, the concentration of propionic acid and butyric acid changed a lot with increasing temperatures from 20 °C to 55 °C, but they were very low and even could not be detectable.

8. pH

pH is another important factor that influences the activities of hydrogen-producing bacteria, and the fermentative hydrogen production, because it may affect the hydrogenase activity as well as the metabolism pathway. It has been demonstrated that in an appropriate range, increasing pH could increase the ability of hydrogen-producing bacteria to produce hydrogen during fermentative hydrogen production, but pH at much higher levels could decrease it with increasing levels. Since most studies were conducted in batch mode without pH control, only the effect of initial pH on fermentative hydrogen production was investigated in these studies. Table 10 summarizes several studies investigating the effect of initial pH on fermentative hydrogen production in batch mode.

As shown in Table 10, there exists certain disagreement on the optimal initial pH for fermentative hydrogen production. For example, the optimal initial pH for fermentative hydrogen production reported by Khanal et al. was 4.5 [105], while that reported by Lee et al. was 9.0 [109]. The possible reason for this disagreement was the difference among these studies in the terms of inoculum, substrate and initial pH range studied.

In addition, sucrose was the most widely used substrate during the investigation of the effect of initial pH on fermentative hydrogen production. Thus, investigating the effect of initial pH on fermentative hydrogen production using organic wastes as substrate is recommended.

Since some studies on fermentative hydrogen production were conducted in batch mode with pH control, while some others were conducted in continuous mode, in these cases, the effect of pH on fermentative hydrogen production was investigated. Table 11 summarizes several studies investigating the effect of pH on fermentative hydrogen production.

As shown in Table 11, there exists certain disagreement on the optimal pH for fermentative hydrogen production. For example, the optimal pH for fermentative hydrogen production reported by Mu et al. was 4.2 [110], while that reported by Zhao and Yu was 7.0 [112]. The possible reason for this disagreement was the difference among these studies in the terms of inoculum, substrate and pH range studied.

In addition, sucrose was the most widely used substrate during the investigation of the effect of pH on fermentative hydrogen production. Thus, investigating the effect of pH on fermentative hydrogen production using organic wastes as substrate is recommended.

9. Conclusions

Several main factors influencing fermentative hydrogen production, including inoculum, substrate, reactor type, nitrogen, phosphate, metal ion, temperature and pH were summarized and analyzed in this review, the effect of each factor on fermentative hydrogen production and the advance in the research of the effect were briefly introduced and discussed, followed by some suggestions for the future work of fermentative hydrogen production. This review showed that there usually existed some disagreements on the optimal condition of a given factor for fermentative hydrogen production, thus more researches in this respect are

recommended. Furthermore, most of the studies on fermentative hydrogen production were conducted in batch mode using glucose and sucrose as substrate, thus more studies on fermentative hydrogen production in continuous mode using organic wastes as substrate are recommended.

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