



Review

Bio-hydrogen production from waste materials

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Accepted 15 September 2005

Abstract

Hydrogen is a valuable gas as a clean energy source and as feedstock for some industries. Therefore, demand on hydrogen production has increased considerably in recent years. Electrolysis of water, steam reforming of hydrocarbons and auto-thermal processes are well-known methods for hydrogen gas production, but not cost-effective due to high energy requirements. Biological production of hydrogen gas has significant advantages over chemical methods. The major biological processes utilized for hydrogen gas production are bio-photolysis of water by algae, dark and photo-fermentation of organic materials, usually carbohydrates by bacteria. Sequential dark and photo-fermentation process is a rather new approach for bio-hydrogen production. One of the major problems in dark and photo-fermentative hydrogen production is the raw material cost. Carbohydrate rich, nitrogen deficient solid wastes such as cellulose and starch containing agricultural and food industry wastes and some food industry wastewaters such as cheese whey, olive mill and bakers yeast industry wastewaters can be used for hydrogen production by using suitable bio-process technologies. Utilization of aforementioned wastes for hydrogen production provides inexpensive energy generation with simultaneous waste treatment. This review article summarizes bio-hydrogen production from some waste materials. Types of potential waste materials, bio-processing strategies, microbial cultures to be used, bio-processing conditions and the recent developments are discussed with their relative advantages.

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Keywords: Bio-hydrogen; Waste bio-processing; Dark and photo-fermentations**Contents**

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1. Introduction

The worldwide energy need has been increasing exponentially, the reserves of fossil fuels have been decreasing, and the combustion of fossil fuels has serious negative effects on environment because of CO₂ emission. For these reasons, many researchers have been working on the exploration of new sustainable energy sources that could substitute fossil fuels. Hydrogen is considered as a viable alternative fuel and “energy carrier” of future. Hydrogen gas is clean fuel with no CO₂ emissions and can easily be used in fuel cells for generation of electricity. Besides, hydrogen has a high energy yield of 122 kJ/g, which is 2.75 times greater than hydrocarbon fuels. The major problem in utilization of hydrogen gas as a fuel is its inavailability in nature and the need for inexpensive production methods.

Demand on hydrogen is not limited to utilization as a source of energy. Hydrogen gas is a widely used feedstock for the production of chemicals, hydrogenation of fats and oils in food industry, production of electronic devices, processing steel and also for desulfurization and re-formulation of gasoline in refineries.

It has been reported that 50 million tonnes of hydrogen are traded annually worldwide with a growth rate of nearly 10% per year for the time being [1]. Based on the National Hydrogen program of the United States, the contribution of hydrogen to total energy market will be 8–10% by 2025 [2]. It was reported by the US Department of Energy (US-DOE) that H₂ power and transport systems will be available in all regions of the United States by the year 2040 [3]. Due to increasing need for hydrogen energy, development of cost-effective and efficient hydrogen production technologies has gained significant attention in recent years.

Conventional hydrogen gas production methods are steam reforming of methane (SRM), and other hydrocarbons (SRH), non-catalytic partial oxidation of fossil fuels (POX) and auto-thermal reforming which combines SRM and POX. Those methods are all energy intensive processes requiring high temperatures (>850 °C). Among other methods developed to improve the existing technologies are the membrane processes, selective oxidation of methane and oxidative dehydrogenation [2].

Biomass and water can be used as renewable resources for hydrogen gas production. Utilization of wide variety of gaseous, liquid and solid carbonaceous wastes was investigated by Kim [4] as renewable sources for formation of hydrogen gas by steam reforming. Despite the low cost of waste materials used, high temperature requirement ($T=1200\text{ }^{\circ}\text{C}$) is still the major limitation for this process. Electrolysis of water may be the cleanest technology for hydrogen gas production. However, electrolysis should be used in areas where electricity is inexpensive since electricity costs account for 80% of the operating cost of H₂ production. In addition, feed water has to be demineralized to avoid deposits on the electrodes and corrosion [2].

Biological hydrogen production is a viable alternative to the aforementioned methods for hydrogen gas production. In accordance with sustainable development and waste minimization

issues, bio-hydrogen gas production from renewable sources, also known as “green technology” has received considerable attention in recent years. Bio-hydrogen production can be realized by anaerobic and photosynthetic microorganisms using carbohydrate rich and non-toxic raw materials. Under anaerobic conditions, hydrogen is produced as a by-product during conversion of organic wastes into organic acids which are then used for methane generation. Acidogenic phase of anaerobic digestion of wastes can be manipulated to improve hydrogen production. Photosynthetic processes include algae which use CO₂ and H₂O for hydrogen gas production. Some photo-heterotrophic bacteria utilize organic acids such as acetic, lactic and butyric acids to produce H₂ and CO₂. The advantages of the later method are higher H₂ gas production and utilization of waste materials for the production. However, the rate of H₂ production is low and the technology for this process needs further development [5].

Production of clean energy source and utilization of waste materials make biological hydrogen production a novel and promising approach to meet the increasing energy needs as a substitute for fossil fuels. On the basis of these facts, this review focuses on potential use of carbohydrate rich wastes as the raw material, microbial cultures, bio-processing strategies and the recent developments on bio-hydrogen production.

2. Types of waste materials

The major criteria for the selection of waste materials to be used in bio-hydrogen production are the availability, cost, carbohydrate content and biodegradability. Simple sugars such as glucose, sucrose and lactose are readily biodegradable and preferred substrates for hydrogen production. However, pure carbohydrate sources are expensive raw materials for hydrogen production. Major waste materials which can be used for hydrogen gas production may be summarized as follows.

2.1. Starch and cellulose containing agricultural or food industry wastes

Many agricultural and food industry wastes contain starch and/or cellulose which are rich in terms of carbohydrate contents. Complex nature of these wastes may adversely affect the biodegradability. Starch containing solid wastes is easier to process for carbohydrate and hydrogen gas formation. Starch can be hydrolyzed to glucose and maltose by acid or enzymatic hydrolysis followed by conversion of carbohydrates to organic acids and then to hydrogen gas. Cellulose containing agricultural wastes requires further pre-treatment. Agricultural wastes should be ground and then delignified by mechanical or chemical means before fermentation. Cellulose and hemicellulose content of such wastes can be hydrolyzed to carbohydrates which are further processed for organic acid and hydrogen gas production. It was reported that there is an inverse relationship between lignin content and the efficiency of enzymatic hydrolysis of agricultural wastes [6]. Fig. 1 depicts a schematic diagram for bio-hydrogen production from cellulose and starch containing agricultural wastes by two stage anaerobic dark and photo-fermentations.

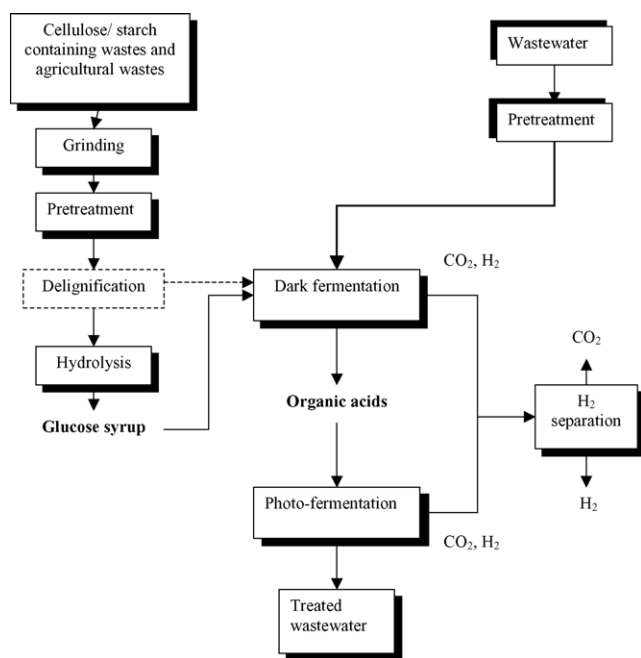


Fig. 1. A schematic diagram for bio-hydrogen production from cellulose/starch containing agricultural wastes and food industry wastewaters.

2.2. Carbohydrate rich industrial wastewaters

Some biodegradable carbohydrate containing and non-toxic industrial effluents such as dairy industry, olive mill, baker's yeast and brewery wastewaters can be used as raw material for bio-hydrogen production. Those wastewaters may require pretreatment to remove undesirable components and for nutritional balancing. Carbohydrate rich food industry effluents may be further processed to convert the carbohydrate content to organic acids and then to hydrogen gas by using proper bio-processing technologies. Fig. 1 shows schematic diagram for bio-hydrogen production from food industry wastewaters by two stage anaerobic dark and photo-fermentations.

2.3. Waste sludge from wastewater treatment plants

The waste sludge generated in wastewater treatment plants contains large quantities of carbohydrate and proteins which can be used for energy production such as methane or hydrogen gas. Anaerobic digestion of excess sludge can be realized in two steps. Organic matter will be converted to organic acids in the first step (acidogenic phase) and the organic acids will be used for hydrogen gas production in the second step by using photo-heterotrophic bacteria.

3. Bio-processes for hydrogen gas production

Major bio-processes utilized for hydrogen gas production can be classified in three categories:

1. Bio-photolysis of water by algae.
2. Dark-fermentative hydrogen production during acidogenic phase of anaerobic digestion of organic matter.
3. Two stage dark/photo-fermentative production of hydrogen.

The metabolic pathways, types and function of enzymes involved in biological hydrogen production for different microbial processes are summarized in details in some recent review articles [7–10].

3.1. Hydrogen gas production from water by algae

Algae split water molecules to hydrogen ion and oxygen via photosynthesis. The generated hydrogen ions are converted into hydrogen gas by hydrogenase enzyme. *Chlamydomonas reinhardtii* is one of the well-known hydrogen producing algae [9,11]. Hydrogenase activity has been detected in green algae, *Scenedesmus obliquus* [12], in marine green algae *Chlorococcum littorale* [13,14], *Playtmonas subcordiformis* [15] and in *Chlorella fusca* [16]. However, no hydrogenase activity was observed in *C. vulgaris* and *Dunaliella salina* [16,17]. The hydrogenase activity of different algae species was compared by Winkler et al. [18] and it was reported that enzyme activity of the *Scenedesmus* sp. (150 nmol/ μ g Chl a.h) is lower than *C. reinhardtii* (200 nmol/ μ g Chl a.h).

Cyanobacterial hydrogen gas evolution involves nitrogen fixing cultures such as non-marine *Anabaena* sp., marine cyanobacter *Oscillatoria* sp., *Calothrix* sp. and non-nitrogen fixing organisms such as *Synechococcus* sp., *Gloebacter* sp. and it was reported that *Anabaena* sp. have higher hydrogen evolution potential over the other cyanobacter species [19]. Heterocystous filamentous *Anabaena cylindrica* is a well-known hydrogen producing cyanobacter [8,19]. However, *A. variabilis* has received more attention in recent years because of higher hydrogen production capacity [20–24]. The growth conditions for *Anabaena* include nitrogen free media, illumination, CO_2 and O_2 . Since nitrogenase enzyme is inhibited by oxygen, hydrogen production is realized under anaerobic conditions. CO_2 is required for some cultures during hydrogen evolution phase [19] although inhibition effects of CO_2 on photo-production of H_2 was also observed [21]. Four to 18% CO_2 concentrations were reported to increase cell density during growth phase resulting in higher hydrogen evolution in the later stage [22]. The use of simple sugars as supplement was reported to promote hydrogen evolution [23]. Recent studies are concentrated on development of hydrogenase and bi-directional hydrogenase deficient mutant of *Anabaena* sp. in order to increase the rate of hydrogen production. At the present time the rate of hydrogen production by *Anabaena* sp. is considerably lower than that obtained by dark or photo-fermentations. [20,24].

The algal hydrogen production could be considered as an economical and sustainable method in terms of water utilization as a renewable resource and CO_2 consumption as one of the air pollutants. However, strong inhibition effect of generated oxygen on hydrogenase enzyme is the major limitation for the process. Inhibition of the hydrogenase enzyme by oxygen can be alleviated by cultivation of algae under sulfur deprivation for 2–3 days to provide anaerobic conditions in the light [15,18,25,26]. Low hydrogen production potential and no waste utilization are the other disadvantages of hydrogen production by algae. Therefore, dark and photo-fermentations are considered to be more advan-

tageous due to simultaneous waste treatment and hydrogen gas production.

3.2. Hydrogen gas production by dark fermentation

3.2.1. Type of organisms and conditions

Many anaerobic organisms can produce hydrogen from carbohydrate containing organic wastes. The organisms belonging to genus *Clostridium* such as *C. butyricum* [27], *C. thermolacticum* [28], *C. pasteurianum* [29,30], *C. paraputrificum* M-21 [31] and *C. bifermentans* [32] are obligate anaerobes and spore forming organisms. *Clostridia* species produce hydrogen gas during the exponential growth phase. In batch growth of *Clostridia* the metabolism shifts from a hydrogen/acid production phase to a solvent production phase, when the population reaches to the stationary growth phase. Investigations on microbial diversity of a mesophilic hydrogen producing sludge indicated the presence of *Clostridia* species as 64.6% [33]. The dominant culture of *Clostridia* can be easily obtained by heat treatment of biological sludge. The spores formed at high temperatures can be activated when required environmental conditions are provided for hydrogen gas production.

The species of the genus enterobacteriaceae have the ability to metabolize glucose by mixed acid or the 2–3 butanediol fermentation. In both patterns, CO₂ and H₂ are produced from formic acid in addition to ethanol and the 2–3 butanediol [34]. Hydrogen production capacity of anaerobic facultative bacterial culture *Enterobacter aerogenes* has been widely studied [27,35–41]. *Enterobacter cloacae* ITT-BY 08 produced 2.2 mol H₂/mol glucose [42]. Hydrogen production from glucose by *E. coli* and *Hafnia alvei* was studied by Podestá et al. [34] and trace amount of hydrogen yield was detected.

Recently, hydrogen producing aerobic cultures such as *Aeromonas* spp., *Pseudomonas* spp. and *Vibrio* spp. were identified. Anaerobic cultures like *Actinomyces* spp., *Porphyromonas* spp. beside to *Clostridium* spp. have been detected in anaerobic granular sludge. The hydrogen yield varied between 1 and 1.2 mmol/mol glucose when the cultures were cultivated under anaerobic conditions [43]. Hydrogen production by *Thermotogales* species and *Bacillus* sp. were detected in mesophilic acidogenic cultures [44].

Hydrogen gas production capacity of some anaerobic thermophilic organisms belonging to the genus *Thermoanaerobacterium* has also been investigated [44–47]. Shin reported *T. thermosaccharolyticum* and *Desulfotomaculum geothermicum* strains producing hydrogen gas in thermophilic acidogenic culture [44]. A hyperthermophilic archeon, *Thermococcus kodakaraensis* KOD1 with 85 °C optimum growth temperature was isolated from a geothermal spring in Japan and identified as a hydrogen producing bacteria [48]. *Clostridium thermolacticum* can produce hydrogen from lactose at 58 °C [28]. Recently, a hydrogen producing bacterial strain *Klebsiella oxytoca* HP1 was isolated from hot springs with maximal hydrogen production rate at 35 °C [49].

Environmental conditions are the major parameters to be controlled in hydrogen production. Medium pH affects hydrogen production yield, biogas content, type of the organic acids pro-

duced and the specific hydrogen production rate. The reported pH range for the maximum hydrogen yield or specific hydrogen production rate is between pH 5.0 and 6.0 [50–54]. However, some investigators report the optimum pH range between 6.8 and 8.0 [28,29,45,48,55] and around pH 4.5 for the thermophilic culture [44]. Most of the studies indicated that final pH in anaerobic hydrogen production is around 4.0–4.8 regardless of initial pH [27,29,45,46,55,56]. The decrease in pH is due to production of organic acids which depletes the buffering capacity of the medium resulting in low final pH [53]. Gradual decreases in pH inhibit hydrogen production since pH affects the activity of iron containing hydrogenase enzyme [57]. Therefore, control of pH at the optimum level is required. Initial pH also influences the extent of lag phase in batch hydrogen production. Composition of the substrate, media composition, temperature and the type of microbial culture are also important parameters affecting the duration of lag phase. Some studies reported that low initial pH of 4.0–4.5 causes longer lag periods such as 20 h [29,53]. High initial pH levels such as 9.0 decrease lag time; however, lower the yield of hydrogen production [45].

The major products in hydrogen production by anaerobic dark fermentation of carbohydrates are acetic, butyric and propionic acids. Formation of lactic acid was observed when lactose and molasses (sucrose) were used as the substrates [28,39,40]. pH also affects the type of organic acids produced. More butyric acid is produced at pH 4.0–6.0. Concentration of acetate and butyrate could be almost equal at pH 6.5–7.0 [50]. Ethanol production was observed depending on the environmental conditions [28,43,45–47,58]. Methane was not detected in most of the hydrogen production studies because of elimination of methane producers by heat digestion of sludge [29,30,58]. However, long retention times may cause methane formation by the mesophilic cultures [44]. Methane production was also observed when sewage sludge was used as the substrate [32,59].

Since the hydrogenase enzyme present in anaerobic organisms oxidizes reduced ferredoxin to produce molecular hydrogen, external iron addition is required for hydrogen production. Liu reported that high iron concentrations (100 mg/L) increases lag phase in batch operations and also composition volatile fatty acids (VFA) may vary as a result of metabolic shift in anaerobic digestion. Ten milligram per liter iron concentration was determined to be the optimum in batch hydrogen production by *C. pasteurianum* from starch [29].

Nitrogen is an essential nutrient for hydrogen production by dark fermentation under anaerobic conditions. Yokoi reported that the highest level of hydrogen (2.4 mol/mol glucose) could be obtained from starch in the presence of 0.1% polypepton. But no hydrogen production was observed when urea or other nitrogen salts were used as nitrogen source [27]. Maximum specific hydrogen production rate was obtained as 178 mL/g VSS d in the presence of 5.64 g/L (NH₄)₂ HCO₃ [29]. Corn-steep liquor which is a waste of corn starch manufacturing process could be used as nitrogen source [61]. Lin reported that the C/N ratio affected hydrogen productivity more than the specific hydrogen production rate [30].

Hydrogen gas producing organisms are strict anaerobes. Therefore, reducing agents such as argon, nitrogen, hydrogen

gas and L-cystine-HCl are used to remove trace amounts of oxygen present in the medium. However, the use of such reducing agents is relatively expensive, and therefore uneconomical for industrial production of hydrogen gas. *Enterobacter aerogenes* is a facultative anaerobe and the amount of hydrogen produced by this culture is comparable to *Clostridium* sp. [36–41]. The culture has the ability to survive in the presence of slight amount of oxygen generated during anaerobic biodegradation. Therefore, utilization of *E. aerogenes* along with *Clostridium* instead of expensive chemical reducing agents was suggested by Yokoi for effective hydrogen gas production by dark fermentation [27,37].

3.2.2. Type of substrates

3.2.2.1. Use of simple sugars. Glucose is an easily biodegradable carbon source, present in most of the industrial effluents and can be obtained abundantly from agricultural wastes. Theoretically bioconversion of 1 mol of glucose yields 12 mol of hydrogen gas (H₂). According to reaction stoichiometry, bioconversion of 1 mol of glucose into acetate yields 4 mol H₂/mol glucose, but only 2 mol H₂/mol glucose is formed when butyrate is the end product. The highest hydrogen yield obtained from glucose is around 2.0–2.4 mol/mol [47,50,56]. Production of butyrate rather than acetate may be one of the reasons for deviations from the theoretical yield. Fang suggested that partial biodegradation of glucose could be another reason for lower yields [50]. However, even when more than 95% glucose was degraded, the yield could be less than 1.7 mol H₂/mol glucose [60]. Therefore, utilization of substrate as an energy source for bacterial growth is the main reason for obtaining the yields lower than theoretical estimations.

Batch and continuous hydrogen gas production from sucrose has been widely studied (Tables 1 and 2). Chen obtained a yield

of 4.52 mol H₂/mol sucrose in a CSTR with 8 h hydraulic residence time [62]. This yield is higher than the other reported studies such as 3.47 mol H₂/mol sucrose in CSTR [54] and 1.5 mol H₂/mol sucrose in UASB [63] at the same HRT. However, the yield from glucose was only 0.91 mol H₂/mole glucose under the same operating conditions in CSTR [64]. Optimization of C/N ratio at 47 provided efficient conversion of sucrose to hydrogen gas with a yield of 4.8 mol H₂/mol sucrose [30]. Similarly, cumulative hydrogen production from sucrose was 300 mL while it was only 140 mL from starch [53]. *Enterobacter cloacae* ITT-BY 08 produced 6 mol H₂/mol sucrose which is the highest yield among the other tested carbon sources [42]. Collet reported maximum hydrogen yield of 3 mol H₂/mol lactose although theoretical yield is 8 mol H₂/mol lactose [28]. The results of these studies indicated that the higher hydrogen yields could be obtained from sucrose compared to other simple sugars. However, the yield per mole of hexose remains almost the same for all types of the disaccharides.

3.2.2.2. Use of starch containing wastes. Starch containing materials are abundant in nature and have great potential to be used as a carbohydrate source for hydrogen production. Tables 1 and 2 summarize the yields and the rates of hydrogen production for batch and continuous operations when starch was used as the substrate. According to the reaction stoichiometry, a maximum of 553 mL hydrogen gas is produced from one gram of starch with acetate as a by-product [45]. However, the yield may be lower than the theoretical value because of utilization of substrate for cell synthesis.

The maximum specific hydrogen production rate was 237 mL H₂/g VSS d when 24 g/L edible corn starch was used as the substrate by *C. pasteurianum* [29]. Zang obtained higher specific

Table 1
Yields and rates of bio-hydrogen production from pure carbohydrates by batch dark fermentations

Organism	Carbon source	SHPR	VHPR	H ₂ yield	% H ₂ yield	H ₂ content in gas mixture (%)	Reference
<i>Klebsiella oxytoca</i> HP1	Glucose (50 mM)	9.6 mmol/g DW h	87.5 mL/L h	1 mol/mol glucose	16.7		[49]
<i>E. cloacae</i> IIT-BT 08	Glucose (1%)		447 mL/L h	2.2 mol/mol glucose			[42]
<i>E. coli</i>	Glucose (20 g/L)			4.73 × 10 ⁻⁸ mol/mol glucose			[34]
<i>H. alvei</i>	Glucose (10 g/L)			5.87 × 10 ⁻⁸ mol/mol glucose			[34]
Sludge compost	Glucose (10 g/L)		147 mL/L h	2.1 mol/mol glucose			[56]
Mixed culture	Glucose (1 g COD/L)			0.9 mol/mol glucose	23	60	[73]
Mixed culture	Sucrose (6 g/L)	9 mL/g VSS h		300 mL/g COD		40	[53]
<i>Klebsiella oxytoca</i> HP1	Sucrose (50 mM)	8.0 mmol/g DW h		1.5 mol/mol sucrose	12.3		[49]
<i>C. pasteurianum</i> (dominant)	Sucrose (20 g COD/L)	4.58 mmol/g VSS h	270 mmol/L d	4.8 mol/mol sucrose		55	[60]
<i>E. cloacae</i> IIT-BT 08	Sucrose (10 g/L)	29.5 mmol/g DW h	660 mL/L h	6 mol/mol sucrose	28	92	[42]
Mixed culture	Sucrose (1 g COD/L)			1.8 mol/mol sucrose	23		[73]
<i>Thermoanaerobacterium</i>	Cellulose (5 g/L)	11.9 mL/g VSS h		102 mL/g cellulose	18		[46]
<i>Clostridium</i> sp.	Microcrystalline cellulose (25 g/L)	0.46 mmol/VSS d		2.18 mmol/g cellulose		60	[55]
<i>E. aerogenes</i>	Starch ^a (20 g glucose/L)	9.68 mmol/g DW h	17.4 mmol/L h	1.09 mol/mol glucose			[41]
<i>Thermoanaerobacterium</i>	Starch (4.6 g/L)	15.2 mL/g VSS h	1.9 mL/h	92 mL/g starch	17	60	[45]
<i>C. pasteurianum</i>	Starch (24 g/L)	9.9 mL/g VSS h	4.2 mL/h	106 mL/g starch	19		[29]
Mixed culture	Potato starch (1 g COD/L)			0.59 mol/mol starch	15		[73]
Mixed culture	Sugar beet juice			1.7 mol H ₂ /mol hexose			[76]

^a Hydrolysate; SHPR, specific hydrogen production rate; VHPR, volumetric hydrogen production rate.

Table 2
Yields and rates of bio-hydrogen production from pure carbohydrates by continuous dark fermentations

Organism	Carbon	SHPR	VHPR	H ₂ yield	% H ₂ content	Reactor	HRT _i (h)	Reference
<i>C. acetobutyricum</i>	Glucose	6 mmol/OD ₆₀₀ h L		2 mol/mol glucose	50	Fed-batch		[77]
Mixed culture	Glucose (20 g COD/L)	20 mmol/g VSS h		1.1 mol/mol glucose		CSTR	4	[64]
Mixed culture	Glucose (13.7 g/L)		376 mmol/L d	1.2 mol/mol glucose	60	Trickling biofilter	4–12	[80]
<i>Clostridia</i> sp.	Glucose (20 g COD/L)	14.2 mmol/g VSS h	359 mmol/L d	1.7 mol/mol glucose	42.6	CSTR	6	[60]
Mixed culture	Glucose (7 g/L)	191 mL/g VSS h		2.1 mol/mol glucose	64	CSTR	6	[50]
Mixed culture	Glucose (20 g/L)		300 mL/L h		60	UASB	20	[78]
<i>Clostridium</i> sp.	Glucose (10 g/L)		640 mL/h		60	AMBR ^a	3.3	[79]
<i>E. aerogenes</i> HO39	Glucose (10 g/L)		850 mL/L h			Fixed film	1	[38]
Mixed culture	Sucrose (20 g COD/L)		105 mol/h	3.47 mol/mol sucrose		CSTR	8	[54]
Mixed culture	Sucrose	340 mL/g VSS h	5.10 L/h L	2.1 mol/mol sucrose	35	CIGSBR ^b	0.5	[74]
Mixed culture	Sucrose (20 g COD/L)	2.2 mmol/g VSS h	270 mmol/L d	1.5 mol/mol sucrose	42	UASB	8	[63]
Mixed culture	Sucrose (20 g COD/L)	3.7 mmol/g VSS h	470 mmol/L d	2.6 mol/mol sucrose	35	SBR	4–12	[75]
<i>Klebsiella oxytoca</i> HP1	Sucrose (50 mM)	15.2 mmol/g DW h	350 mL/L h	3.6 mol/mol sucrose		CSTR	5	[49]
Mixed culture	Sucrose (20g COD/L)	35 mmol/g VSS h	20.8 L/L d	1.48 mol/mol sucrose	42	CSTR	2	[62]
<i>C. butyricum</i> + <i>E. aerogenes</i>	Starch (2%)	NA	800 mL/L h	2.5 mol/mol glucose		CSTR	2	[37]
<i>C. butyricum</i> + <i>E. aerogenes</i>	Starch (2%)	NA	1300 mL/L h	2.6 mol/mol glucose		Immobilized ^c	0.75	[37]
<i>Thermococcus kodakaraensis</i> KOD1	Starch (5 g/L)	14.0 mmol/g DW h	9.46 mmol/L h	3.33 mol/mol starch	<10	Gas-lift fermenter	5	[48]
Mixed culture	Wheat starch (10 g/L)		131 mL/L h	0.83 mol/mol starch d	50.3	CSTR	12	[72]
Mixed culture	Starch (6 kg starch/m ³)	97.5 mL/g VSS h	1497 L/m ³ d	1.29 L/g starch COD	61	CSTR	20	[52]
<i>C. termolacticum</i>	Lactose (29 mmol/L)	5.74 mmol/g DW h	2.58 mmol/L h	3 mol/mol lactose	86	CSTR	5–35	[28]

^a Anaerobic membrane bioreactor.

^b CIGBR, carrier induced granular bed reactor.

^c Immobilization on porous glass beads; SHPR, specific hydrogen production rate; VHPR, volumetric hydrogen production rate.

yield of 480 mL H₂/g VSS d with 4.6 g/L starch concentration at 37 °C using a mixed sludge. Thermophilic conditions did not improve the production rate yielding 365 mL H₂/g VSS d with *Thermoanaerobacterium* at 55 °C [45].

Yokoi used dried sweet potato starch residue for hydrogen production by the mixed culture of *C. butyricum* and *E. aerogenes*. Hydrogen yield obtained in long term repeated batch operations was 2.4 mol H₂/mol glucose from 2.0% starch residue containing wastewater [27].

3.2.2.3. Use of cellulose containing wastes. Cellulose is the major constitute of plant biomass and highly available in agricultural wastes and industrial effluents such as pulp/paper and food industry. Hydrogen gas production potential from microcrystalline cellulose at mesophilic conditions with heat-digested sludge was investigated by Lay [55]. Increasing cellulose concentration resulted in lower yields with the maximum value of 2.18 mol H₂/mol cellulose with 12.5 g/L cellulose concentration. However, 25 g/L cellulose concentration provided the highest specific hydrogen production rate of 11.16 mmol/g VSS d. Liu reported that cellulose is converted to hydrogen with a higher rate at 37 °C, but more hydrogen was accumulated at thermophilic range. The maximum hydrogen yield obtained in this study was 102 mL/g cellulose which is only 18% of the theoretical yield [46]. Low yield was explained as partial hydrolysis of cellulose. Taguchi hydrolyzed the cellulose and used the hydrolysate

for fermentation by a *Clostridium* sp. During an 81 h period of stationary culture, the organisms consumed 0.92 mmol glucose/h and produced 4.10 mmol H₂/h [65]. The same culture was also used for hydrogen production from pure xylose or glucose and enzymatic hydrolysate of Avicel cellulose or xylan. The hydrogen yield from the hydrolysate was higher than that of carbohydrates as 19.6 and 18.6 mmol H₂ per gram of substrate consumed, respectively [66].

3.2.2.4. Use of food industry wastes and wastewater. Food industry wastes constitute a major fraction of the municipal solid wastes. Landfilling, composting and incineration are the conventional approaches for the solid waste management. However, high carbohydrate content in form of simple sugars, starch and cellulose makes the solid food wastes a potential feedstock for biological hydrogen production. The problem with the food waste is the variations in carbohydrate and protein types and concentrations in the mixture. Each component requires different environmental and bio-processing conditions for hydrogen gas production. Table 3 summarizes hydrogen gas production from different wastewaters and solid wastes.

The feasibility of biological hydrogen production from organic fraction of municipal solid wastes (OFMSW) was investigated by Lay et al. under mesophilic conditions by using mixed anaerobic bacterial flora. At high F/M ratio (0.4 g OFMSW/g biomass), the pre-treated digested sludge had a high

Table 3
Yields and rates of bio-hydrogen production from different waste materials by dark fermentation

Organism	Carbon source	SHPR	VHPR	$Y_{P/S}$ yield coefficient	% H ₂ content	Reference
Mixed culture	OFMSW	16.8 mL/g VSS h	117 mL/g TVS h	150 mL/g OFMSW	66	[51]
<i>Thermoanaerobacterium</i>	Food waste (6 gVS/L)	12 mL/g VSS h		1.8 mol/mol hexose	55	[44]
Mesophilic mixed culture	Food waste (3% VS)	0.7 mL/g VSS h		0.05 mol/mol hexose	1	[44]
Mixed culture	Food waste (3% VS)	111 mL/g VSS h				[59]
Mixed culture	Potato Ind. WW (21 g COD/L)			2.8 L/L WW	60	[69]
Mixed culture	Apple (9 g COD/L)			0.9 L/L WW	60	[69]
Mixed culture	Domestic WW			0.01 L/L WW	23	[69]
<i>E. aerogenes</i>	Molasses (2% sucrose)	36 mmol/L culture h	138 mL/L h	1.5 mol/mol sucrose	60	[39]
Mixed culture	Rice winery WW (36 g COD/L)	389 mL/g VSS h	159 mL/L h	2.14 mol/mol hexose	53–61	[58]
Mixed culture	Biosolid			1.2 mg/g COD		[70]
Mixed culture	Filtrate			15 mg/g COD		[70]
<i>C. butyricum</i> + <i>E. aerogenes</i>	Sweet potato starch residue (0.5%)			2.4 mol/mol glucose		[27]
<i>C. butyricum</i> + <i>E. aerogenes</i>	Sweet potato starch residue (2%)			2.7 mol/mol glucose		[61]

OFMSW, organic fraction of solid waste; SHPR, specific hydrogen production rate; VHPR, volumetric hydrogen production rate.

hydrogenic activity with 43 mL/g VSS h specific production rate and 125 mL/g TVS h production potential [51]. Kim obtained 111.2 mL H₂/g VSS h when food waste was used as sole substrate. Addition of sewage sludge onto food waste as a rich protein source did not improve the production rate [59]. Similarly, hydrogen production potential of carbohydrate rich high solid organic waste (HSOW) was 20 times larger than those of fat rich HSOW and protein rich HSOW. This is probably because of the consumption of hydrogen gas to form ammonium using nitrogen generated from biodegradation of protein rich solid wastes [67]. Shin reported higher production potential and specific H₂ production rates from food wastes under thermophilic conditions as compared to the mesophilic processes [44]. The effect of dilution rate (HRT) on hydrogen production from food wastes was studied by Han and 58% COD reduction, 70% hydrogen formation efficiency, over 100 L cumulative H₂ gas were obtained at optimum dilution rate of 4.5 d⁻¹ or HRT of 5.3 h [68].

Food processing industrial wastewaters are carbohydrate rich effluents. Ginkel studied hydrogen production from confectioners, apple and potato processor industrial effluents and also from domestic wastewater. The highest production yield was obtained as 0.21 L H₂/g COD from potato processing wastewater [69]. Molasses is another carbohydrate rich substrate and it is a good source of sucrose. The maximum and available rate of hydrogen production in continuous operation with *E. aerogenes* strain E.82005 was 36 and 20 mmol H₂/L h, respectively. The available yield was 1.5 mol H₂/mol sugar expressed in terms of sucrose [39]. Immobilization of cultures on polyurethane foam increased the yield to 2.2 mol H₂/mol sugar [40].

3.2.2.5. Use of waste sludge. Biosolids (sludges) from wastewater treatment plants contain large amounts of polysaccharides and proteins. Hydrogen yields of 1.2 mg H₂/g COD [70] and 0.6 mol/kg COD_i [71] were reported when sludge was used as the raw material. However, higher hydrogen yields (15 mg H₂/g COD) were obtained from the filtrate [70]. Pre-treatment of the sludge increased the soluble COD enhancing the hydrogen yield (0.9 mmol/g dried sludge) [32].

3.3. Hydrogen gas production by photo-fermentations

3.3.1. Types of organisms and the conditions

Some photo-heterotrophic bacteria are capable of converting organic acids (acetic, lactic and butyric) to hydrogen (H₂) and carbon dioxide (CO₂) under anaerobic conditions in the presence of light. Therefore, the organic acids produced during the acidogenic phase of anaerobic digestion of organic wastes can be converted to H₂ and CO₂ by those photosynthetic anaerobic bacteria. Hydrogen gas production capabilities of some purple photosynthetic bacteria such as *Rhodobacter spheroides* [10,81–90], *Rhodobacter capsulatus* [91–94], *Rhodovulum sulfidophilum* W-1S [95,96] and *Rhodospseudomonas palustris* [97] have been investigated to some extent. Photoproduction of hydrogen from CO or other organic acids by carbon-monoxide dependent dehydrogenase (CODH) enzyme containing cultures such as *Rhodospirillum rubrum* and *Rhodospseudomonas palustris* P4 has also been reported [98–100].

The optimum growth temperature and pH for the photosynthetic bacteria is in the range of 30–35 °C and pH_{opt} 7.0, respectively [81,82,87,88,91,100–102]. The hydrogen production takes place under anaerobic conditions with light illumination. The organisms prefer organic acids as carbon source such as acetic [92,97–99], butyric [92], propionic [94], lactic [81,86,90,91,103] and malic acid [82]. However, other carbohydrates [95,104] and industrial effluents may also be used for hydrogen gas production by photosynthetic bacteria [84,85,88,89,105]. Table 4 summarizes the yields and the rates of hydrogen production from different organic acids by the photo-fermentative organisms. Hydrogen production rates vary depending on the light intensity, carbon source and the type of microbial culture. On the basis of available literature the highest conversion efficiency was obtained using lactic acid as the carbon source [81,90,91].

Nitrogenase is the key enzyme that catalyzes hydrogen gas production by photosynthetic bacteria. The activity of the enzyme is inhibited in the presence of oxygen, ammonia or at high N/C ratios [83]. Therefore, the process requires ammonium limited and oxygen free conditions [106,107]. Hydrogen

Table 4
Yields and rates of bio-hydrogen production from organic acids by photo-fermentations

Organic acid	Organism	Concentration	Light intensity	Conversion efficiency (%)	LCE ^a (%)	H ₂ yield ^b	SHPR	VHPR	Process	Reference
Acetate	<i>Rhodospseudomonas</i>	22 mM	680 $\mu\text{mol photons/m}^2 \text{ s}$	72.8	0.9			25.2 mL H ₂ /L h	Batch	[97]
	<i>R. palustris</i>	22 mM	480 $\mu\text{mol photons/m}^2 \text{ s}$	14.8	0.1			2.2 mL H ₂ /L h	Batch	[97]
	<i>R. palustris</i>		2500 lux	60–70		2.8	9.8 mL/g cell h	1.6 mL H ₂ /L h	Batch	[99]
	<i>R. capsulata</i>	4 g/L	200 W/m ²	76.5		1.1	22 mL/g VSS h	0.88 mL/h	Batch	[92]
	<i>R. capsulata</i>	1.8 g/L	4170 lux	32.6	4.2		19.07 mL/g DW h		Batch	[93]
Lactate	<i>Rhodospseudomonas</i>	50 mM	680 $\mu\text{mol photons/m}^2 \text{ s}$	9.6	0.4			10.7 mL H ₂ /L h	Batch	[97]
	<i>R. palustris</i>	50 mM	480 $\mu\text{mol photons/m}^2 \text{ s}$	12.6	0.5			9.1 mL H ₂ /L h	Batch	[97]
	<i>R. sphaeroides</i> RV	100 mM	3klx	80			75 mL/g DW h	1.5 L/L d	CSTR	[90]
	<i>R. capsulatus</i> IR3	30 mmol	120 W	84.8					Batch	[91]
	<i>R. sphaeroides</i> GL-1	20 mM	300 W/m ²	86			0.2 mL/mL PU matrix h		^c	[81]
Butyrate	<i>Rhodospseudomonas</i>	27 mM	680 $\mu\text{mol photons/m}^2 \text{ s}$	8.4	0.3			7.6 mL H ₂ /L h	Batch	[97]
	<i>R. capsulata</i>	1 g/L	200 W/m ²	67.6		2.8	32 mL/g VSS h	1.28 mL/h	Batch	[92]
Malate	<i>Rhodospseudomonas</i>	15 mM	680 $\mu\text{mol photons/m}^2 \text{ s}$	6.6				1.1 mL H ₂ /L h	Batch	[97]
	<i>R. palustris</i>	15 mM	480 $\mu\text{mol photons/m}^2 \text{ s}$	36	0.3			5.8 mL H ₂ /L h	Batch	[97]
	<i>R. sphaeroides</i>	15 mM	200 W/m ²				2.4 mL/g DW h	12 mL/L h	Batch	[82]
	<i>R. sphaeroides</i>	7.5 mM	150–250 W/m ²	35–45			18 mL/g DW h	5 mL H ₂ /L h	Batch	[10]
PHB ^d	<i>R. sulfidophilum</i>		190 W/m ²					33 mL/L h	Batch	[95]
Succinate	<i>R. sulfidophilum</i>	50 mM	190 W/m ²					26.6 mL/L h	Batch	[95]

^a Light conversion efficiency.

^b H₂ yield mol/mol substrate.

^c Immobilized on polyurethane foam.

^d PHB, poly-hydroxy butyrate; 210 $\mu\text{mol photons/m}^2 \text{ s} = 190 \text{ W/m}^2$.

production by *R. sphaeroides* is completely inhibited at ammonia concentrations above 2 mM [101]. Hydrogen gas production was lower in the presence of ammonia salts, while proteins such as albumin, glutamate and yeast extract as a nitrogen source enhanced the production [99,107]. The metabolism shifts to utilization of organic substance for cell synthesis rather than hydrogen production in the presence of high nitrogen concentrations resulting in excess biomass growth and reduction in light diffusion [90,99]. However, hydrogen production activity can be recovered after ammonia is consumed. It was reported that presence of carbonate enhanced ammonia removal and stimulated hydrogen production [107]. Two stage ammonia removal and hydrogen production process has been suggested for hydrogen production from high level ammonia containing wastewater [90].

Hydrogenase enzyme in photo-fermentative bacteria is an uptake hydrogenase which utilizes hydrogen gas and therefore is antagonistic to nitrogenase activity [10]. Uptake hydrogenase activity should be limited for enhanced hydrogen gas production. Hydrogenase deficient mutant cultures of photo-fermentative bacteria could produce 2–3 times more hydrogen [87].

One of the parameters affecting the performance of photo-fermentation is the light intensity. Increasing light intensity has

a stimulatory affect on hydrogen yield and production rate, but has an adverse effect on the light conversion efficiency [93,97]. Kondo found that the reduced pigment mutant of *R. sphaeroides* MTP4 produces hydrogen more efficiently under high light intensity as compared to the wild type [86,108]. Light intensity might also affect the consumption rates of organic acids. Shi stated that butyrate consumption requires higher light intensities (4000 lux) as compared to acetate and propionate [93].

Hydrogen production under dark conditions is usually lower than that of the illuminated conditions [83,99]. However, alternating 14 h light/10 h dark cycles yielded slightly higher hydrogen production rates and cell concentrations as compared to continuous illumination [83]. Similarly, Wakayama reported that hydrogen production rate during 30 min dark/light cycle was 22 L/m² d which was twice as much as that obtained by illuminated culture during a 12 h cycle under the same conditions [109].

3.3.2. Types of substrates

Utilization of industrial effluents for hydrogen gas production by photosynthetic bacteria is possible although, these cultures prefer organic acids as carbon sources. One of the

Table 5
Yields and rates of bio-hydrogen production from food industry wastewaters by photo-fermentations

Wastewater	Dilution (%)	Organism	Light intensity	H ₂ yield	HPR	Operation	Reference
Sugar refinery effluent + malic acid	20	<i>R. sphaeroides</i> OU 001	200 W/m ²	13.44 L/mol C	5 mL/L culture h	Batch	[85]
Sugar refinery effluent + malic acid	20	<i>R. sphaeroides</i> OU 001	200 W/m ²	11.67 L/mol C	3 mL/L culture h	Continuous	[85]
Olive mill WW	2	<i>R. sphaeroides</i> OU 001	200 W/m ²		4 mL/L culture h	Batch	[84]
Tofu WW	ND ^a	<i>R. sphaeroides</i>	8klx	0.24 mL/mg carbohydrate	2.1 L/h m ² gel	Immobilized	[89]
Tofu WW	ND ^a				15.9 mL/L h	Batch	[89]
Tofu WW	ND ^a	<i>R. sphaeroides</i>	8500 lx		0.393 mL/mg DW h	Immobilized	[88]

^a ND, no dilution.

major problems in hydrogen gas production from industrial effluents is the color of wastewaters, which could reduce the light penetration. High ammonia concentration is another problem which inhibits the nitrogenase enzyme reducing the hydrogen productivity. High organic matter content (COD) and presence of some toxic compounds (heavy metals, phenolics and PAH) in industrial effluents may require pre-treatment before hydrogen gas production.

Table 5 summarizes hydrogen production studies from some food industry wastewaters by photo-fermentation. Photo-production of hydrogen from pre-treated sugar refinery wastewater (SRWW) was studied by Yetis in a column photo-bioreactor using *R. sphaeroides* OU 001 [85]. The hydrogen production rate was 3.8 mL/L h at 32 °C in batch operation with 20% diluted SRWW. Addition of malic acid (20 g/L) into SRWW enhanced the production rate to 5 mL/L h. Eroglu reported that high dilutions (3–4%) of olive mill waste (OMW) are necessary to alter the inhibitory effects of high organic content and dark color of OMW. Two percent dilution resulted in the highest hydrogen production potential of 13.9 L H₂/L WW at 32 °C with *R. sphaeroides* OU 001 and around 35% COD reduction was observed [84].

Tofu wastewater is a carbohydrate and protein rich effluent. Hydrogen yield from tofu wastewater (1.9 L/L wastewater at 30 °C) was comparable to the yield from glucose (3.6 L/L wastewater) by immobilized *R. sphaeroides* RV on agar gels. No ammonia inhibition (2 mM) was observed and 41% of TOC was removed [89]. Fifty percent dilution of the wastewater increased the yield to 4.32 L/L wastewater and TOC removal efficiency to 66% [88].

Singh investigated the hydrogen gas production from potato starch, sugar cane, juice and whey by using *Rhodospseudomonas* sp. Among the three substrates sugar cane juice yielded the maximum level of hydrogen production (45 mL/mg DW h) as compared to potato (30 mL/mg DW h) and whey (25 mL/mg DW h) [105]. On the contrary, no hydrogen production from raw starch was observed by salt-tolerant photosynthetic bacteria *Rhodobium marinum* [104].

3.3.3. Photo-bioreactors for bio-hydrogen production

The major types of photo-bioreactors developed for hydrogen production are tubular, flat panel and bubble column reactors.

The features of these photo-bioreactors have been reviewed by Akkerman et al. [110] and the importance of photochemical efficiency (theoretical maximum 10%) in hydrogen production was strongly emphasized. High illuminations cause lower light conversion yields, but higher hydrogen production rates. However, excess light could also cause photo-inhibition resulting in decreases in hydrogen production rate. A mutant type photosynthetic bacteria have been developed to increase the light conversion efficiency and hence hydrogen production rate [111]. Although an improvement was observed by mutant type, the light conversion efficiency was around 6% which is still less than theoretical efficiency. El-Shishtawy reported 9.23% maximum light conversion efficiency by using light-induced and diffused photo-bioreactor (IDPBR) at 300 W/m² light intensity [112]. The width of the culture significantly affected the productivity which reached to 7577 mL H₂/m² h or 50 mL/L culture h hydrogen production rate with 1 cm culture width. Similar results were observed by Nakada in a photo-bioreactor composed of four compartments aligned along the light penetration axis [113]. The efficiency of the conversion of light to hydrogen increased with the depth in the reactor and 1 cm depth showed the highest efficiency.

In relating to solar hydrogen production, the light conversion efficiency could be less during mid-day because of high light intensity (1.0 kW/m²). In addition, a delay of 2–4 h was observed in maximum hydrogen production rate (3.4 L H₂/m² h) after the highest light intensity at noon with an average light conversion efficiency of 1.4% [103]. Wakayama developed a light shade bands photo-bioreactor system to improve the solar hydrogen production efficiency. 3.5% light conversion efficiency at mid-day with over 0.8 kW/m² light intensity was obtained in photo-bioreactors with light shade bands whereas photo-inhibition was observed at 0.4 kW/m² in the ones without shade bands [114].

The other important parameter to be controlled in photo-bioreactors is mixing. Argon gas was commonly used for mixing and providing anaerobic conditions in photo-bioreactors although not cost-effective. It was observed that continuous argon sparging inhibited the growth of *Rhodospseudomonas* in a pneumatically agitated photo-bioreactor (Fig. 2a) because of CO₂ loss whereas re-circulation provided better growth of the culture [115]. A novel flat-panel airlift photo-bioreactor with baffles (Fig. 2b) was developed by Degen et al. [116]. It was

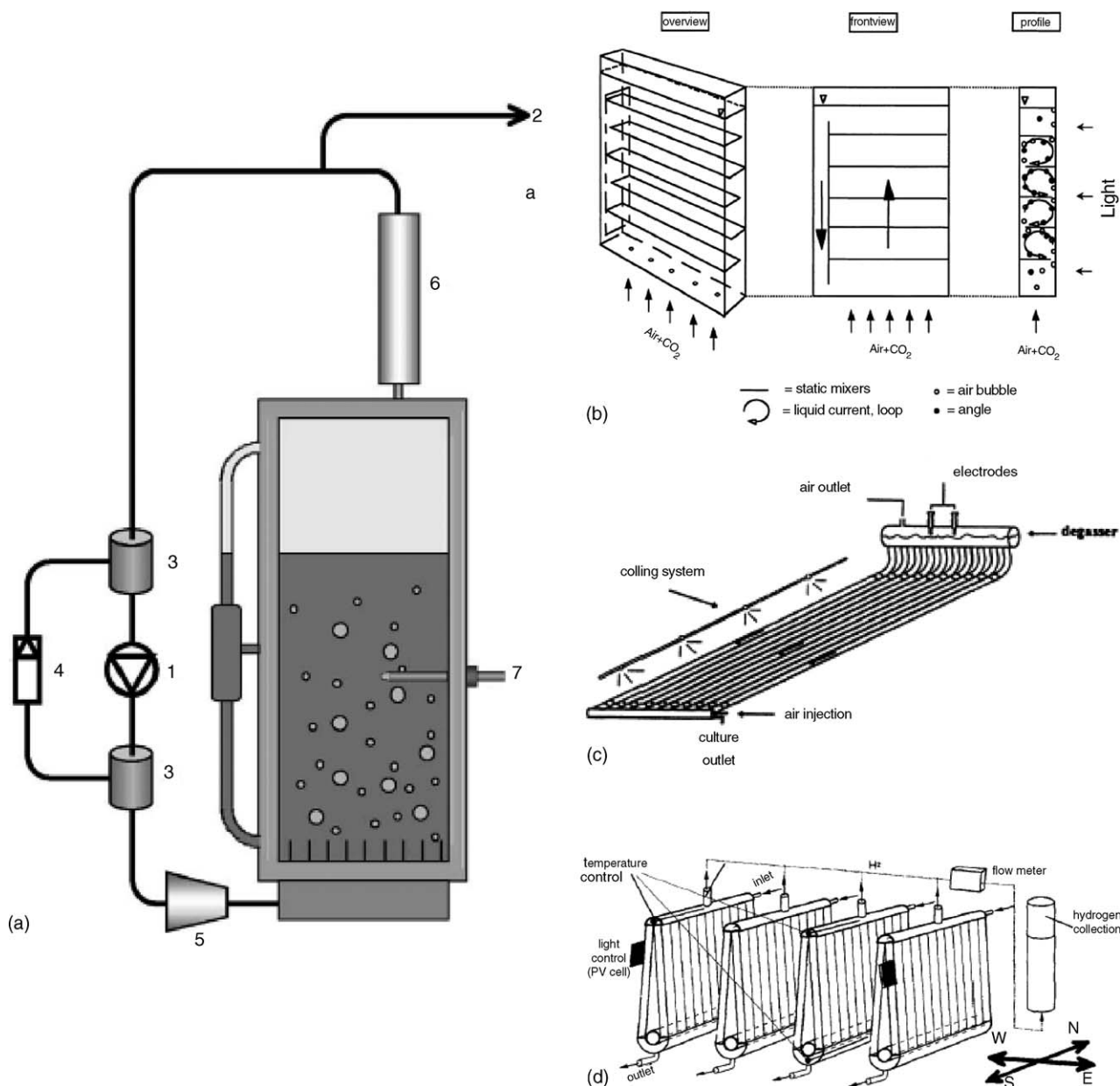


Fig. 2. Some configurations for photo-bioreactors used for hydrogen production. (a) Photo-bioreactor with gas recirculation [115]: (1) membrane gas pump, (2) gas bag for collection of produced gas, (3) two 1 L pressure vessels, (4) pressure valve, (5) mass flow controller, (6) condenser and (7) pH/redox electrode. (b) Flat panel airlift (FPA) photo-bioreactor [116]. (c) Multi-tubular (Tredici) photo-bioreactor [117] and (d) a modular outdoor photo-bioreactor [118].

observed that both installation of baffles for better mixing and reduction in the light path provides a significant increase in the biomass productivity. Although this photo-bioreactor was used for cultivation of *Chlorella vulgaris*, it may also be used in hydrogen gas production.

Tredici type multi-tubular photo-bioreactors (Fig. 2c) was used for hydrogen gas production in the presence and absence of light by using *Spirulina* [117]. Tubular reactors are made up of parallel transparent tubes filled with water. The system is inclined with a 10–30% slope to allow gas bubbles to rise. A modification of tubular reactor was developed by Modigell as a modular outdoor photo-bioreactor (Fig. 2d) [118]. The hydrogen production rate from lactate reached $2 \text{ L/m}^2 \text{ h}$ with light conversion efficiency of 2% in outdoor experiments.

3.4. Hydrogen gas production by sequential dark and photo-fermentation

Sequential dark and photo-fermentation is rather a new approach in biological hydrogen gas production. There is limited number of studies carried out on sequential hydrogen gas production system. Table 6 summarizes literature studies on sequential and combined dark and photo-fermentations for hydrogen production. The sequential production system has certain advantages over single stage dark or photo-fermentation processes. The effluent of dark fermentation in hydrogen production provides sufficient amount of organic acids for the photo-fermentation as mentioned in previous sections. Therefore, the limitation by the organic acid availability would be

Table 6
Yields and production rates of bio-hydrogen by the sequential and combined dark–photo fermentations

Fermentation type	Organisms	Carbon source	Organic acid	Total H ₂ yield (mol/mol glucose)	SHPR	Reference
Sequential dark–photo-fermentation	<i>C. butyricum</i> , <i>E. aerogenes</i> , <i>Rhodobacter</i> sp. M-19	Sweet potato starch residue	Acetic, butyric, lactic	7		[27]
	<i>C. butyricum</i> , <i>E. aerogenes</i> , <i>Rhodobacter</i> sp. M-19	Starch manufacturing wastes	Acetic, butyric, lactic	7.2		[53]
	<i>Lactobacillus amylovorus</i> , <i>R. marinum</i> A-501	Algal biomass (<i>D. tertiolecta</i>)	Lactic acid		2.47 mmol/L culture h	[120]
	Mixed anaerobic culture, <i>R. sphaeroides</i> RV	Solid waste	Lactic acid		~110 mL/g DW h	[119]
Combined dark–photo-fermentation	<i>C. butyricum</i> , <i>Rhodobacter</i> sp. M-19	Starch		6.6		[101]
	<i>Lactobacillus amylovorus</i> , <i>R. marinum</i> A-501	Algal biomass (<i>D. tertiolecta</i>)	Lactic acid		1.55 mmol/L culture h	[120]
	<i>V. fluvialis</i> , <i>R. marinum</i> A-501	Algal biomass (<i>C. reinhardtii</i>)	Lactic acid		1.18 mmol/L culture h	[120]

eliminated. Higher hydrogen production yields can be obtained when two systems are combined [61,101]. Further utilization of organic acids by photo-fermentative bacteria could provide better effluent quality in terms of COD. However, the system should be well-controlled to provide optimum media composition and environmental conditions for the two microbial components of the process [27,61,101,119]. The ammonia concentration and C/N ratio in the effluent of anaerobic fermentation should not be at the inhibitory level for the photosynthetic bacteria [102,119]. Dilution and neutralization of dark fermentation effluents are required before photo-fermentation to adjust the organic acid concentration and the pH7 for the optimal performance of photosynthetic bacteria [119,120].

Bio-hydrogen production by co-culture of anaerobic and photosynthetic bacteria in single stage has also been studied. Yokoi obtained higher hydrogen production yield (4.5 mol/mol glucose) by co-culture of *C. butyricum* and *Rhodobacter* sp. as compared to single stage dark fermentation (1.9 mol/mol glucose) and sequential two step fermentation (3.7 mol/mol glucose) of starch [101]. Similarly, higher hydrogen yields from different substrates were reported by co-cultures of *R. marinum* and *V. fluvialis* compared to *R. marinum* alone [104]. Better hydrogen yield (60%), but lower production rate was observed in combined fermentation and hydrogen gas production by *Lactobacillus amylovorus* and *R. marinum* from starch accumulating algae in comparison to sequential two stage fermentation (45%) [120]. In addition, pH of the mixed fermentation remained around pH 7 which is considered as an advantage over the two stage fermentation process. However, the differences in organic acid production/consumption rates, and therefore potential accumulation of organic acids in the media, decreases in light penetration because of suspended solids are the major problems in mixed fermentation processes.

4. Conclusions

Hydrogen is considered as the ‘energy for future’ since it is a clean energy source with high energy content as compared to hydrocarbon fuels. Unlike fossil fuels, petroleum, natural gas

and biomass, hydrogen is not readily available in nature. Therefore, new processes need to be developed for cost-effective production of hydrogen. Chemical methods such as steam reforming of hydrocarbons and partial oxidation of fossil fuels operate at high temperatures, and therefore are energy intensive and expensive. Biological methods offer distinct advantages for hydrogen production such as operation under mild conditions and specific conversions. However, raw material cost is one of the major limitations for bio-hydrogen production. Utilization of some carbohydrate rich, starch or cellulose containing solid wastes and/or some food industry wastewaters is an attractive approach for bio-hydrogen production.

Among the various methods used for bio-hydrogen production are: (a) water splitting by photosynthetic algae, (b) dark fermentation of carbohydrate rich wastes and (c) photo-fermentation of organic acid rich wastewaters. Algal production of hydrogen is rather slow, requires sunlight and is inhibited by oxygen. Hydrogen is produced as a by-product during acidogenic phase of anaerobic digestion of organic wastes which is known as the dark fermentation. The yield of hydrogen production by dark fermentation is low and the rate is slow. Organic acids produced during the dark fermentation of carbohydrate rich wastes may be converted to hydrogen and CO₂ by photo-heterotrophic bacteria. The process requires special organisms, light and strict control of the environmental conditions. Sequential or combined bio-processes of dark and photo-fermentations seem to be the most attractive approach resulting in high hydrogen yields for hydrogen production from carbohydrate rich wastes.

The major problems in bio-hydrogen production from wastes are the low rates and yields of hydrogen formation. Large reactor volumes are required for bio-hydrogen production due to low hydrogen production rates. Low yields and the rates of hydrogen formation may be overcome by selecting and using more effective organisms or mixed cultures, developing more efficient processing schemes, optimizing the environmental conditions, improving the light utilization efficiency and developing more efficient photo-bioreactors. Due to inhibition of bio-hydrogen production by oxygen and ammonium-nitrogen,

microbial growth and hydrogen formation steps may need to be separated in order to improve the hydrogen productivity. Considerable research and development studies are needed to improve the 'state of the art' in bio-hydrogen production.

Acknowledgement

This study was supported by the research funds of Dokuz Eylul University, Izmir, Turkey.

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