

1 **Biohydrogen production from anaerobic digestion and its potential as renewable energy**

2 Mohd Atiqueuzzaman Khan^a, Huu Hao Ngo^{a*}, Wenshan Guo^a, Yiwen Liu^a, Xinbo Zhang^b, Jianbo
3 Guo^b, Soon Woong Chang^c, Dinh Duc Nguyen^c, Jie Wang^d

4
5 ^a*Centre for Technology in Water and Wastewater, School of Civil and Environmental Engineering,*
6 *University of Technology Sydney, Sydney, NWS 2007, Australia*

7 ^b*Department of Environmental and Municipal Engineering, Tianjin Key Laboratory of Aquatic*
8 *Science and Technology, Tianjin Chengjian University, Jinjing Road 26, Tianjin 300384, China.*

9 ^c*Department of Environmental Energy & Engineering, Kyonggi University, 442-760, Republic of*
10 *Korea.*

11 ^d*School of Environmental and Chemical Engineering, Tianjin Polytechnic University, Tianjin,*
12 *300387, China*

13

14 * Corresponding author: School of Civil and Environmental Engineering, University of Technology, Sydney
15 (UTS), P.O. Box 123, Broadway, NSW 2007, Australia. Tel.: +61 2 9514 2745; Fax: +61 2 9514 2633. *E-mail*
16 *address:* ngohuuhaio121@gmail.com

17

18 **Abstract**

19 The current demand-supply scenario for fossil fuels requires an alternative energy source
20 with cleaner combustion products whilst production of hydrogen from anaerobic digestion
21 involves the utilization of waste materials and zero emission of greenhouse gasses. However,
22 large scale industrial application has yet not been implemented due to numerous challenges in
23 its production, storage, and transportation. This review study demonstrates that production of
24 hydrogen from anaerobic digestion is potentially a worthy alternative regarding energy
25 density, environmental impact, and cost. Moreover, dependence on fossil fuel systems in the
26 future could be minimized when biohydrogen production is feasible from renewable energy
27 sources.

28 **Keywords** Biohydrogen, hydride, volatile fatty acids, biomass, renewable energy, anaerobic
29 digestion

30 **1. Introduction**

31 **1.1 Background**

32 The world is looking for sustainable energy sources in order to replace the continuous
33 depletion of fossil fuel reserve and exponential rise in energy demand. Hydrogen could be
34 considered a worthy alternative to conventional fossil fuel energy sources due to its high
35 energy density (143 MJ kg^{-1}) [1] and clean combustion product (water vapor only) [2, 3].
36 Unfortunately, some technical issues have hindered the application of this technology on an
37 industrial scale. These include process operation storage and transportation [4], cost of
38 production [2] and process optimization [5-7]. As raw materials, fossil fuels are utilized in
39 conventional methods of hydrogen production such as steam reforming of natural gas, partial
40 oxidation of hydrocarbon or coal gasification [8]. None of these processes, however, are cost-
41 effective because they involve both the utilization of fossil fuels and have a destructive
42 impact on the environment by producing greenhouse gasses. Abbasi and Abassi [2] noted that
43 2.5–5 tones of CO_2 emission per ton of hydrogen is produced from fossil fuel sources.
44 Hydrogen production from biomass is a renewable source of energy which is both sustainable
45 and furthermore the combustion product poses no danger to the environment. Over the past
46 few years, the anaerobic digestion process has the major focus of producing methane
47 containing biogas from waste materials [9-11]. Recent studies have proven the technical
48 feasibility to produce volatile fatty acid (VFA) and biohydrogen from anaerobic digestion
49 [12-16]. Although such technical feasibility has been proven, biohydrogen production from
50 anaerobic digestion has to date not been employed in large scale industrial production.

51

52 The aim of this literature review is to focus on the major challenges involved in biohydrogen
53 production from anaerobic digestion [17-21]. However, there have been some reasons put
54 forward why the industrial production of biohydrogen is not yet feasible. These challenges

55 include sustainability in the production from different substrates, identifying the process
56 inhibition conditions and avoiding them, optimizing common process parameters and finally
57 the safety and economic challenges involved in creating a hydrogen storage system. Different
58 bioreactor design and arrangements have been employed for biohydrogen production. The
59 bioreactor arrangement along with process operating conditions (with different types of
60 substrates) has been noted. The second part of the literature review includes the potential of
61 biohydrogen as an alternative energy source. It includes the availability of fossil fuels in
62 contrast to the current demand scenario, energy density and other fuel properties of hydrogen
63 compared to common fossil fuels, the impact on the environment and overall cost comparison
64 of biohydrogen production compared to conventional energy sources. Previous review
65 articles discuss the potential of hydrogen as fuel, but to the best of our knowledge this is the
66 first review study that focuses on: firstly, the production challenges of anaerobic biohydrogen
67 production; and secondly, the potential of this fuel to be better than conventional energy
68 sources.

69

70 **1.2 Technical overview**

71 Anaerobic digestion is a series of biochemical reactions by which organic materials are
72 converted into a mixture of methane and carbon dioxide by microorganisms in the absence of
73 oxygen [22]. The process includes four different stages, namely hydrolysis, acidogenesis,
74 acetogenesis and the final stage of methanogenesis [23].

75

76 The initial stage of hydrolysis involves the transformation of insoluble organic materials in
77 the substrate into their soluble derivatives. Compounds with higher molecular mass such as
78 cellulose, hemicellulose, polysaccharides, proteins, and fats are converted into amino acids,

79 sugars and fatty acids [24]. Extracellular enzymes secreted by different types of
80 microorganisms enable the biodegradation of large molecules during this stage [22].

81

82 Acidogenesis is referred as the hydrogen production stage derived from anaerobic digestion.
83 During this phase, the monomers and soluble derivatives of organic component in the
84 substrate are converted into short chain volatile fatty acids (VFAs), hydrogen, carbon
85 dioxide, alcohols and acetates [25]. According to different microbial proportions, acidogenic
86 fermentation could be classified into three different types [26]. The butyric-type fermentation
87 produces butyric acid and acetic acid along with CO₂ and H₂. Propionic-type fermentation
88 involves no significant CO₂ and H₂ production in the production of propionic acid, acetic
89 acid, and valeric acid. Finally, ethanol-type fermentation has major production components of
90 ethanol and acetic acid along with small amounts of CO₂ and H₂ [26].

91

92 The products from the initial two stages cannot be consumed directly by the methanogens in
93 the final stage. They are converted into acetate, hydrogen and carbon dioxide in the third
94 stage of the anaerobic process [22]. The third stage of acetogenesis plays a critical role in
95 biohydrogen production since the volatile fatty acids produced in the first two stages are
96 converted into acetate and hydrogen. During this process, the final electron acceptors are
97 protons that are eventually converted back into hydrogen after receiving electrons [27].

98 Bundhoo and Mohee. [28] reported that a high concentration of hydrogen in this stage
99 inhibits the conversion of long chain fatty acids. The authors also contended that high partial
100 pressure of hydrogen causes a metabolic shift in the production of lactate, ethanol, acetone
101 and butanol. Later, Hydrogen is consumed during the conversion of ethanol to acetic acid
102 where high partial pressure of hydrogen is thermodynamically favorable [29].

103

104 The final stage of anaerobic digestion involves the production of methane and carbon dioxide
 105 by methanogenic Archaea. Two different groups of microorganisms are operating during this
 106 stage. The acetotrophic group of methanogens consumes the acetates and converts them into
 107 methane and carbon dioxide while the hydrogenotrophic group converts hydrogen and carbon
 108 dioxide into methane [30]. The hydrogenotrophic group of methanogens consumes the
 109 hydrogen produced as an electron donor for the reduction of carbon dioxide [31]. During
 110 anaerobic biohydrogen production, the hydrogenotrophic group of methanogens acts as the
 111 hydrogen consumer in the final stage. Their activities are required to be suppressed to ensure
 112 maximum biohydrogen production based on the anaerobic process.

113 **2. Bioreactors for biohydrogen production**

114 The technology involved in biohydrogen production has been evolving, and different types of
 115 bioreactors with varied arrangements have produced feasible results. Some research attempts
 116 have successfully tested the technical feasibility of biohydrogen production through
 117 anaerobic digestion. Table 1 summarizes the type of bioreactor arrangements, relevant
 118 process operating conditions, maximum biohydrogen production rate, and the yield from each
 119 process.

120 Table 1: Different types of bioreactors for biohydrogen production

Substrate	Operating conditions	Type of bioreactor	Maximum biohydrogen production rate	Maximum biohydrogen yield	Reference
Cheese whey	-	Batch Fermenter	6.35 ± 0.2 mol-H ₂ /mol-lactose	-	[18]
Food waste	35± 1 °C	STR	1.67 - 1.73	-	[17]

	pH = 6.0 ± 6.9		H ₂ /mol-hexose		
Anaerobic sludge	70 °C pH = 7.0-8.0	CSTR	1.11 mol-H ₂ /mol-hexose	-	[32]
Municipal sewage glucose	37 °C pH = 5.0	UASB	1.44 ± 0.1 mol-H ₂ /mol-hexose	-	[33]
Activated sludge	35 ± 1 °C	ESBG	1.7 mol-H ₂ /mol-hexose		[34]
Heat-treated sludge	35 °C pH = 5.5	ASBR	0.79 ± 0.03 mol-H ₂ /mol-hexose	-	[35]
Tofu-processing waste	60 °C pH = 5.5	CSTR	500 ml H ₂ L ⁻¹ h ⁻¹	2.3 mol H ₂ /mol glucose	[36]
Municipal wastes	pH = 4.65–5.87	TBSBR	67.67 ml H ₂ L ⁻¹ h ⁻¹	1.67 mol H ₂ /mol glucose	[19]
Cow dung compost	33.5 °C pH = 5	CSTBR	-	2.15 mol H ₂ /mol glucose	[37]
Clostridium butyricum (glucose)	37 °C pH = 5	Anaerobic continuous stirred tank reactor (ACSTR)	-	1.3 mol H ₂ /mol glucose	[38]
Wastewater (glucose)	37 °C pH = 5.5	Anaerobic sequencing batch reactor (ASBR)	-	2.89 ± 0.18 mol H ₂ /mol glucose	[39]

Municipal sewage	40 °C	Fluidized bed reactor (FBR)	1800 ml H ₂ L ⁻¹ h ⁻¹	4.26+/- 0.04 mol H ₂ /mol sucrose	[40]
Activated sludge	55 °C pH = 7.0	Batch	-	1.25 mol H ₂ /mol glucose	[41]
Anaerobic digested sludge (from distillery wastewater)	37 °C pH = 5.5	Batch	-	1000 ml H ₂ /L medium	[42]
Clostridium butyricum CGS2 (starch)	37 °C pH = 5.5	CSTR	-	2.3 mol H ₂ /mol hexose	[43]

121

122 Immobilized bioprocess system has proven to be useful to enhance the production of
123 biohydrogen through dark fermentation. For example, an yield of 2.1 mol/mol glucose was
124 achieved in batch operation from waste wheat. The bioreactor assembly included a metal
125 mesh assembly with covered plastic scouring sponge [44]. Another experiment showed
126 1.50 mol H₂/mol glucose yield of biohydrogen during batch fermentation [45]. Using corn
127 stalk as carrier, this immobilized mixed culture technology produced 62.5 % yield compared
128 to the suspended fermentation. Another experiment performed by Bai et al. [46] achieved a
129 yield of 1.8 mol-H₂/mol-hexose by performing immobilization technology on a thin film and
130 attached on a carrier by polymer.

131

132 More recent approaches include the combination of Bio Electrochemical Systems (BES) and
133 anaerobic digestion to produce biohydrogen [47, 48]. The Microbial Electrolysis Cell (MEC)
134 method has the advantages of no downstream processing for hydrogen purification and low

135 energy requirement (0.6 - 1 kWh/m³H₂) compared to the conventional energy input for
 136 electrolysis (4.5–50.6 kWh/m³ H₂) [49]. The process also makes it possible to completely
 137 recover produced biohydrogen and a higher yield (up to 8.55 mol H₂/mol-glucose at 0.6 V)
 138 [50] compared to 4 mol H₂/mol-glucose from dark fermentation [49].
 139
 140 Wu et al. [50] in their study used a single-chamber microbial electrolysis cell (MEC) to
 141 produce hydrogen using the effluent from an anaerobic baffled reactor (ABR). Their results
 142 included an impressive 99.0 ± 0.3% total COD removal efficiency, 1.31 ± 0.04 m³H₂/m³d
 143 hydrogen production, 2.78 ± 0.11 mLH₂/mg COD hydrogen yield and 138.63 ± 3.11%
 144 electrical energy efficiency. Other research studies have included a combination of MEC and
 145 MFC (Microbial Fuel Cell) in hydrogen production where MFC supplies the power required
 146 to operate the MEC. The following table (Table 2) includes MEC-MFC packages designed to
 147 treat the effluent from upstream processes that are not suitable to achieve the maximum
 148 hydrogen production rate and yield. This table lists the recent Bioelectrochemical system
 149 (BES) that has been developed for biohydrogen production.

150

151 Table 2: Bioelectrochemical system (BES) integration for different processes. (Modified
 152 from [52])

Feedstock	Design	Description	Results	Additional Information	References
Synthetic wastewater	BioH ₂ -MFC	Hydrogen-producing biofermenter (HPB)(2 L) + MFC (single chamber type, 100 mL)	Fermentation: 2.85 mol H ₂ mol ⁻¹ glucose, MFC: Maximum energy recovery, 559 J/L and	Integrated system for biohydrogen and effluent polishing with MFC	[53]

removal
efficiency of
97%

Vegetable wastewater	BioH ₂ -MFC	Acidogenic sequencing batch biofilm reactor + MFC	Maximum power density 111.76 mW/m ² . Hydrogen production 2.46 mmol H ₂ h ⁻¹	80% of VFA consumed in MFC	[54]
Molasses wastewater	BioH ₂ -MEC	Ethanol-type batch fed dark fermentation +MEC	1.41 ± 0.08 m ³ H ₂ m ⁻³ reactor/d at 0.6 V	Integrated Bio H ₂ effluent (~3250 mg C OD L ⁻¹) generated hydrogen	[55]
Synthetic media with acetate (100 mM of phosphate buffer)	MEC-MFC coupled system	MEC: two-chamber (450 mL each) MFC: single chamber (350 mL)	H ₂ production rate 14.9 ± 0.4 mL L ⁻¹ d ⁻¹ and yield 1.60 ± 0.08 mol-H ₂ mol-acetate ⁻¹	MFC provided external power for MEC	[56]

153

154 The synergy of a MEC-bioreactor combined system offers maximum utilization of organic
 155 content in the substrate, and theoretically, it could achieve production up to 12 mol H₂/per
 156 mol glucose [57]. Not many research studies have been done using combined bioreactor
 157 arrangement with MEC. Furthermore, most industrial treatment processes can not ensure the
 158 same type and composition of waste materials for anaerobic digestion. Consequently, a

159 generic model of anaerobic digestion should be designed using a suitable microbial strain,
 160 and metabolic pathway for high hydrogen yield. In this regard, the knowledge obtained from
 161 specific bioreactor arrangements and specific treatment processes could be employed in the
 162 generic model. More research initiatives are needed for the generic model where the
 163 emphasis is on anaerobic hydrogen production.

164 3. Challenges facing biohydrogen production

165 3.1 Sustainability in biohydrogen production from different substrates

166 The carbon content in different types of waste materials differs widely, and so does the
 167 biohydrogen production yield. A variable rate and yield cannot necessarily ensure
 168 sustainability in production. The biohydrogen production rate and yield also differ from
 169 anaerobic processes [58-62]. Table 3 summarizes the highest hydrogen yields derived from
 170 various types of biomass.

171

172 Table 3: Biohydrogen production from different substrates

Biomass	Inoculum	Operating conditions	Highest H₂ yield	Highest H₂ production rate	Reference
Cornstalk	Aerated microbial consortium	Pre-treating microbe additives (25 °C, 15 days)	176 mL H ₂ g ⁻¹ DB	18 mL H ₂ g ⁻¹ DB h ⁻¹	[63]
Lawn grass	Mixed culture dominated by <i>C. pasteurianu</i>	Pre-Treating 4% HCl (30 min, boiling)	72.21 mL H ₂ g ⁻¹ DB	1.72 mL H ₂ g ⁻¹ DB h ⁻¹	[14]

<i>m</i>					
Sugarcane bagasse	<i>C. butyricum</i>	0.5% H ₂ SO ₄ (60 min, n,121 °C, 1.47 bar)	44 mL H ₂ m mol ⁻¹ TS ^a	4.7 mL H ₂ h ⁻¹	[64]
Rice straw	<i>T. neapolitana</i>	10% NH ₄ OH (60 min, 121 °C) and 1% H ₂ SO ₄ (50 min, n, 121 °C)	77.1 mL H ₂ g ⁻¹ DB ^d	0.19 mL H ₂ h ⁻¹	[65]
Corn stalk	<i>T. thermosaccharolyticum</i> W 16	60 °C Batch	89.3 mL H ₂ g ⁻¹ dry biomass	-	[66]
Wheat straw	<i>C. saccharolyticus</i>	70 °C Batch	44.68 mL H ₂ g ⁻¹ dry biomass	-	[67]
Corn stalk	<i>C. butyricum</i>	36 °C Batch	92.9 mL H ₂ g ⁻¹ dry biomass	-	[68]
	<i>Bacillus licheniformis</i>	35 °C pH 6.0 (Pretreatment with 2% NaOH)	185 ml/l and 82.5 ml/g substrate	-	[69]
Rice straw	Waste water sludge	55 °C Batch	24.8 mL H ₂ g ⁻¹ dry biomass	-	[6]
Microalgae	Enriched functional consortia	30 °C Batch	25.1 mL H ₂ g ⁻¹ dry biomass	-	[70]
<i>L. japonica</i>	Anaerobic	35 °C Batch	71.4 mL H ₂	-	[71]

	mixed culture		g^{-1} dry biomass		
Water hyacinth, beverage waste water	Pig slurry	45°C Batch	13.65 mL H ₂ g^{-1} dry feedstock	-	[72]
Mixture of corn husk, hut shell, rice husk	Buffalo dung compost	37°C Batch	65.78 mL H ₂ g^{-1} TVS	-	[73]
Waste papers	<i>R. albus</i>	37°C Batch	42.8– 282.7 mL H ₂ g^{-1} dry biomass	-	[74]
Switchgrass	<i>C.</i> <i>saccharolyti</i> <i>cus</i>	65°C Batch	310 mL H ₂ g $^{-1}$ dry biomass	-	[75]
Deoiled Jatropha waste	Anaerobic mixed culture	55°C ASBR	8.7 mL H ₂ g ⁻ ¹ VS	-	[76]
<i>L. japonica</i>	Anaerobic digester sludge	35°C ASBR	61.3 mL H ₂ g^{-1} dry biomass	-	[77]

173

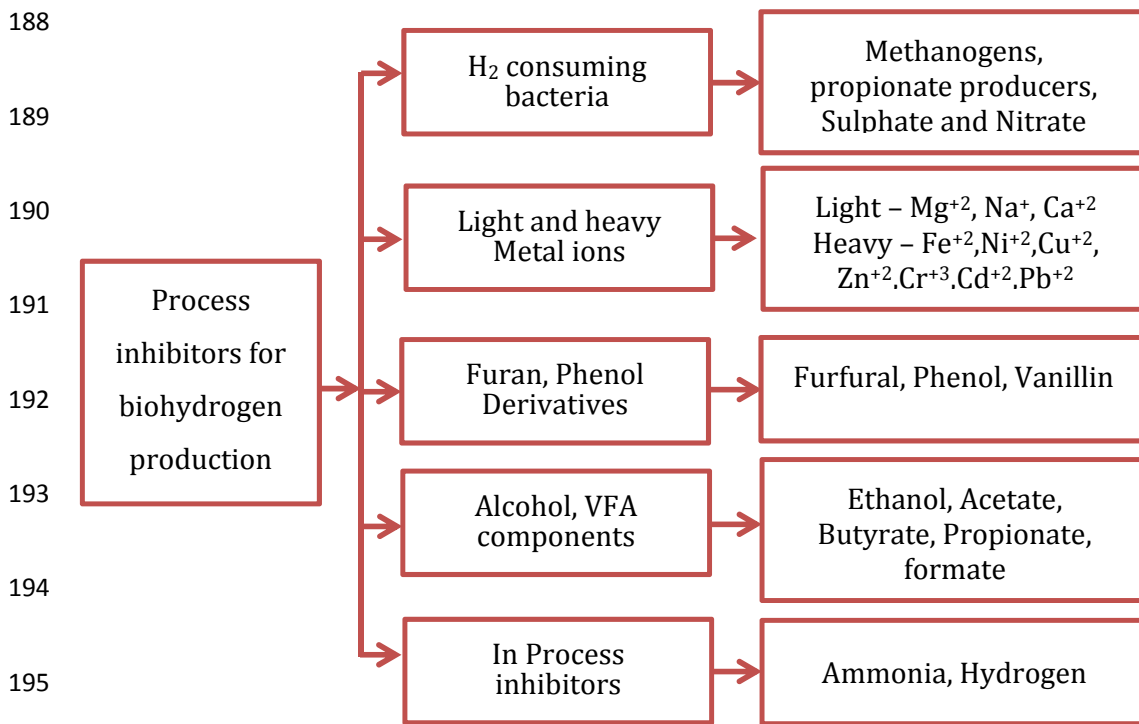
174 The studies listed above have two major limitations: firstly, they have produced feasible
175 results based on fixed type of substrate, specific reactor design and operational parameters,
176 not for generic purposes; and secondly, the production processes were only conducted in
177 laboratories. As a result, there was no discussion regarding the production and relevant
178 downstream processing challenges involved in large scale production.

179

180

181 **3.2 Challenges in controlling process inhibition**

182 Some process inhibition conditions can seriously compromise biohydrogen production during
183 the anaerobic digestion process. The types of microorganism in biohydrogen production
184 depend mainly on the type and composition of organic content in the substrate. The metabolic
185 pathway for biohydrogen production is then defined by the microorganisms present in the
186 system. Figure 1 summarizes several factors that work against biohydrogen production and
187 make the process unfeasible for large scale industrial application.



196

197 Figure 1: Inhibitors that discourage biohydrogen production

198 **3.2.1 Hydrogen consuming bacteria**

199 The final stage of anaerobic digestion involves the production of biomethane from the
200 reduction of CO₂ using H₂ as the electron donor. The biohydrogen produced is consumed
201 through the process known as hydrogenotrophic methanogenesis, which decreases the overall

202 yield of biohydrogen [23]. Hydrogen is also consumed by the alcohol-producing bacteria,
203 where it is consumed as an electron donor both in the form molecule or hydrogen equivalents
204 (NADH_2 ; potential H_2). During dark fermentation, lactate is degraded by propionate
205 producers such as *Clostridium propionicum* and *Clostridium homopropionicum* through the
206 consumption of NADH [28, 29].

207

208 Sulfate reducing bacteria (SRB) use different types of substrate as the donor of electrons and
209 cause the sulfates to be reduced to sulfides. SRBs perform their action simultaneously
210 through partial oxidation (to acetic acid and CO_2) or complete oxidation (to CO_2 and HCO_3^-)
211 [28]. Of the different types of SRB, hydrogenotrophic SRB constitute the major consumers of
212 biohydrogen because they consume biohydrogen as an electron donor [78].

213

214 Another hydrogen consumer is Nitrate reducing bacteria (NRB) who can be either
215 heterotrophic or autotrophic. Autotrophic NRBs utilize biohydrogen as electron donor to produce
216 ammonia [20]. Besides, NRBs can also decrease biohydrogen production by producing ammonia;
217 it has been proven through experiments that high concentrations of NH_3 and NH_4^+ have toxic
218 and repressing effects in biohydrogen production through anaerobic process [79].

219

220 **3.2.2 Light and heavy metal ions**

221 Metals ions have been referred to as an important requirement for bacterial growth and enhanced
222 enzyme and co-enzyme activity [80]. However, research studies have also revealed that a high
223 concentration of metal ions can seriously discourage biohydrogen production [81]. Magnesium
224 ion is referred to as a cellular protein builder and activator and cofactor for enzymes that help
225 in the production of biohydrogen [80]. Yet, Bao et al. [82] suggested an inhibitory level of
226 20.0 mg/L Mg^{+2} suppressed the biohydrogen production from starch. Similarly, Na^+ and Ca^{+2}

227 have also been reported as micronutrients that enhance bacterial growth and cell retention
228 [83, 84]. Elsewhere, research studies have indicated that 2000 mg/L and 100 mg/L are the
229 inhibitory thresholds for Na^+ and Ca^{+2} , respectively [28, 85].

230

231 Iron has been identified as an important element to support bacterial growth, biosynthesis of
232 enzymes and to reduce the sulphide inhibition for hydrogen production [82, 86]. But similar
233 to sodium, magnesium and calcium ions, Fe^{+2} yields an inhibitory effect when the
234 concentration reaches 100 mg/L [87]. Several other metal ions have also been reported as
235 exerting inhibitory effects in biohydrogen production, for instance, 1600 mg/L for Ni^{+2} [88],
236 15, 3 and 0 mg/L for Cr, Cu, and Zn, respectively [89].

237

238 Bundhoo & Mohee [28] mentioned that dilution and precipitation as sulphides are two
239 common methods used to control metal ion concentration below the threshold level.
240 Additionally, adsorbents like activated carbons and organic ligands also could be utilized to
241 control the heavy metal ion concentrations during the dark fermentation.

242

243 **3.2.3 Furan, phenol derivatives from pre-treatment**

244 Pre-treatment processes are applied to anaerobic digestion to break up the crystalline
245 structure of cellulose and accelerate the rate of hydrolysis of the substrates [23, 90]. During
246 the pre-treatment process toxic by-products such as phenolic compounds and furan
247 derivatives are produced that have been identified to have inhibitory effect on biohydrogen
248 production [28]. The major components of furan derivatives are referred to as furfuraldehyde
249 (furfural) and hydroxymethylfurfural (HMF). Furan derivatives affect enzymic activities, cell
250 membrane function, and glycolysis of fermentative bacteria during biohydrogen production
251 [91]. Operating processes at high temperature and pressure trigger furfural production while

252 the degradation of pentose encourages the production of HMF [28]. A threshold range of 2-
253 4 g/l concentration of furfural has been identified to decrease the production of biohydrogen
254 from 29 – 63% [92].

255

256 Major phenolic components include vanillin and syringaldehyde during degradation of lignin
257 or acid hydrolysis [93]. They have been reported to affect cell membranes by either by
258 increasing their permeability or simply by damaging the membranes. As a result, the absence
259 of protective barrier of the cell cytoplasm exposes the cell to extracellular toxic compounds
260 [28, 93].

261

262 Production of furan and phenolic components cannot be avoided during pre-treatment, but
263 their concentration could be lowered to reduce the inhibitory effect. In this connection,
264 physical and chemical detoxification process may be performed by alkalination, washing and
265 removal by subjecting the pre-hydrolysate to ion exchange resin [35], whereas biological
266 detoxification involves treating the hydrolysate with enzymes or fungus [28].

267

268 **3.2.4 Alcohol and VFA components**

269 During the stage of acidogenesis in anaerobic digestion, volatile fatty acids (VFAs) and
270 alcohols are being produced. The rate of initial hydrolysis and acidogenesis is higher
271 compared to the final stage of methanogenesis where VFAs are consumed by the
272 methanogens. As a result, the high concentration of accumulated VFA causes a sharp
273 decrease in overall pH of the reactor [24, 94, 95]. The pH imbalance due to the production
274 affects the growth of HPB and reduces the production of biohydrogen. Specifically, it has
275 been asserted that ethanol does not suppress biohydrogen production compared to the VFA
276 components [96].

277 Bundhoo & Mohee [28] explained the mechanism by which the volatile fatty acids perform
278 their inhibition in biohydrogen production. During anaerobic digestion, fatty acids produced
279 in the stage of acidogenesis may be present in either ionized or non-ionized forms. The
280 ionized acid increase the ionic strength of the medium and eventually affects biohydrogen
281 production as it shifts the metabolic pathway from acidogenesis to solventogenesis (where
282 VFA is converted to organic solvents, namely butanol, acetone, and ethanol). The non-
283 ionized acids penetrate to the cell membrane, dissociate due to the higher intracellular pH and
284 finally, the H^+ concentration is increased. Cell death and suppression in biohydrogen
285 production is caused by the pH imbalance caused from the influx of protons.

286

287 **3.2.5 In process inhibitors**

288 Ammonia, generated by either NRB or nitrogen-containing compounds is considered to be a
289 source of nutrient for bacterial growth only to a certain extent, and high concentrations of
290 NH_3 and NH_4^+ have been reported as proving toxic and inhibiting biohydrogen production
291 [79, 97]. Ammonia passively diffuses into microbial cells and results abnormal cell
292 ectoplasm, imbalance in sodium potassium exchange and finally changes the pH value, which
293 affects the stability of the cell [98]. As a consequence, an imbalance in intercellular pH is
294 observed and this results in reduced biohydrogen production [28].

295

296 A high concentration of produced biohydrogen in the liquid phase results in high partial
297 pressure of hydrogen which causes biohydrogen production to decline [20]. During anaerobic
298 digestion, the reduction of protons to hydrogen is thermodynamically favorable when the
299 partial pressure of hydrogen is low. Continuous gas release, larger headspace volume,
300 vacuum stripping or the gas sparging process could be applied to avoid this problem [28].
301 Subsequently, avoiding process inhibition conditions could be the basis for increasing the

302 production of biohydrogen. Carbon content, type and composition of the feed material,
303 operating conditions, and pretreatment processes define what particular process inhibition
304 conditions need to be avoided during anaerobic hydrogen production.

305

306 **3.3 Challenges in process optimization**

307 Optimizing the anaerobic digestion for biohydrogen production could be classified into two
308 major categories: for biohydrogen production only [99]; and for simultaneous production of
309 biomethane and biohydrogen [23]. Some research studies have set out to establish the optimal
310 values of common process parameters for biohydrogen production [24, 100]. The most
311 common process parameters include temperature, pH, retention time (both HRT and SRT),
312 organic loading rate and specific chemical additives that enhance the biohydrogen
313 production. These parameters are explained in more detail below.

314

315 **3.3.1 Temperature**

316 So far, no research study has directly compared the relative biohydrogen production rate
317 using different process temperatures. The work of Zhong et al. [21] mentions 131.5 ml H₂/g-
318 COD_{removed} at 60 °C compared to 116.5 ml H₂/g-COD_{removed} at 40 °C. These findings could
319 be explained from the assertion that high temperature makes a high rate of acclimatization
320 and initial hydrolysis possible during anaerobic digestion [21, 101]. Results also documented
321 that the activity of the enzymes was higher at a high temperature [21]. As mentioned
322 previously, the products of initial hydrolysis (volatile fatty acids) act as precursors to
323 hydrogen production in the next two stages. In this scenario, the high rate of hydrolysis
324 favors hydrogen production.

325

326 For biohydrogen production, it is necessary to inhibit the activities of hydrogen consuming
327 bacteria such as methanogens. Two common processes to inhibit methanogenic activity are
328 heat and load shock treatment [24]. Jariyaboon et al. [101] has reported a temperature of
329 100 °C for sludge treatment (30 minutes) in a thermophilic two-stage anaerobic digestion
330 processing skim latex serum.

331 In this context, the major challenge in selecting the best operating temperature is considering
332 the trade-off between the cost associated in maintaining high temperature and the added
333 amount of revenue for additional biohydrogen produced at that temperature. More research
334 studies are needed to generalize the range of process operating temperatures in connection to
335 the production rate and yield of biohydrogen.

336

337 **3.3.2 pH**

338 The metabolic pathway and growth rate of microorganisms are both defined by the pH of a
339 reactor during biohydrogen production. Any change in hydrogen ion concentration results in
340 a change in pH and eventually leads to a change in redox potential. These series of events can
341 trigger a change in the rate of receiving electrons by the protons and finally the rate of
342 biohydrogen production [102]. Unlike temperature, a certain pH range has proven to favor
343 biohydrogen production. The pH 5.5-6.8 range has been reported to be ideal for biohydrogen
344 production [103], whereas a value of 4.5 seems to have an inhibitory effect on biohydrogen
345 production [102]. Same study recorded a pH of 5.5 ensured maximum biohydrogen
346 production (1.47 mol H₂/mol hexose) from noodle manufacturing wastewater [102].

347

348 The high rate of hydrolysis enables faster production of volatile fatty acids in the initial stage.
349 If the rates of acidogenesis and acetogenesis are slow compared to hydrolysis, then volatile
350 fatty acids could accumulate in the bioreactor. The accumulation of VFA could result a sharp

351 decrease in the overall pH of the reactor and eventually inhibit biohydrogen production [24].
352 Although the pH range is common for substrates with substrates with different values of
353 organic content, maintaining the reactor pH can be a challenging option. In a laboratory scale
354 study, controlling the pH of a reactor is relatively easy since the required amount of chemical
355 additives is less compared to the demands of large scale production where more cost-
356 effective methods are required to control the system's pH level.

357

358 **3.3.3 Retention time**

359 Regarding biohydrogen production, optimum values of hydraulic, solid retention time mainly
360 depend on the bioreactor arrangement and type and composition of the substrate [12, 104,
361 105]. The aim of regulating HRT for a particular bioreactor should be to retain the hydrogen-
362 producing bacteria while washing out the methane-producing bacteria. As a result, inhibition
363 of homoacetogens, hydrogenotrophic methanogens, and prevention the washout of HPB both
364 needs to be ensured [24].

365

366 The results of a recent study mentioned HRT values between 3 to 6 hours are suitable for
367 maximum biohydrogen production rate (25.9 L H₂/L-d) from granular hydrogen-producing
368 mixed cultures fed with galactose. In a thermophilic anaerobic co-digestion (80:20 mixtures
369 from municipal solid waste and food waste), maximum daily yield of biohydrogen production
370 was 2.51 L H₂/L reactor, at an SRT of 1.9 days [12]. Another experiment conducted by
371 Kumar et al. [104] specified 6-18 h as the optimum HRT for the maximum biohydrogen
372 production rate and yield (4.49 L/L/d and 1.62 mol/mol glucose, respectively).

373 In summary, longer SRT and shorter HRT have proven to be useful for the production of
374 biohydrogen, but the specific process values would vary for different types of substrates.

375 Hence, determining the HRT and SRT values for an anaerobic system processing different
376 types of substrates could be particularly challenging.

377

378 **3.3.4 Organic Loading Rate**

379 The organic content present in the substrate is later converted into molecular hydrogen during
380 anaerobic digestion. As a result, for a specific bioreactor design, the organic loading rate
381 needs to be optimized to ensure the maximum hydrogen production rate and yield. The initial
382 increase in organic loading increases the production of biohydrogen. Shen et al. [106]
383 performed an experiment to study fermentative hydrogen production at different loading rates
384 (4.0, 6.0, 13, 22 and 30 g COD L⁻¹ d⁻¹). The hydrogen production rate rose from
385 0.020 ± 0.004 to 0.196 ± 0.015 mol d⁻¹ L⁻¹ when the OLR increased from 4 to 22
386 g COD L⁻¹ d⁻¹. Later a further increase to the OLR at 30 g COD L⁻¹ d⁻¹ reduced the
387 production to 0.160 ± 0.003 mol d⁻¹ L⁻¹. The maximum value of organic loading rate is
388 dependent on the reactor type, arrangement, and type of organic substrate [24]. A novel
389 bioreactor arrangement in this connection was utilized by [107] where an integrated
390 biohydrogen reactor clarifier was coupled with CSTR. The results showed an optimum OLR
391 of 103 gCOD/L-d for maximum hydrogen production of 2.8 mol /mol glucose.

392

393 Unfortunately, increasing the loading rate can cause problems like severe membrane fouling
394 [106], reactor instability and inhibition of hydrogen production [107]. Results from the
395 experiment conducted by Hafez et al. [107] also revealed that an optimum F/M ratio (4.4–
396 6.4 gCOD/gVSS-d) should be maintained to ensure the maximum biohydrogen production
397 without any deviation in the metabolic pathways or microbial shifts from biological hydrogen
398 production. Finally, for given bioreactors type it needs to be optimized with a consideration
399 that the maximum hydrogen production process must be stable.

400 3.3.5 The OLR-HRT relationship

401 The optimum value of OLR and HRT depend on each other for a given anaerobic process.
402 Zhang et al. [105] carried out an experiment aiming to discover the effect of OLRs and HRTs
403 on hydrogen production by halophilic hydrogen-producing bacterium (HHPB). One set of
404 results included maximum 1.1 mol-H₂/mol-glucose at OLR of 20 g-glucose/L/day and HRT
405 of 12 hours. Whereas increasing the OLR from 20 to 60 g-glucose/L-reactor/day at HRT 6 h,
406 the hydrogen production rate increased for the same experiment.

407

408 Another experiment was carried out to study the effects on HRT and OLR in biohydrogen
409 production based on lactate type fermentation [108]. The experiment was performed in
410 OLRs: 10, 15, 20 and 40 g/L/day and hydraulic retention times were 6, 12 and 24 hours. The
411 study concluded an OLR of 40 g/L/day and HRT of 12 days for maximum yield of
412 continuous hydrogen production.

413 The experiment performed by Hafez et al. [107] included the application of six different
414 organic loading rates (from 6.5 gCOD/L-d to 206 gCOD/L-d) with a gravity settler where
415 solid retention time (SRT) was decoupled from the hydraulic retention time (HRT). At HRT
416 8 hours, 103 g COD/L-d was the optimum value of OLR for maximum hydrogen yield
417 (2.8 mol H₂/mol glucose).

418

419 The trend concerning the hydrogen yield and production rate from this experiment suggests
420 that the optimization approach should include one parameter that keeps the other condition
421 constant. The best combination of values for both parameters should be identified for
422 maximum biohydrogen production and yield.

423

424 3.3.6 Chemical additives of special treatment process

425 Different chemical additives have been proven to increase the efficiency of anaerobic
426 digestion, specifically the generation of methane. So far, only a few studies have observed the
427 effect of chemical additives on enhancing biohydrogen production derived from anaerobic
428 digestion.

429 Apart from the conventional anaerobic system, heat pretreatment has been observed to
430 enhance biohydrogen production. Results from one experiment included an increase from
431 14 ml H₂/gVS to 69.6 ml H₂/gVS when the inoculum was heat treated in a thermophilic
432 scenario [109]. Additionally, metal additives like FeSO₄ can effectively increase the
433 production of biohydrogen. Lee et al. [110] showed a H₂ production rate of 41.6 l/day using
434 10.9 mg FeSO₄/l H₂ which is 1.59 times higher compared to the production rate achieved at
435 2.7 mg FeSO₄/l.

436 A combination of pretreatment and adding chemicals in the first stage of anaerobic digestion
437 has increased the level of biohydrogen production. Muñoz & Steinmetz [111] carried out a
438 set of 21 set of experiments to observe the influence of pretreatment and chemical addition on
439 biohydrogen production. Heat shock as microwave (5min @800W) was proven to be
440 effective in that it produced a yield of 0.96 mol H₂/mol glucose compared to the yield of 0.62
441 mol H₂/mol glucose achieved by heat treatment at 90° C using water bath. The same set of
442 experiment used chemical additives like Bromoethanosulfonate (BES), Fe⁺³, HCl and
443 Chloroform (CHCl₃) for selective inhibition of methanogenesis. A yield of 0.52 mol H₂/mol
444 glucose was achieved at BES concentration of 7mM but no environmental impact assessment
445 is yet available for BES and its by-products.

446 Implementing any standalone or combination physical of chemical treatment process is
447 dependent on two main criteria: Firstly, the cost-benefit analysis and energy balance should

448 be performed when any pretreatment process is applied. The increase in revenue earned from
449 biohydrogen should exceed the cost associated with the treatment process. Secondly, the
450 environmental impact assessment should be performed for the selective methanogenic
451 inhibitors and their potential by-products. The number of research studies, in this case, is
452 limited, and any potential increase in biohydrogen production is still not well known.
453 Challenges lie ahead to find the optimum combination of physical and chemical treatment
454 process that would significantly increase the production of biohydrogen.

455

456 **3.4 Challenges on hydrogen storage system**

457 Developing a safe, reliable and economically feasible storage system for hydrogen is
458 currently the biggest challenge for the widespread industrial application of hydrogen as a
459 fuel. However, the envisaged storage system depends on the type of application. Gravimetric
460 density is the dominating factor for automobile applications since volume of storage is
461 limited, and a feasible driving range is expected [112]. For hydrogen transportation, process
462 safety and high density of hydrogen are the predominant conditions for hydrogen storage.
463 Figure 2 summarizes the basic categories of hydrogen storage system and their corresponding
464 issues and applications.

465

466

467

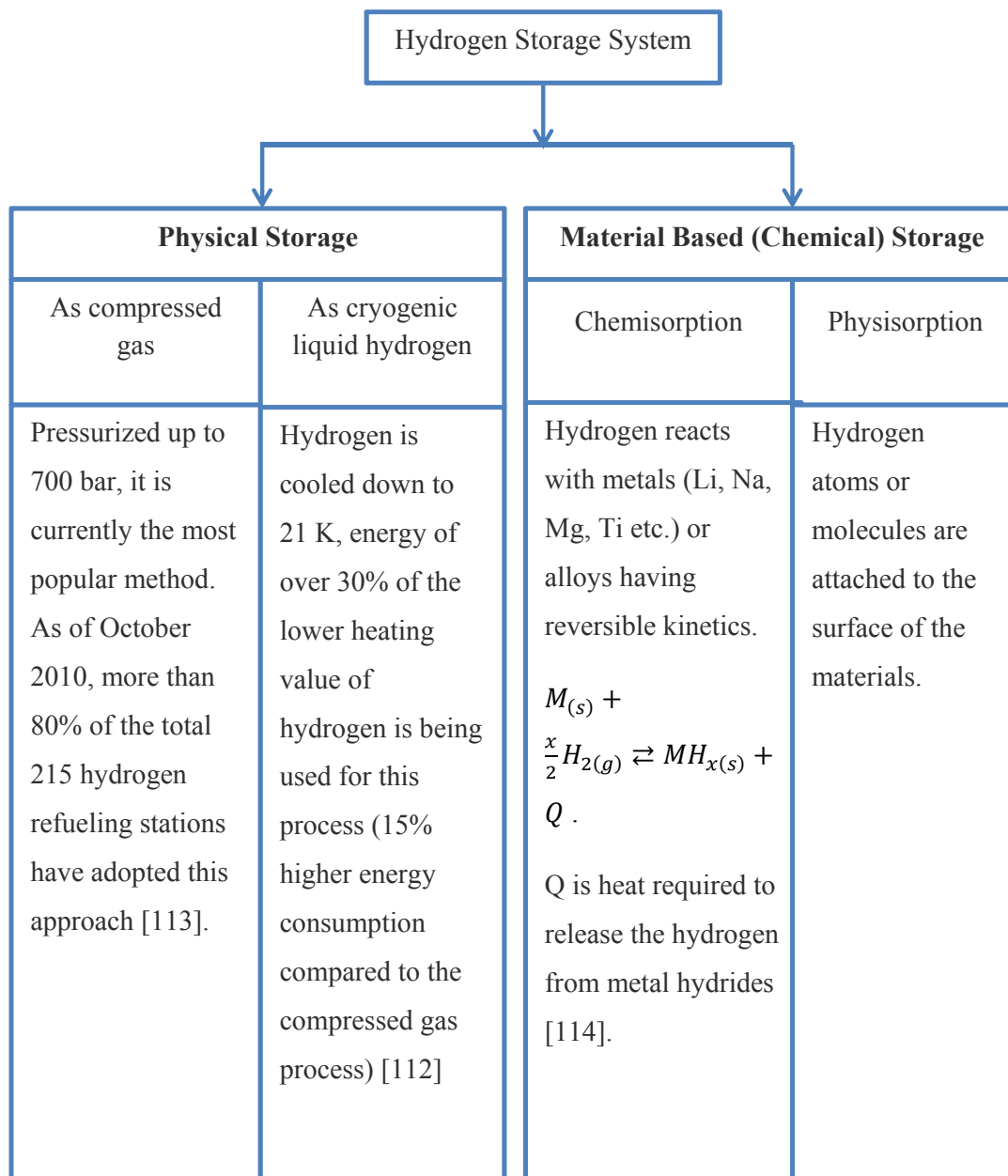
468

469

470

471

472



473

474 Figure 2: Basic categories of hydrogen production system

475 The common hydrogen storage techniques mentioned suffer from some technical difficulties.

476 These disadvantages are as follows:

- 477 i) Low storage density is the main physical storage problem for hydrogen as compressed
 478 gas. According to Zhang et al. [112], the gravimetric densities of hydrogen are 5.5 and
 479 4.6 wt. % at 350 and 700 bars, respectively. This outcome implies the compressed

480 storage system of hydrogen as gas is not feasible for automobiles [112, 115].
481 Challenges lie ahead to identify a suitable material that can meet the targets of
482 volumetric capacity in addition to stress and safety for high pressure storage tanks.

483 ii) Low pressure liquid hydrogen storage system has advantages including high fuel
484 density and low cost compared to the pressurized storage as compressed gas but has a
485 technical limitation due to the evaporative loss of hydrogen [4]. Additionally, the
486 expensiveness of high energy consumption during the liquefaction process is a major
487 hurdle for this process [112].

488 iii) Boiling-off for liquid hydrogen storage is another problem where the system can lose
489 up to 2-3% hydrogen in a single day [112]. This scenario not only contributes to the
490 fueling frequency and cost but also poses a potential threat to safety when the storage
491 system is located in a confined space.

492 iv) Hydrogen embrittlement is a general issue for hydrogen storage where materials in
493 continuous contact with hydrogen become brittle. Lone hydrogen atoms are diffused
494 into the metal structure, and the recombined hydrogen molecules atoms create pressure
495 from the cavity and initiate a crack in the metal [1]. The single metal or alloy with the
496 ability to avoid embrittlement is yet to be identified.

497 v) Pure metal hydrides have individual drawbacks in the hydrogen storage system, for
498 instance: NaH and CaH₂ have good reversible kinetics but low wt. % (4.2 and 4.8,
499 respectively) of hydrogen, MgH₂ and LiH has poor reversibility, and AlH₃ production
500 demands high pressure [116]. As a result, recent research focus is more inclined to
501 finding the best composition for a metal alloy that makes hydride production possible,
502 and reversible reaction kinetics can be applied for hydrogen recovery.

503 vi) The most recent research approaches include the formation of multi-cation
504 borohydrides $MM'(BH_4)_n$ including ZrLi(BH₄)₅, ZrLi₂(BH₄)₆, LiK(BH₄)₂, LiSc(BH₄)₄

505 [112, 117] and amides $M(NH_2)_x$ due to their high storage capacity and low operating
 506 temperature [112]. However, their application is limited due to poor absorption capacity
 507 and costs involved in adsorption/desorption [118].

508 Apart from the technical challenges that are evident in hydrogen storage, cost and practicality
 509 have not made widespread industrial application feasible. The following table includes the
 510 current and projected target for H₂ storage systems.

511 Table 4: Target and current status of H₂ storage technologies [119]

Storage targets	Gravimetric kWh/kg(kgH₂/kg system)	Volumetric kWh/L(kgH₂/L system)	Costs\$/kWh(\$ /kgH₂)
2017	1.8 (0.055)	1.3 (0.040)	\$12 (\$400)
Ultimate	205 (0.075)	2.3 (0.070)	\$8 (\$266)
Projected H₂ storage system performance	Gravimetric (kWh/kg)	Volumetric (kWh/L)	Costs (\$/kWh)
700 bar compressed (Type IV)	1.5	0.8	17
350 bar compressed (Type IV)	1.8	0.6	13
Metal hydride (NaAlH ₄)	0.4	0.4	TBD
Sorbent (MOF-5, 100 bar)	1.1	0.7	16
Chemical hydrogen storage (AB-50 wt. %)	1.7	1.3	16

512

513 **4. Potential of biohydrogen as renewable energy**

514 **4.1 Predicting the availability of fossil fuels in the future**

515 The current usage of fossil fuel is approximately 89 million barrels per day according to the
516 statistics published by the International Energy Agency (IEA) [1]. The Intergovernmental
517 Panel on Climate Change (IPCC) has reported that 275 GtC of fossil fuels could be produced
518 from the total reserve of 746 GtC for the remainder of this century only if the anthropogenic
519 climate change is controlled to ≤ 2 °C [120]. However, it is particularly difficult to project the
520 demand for fossil fuels in the future since the level of demand is dependent on fuel cost and
521 supply. Asafu et al. [121] carried out a pooled mean group analysis to predict economic
522 growth and fossil and non-fossil fuel consumption. They concluded an annual worldwide
523 growth rate of 2.41% and 3.62% for annual fossil fuel and non-fossil fuel energy sources,
524 respectively.

525

526 The availability of fossil fuels in the future is subjected to three major uncertainties:
527 technical, economic and political situations [122]. Technical advances like hydraulic
528 fracturing (fracking), deep water seismic survey, long-wall mining, etc., have increased the
529 amount of fossil fuel obtained from reservoirs. Conversely, the cost involved in these
530 advanced technologies should be considered too. Research studies have shown that the cost
531 can increase from 7.65 to 15.2 million USD when the oil well depth changes from 2400 to
532 4600 meters [123]. Furthermore, recovering oil and gas from hydraulic cracking has negative
533 environmental and economic factors such as water and air pollution, use of additional
534 infrastructure and high water consumption (6 million gallons of pressurized water with
535 chemicals) [124]. Under these circumstances, technological advances are not always
536 sustainable regarding cost and the long term impact on the environment.

537 As a result, relying on the fossil fuel reserve is not the best possible option when considering
 538 energy sustainability over the long term. In 2012, fossil fuel contributed to 68% of the total
 539 electricity generated worldwide. On the other hand, the overall renewable energy production
 540 was 4862 TWh [125]. By 2018 it is predicted that overall renewable energy will reach 6851
 541 TWh [125]. It can be stated here that the gradual depletion of fossil fuel resources, expensive
 542 recovery and exponential growth in demand poses a serious threat to the sustainability of
 543 energy supplies. Biohydrogen production could be the key to achieving sustainable sources of
 544 renewable energy in the future.

545 4.2 Energy density and fuel properties

546 Compared to conventional fossil fuel energy sources, hydrogen is considered to be an
 547 important alternative because of its high energy density. The average energy density of
 548 hydrogen per mass content is equal to 143 MJ kg^{-1} , which is about three times more
 549 compared to the energy densities of fossil fuels [1]. It has been reported that 10% blend of
 550 hydrogen with natural gas in internal combustion engines can reduce up to 95% greenhouse
 551 gas emissions [18]. Table 5 compares the energy densities of some common fuels.

552 Table 5: Energy density of some common fuels (Modified from [1, 126]).

Fuel	Process conditions	Energy per kilogram (MJ kg⁻¹)
Hydrogen (liquid)	Liquid	143
Hydrogen (compressed, 700 bar)	Compressed at 700 bar	143
Hydrogen (ambient pressure)	Ambient pressure	143
Methane (ambient pressure)	Ambient pressure	55.6

Fuel	Process conditions	Energy per kilogram (MJ kg⁻¹)
Natural gas (liquid)	Liquid	53.6
Natural gas (compressed, 250 bar)	Compressed at 250 bar	53.6
Natural gas	-	53.6
Liquefied natural gas (LNG)		19.9
Compressed natural gas (CNG)		19.9
LPG propane	-	49.6
LPG butane	-	49.1
Gasoline (petrol)	-	46.4
Biodiesel oil	-	42.2
Diesel	-	45.4
Methanol		22.7
Ethanol		29.7

553

554 Considering an alternative source of energy, hydrogen has a great advantage over other
555 renewable sources. Methanol or ethanol has an energy density of 19.98 which is significantly
556 less than hydrogen. Also, the flash points of methanol and ethanol are 11 and 13 °C,
557 respectively, and this means the alcohol storage tank would have a flammable atmosphere
558 even at an ambient temperature. Table 6 below presents an additional list of the properties of
559 hydrogen and some other common fuels that are important to evaluate their potential as fuel.

560

561 Table 6: Comparison of common fuel properties between gasoline, methane, and hydrogen
562 (modified from [126, 127]).

Property	Gasoline	Methane	Hydrogen
-----------------	-----------------	----------------	-----------------

Minimum ignition energy (mJ)	0.2	0.2	0.017
Diffusion coefficient in air (cm ² /s)	0.05	0.16	0.610
Specific heat at constant pressure (J/kg K)	1.20	2.22	14.89
Flammable limit in air (vol%)	1.0–7.6	5.3–15.0	4.0–75.0
Flammable energy in air (MJ)	0.24	0.29	0.02
Flammable temperature (K)	501–744	813	858
Flame temperature in air (K)	2470	2148	2318
Explosion limit in air (vol%)	1.1–3.3	6.3–14.0	13.0–59.0

563

564 In addition to the energy density, two other properties of hydrogen have made this more
565 sensitive to ignition. Firstly, the minimum ignition energy (minimum energy required to
566 ignite an optimum air-fuel mixture) for hydrogen has been recorded as 0.017 mJ compared to
567 the value of 0.2 mJ for hydrocarbons [126]. Secondly, the flammable limit for hydrogen is 4-
568 75 % (v/v) compared to the value of 1.4–7.4 % (v/v) for gasoline which explains why
569 hydrogen could detonate readily compared to hydrocarbons [126].

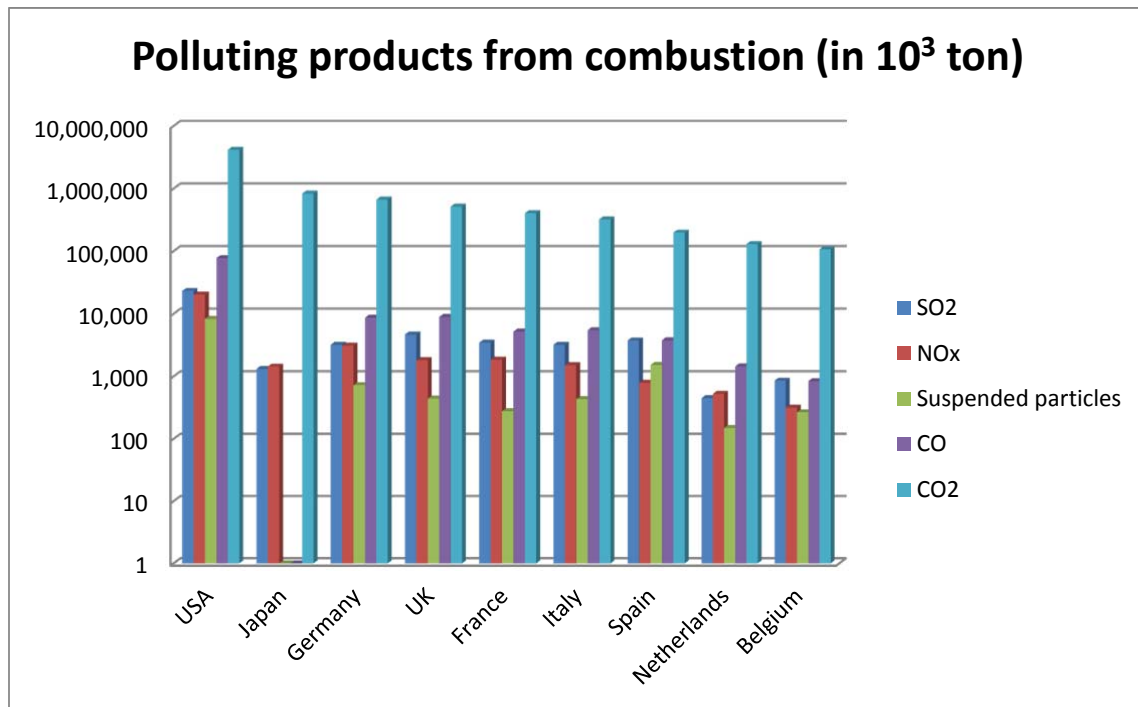
570

571 **4.3 Effect on the environment**

572 Conservation of the environment and sustainability are now the most prioritized section in
573 aspects of any fossil fuel's evaluation. The primary product from the combustion of fossil
574 fuels is CO₂ and CO where additional impurities' supply of air also leads to the production of
575 NO_x and oxides of sulfur [127]. In contrast, the only possible product from the combustion
576 of hydrogen is water vapor which has no significant direct effect on the environment or
577 human health. The statistics related to the emission of pollutants, such as SO_x, NO_x, carbon
578 monoxide and carbon dioxide emphasize the superiority of hydrogen over fossil fuels. To

579 provide an idea of the effects of air pollution, Figure 3 lists the common industrial pollutants
580 that are now typical in industrialized countries.

581



582

583 Figure 3: Comparison between the main pollution emissions in the industrialized
584 countries (Data adapted from [127]).

585

586 Nicoletti et al. [127] compared the weighted percentages of pollutants in combustion flue gas
587 concerning hydrogen, carbon, methane and octane. From the results listed in Table 7, it is
588 clearly evident that the combustion of hydrogen offers zero emissions regarding CO₂ and SO₂
589 apart from the emission of nitrogen oxides. The formation of NO_x is a function of flame
590 temperature and duration [128]. Considering the broad flammability range of hydrogen, its
591 combustion can be influenced how an engine has been designed, so the aim should be to
592 reduce NO_x emissions.

593

594 Table 7: Weighted percentages of pollutants in combustion flue gas for common fuels

Fuel	kg Pollutants /kg of fuel				
	CO ₂	SO ₂	NO _x	Un-burnts, particulates	H ₂ O
H ₂	0	0	0.016	0	7
C	1.893	0.012	0.008	0.1	0.633
CH ₄	2.75	0.03	0.0075	0	2.154
C ₈ H ₁₈	3.09	0.010	0.0115	0.85	1.254

595

596 The table does not include pollutants like Volatile Organic Materials (VOCs), radioactive
 597 materials and heavy metals that may be present with fossil fuels during combustion. Impacts
 598 on the environment that are additional to greenhouse gas emissions have been mentioned by
 599 Khan et al. [23]. For example, methane containing biogas is produced by the process of
 600 anaerobic digestion. Besides combustion, methane could be present in the liquid effluent that
 601 leads to environmental problems like eutrophication, marine aquatic eco-toxicity, freshwater
 602 aquatic eco-toxicity, terrestrial eco-toxicity, human toxicity, etc. Since hydrogen is not
 603 soluble in water, the production of hydrogen from anaerobic digestion could eliminate these
 604 serious environmental problems.

605

606 The advantage of hydrogen as a clean energy source is evident in that it reduces the release of
 607 pollutants into the environment. To limit the rise of global average temperature < 2°C,
 608 maximum allowable emission limit carbon dioxide should be around 565-886 billion tones
 609 until 2050 [129]. Achieving this target would only be possible if more emphasis is given on
 610 developing and employing alternative energy sources like hydrogen.

611 **4.4 Estimating cost of hydrogen compared to conventional energy sources**

612 A typical cost analysis model includes the energy supply system (production cost, production
613 level, available resources, etc.), energy markets (fuel prices, price adjustment), consumer
614 choice behavior (consumer utility, fuel demand, vehicle adoption) and refueling infrastructure
615 [130]. The following discussion will focus on the production and storage cost of hydrogen as
616 fuel.

617

618 Hydrogen production on an industrial scale is not considered to be an economically feasible
619 option. The sustainability of hydrogen production has been criticized because the production
620 process from carbon sources requires fossil fuels as raw materials and external costs are
621 associated with carbon capture and storage. Furthermore, production of hydrogen from
622 anaerobic digestion is also variable as different anaerobic process contains substrates
623 containing different carbon composition. Some cost comparisons have been done regarding
624 different hydrogen production processes [131], but it has been particularly challenging to
625 compare hydrogen production costs to continuously changing fuel and oil prices. Table 8
626 contains the summary of overall hydrogen production cost from different raw materials and
627 processes.

628 Table 8: Cost of hydrogen production using different energy sources

Raw material	Process	Production cost (\$/kgH₂)	References
Natural gas	Steam Methane Reforming	0.75	[132]
Natural gas	Steam Methane Reforming (with carbon capture & storage)	2.67	[133]
Nuclear	Electrolysis	2.4	

Nuclear	High Temperature Electrolysis	3.5	[134]
Nuclear	Copper–chlorine	1.7	
Nuclear	Sulfur–iodine cycle	1.9	[135]
Coal	Gasification (with carbon capture & storage)	1.8	
Solar	Electrolysis	7.7	[136]
Solar	Photovoltaic electrolysis	9.1	[137]
Solar	Photoelectrochemical	3.5	[138]
Wind	Electrolysis	7.2	[135]
Wind	Electrolysis	7.3	[139]
Biomass	Gasification	1.65	[140]
Biomass	Gasification	1.4–2	[141]
Biomass	Pyrolysis	1.3–2.2	
Biomass	Gasification	4.60–7.86	[142]
Geothermal	Steam electrolysis	1–2.6	[143]

629

630 From Table 8, it is evident that the lowest production cost for steam methane reforming
631 process is 0.75 USD per kg of hydrogen whereas the maximum retail price of gasoline was
632 3.5 USD per gallon considering the time from 1994 to 2011. However, the costs associated
633 with carbon capture and storage from CO₂ produced from gasoline has not been accounted for
634 in this calculation. Additionally, it is expected that by the year 2030 the supply/demand gap
635 in global oil and gas production will increase when demand for energy will rise by 60%
636 [131]. It will, therefore, be predicted that oil and gas prices will rise over time in the next few
637 decades.

638

639 The costs involved in carbon capture and storage are variable since the type of carbon
640 capture, and storage process differ, and the expenses required for building the infrastructure
641 for CCS also vary. The US National Energy Technology Laboratory has estimated USD 16/t
642 CO₂ for carbon capture and storage [144]. Given the current trend of fossil fuel usage, global
643 CO₂ emissions could rise to 44 billion tones by 2040 [145], and it means that approximately
644 \$704 billion USD will have to be spent on capture and storage technologies and processes.
645 Such costs can only be reduced if the increased demand for energy is satisfied by the
646 production of biohydrogen from renewable energy sources.

647

648 An average of 50 million metric tons of hydrogen is produced worldwide annually where 76–
649 77% of the produced hydrogen is converted from natural gas and oil (Naphtha), 19–20% is
650 produced from coal, and the remaining 3–4% is produced from renewable sources [131]. As
651 the majority of hydrogen production involves the usage of fossil fuel as raw materials,
652 production costs have not been competitive enough compared to the traditional energy
653 sources such as gasoline or petrol. For example, Lee [146] performed cost benefit analysis
654 and evaluation of financial feasibility of full commercialization of biohydrogen. The study
655 was performed on cost-capacity scaling methods for different biohydrogen production plants.
656 Their final results showed 2.20, 3.37, and 3.85 benefit/cost ratios for three different scenarios
657 respectively. Additionally, Internal Rate of Return (IRR) and External Rate of Return (ERR)
658 were calculated to 42.45%, 58.71%, 62.77%, and 14.40%, 16.05%, and 16.53% in payback
659 periods of 11.33, 8.95 and 8.52 years.

660

661 Another study involved the cost benefit analysis of carbon footprint from hydrogen fueled
662 scooter and Internal Combustion Engine (ICE) scooters [147]. The experiment came up with
663 a result that the hydrogen fueled scooter from Steam Methane Reforming (SMR) process has

664 the smallest carbon emission (0.0115 kg CO_2). As a result, the cost involved in carbon
665 capture and storage would be less compared to the biohydrogen production process [148].
666 Same study measured the total life cycle and came up with a result that the total life cycle
667 cost (excluding fixed costs i.e. cost of the vehicle, hydrogen production unit, etc.) is
668 maximum (USD 6632) for the hydrogen fueled scooter whereas the ICE scooters have an
669 amount of USD 4233. However, the reason was quite obvious as the hydrogen fueled scooter
670 was using the SMR process. As a result, hydrogen fuel generation from biomass could be an
671 interesting research option to reduce the total life cycle costs for hydrogen fueled vehicles.

672

673 On the other hand, some cost benefit analysis has produced negative results leaving the
674 concept of hydrogen fueled vehicles not being a feasible option. Ito & Managi [149]
675 investigated the economic validity of diffusion of hydrogen Fuel Cell Vehicles (FCV) and all
676 Electric Vehicle (EV) in Japan. The differences between net present value between benefit
677 and cost were studied to find out the economically feasible option between these two. The
678 highest net positive value (NPV) was – 19 billion dollar based on 5 million FCV vehicle
679 diffusion scenarios. However, the major limitation of this study lies in the calculation of total
680 cost estimation. The authors estimated the differences in vehicle purchase and operating costs
681 for FCV and Internal Combustion Vehicles (ICV) and added the differences to find out the
682 total cost. Surely, the actual cost was not reflected during the cost benefit analysis of this
683 study.

684

685 Regarding energy storage and transport, hydrogen fuel could be considered as an
686 economically favorable option for term storage. A study compared the cost comparison of
687 pumped-hydro, hydrogen storage and compressed air energy [150]. This calculation included
688 the average discounted electricity generation cost, termed as “Levelized Electricity cost”

689 (LEC) for three different energy sources. For a long term storage scenario, the findings
690 included a reduction of 70% LEC for hydrogen storage compared to 10% and 20% reductions
691 for pumped hydro and compressed air storage, respectively. This research study suggested
692 that by 2030 hydrogen storage would emerge as the best source of energy for all storage-
693 discharge paths.

694 In a summary, the available cost benefit analyses for hydrogen fuel have been subjected to
695 specific usage conditions, but the major limitation is the exclusion of carbon capture and
696 storage cost when fossil fuels are used for hydrogen generation. Therefore, cost benefit
697 analysis for biohydrogen production could be a future scope of research for improved
698 economic feasibility.

699 **5. Conclusion**

700 The potential of biohydrogen regarding energy efficiency, environmental impact and cost-
701 effectiveness has been described in detail in this paper. It is evident that biohydrogen is one
702 of the best – if not the best - alternatives to fossil fuel energy. Biohydrogen production also
703 offers an added advantage of not posing a threat to the environment during production, and
704 there are zero external costs for carbon capture and storage. Unfortunately, proposed large
705 scale industrial production has been hindered by some important technical and economic
706 challenges. The knowledge garnered from existing research studies could be utilized to
707 design a generic production model that makes the production of biohydrogen profitable and
708 sustainable.

709

710 **Acknowledgements**

711

712 This work was supported by the Centre for Technology in Water and Wastewater (CTWW),

713 School of Civil and Environmental Engineering (CEE), University of Technology Sydney
714 (UTS) and Australian Postgraduate Award.

715 **6. References**

- 716 [1] K. Mazloomi, C. Gomes, Hydrogen as an energy carrier: Prospects and challenges,
717 *Renew. Sust. Energ. Rev.* 16(5) (2012) 3024-3033.
- 718 [2] T. Abbasi, S.A. Abbasi, 'Renewable' hydrogen: Prospects and challenges, *Renew. Sust.*
719 *Energ. Rev.* 15(6) (2011) 3034-3040.
- 720 [3] A. Marone, G. Izzo, L. Mentuccia, G. Massini, P. Paganin, S. Rosa, C. Varrone, A.
721 Signorini, Vegetable waste as substrate and source of suitable microflora for bio-hydrogen
722 production, *Renew. Energ.*, 68 (2014) 6-13.
- 723 [4] S.M. Aceves, G. Petitpas, F. Espinosa-Loza, M.J. Matthews, E. Ledesma-Orozco, Safe,
724 long range, inexpensive and rapidly refuelable hydrogen vehicles with cryogenic pressure
725 vessels, *Int. J. Hydrogen Energ.*, 38(5) (2013) 2480-2489.
- 726 [5] H. Argun, F. Kargi, Bio-hydrogen production by different operational modes of dark and
727 photo-fermentation: An overview, *Int. J. Hydrogen Energ.*, 36(13) (2011) 7443-7459.
- 728 [6] C.-C. Chen, Y.-S. Chuang, C.-Y. Lin, C.-H. Lay, B. Sen, Thermophilic dark fermentation
729 of untreated rice straw using mixed cultures for hydrogen production, *Int. J. Hydrogen*
730 *Energ.*, 37(20) (2012) 15540-15546.
- 731 [7] D. Ghosh, I.F. Sobro, P.C. Hallenbeck, Optimization of the hydrogen yield from single-
732 stage photofermentation of glucose by *Rhodobacter capsulatus* JP91 using response surface
733 methodology, *Bioresource Technol.*, 123 (2012).
- 734 [8] R. Kothari, D. Buddhi, R.L. Sawhney, Comparison of environmental and economic
735 aspects of various hydrogen production methods, *Renew. Sust. Energ. Rev.* 12(2) (2008) 553-
736 563.

737 [9] C. Mao, Y. Feng, X. Wang, G. Ren, Review on research achievements of biogas from
738 anaerobic digestion, *Renew. Sust. Energ. Rev.* 45 (2015) 540-555.

739 [10] F. Passos, S. Astals, I. Ferrer, Anaerobic digestion of microalgal biomass after
740 ultrasound pretreatment, *Waste Manage.* 34(11) (2014) 2098-2103.

741 [11] Y. Shen, J.L. Linville, M. Urgan-Demirtas, R.P. Schoene, S.W. Snyder, Producing
742 pipeline-quality biomethane via anaerobic digestion of sludge amended with corn stover
743 biochar with in-situ CO₂ removal, *Appl. Energ.* 158 (2015) 300-309.

744 [12] R. Angeriz-Campoy, C.J. Alvarez-Gallego, L.I. Romero-Garcia, Thermophilic anaerobic
745 co-digestion of organic fraction of municipal solid waste (OFMSW) with food waste (FW):
746 Enhancement of bio-hydrogen production, *Bioresource Technol.*, 194 (2015) 291-6.

747 [13] J. Cheng, M. Zhu, A novel anaerobic co-culture system for bio-hydrogen production
748 from sugarcane bagasse, *Bioresource Technol.*, 144 (2013).

749 [14] M. Cui, J. Shen, Effects of acid and alkaline pretreatments on the biohydrogen
750 production from grass by anaerobic dark fermentation, *Int. J. Hydrogen Energ.*, 37(1) (2012)
751 1120-1124.

752 [15] M.H. Hwang, N.J. Jang, S.H. Hyun, I.S. Kim, Anaerobic bio-hydrogen production from
753 ethanol fermentation: the role of pH, *J. Biotechnol.* 111(3) (2004) 297-309.

754 [16] P. Intanoo, P. Chaimongkol, S. Chavadej, Hydrogen and methane production from
755 cassava wastewater using two-stage upflow anaerobic sludge blanket reactors (UASB) with
756 an emphasis on maximum hydrogen production, *Int. J. Hydrogen Energ.*, 41(14) (2016)
757 6107-6114.

758 [17] C. Moon, S. Jang, Y.-M. Yun, M.-K. Lee, D.-H. Kim, W.-S. Kang, S.-S. Kwak, M.-S.
759 Kim, Effect of the accuracy of pH control on hydrogen fermentation, *Bioresource Technol.*,
760 179 (2015) 595-601.

- 761 [18] A.K. Patel, N. Vaisnav, A. Mathur, R. Gupta, D.K. Tuli, Whey waste as potential
762 feedstock for biohydrogen production, *Renew. Energ.*, 98 (2016) 221-225.
- 763 [19] R.G. Puhulwella, L. Beckers, F. Delvigne, A.S. Grigorescu, P. Thonart, S. Hiligsmann,
764 Mesophilic biohydrogen production by *Clostridium butyricum* CWBI1009 in trickling
765 biofilter reactor, *Int. J. Hydrogen Energ.*, 39(30) (2014) 16902-16913.
- 766 [20] I. Valdez-Vazquez, H.M. Poggi-Varaldo, Hydrogen production by fermentative
767 consortia, *Renew. Sust. Energ. Rev.* 13(5) (2009) 1000-1013.
- 768 [21] J. Zhong, D.K. Stevens, C.L. Hansen, Optimization of anaerobic hydrogen and methane
769 production from dairy processing waste using a two-stage digestion in induced bed reactors
770 (IBR), *Int. J. Hydrogen Energ.*, 40(45) (2015) 15470-15476.
- 771 [22] K.F. Adekunle, J.A. Okolie, A Review of Biochemical Process of Anaerobic Digestion,
772 *Adv. Biosci. Biotechnol.* 06(03) (2015) 205-212.
- 773 [23] M.A. Khan, H.H. Ngo, W.S. Guo, Y.W. Liu, J.L. Zhou, J. Zhang, S. Liang, B.J. Ni, X.B.
774 Zhang, J. Wang, Comparing the value of bioproducts from different stages of anaerobic
775 membrane bioreactors, *Bioresource Technol.*, 214 (2016) 816-25.
- 776 [24] M.A. Khan, H.H. Ngo, W.S. Guo, Y. Liu, L.D. Nghiem, F.I. Hai, L.J. Deng, J. Wang, Y.
777 Wu, Optimization of process parameters for production of volatile fatty acid, biohydrogen
778 and methane from anaerobic digestion, *Bioresource Technol.*, 219 (2016) 738-48.
- 779 [25] P. Kumaran, D. Hephzibah, R. Sivasankari, N. Saifuddin, A.H. Shamsuddin, A review
780 on industrial scale anaerobic digestion systems deployment in Malaysia: Opportunities and
781 challenges, *Renew. Sust. Energ. Rev.* 56 (2016) 929-940.
- 782 [26] P. Kandyliis, A. Bekatorou, K. Pissaridi, K. Lappa, A. Dima, M. Kanellaki, A.A.
783 Koutinas, Acidogenesis of cellulosic hydrolysates for new generation biofuels, *Biomass*
784 *Bioenerg.* 91 (2016) 210-216.

785 [27] A. Bar-Even, Does acetogenesis really require especially low reduction potential?, *BBA.*
786 *Bioenergetics* 1827(3) (2013) 395-400.

787 [28] M.A.Z. Bundhoo, R. Mohee, Inhibition of dark fermentative bio-hydrogen production: A
788 review, *Int. J. Hydrogen Energ.*, 41(16) (2016) 6713-6733.

789 [29] N.M.C. Saady, Homoacetogenesis during hydrogen production by mixed cultures dark
790 fermentation: Unresolved challenge, *Int. J. Hydrogen Energ.*, 38(30) (2013) 13172-13191.

791 [30] L. Andre, M. Ndiaye, M. Pernier, O. Lespinard, A. Paus, E. Lamy, T. Ribeiro, Methane
792 production improvement by modulation of solid phase immersion in dry batch anaerobic
793 digestion process: Dynamic of methanogen populations, *Bioresource Technol.*, 207 (2016)
794 353-60.

795 [31] F. Sarmiento, J.A. Leigh, W.B. Whitman, Genetic systems for hydrogenotrophic
796 methanogens, *Meth. Enzymol.* 494 (2011) 43-73.

797 [32] Y. Zhang, F. Zhang, M. Chen, P.-N. Chu, J. Ding, R.J. Zeng, Hydrogen supersaturation
798 in extreme-thermophilic (70 °C) mixed culture fermentation, *Appl. Energ.* 109 (2013) 213-
799 219.

800 [33] B. Si, Z. Liu, Y. Zhang, J. Li, X.-H. Xing, B. Li, N. Duan, H. Lu, Effect of reaction
801 mode on biohydrogen production and its microbial diversity, *Int. J. Hydrogen Energ.*, 40(8)
802 (2015) 3191-3200.

803 [34] X. Wang, J. Ding, N.-Q. Ren, B.-F. Liu, W.-Q. Guo, CFD simulation of an expanded
804 granular sludge bed (EGSB) reactor for biohydrogen production, *Int. J. Hydrogen Energ.*,
805 34(24) (2009) 9686-9695.

806 [35] G. Kumar, P. Bakonyi, S. Periyasamy, S.H. Kim, N. Nemestóthy, K. Bélafi-Bakó,
807 Lignocellulose biohydrogen: Practical challenges and recent progress, *Renew. Sust. Energ.*
808 *Rev.* 44 (2015) 728-737.

809 [36] M.-S. Kim, D.-Y. Lee, Fermentative hydrogen production from tofu-processing waste
810 and anaerobic digester sludge using microbial consortium, *Bioresource Technol.*, 101(1,
811 Supplement) (2010) S48-S52.

812 [37] X. Wu, H. Yang, L. Guo, Effect of operation parameters on anaerobic fermentation
813 using cow dung as a source of microorganisms, *Int. J. Hydrogen Energ.*, 35(1) (2010) 46-51.

814 [38] D. Karadag, J.A. Puhakka, Direction of glucose fermentation towards hydrogen or
815 ethanol production through on-line pH control, *Int. J. Hydrogen Energ.*, 35(19) (2010) 10245-
816 10251.

817 [39] S.R. Chaganti, B. Pendyala, J.A. Lalman, S.S. Veeravalli, D.D. Heath, Influence of
818 linoleic acid, pH and HRT on anaerobic microbial populations and metabolic shifts in ASBRs
819 during dark hydrogen fermentation of lignocellulosic sugars, *Int. J. Hydrogen Energ.*, 38(5)
820 (2013) 2212-2220.

821 [40] C.-N. Lin, S.-Y. Wu, J.-S. Chang, J.-S. Chang, Biohydrogen production in a three-phase
822 fluidized bed bioreactor using sewage sludge immobilized by ethylene–vinyl acetate
823 copolymer, *Bioresource Technol.*, 100(13) (2009) 3298-3301.

824 [41] B. Baghchehsaraee, G. Nakhla, D. Karamanev, A. Margaritis, Fermentative hydrogen
825 production by diverse microflora, *Int. J. Hydrogen Energ.*, 35(10) (2010) 5021-5027.

826 [42] E. Wicher, K. Seifert, R. Zagrodnik, B. Pietrzyk, M. Laniecki, Hydrogen gas production
827 from distillery wastewater by dark fermentation, *Int. J. Hydrogen Energ.*, 38(19) (2013)
828 7767-7773.

829 [43] T. Doi, H. Matsumoto, J. Abe, S. Morita, Application of rice rhizosphere microflora for
830 hydrogen production from apple pomace, *Int. J. Hydrogen Energ.*, 35(14) (2010) 7369-7376.

831 [44] B. Kirli, I.K. Kapdan, Selection of microorganism immobilization particle for dark
832 fermentative biohydrogen production by repeated batch operation, *Renew. Energ.*, 87, Part 1
833 (2016) 697-702.

834 [45] Z. Ma, C. Li, H. Su, Dark bio-hydrogen fermentation by an immobilized mixed culture
835 of *Bacillus cereus* and *Brevumdimonas naejangsanensis*, *Renew. Energ.*, 105 (2017) 458-
836 464.

837 [46] M.-D. Bai, Y.-C. Chao, Y.-H. Lin, W.-C. Lu, H.-T. Lee, Immobilized biofilm used as
838 seeding source in batch biohydrogen fermentation, *Renew. Energ.*, 34(8) (2009) 1969-1972.

839 [47] S.N.A. Rahman, M.S. Masdar, M.I. Rosli, E.H. Majlan, T. Husaini, S.K. Kamarudin,
840 W.R.W. Daud, Overview biohydrogen technologies and application in fuel cell technology,
841 *Renew. Sust. Energ. Rev.* 66 (2016) 137-162.

842 [48] G.C. Premier, J.R. Kim, J. Massanet-Nicolau, G. Kyazze, S.R.R. Esteves, B.K.V.
843 Penumathsa, J. Rodríguez, J. Maddy, R.M. Dinsdale, A.J. Guwy, Integration of biohydrogen,
844 biomethane and bioelectrochemical systems, *Renew. Energ.*, 49 (2013) 188-192.

845 [49] A. Kadier, M.S. Kalil, P. Abdeshahian, K. Chandrasekhar, A. Mohamed, N.F. Azman,
846 W. Logroño, Y. Simayi, A.A. Hamid, Recent advances and emerging challenges in microbial
847 electrolysis cells (MECs) for microbial production of hydrogen and value-added chemicals,
848 *Renew. Sust. Energ. Rev.* 61 (2016) 501-525.

849 [50] S. Cheng, B.E. Logan, High hydrogen production rate of microbial electrolysis cell
850 (MEC) with reduced electrode spacing, *Bioresource Technol.*, 102(3) (2011) 3571-3574.

851 [51] T. Wu, G. Zhu, A.K. Jha, R. Zou, L. Liu, X. Huang, C. Liu, Hydrogen production with
852 effluent from an anaerobic baffled reactor (ABR) using a single-chamber microbial
853 electrolysis cell (MEC), *Int. J. Hydrogen Energ.*, 38(25) (2013) 11117-11123.

854 [52] A.J. Guwy, R.M. Dinsdale, J.R. Kim, J. Massanet-Nicolau, G. Premier, Fermentative
855 biohydrogen production systems integration, *Bioresource Technol.*, 102(18) (2011) 8534-
856 8542.

857 [53] Y. Sharma, B. Li, Optimizing energy harvest in wastewater treatment by combining
858 anaerobic hydrogen producing biofermentor (HPB) and microbial fuel cell (MFC), *Int. J.*
859 *Hydrogen Energ.*, 35(8) (2010) 3789-3797.

860 [54] G. Mohanakrishna, S. Venkata Mohan, P.N. Sarma, Utilizing acid-rich effluents of
861 fermentative hydrogen production process as substrate for harnessing bioelectricity: An
862 integrative approach, *Int. J. Hydrogen Energ.*, 35(8) (2010) 3440-3449.

863 [55] L. Lu, N. Ren, D. Xing, B.E. Logan, Hydrogen production with effluent from an
864 ethanol-H₂-coproducing fermentation reactor using a single-chamber microbial electrolysis
865 cell, *Biosens. Bioelectron.*, 24(10) (2009) 3055-3060.

866 [56] M. Sun, G.P. Sheng, L. Zhang, C.R. Xia, Z.X. Mu, X.W. Liu, H.L. Wang, H.Q. Yu, R.
867 Qi, T. Yu, M. Yang, An MEC-MFC-coupled system for biohydrogen production from
868 acetate, *Environ. Sci. Technol.*, 42(21) (2008) 8095-8100.

869 [57] K.-Y. Show, D.-J. Lee, J.-S. Chang, Bioreactor and process design for biohydrogen
870 production, *Bioresource Technol.*, 102(18) (2011) 8524-8533.

871 [58] E. Kan, Effects of pretreatments of anaerobic sludge and culture conditions on hydrogen
872 productivity in dark anaerobic fermentation, *Renew. Energ.*, 49 (2013) 227-231.

873 [59] S.R. Chaganti, D.-H. Kim, J.A. Lalman, Dark fermentative hydrogen production by
874 mixed anaerobic cultures: Effect of inoculum treatment methods on hydrogen yield, *Renew.*
875 *Energ.*, 48 (2012) 117-121.

876 [60] C.-H. Lay, B. Sen, S.-C. Huang, C.-C. Chen, C.-Y. Lin, Sustainable bioenergy
877 production from tofu-processing wastewater by anaerobic hydrogen fermentation for onsite
878 energy recovery, *Renew. Energ.*, 58 (2013) 60-67.

879 [61] S. Ma, H. Wang, Y. Wang, H. Bu, J. Bai, Bio-hydrogen production from cornstalk
880 wastes by orthogonal design method, *Renew. Energ.*, 36(2) (2011) 709-713.

- 881 [62] S.D.M. Lucas, G. Peixoto, G. Mockaitis, M. Zaiat, S.D. Gomes, Energy recovery from
882 agro-industrial wastewaters through biohydrogen production: Kinetic evaluation and
883 technological feasibility, *Renew. Energ.*, 75 (2015) 496-504.
- 884 [63] Y.-T. Fan, Y. Xing, H.-C. Ma, C.-M. Pan, H.-W. Hou, Enhanced cellulose-hydrogen
885 production from corn stalk by lesser panda manure, *Int. J. Hydrogen Energ.*, 33(21) (2008)
886 6058-6065.
- 887 [64] S. Pattra, S. Sangyoka, M. Boonmee, A. Reungsang, Bio-hydrogen production from the
888 fermentation of sugarcane bagasse hydrolysate by *Clostridium butyricum*, *Int. J. Hydrogen*
889 *Energ.*, 33(19) (2008) 5256-5265.
- 890 [65] T.-A.D. Nguyen, K.-R. Kim, M.S. Kim, S.J. Sim, Thermophilic hydrogen fermentation
891 from Korean rice straw by *Thermotoga neapolitana*, *Int. J. Hydrogen Energ.*, 35(24) (2010)
892 13392-13398.
- 893 [66] L. Zhao, G.-L. Cao, A.-J. Wang, W.-Q. Guo, H.-Y. Ren, N.-Q. Ren, Simultaneous
894 saccharification and fermentation of fungal pretreated cornstalk for hydrogen production
895 using *Thermoanaerobacterium thermosaccharolyticum* W16, *Bioresource Technol.*, 145
896 (2013) 103-107.
- 897 [67] G. Ivanova, G. Rákhely, K.L. Kovács, Thermophilic biohydrogen production from
898 energy plants by *Caldicellulosiruptor saccharolyticus* and comparison with related studies,
899 *Int. J. Hydrogen Energ.*, 34(9) (2009) 3659-3670.
- 900 [68] Z.-X. Song, X.-H. Li, W.-W. Li, Y.-X. Bai, Y.-T. Fan, H.-W. Hou, Direct bioconversion
901 of raw corn stalk to hydrogen by a new strain *Clostridium* sp. FS3, *Bioresource Technol.*, 157
902 (2014) 91-97.
- 903 [69] K. Bala-Amutha, A.G. Murugesan, Biohydrogen production using corn stalk employing
904 *Bacillus licheniformis* MSU AGM 2 strain, *Renew. Energ.*, 50 (2013) 621-627.

905 [70] K.-L. Ho, D.-J. Lee, A. Su, J.-S. Chang, Biohydrogen from lignocellulosic feedstock via
906 one-step process, *Int. J. Hydrogen Energ.*, 37(20) (2012) 15569-15574.

907 [71] X. Shi, K.-W. Jung, D.-H. Kim, Y.-T. Ahn, H.-S. Shin, Direct fermentation of *Laminaria*
908 *japonica* for biohydrogen production by anaerobic mixed cultures, *Int. J. Hydrogen Energ.*,
909 36(10) (2011) 5857-5864.

910 [72] C.-H. Lay, B. Sen, C.-C. Chen, J.-H. Wu, S.-C. Lee, C.-Y. Lin, Co-fermentation of water
911 hyacinth and beverage wastewater in powder and pellet form for hydrogen production,
912 *Bioresource Technol.*, 135 (2013) 610-615.

913 [73] R.S. Prakasham, T. Sathish, P. Brahmaiah, C. Subba Rao, R. Sreenivas Rao, P.J. Hobbs,
914 Biohydrogen production from renewable agri-waste blend: Optimization using mixer design,
915 *Int. J. Hydrogen Energ.*, 34(15) (2009) 6143-6148.

916 [74] I. Ntaikou, E. Koutros, M. Kornaros, Valorisation of wastepaper using the
917 fibrolytic/hydrogen producing bacterium *Ruminococcus albus*, *Bioresource Technol.*,
918 100(23) (2009) 5928-5933.

919 [75] S. Talluri, S.M. Raj, L.P. Christopher, Consolidated bioprocessing of untreated
920 switchgrass to hydrogen by the extreme thermophile *Caldicellulosiruptor saccharolyticus*
921 DSM 8903, *Bioresource Technol.*, 139 (2013) 272-279.

922 [76] G. Kumar, C.-Y. Lin, Biogenic Hydrogen Conversion of De-Oiled *Jatropha* Waste via
923 Anaerobic Sequencing Batch Reactor Operation: Process Performance, Microbial Insights,
924 and Reduction Efficiency, *Scientific World J.*, 2014 (2014) 9.

925 [77] X. Shi, D.-H. Kim, H.-S. Shin, K.-W. Jung, Effect of temperature on continuous
926 fermentative hydrogen production from *Laminaria japonica* by anaerobic mixed cultures,
927 *Bioresource Technol.*, 144 (2013) 225-231.

928 [78] Y. Chen, J.J. Cheng, K.S. Creamer, Inhibition of anaerobic digestion process: A review,
929 *Bioresource Technol.*, 99(10) (2008) 4044-4064.

930 [79] O. Yenigün, B. Demirel, Ammonia inhibition in anaerobic digestion: A review, *Process*
931 *Biochem.*, 48(5–6) (2013) 901-911.

932 [80] P. Sinha, A. Pandey, An evaluative report and challenges for fermentative biohydrogen
933 production, *Int. J. Hydrogen Energ.*, 36(13) (2011) 7460-7478.

934 [81] J. Wang, W. Wan, Factors influencing fermentative hydrogen production: A review, *Int.*
935 *J. Hydrogen Energ.*, 34(2) (2009) 799-811.

936 [82] M.D. Bao, H.J. Su, T.W. Tan, Dark fermentative bio-hydrogen production: Effects of
937 substrate pre-treatment and addition of metal ions or L-cysteine, *Fuel*, 112 (2013) 38-44.

938 [83] Z. Yuan, H. Yang, X. Zhi, J. Shen, Increased performance of continuous stirred tank
939 reactor with calcium supplementation, *Int. J. Hydrogen Energ.*, 35(7) (2010) 2622-2626.

940 [84] X. Hao, M. Zhou, H. Yu, Q. Shen, L. Lei, Effect of Sodium Ion Concentration on
941 Hydrogen Production from Sucrose by Anaerobic Hydrogen-producing Granular Sludge,
942 *Chinese J. Chem. Eng.*, 14(4) (2006) 511-517.

943 [85] M.-J. Lee, T.-H. Kim, B. Min, S.-J. Hwang, Sodium (Na⁺) concentration effects on
944 metabolic pathway and estimation of ATP use in dark fermentation hydrogen production
945 through stoichiometric analysis, *J. Environ. Manage.*, 108 (2012) 22-26.

946 [86] H. Alshiyab, M.S. Kalil, A.A. Hamid, W.M. Wan Yusoff, Effect of salts addition on
947 hydrogen production by *C. acetobutylicum*, *Pak. J. Biol. Sci.*, 11(18) (2008) 2193-2200.

948 [87] S. Srikanth, S.V. Mohan, Regulatory function of divalent cations in controlling the
949 acidogenic biohydrogen production process, *RSC Adv.*, 2(16) (2012) 6576-6589.

950 [88] C. Li, H.H.P. Fang, Inhibition of heavy metals on fermentative hydrogen production by
951 granular sludge, *Chemosphere*, 67(4) (2007) 668-673.

952 [89] C.-Y. Lin, S.-H. Shei, Heavy metal effects on fermentative hydrogen production using
953 natural mixed microflora, *Int. J. Hydrogen Energ.*, 33(2) (2008) 587-593.

- 954 [90] Z. Trad, J.-P. Fontaine, C. Larroche, C. Vial, Multiscale mixing analysis and modeling
955 of biohydrogen production by dark fermentation, *Renew. Energ.*, 98 (2016) 264-282.
- 956 [91] R. Lin, J. Cheng, L. Ding, W. Song, J. Zhou, K. Cen, Inhibitory effects of furan
957 derivatives and phenolic compounds on dark hydrogen fermentation, *Bioresource Technol.*,
958 196 (2015) 250-255.
- 959 [92] B.M. Haroun, G. Nakhla, H. Hafez, F.A. Nasr, Impact of furfural on biohydrogen
960 production from glucose and xylose in continuous-flow systems, *Renew. Energ.*, 93 (2016)
961 302-311.
- 962 [93] A. Barakat, F. Monlau, J.-P. Steyer, H. Carrere, Effect of lignin-derived and furan
963 compounds found in lignocellulosic hydrolysates on biomethane production, *Bioresource*
964 *Technol.*, 104 (2012) 90-99.
- 965 [94] J.-H. Park, S.-H. Lee, H.-J. Ju, S.-H. Kim, J.-J. Yoon, H.-D. Park, Failure of
966 biohydrogen production by low levels of substrate and lactic acid accumulation, *Renew.*
967 *Energ.*, 86 (2016) 889-894.
- 968 [95] M. Li, Y. Zhao, Q. Guo, X. Qian, D. Niu, Bio-hydrogen production from food waste and
969 sewage sludge in the presence of aged refuse excavated from refuse landfill, *Renew. Energ.*,
970 33(12) (2008) 2573-2579.
- 971 [96] B. Wang, W. Wan, J. Wang, Inhibitory effect of ethanol, acetic acid, propionic acid and
972 butyric acid on fermentative hydrogen production, *Int. J. Hydrogen Energ.*, 33(23) (2008)
973 7013-7019.
- 974 [97] G.H. Zheng, L. Wang, Z.H. Kang, Feasibility of biohydrogen production from tofu
975 wastewater with glutamine auxotrophic mutant of *Rhodobacter sphaeroides*, *Renew. Energ.*,
976 35(12) (2010) 2910-2913.
- 977 [98] G.D. Sprott, K.M. Shaw, K.F. Jarrell, Ammonia/potassium exchange in methanogenic
978 bacteria, *J. biol. Chem.*, 259(20) (1984) 12602-12608.

979 [99] H. Watanabe, H. Yoshino, Biohydrogen using leachate from an industrial waste landfill
980 as inoculum, *Renew. Energ.*, 35(5) (2010) 921-924.

981 [100] J. Carrillo-Reyes, M.A. Cortés-Carmona, C.D. Bárcenas-Ruiz, E. Razo-Flores, Cell
982 wash-out enrichment increases the stability and performance of biohydrogen producing
983 packed-bed reactors and the community transition along the operation time, *Renew. Energ.*,
984 97 (2016) 266-273.

985 [101] R. Jariyaboon, O.T. S, P. Kongjan, Bio-hydrogen and bio-methane potentials of skim
986 latex serum in batch thermophilic two-stage anaerobic digestion, *Bioresource Technol.*, 198
987 (2015) 198-206.

988 [102] B. Ruggeri, T. Tommasi, S. Sanfilippo, BioH₂ & BioCH₄ Through Anaerobic
989 Digestion, 2015 ed., Springer-Verlag, London, 2015.

990 [103] I.C. Liu, L.-M. Whang, W.-J. Ren, P.-Y. Lin, The effect of pH on the production of
991 biohydrogen by clostridia: Thermodynamic and metabolic considerations, *Int. J. Hydrogen*
992 *Energ.*, 36(1) (2011) 439-449.

993 [104] G. Kumar, J.-H. Park, M.-S. Kim, D.-H. Kim, S.-H. Kim, Hydrogen fermentation of
994 different galactose–glucose compositions during various hydraulic retention times (HRTs),
995 *Int. J. Hydrogen Energ.*, 39(35) (2014) 20625-20631.

996 [105] S. Zhang, Y. Lee, T.H. Kim, S.J. Hwang, Effects of OLRs and HRTs on hydrogen
997 production from high salinity substrate by halophilic hydrogen producing bacterium (HHPB),
998 *Bioresource Technol.*, 141 (2013) 227-32.

999 [106] L. Shen, Y. Zhou, B. Mahendran, D.M. Bagley, S.N. Liss, Membrane fouling in a
1000 fermentative hydrogen producing membrane bioreactor at different organic loading rates, *J.*
1001 *Membrane Sci.*,360(1-2) (2010) 226-233.

1002 [107] H. Hafez, G. Nakhla, M.H. El. Naggat, E. Elbeshbishy, B. Baghchehsaraee, Effect of
1003 organic loading on a novel hydrogen bioreactor, *Int. J. Hydrogen Energ.*, 35(1) (2010) 81-92.

1004 [108] T.H. Kim, Y. Lee, K.H. Chang, S.J. Hwang, Effects of initial lactic acid concentration,
1005 HRTs, and OLRs on bio-hydrogen production from lactate-type fermentation, *Bioresource*
1006 *Technol.*, 103(1) (2012) 136-41.

1007 [109] G. Luo, L. Xie, Z. Zou, W. Wang, Q. Zhou, H. Shim, Anaerobic treatment of cassava
1008 stillage for hydrogen and methane production in continuously stirred tank reactor (CSTR)
1009 under high organic loading rate (OLR), *Int. J. Hydrogen Energ.*, 35(21) (2010) 11733-11737.

1010 [110] D. Lee, Y. Li, Y. Oh, M. Kim, T. Noike, Effect of iron concentration on continuous H₂
1011 production using membrane bioreactor, *Int. J. Hydrogen Energ.*, 34(3) (2009) 1244-1252.

1012 [111] K.P. Muñoz, H. Steinmetz, Evaluation of Pre-treatment on the First Stage of an
1013 Anaerobic Digester for Enhancing Bio-hydrogen Production and its Associated Energy
1014 Balance, *Energy Procedia*, 29 (2012) 469-479.

1015 [112] F. Zhang, P. Zhao, M. Niu, J. Maddy, The survey of key technologies in hydrogen
1016 energy storage, *Int. J. Hydrogen Energ.*, 41(33) (2016) 14535-14552.

1017 [113] J. Zheng, X. Liu, P. Xu, P. Liu, Y. Zhao, J. Yang, Development of high pressure
1018 gaseous hydrogen storage technologies, *Int. J. Hydrogen Energ.*, 37(1) (2012) 1048-1057.

1019 [114] M.V. Lototsky, V.A. Yartys, B.G. Pollet, R.C. Bowman Jr, Metal hydride hydrogen
1020 compressors: A review, *Int. J. Hydrogen Energ.*, 39(11) (2014) 5818-5851.

1021 [115] S. Satyapal, J. Petrovic, C. Read, G. Thomas, G. Ordaz, The U.S. Department of
1022 Energy's National Hydrogen Storage Project: Progress towards meeting hydrogen-powered
1023 vehicle requirements, *Catal. Today*, 120(3-4) (2007) 246-256.

1024 [116] Y. Song, New perspectives on potential hydrogen storage materials using high
1025 pressure, *Phys. Chem. Chem. Phys.*, 15(35) (2013) 14524-14547.

1026 [117] H.W. Li, S. Orimo, Y. Nakamori, K. Miwa, N. Ohba, S. Towata, A. Züttel, Materials
1027 designing of metal borohydrides: Viewpoints from thermodynamical stabilities, *J. Alloy.*
1028 *Compd.*, 446-447 (2007) 315-318.

1029 [118] I.P. Jain, P. Jain, A. Jain, Novel hydrogen storage materials: A review of lightweight
1030 complex hydrides, *J. Alloy. Compd.*, 503(2) (2010) 303-339.

1031 [119] N.T. Stetson, Hydrogen Storage Overview, U.S. Department of Energy, Annual Merit
1032 Review and Peer Evaluation Meeting, 2012.

1033 [120] R. Heede, N. Oreskes, Potential emissions of CO₂ and methane from proved reserves
1034 of fossil fuels: An alternative analysis, *Global Environ. Chang.*, 36 (2016) 12-20.

1035 [121] J. Asafu-Adjaye, D. Byrne, M. Alvarez, Economic Growth, Fossil Fuel and Non-Fossil
1036 Consumption: A Pooled Mean Group Analysis using Proxies for Capital, *Energ. Econ.*,
1037 (2016).

1038 [122] N. Bauer, J. Hilaire, R.J. Brecha, J. Edmonds, K. Jiang, E. Kriegler, H.-H. Rogner, F.
1039 Sferra, Assessing global fossil fuel availability in a scenario framework, *Energy*, 111 (2016)
1040 580-592.

1041 [123] M.Z. Lukawski, R.L. Silverman, J.W. Tester, Uncertainty analysis of geothermal well
1042 drilling and completion costs, *Geothermics*, 64 (2016) 382-391.

1043 [124] S.H.M.M. Mohammed, Identifying Cost Centers and Environmental Impacts Needs
1044 Assessment for Fracking Life Cycle in the United States, *Procedia Eng.*, 145 (2016) 444-451.

1045 [125] F. Behrouzi, M. Nakisa, A. Maimun, Y.M. Ahmed, Global renewable energy and its
1046 potential in Malaysia: A review of Hydrokinetic turbine technology, *Renew. Sust. Energ.*
1047 *Rev.* 62 (2016) 1270-1281.

1048 [126] G.R. Astbury, A review of the properties and hazards of some alternative fuels, *Process*
1049 *Saf. Environ.*, 86(6) (2008) 397-414.

1050 [127] G. Nicoletti, N. Arcuri, G. Nicoletti, R. Bruno, A technical and environmental
1051 comparison between hydrogen and some fossil fuels, *Energ. Convers. Manage.*, 89 (2015)
1052 205-213.

1053 [128] P. Geng, Q. Tan, C. Zhang, L. Wei, X. He, E. Cao, K. Jiang, Experimental
1054 investigation on NO_x and green house gas emissions from a marine auxiliary diesel engine
1055 using ultralow sulfur light fuel, *Sci. Total Environ.*, 572 (2016) 467-475.

1056 [129] M.H.M. Ashnani, T. Miremadi, A. Johari, A. Danekar, Environmental Impact of
1057 Alternative Fuels and Vehicle Technologies: A Life Cycle Assessment Perspective, *Procedia*
1058 *Environ. Sci.*, 30 (2015) 205-210.

1059 [130] E. Shafiei, B. Davidsdottir, J. Leaver, H. Stefansson, E.I. Asgeirsson, Energy,
1060 economic, and mitigation cost implications of transition toward a carbon-neutral transport
1061 sector: A simulation-based comparison between hydrogen and electricity, *J. Clean. Prod.*, 141
1062 (2017) 237-247.

1063 [131] A. Bakenne, W. Nuttall, N. Kazantzis, Sankey-Diagram-based insights into the
1064 hydrogen economy of today, *Int. J. Hydrogen Energ.*, 41(19) (2016) 7744-7753.

1065 [132] P. Parthasarathy, K.S. Narayanan, Hydrogen production from steam gasification of
1066 biomass: Influence of process parameters on hydrogen yield – A review, *Renew. Energ.*, 66
1067 (2014) 570-579.

1068 [133] I. Dincer, Green methods for hydrogen production, *Int. J. Hydrogen Energ.*, 37(2)
1069 (2012) 1954-1971.

1070 [134] C. Acar, I. Dincer, Comparative assessment of hydrogen production methods from
1071 renewable and non-renewable sources, *Int. J. Hydrogen Energ.*, 39(1) (2014) 1-12.

1072 [135] Z.L. Wang, G.F. Naterer, K.S. Gabriel, R. Gravelins, V.N. Daggupati, Comparison of
1073 sulfur–iodine and copper–chlorine thermochemical hydrogen production cycles, *Int. J.*
1074 *Hydrogen Energ.*, 35(10) (2010) 4820-4830.

1075 [136] J. Nowotny, T.N. Veziroglu, Impact of hydrogen on the environment, *Int. J. Hydrogen*
1076 *Energ.*, 36(20) (2011) 13218-13224.

1077 [137] R. Bhandari, C.A. Trudewind, P. Zapp, Life cycle assessment of hydrogen production
1078 via electrolysis – a review, *J. Clean. Prod.*, 85 (2014) 151-163.

1079 [138] C. Acar, S. Ghosh, I. Dincer, C. Zamfirescu, Evaluation of a new continuous type
1080 hybrid photo-electrochemical system, *Int. J. Hydrogen Energ.*, 40(34) (2015) 11112-11124.

1081 [139] J.-J. Hwang, Sustainability study of hydrogen pathways for fuel cell vehicle
1082 applications, *Renew. Sust. Energ. Rev.* 19 (2013) 220-229.

1083 [140] S. Koumi Ngoh, D. Njomo, An overview of hydrogen gas production from solar
1084 energy, *Renew. Sust. Energ. Rev.* 16(9) (2012) 6782-6792.

1085 [141] C.M. Kalamaras, A.M. Efstathiou, Hydrogen Production Technologies: Current State
1086 and Future Developments, *Conference Papers in Energy* 2013 (2013) 9.

1087 [142] K. Dowaki, T. Ohta, Y. Kasahara, M. Kameyama, K. Sakawaki, S. Mori, An economic
1088 and energy analysis on bio-hydrogen fuel using a gasification process, *Renew. Energ.*, 32(1)
1089 (2007) 80-94.

1090 [143] C. Yilmaz, M. Kanoglu, A. Bolatturk, M. Gadalla, Economics of hydrogen production
1091 and liquefaction by geothermal energy, *Int. J. Hydrogen Energ.*, 37(2) (2012) 2058-2069.

1092 [144] A. Martínez Arranz, Hype among low-carbon technologies: Carbon capture and storage
1093 in comparison, *Global Environ. Chang.*, 41 (2016) 124-141.

1094 [145] W.L. Theo, J.S. Lim, H. Hashim, A.A. Mustafa, W.S. Ho, Review of pre-combustion
1095 capture and ionic liquid in carbon capture and storage, *Appl. Energ.* 183 (2016) 1633-1663.

1096 [146] D.-H. Lee, Cost-benefit analysis, LCOE and evaluation of financial feasibility of full
1097 commercialization of biohydrogen, *Int. J. Hydrogen Energ.*, 41(7) (2016) 4347-4357.

1098 [147] C.-C. Chang, F.-L. Wu, W.-H. Lai, M.-P. Lai, A cost-benefit analysis of the carbon
1099 footprint with hydrogen scooters and electric scooters, *Int. J. Hydrogen Energ.*, 41(30) (2016)
1100 13299-13307.

1101 [148] M. Lakshmikandan, A.G. Murugesan, Enhancement of growth and biohydrogen
1102 production potential of *Chlorella vulgaris* MSU-AGM 14 by utilizing seaweed aqueous
1103 extract of *Valoniopsis pachynema*, *Renew. Energ.*, 96, Part A (2016) 390-399.

1104 [149] Y. Ito, S. Managi, The potential of alternative fuel vehicles: A cost-benefit analysis,
1105 *Res. Transp. Econ.*,50 (2015) 39-50.

1106 [150] F. Klumpp, Comparison of pumped hydro, hydrogen storage and compressed air
1107 energy storage for integrating high shares of renewable energies—Potential, cost-comparison
1108 and ranking, *J. Energ. Storag.*, 8 (2016) 119-128.