

Production of hydrogen from biomass and its separation using membrane technology

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Abstract:

Hydrogen is an important raw material for chemical industry and feasible renewable energy carrier that could replace fossil fuels. However, the specie seldom exists in a form of pure H₂. Therefore, to obtain hydrogen in volumes suitable to be used as a raw material it is necessary to decompose hydrogen-rich compounds. The carbohydrate-rich biomass can be an important source of hydrogen by applying the process of dark fermentation. In this paper potential ways of hydrogen production from organic wastes (biomass) by means of dark fermentation are reviewed and discussed. The bacteria used for dark fermentation are enlisted, characterized and compared. The pretreatment processes and various reactor designs are analyzed and discussed. The hydrogen separation by membrane method (which can provide the most pure hydrogen) are presented.

The paper describes recent achievements in optimizing parameters, conditions and reactors used to industrialize dark fermentation.

Keywords: dark fermentation, hydrogen, inoculum pretreatments, membrane separation

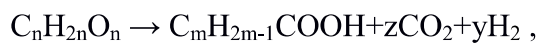
1. Background

Dark fermentation is a branch of science and technology which is developing very rapidly in every step of the process different substrates [1,2,3,5,8], including crop residues (such as corn [2, 3], bagasse[3,4], carrots [5], Jerusalem artichoke roots [5], maize flour [5], oats [5]), potatoes [1, 5], sugarbeet residues [2,6], wheat flour [7], rapeseed oil cakes [5], sunflower oil cakes [5], grape marc[8], vegetable waste from restaurants [8,9], fruit peels (orange peels and banana peels) [8], animal waste e.g. cow manure [7], chicken meat [8], fish residues [5,8], food residues like kitchen waste [5,7,8,10,], sewage wastes [1, 2, 5, 8, 11], and other biodegradation methods leading to hydrogen production [10]. Sambusti et al. [12] and Saiffudin et al. [13] reviewed dark fermentation taking into account one kind of substrate i.e. algae. Ghimire et al. [14] compared different substrates and parameters. Bundhoo et al. [15] and Wong et al. [16] analyzed role of pretreatments and parameters effecting the process. Elsharnouby et al [17] analyzed bacterial monocultures used for dark fermentation.

This review summarizes the role of substrates, bacteria and pretreatments, including parameters and reactors. In the article all the earlier steps and design of the dark fermentation process mentioned above are analyzed. Additionally in the article membrane separation methods are discussed.

45 Standard dark fermentation is an anaerobic process, which leads to the decomposition of
1 46 sugar molecules (usually hexoses) into low-weight organic-acid, hydrogen and carbon
2 47 dioxide. Hexoses and/or pentoses often originate from hydrolysis of higher carbohydrates
3 48 such as starch, molasses and cellulose [15]. The great interest in dark fermentation based on
4 49 different types of carbohydrates is generated because of the widespread availability of
5 50 carbohydrate-rich materials (e.g. paper, wood, grass straws) with high hydrogen content and
6 51 the low number of inhibiting byproducts that can occur during the process, together with the
7 52 low amount of energy needed for bacteria to digest glucose.

11 53 According to Hallenbeck[19] and Gottshalk [20] dark fermentation can be a one-stage
12 54 process, when the substrate contains simple sugars. Then, the general route of dark
13 55 fermentation in the presence of water (for example, glucose or sucrose) is as follows [4, 5] as
14 56 in equation:



17
18
19 where: $n = m+z = 5,6, 12, \dots$; $y=0.5(n-m)$, $y = 2$ or 4 ; $z=n-m-1$.

21 57 Extended dark fermentation includes other biomaterials used successfully in anaerobic
22 58 digestion, like fats, proteins, in addition to pure carbohydrates [14,22–25]. In the case of dark
23 59 fermentation of substrates with high protein content the process can be disrupted due to high
24 60 nitrogen and resulting high ammonia concentration inhibiting hydrogen generation [14,22],
25 61 however, due to Alibardi et al. [26], proteins does not influence on dark fermentation process.
26 62 Fatty acids are substrates with high potential for dark fermentation and high efficiency
27 63 (hydrogen yield for sugars is around 0.33 but 0.38 for glycerol) [10,25,27,28].

31 64 Dark fermentation is related to methane fermentation, but the standard process is limited to
32 65 hydrolysis and acidogenesis. Hydrogen production is optimized during acidogenesis under
33 66 low pH conditions. Processes leading to methanogenesis are at least partly inhibited. In the
34 67 case of dark fermentation process led by acidogenic bacteria (like Clostridium) methanogenic
35 68 processes can be inhibited by special pretreatment of inoculum. Extended fermentation may
36 69 rely on more stages, i.e. hydrolysis, acidogenesis and acetogenesis, but again it obstructs
37 70 methanogenesis.

41 71 2. Bacteria promoting dark fermentation

42 72 Anaerobic microorganisms generate hydrogen using hydrogenase enzymes. Anaerobic
43 73 bacteria produce hydrogen as by-product of their metabolism. The presence of hydrogenase
44 74 enzymes was proven in 1931 in Escherichia coli. Hydrogenases are enzymes that stimulate
45 75 production and recycling of hydrogen in bacteria[20]. Anaerobic bacteria produce hydrogen
46 76 as a by-product of their metabolism. The most common anaerobic bacteria enzymes are: [Fe]-
47 77 hydrogenase, [NiFe]-hydrogenase, [NiFeSe]-hydrogenase[30]. [Fe]-hydrogenase catalyses
48 78 generation of hydrogen, while [NiFe]-hydrogenase uptakes generated hydrogen, and
49 79 [NiFeSe]-hydrogenase is bidirectional. [NiFe]-hydrogenase is 100 fold less active than [Fe]-
50 80 hydrogenase, therefore more generated hydrogen is excreted from the organism than is
51 81 adsorbed back[20]. Hallenbeck pointed out that hydrogen can be generated by both: [Fe]-
52 82 hydrogenase and [NiFe]-hydrogenase[15]. Morra et al.[31] reported existence of [FeFe]-
53 83 hydrogenase enzyme in strict and facultative bacteria.

59 84 Dark fermentation can be stimulated by anaerobic bacteria of several different phyla,
60 85 families, genus and species, belonging to Gram-positive or Gram negative groups. According

86 to Zajic et al.[18] there are several bacteria that produce hydrogen. Bacteria producing
87 hydrogen are from a group of endospore-forming rods Bacillaceae (genuses Clostridium,
88 Bacillus), Gram negative facultatively anaerobic rods (Enterobacteria, Vibrionaceae) and
89 cocci (Veillonellaceae) [32], Gram positive cocci (Micrococcaceae), Peptococcaceae, Gram
90 positive asporogenous rod-shaped bacteria (lactobacillae). Unfortunately, the majority of
91 these bacteria produce hydrogen in amounts considered unsuitable for use in full-scale dark
92 fermentation plants. Hydrogen is produced most efficiently by species of Clostridium,
93 Bacillus, Enterobacter, and some thermophilic bacteria like Thermocellum and Thermatoga.

94 The role of these bacteria, strict bacteria (Clostridium) and facultative bacteria
95 (Enterobacter, Bacillus) will be described below.

96 (a) Clostridium

97 One of the most relevant and the most efficient hydrogen producing groups of bacteria
98 is Clostridium. An important feature of Clostridium is its ability to form protective spores.
99 The protective spores allow surviving harsh conditions, like extreme temperature, low or high
100 pH and chemical agents[33]. Another characteristic of clostridia is lack of cytochrome [34].
101 Therefore, inoculums containing Clostridium can be pretreated by means of heat, determined
102 pH or chemicals for increase of the hydrogen production rate and to remove other bacteria.
103 Hydrogen producing Clostridium are: Clostridium acetobutylicum, Clostridium botulinum,
104 Clostridium butylicum, Clostridium butyricum, Clostridium cellobioparum, Clostridium
105 cellulosolvens, Clostridium dissolvens Clostridium fossicularum, Clostridium hydrogenicus,
106 Clostridium kluyveri, Clostridium oedematis-maligni, Clostridium pasteurianum, Clostridium
107 sporogenes, Clostridium tetani, Clostridium tetanomorphum, Clostridium thermocellum,
108 Clostridium thermosaccharolyticum, Clostridium welchii, Clostridium werni. Among these
109 bacteria, beside thermophilic and mesophilic, also psychrophilic species appear like
110 Clostridium algidixylanolyticum[35]. The most efficient hydrogenic bacteria are Clostridium
111 butylicum, Clostridium butyricum, Clostridium kluyveri and Clostridium pasteurianum [36].
112 A monoculture of Clostridium sp. can produce from 1.61-2.36 mol H₂ mol⁻¹glucose[36].
113 Clostridia belong to strict anaerobic bacteria, the most important bacteria in mixtures which
114 task is to produce hydrogen with the highest possible efficiency. Despite the high yield of
115 hydrogen production clostridium are very fragile to oxygen and to various form of
116 substrate[28]. Some clostridium like Clostridium sp. strain No. 2 are able to convert glucose
117 and xylose with similar efficiencies [37]. There are attempts to reduce oxygen sensitivity by
118 using them in mixtures with other less air sensitive groups of bacteria termed facultative.

119 (b) Bacillus

120 Bacillus is another group of bacteria which like clostridium are made up of endospore
121 forming rods. The most commonly used are Bacillus macerans (acetoethylicus), Bacillus
122 cloacae (Enterobacter cloacae), Bacillus macerans, Bacillus polymyxa. Kumar et al. [27]
123 isolated Bacillus licheniformis from cattle manure. The hydrogen yield of dark fermentation
124 with Bacillus licheniformis was 0.37 mol H₂ mol⁻¹ glucose in semi-continuous process and
125 1.1 mol hydrogen mol glucose in batch mode [28]. The hydrogen yields for Bacillus
126 coagulants from carbohydrates like cellobiose (5.6 mol H₂ mol⁻¹ cellobiose), L-arabinose
127 (1.9 mol H₂ mol⁻¹ L-arabinose), D-xylose (1.2 mol H₂ mol⁻¹ D-xylose) [29] are higher than in
128 the case of bacteria from the Enterobacter group (Citrobacter freundii, Enterobacter cloacae).

131 **c)Enterobacter**

1 132 According to Zajic et al.[18] the family of Enterobacterae includes bacteria from seven
 2 133 groups. The genres are: Escherichia coli (Genus I), Citrobacter intermedius (Genus II),
 3 134 Salmonella enteritidis (Genus III), Genus IV (Enterobacter (Aerobacter) aerogenes),
 4 135 Enterobacter sp, Aerobacter cloacae, Aerobacter indologenes).

5 136 Enterobacterae is a group of bacteria that grow anaerobically or aerobically depending
 6 137 on pH value. Enterobacterae are anaerobic bacteria of high air-resistivity. Although oxygen
 7 138 blocks the growth of bacteria it does not decrease hydrogen yield. Therefore, Enterobacter is
 8 139 often used in mixed cultures, which are more sensitive to oxygen. They are used more rarely
 9 140 in monoculture due to lower hydrogen yield than in the case of Clostridium. According to
 10 141 Yokoi et al [30] for the Enterobacter aerogenes strain HO-39 hydrogen yield depends on the
 11 142 kind of carbohydrate substrate, i.e. from 0.83 moles of hydrogen for a mole of lactose to 2.16
 12 143 mole of hydrogen for a mole of maltose. More detailed analysis of hydrogen production rate
 13 144 and yield are presented in Table 1.

14 145
 15 146 **Table 1.** Dependence of hydrogen production rate and yield from carbohydrate in case of Enterobacter
 16 147 aerogenes strain HO-39[31]
 17 148

Carbohydrate	Hydrogen production rate (ml H ₂ l ⁻¹ substrate medium)	Hydrogen yield (mol H ₂ mol ⁻¹ substrate)
Glucose	1.243	1.00
Galactose	1.181	0.95
Fructose	1.094	0.88
Mannose	1.218	0.98
Mannitol	2.066	1.68
Sucrose	1.237	1.89
Maltose	1.343	2.16
Lactose	0.514	0.83

18 149
 19 150 Bacteria can produce hydrogen in the wide range of pH value from 4.00 to 7.8 [31].
 20 151 Therefore, Enterobacter aerogenes in relation to other bacteria can be considered as
 21 152 insensitive to pH change [32]. Ren et al. [33] extended the experiments of Yokoi et al. [31] to
 22 153 ramnose, arabinose, mannose, xylose and galactose, Table 2.

23 154
 24 155 **Table 2** Dependence of hydrogen production rate and yield from carbohydrate in case of Enterobacter
 25 156 aerogenes [31, 33]
 26 157

Carbohydrate	Hydrogen production rate (ml H ₂ l ⁻¹ substrate medium)	Hydrogen yield (mol H ₂ mol ⁻¹ substrate)
Xylose	1.77	0.79
Galactose	2.35	1.26
Ramnose	1.25	0.56
Mannose	2.42	1.3
Arabinose	1.81	0.81

27 158
 28 159 Productivity of hydrogen from pure compounds like sucrose and glucose in the case of
 29 160 Enterobacter cloacae is higher than for Bacillus or Citrobacter. Hydrogen yields from sucrose

161 are: 5.6 mol H₂ mol⁻¹ sucrose, and 2.8 mol H₂ mol⁻¹ of glucose[35]. Mandal et al.[36] obtained
162 a pure culture of Enterobacter cloacae using malt yeasts, and glucose mixture substrate of
163 hydrogen yield 3.9 mol H₂ mol⁻¹ glucose.

164 In the case of Citrobacter intermedium hydrogen yield is 1 mol H₂ mol⁻¹ of glucose
165 [18]. Hydrogen yields for Citrobacter freundii was found to be equal 5.4 mol H₂ mol⁻¹ sucrose
166 and 2.4mol H₂ mol⁻¹ glucose.

168 **d) other bacteria**

169 A special group of bacteria is hydrogenic bacteria i.e. a genetically modified form of
170 previously mentioned bacteria. Modification of bacteria is aiming to reduce or remove the
171 possibility of hydrogenase uptake that leads to the recycling of hydrogen generated earlier,
172 and to optimize the activity of hydrogenase. There are eighty types of hydrogenases[37]. The
173 gene responsible for hydrogenase in Enterobacter cloacae was isolated and then transferred to
174 non hydrogen producing Escherichia coli BL-21. It is known that Enterobacter can produce
175 up to 6 mol of hydrogen/mol of sucrose [49]. The modification of Escherichia coli genes help
176 them to produce even up to 1 μM of H₂ per minute[20]. Another aim is to block other
177 competitive reductases.

178 According to Fang et al. [39], the bacteria most frequently modified for hydrogen production
179 are Escherichia coli, Clostridium, Citrobacter and Klebsiella.

180 **2.1. Optimal temperature conditions for bacteria**

181 Bacteria can be classified taking into account optimal temperature of culture growth as
182 extra thermophilic, thermophilic, mesophilic and psychrophilic. Thermophilic bacteria can
183 produce hydrogen in the range from 45-90°C but their optimum is usually from 55°C to 60°C.
184 Mesophilic bacteria can work in temperature from 25°C to 45°C with an optimum range
185 between 33°C -37°C. Psychrophilic bacteria sustain in low temperatures from 5°C -25°C with
186 optimum usually between 20°C -25 °C.

187 Some extreme thermophiles like Thermotoga neapolitana or Caldicellulosiruptor
188 saccharolyticus in pure cultures can produce hydrogen from potato starch with the yield from
189 2.5-3.8 mol H₂ mol⁻¹ glucose[40]. The hydrogen productivity of psychrophilic bacteria is
190 much smaller therefore dark fermentation using this type of bacteria is investigated very
191 seldom. The psychrophilic bacteria can be an efficient method for hydrogen production in
192 high mountainous and high latitude regions [35,52]. Dębowski et al. [23] obtained biogas
193 containing 65.2-69.1% of hydrogen and production from 1587.47 - 3087.57 ml H₂ g⁻¹ biomass
194 using these bacteria.

195 **2.2. Inoculum pretreatment method**

196 Inoculum pretreatment is a way of preparing the culture of injected bacteria to special task.
197 There are several pretreatment methods: thermal, acid/base, aeration, microwave,
198 ultrasonication and chemical supplementation.

199 *a) Thermal pretreatment.*

200 Thermal pretreatment methods include: heat shock, sterilization, freezing and thawing. Heat
201 shock method is a method of boiling or drying of inoculum. The pretreatment is often used for
202 preparing mixed-culture systems for hydrogen production [53,54]. According to Zhu and
203 Beland [55] temperature range for heat-shock method should be between 80°C and 104°C and

204 exposure time from 15 to 120 min. On the other hand, Akobi et al.[56] preheated inoculum for
1 205 70°C for 30 minutes. Mixed cultures of Clostridium are boiled to a temperature of 100°C for
2 206 15 min [45]. However, according to Kotay and Das [29] heat treatment for 20 min, in
3 207 temperature 121°C decreased competitive microbial cultures to 2% after heat pretreatment.
4 208 The heat-shock method disables hydrogenotrophic bacteria that uptake generated hydrogen
5 209 and compile anaerobic bacteria like clostridium [46]. The non-spore forming methanogens
6 210 should be removed from system after pretreatment [47]. In the case of clostridium species
7 211 heat shock pretreatment leads most often to the butyric type of fermentation [48]. Logan et al.
8 212 [49] used drying of mixed culture in samples thickness of 1 cm thick in an aluminum pan for
9 213 2 h at T=104°C. Then samples were sieved through a mesh (850 μm) and stored in bottles at a
10 214 temperature of 4°C. Zhu et al. [46] states that methanogenesis inhibits hydrogenesis if
11 215 methane content in biogas is above 2%. Boiling of clostridium reduces activity of uptake
12 216 hydrogenase [62–64]. The method can be used for both mesophilic and thermophilic
13 217 conditions Zhang and Shen[65] placed inoculum in cracked cereal baked for 2 h and then boil
14 218 for 30 min. Mu et al. [48] used heat shock at temperature 102°C for 90 min for anaerobic
15 219 sludge from wastewater fermentation obtaining a yield of 2 mole H₂/mole glucose.

20 220 Chaganti et al. [50] applied heat shock at 90°C for 30 min which achieved a hydrogen
21 221 yield 2.84 mol/mole of glucose. Sterilization or pasteurization is a method that could be
22 222 performed by twice heating of activated sludge for 20 min at a temperature of 80°C and then
23 223 boiled anaerobically digested sludge for 15 min [24]. Palazzi et al. [51] used sterilization with
24 224 autoclaving at 120°C for 20 min. For Hawkes et al.[52], a heat-shock of 100°C for 1 h is the
25 225 most suitable in the case of agricultural soil used as an inoculum source. Lag time after heat
26 226 treating depends on the origin of inoculum. For municipal sludge optimum lag time is 2 days
27 227 while for microcrystalline cellulose is 4 days [36,69,70]. In the case of freezing and thawing
28 228 method, inoculum is firstly frozen to -10°C and kept for 24 h and then thawed up to 30°C
29 229 slowly over 6 hours. Kotay and Das [55] applied freezing of sample to -20°C for 6 h and then
30 230 thawed up to 20°C for 6 h, obtaining a yield of 6.5 ml H₂ g⁻¹ COD.

35 231 36 232 *b) acid pretreatment*

37 233 The acid pretreatment applies usually strong acids like acid chloride. The acidic pretreatment
38 234 is performed at pH from 3.0 to 5.0 [32] for 24 h. Then pH is adjusted to the level of 7.0 by
39 235 hydroxide solution like 0.1M NaOH [44,72]. Chaganti et al. [50] used 2.0 M HCl
40 236 pretreatment at pH 3.0 and inoculum was incubated for 24 h at temperature 37°C. According
41 237 to Ruggeri et al. [57] the acidic pretreatment led to hydrogen concentration increase between
42 238 50-70% in outflow. Methanogenesis does not occur or occurs in minimal amounts.
43 239 Furthermore, acid pretreatment in the case of clostridium can lead to mixed acetic and butyric
44 240 fermentation [58]. Acid pretreatments in particular improve conditions of dark fermentation
45 241 with the use of Clostridium sp. [59]. Mu et al. [60] improved hydrogen yield from wastewater
46 242 by applying the addition of up to 1.3 mol H₂ mol⁻¹ glucose, in contrast to Ruggeri et al's
47 243 experiment. [59] where 0.42 mol H₂ mol⁻¹ glucose was added.

48 244 Chaganti et al.[50] achieved a hydrogen yield of 3 mol H₂ mol⁻¹ glucose. According to Hu
49 245 and Chen [21] acidic pretreatment reduces the methanogenic phase of clostridium to
50 246 negligible amounts in the case of sewage sludge. Methanogenic yield of clostridium granules
51 247 is reduced to 61 ml CH₄ g⁻¹ glucose.
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250 *c) base pretreatment*

251 Under base or alkali-pretreatment conditions, inoculum is kept at pH = 10-13 for 24[h][43].
252 The pH~10-13 is obtained by adding hydroxides usually 1.0-4.0M solution of sodium
253 hydroxide. However Kim et al.[77] also used potassium hydroxide, magnesium hydroxide and
254 calcium hydroxide. After pre-treatment pH is lowered to the level of pH =7.0 by addition of a
255 strong acid such as 0.1M HCl [75]. Mu et al. [60] obtained a hydrogen yield of 0.48 mol H₂
256 mol⁻¹ glucose from wastewater, lower than using acid or heat shock pre-treatment. The
257 alkaline method leads to mixed butyric and acetic fermentation in similar ratios[78]. The
258 inoculums, in order to start hydrogen production, need to be kept at pH from 5.0-5.5 by
259 continuous addition of alkali to maintain stable pH [79]. Chaganti et al. [66] used base
260 treatment with 3.0M NaOH to keep inoculums at pH=11 and then left 24 h for incubation at
261 37°C. The yield achieved was 2.8 mole of H₂ per mole of glucose. The alkali pretreatment
262 inhibits methanogenic behavior of bacteria according to ref. [75].

263
264 *d)aeration*

265 Aeration can be used for anaerobic bacteria quite resistant to oxygen like Enterobacter. Yokoi
266 et al. [19, 49] used 12 h aeration in temperature 30°C in the basal medium of glucose
267 polypepton at pH 6.5. Palazzi et al. [67] used aerobic pretreatment in 37°C for 12 h in rotary
268 shakers; later, the bacteria cells were moved to stationary phase. Bagley and Kramer [81]
269 treated the sample with air for 1 h before placement in the reactor. Zhu and Beland [55]
270 continued pretreatment for 0.5 h and obtained yield 4.7 mol H₂ mol⁻¹ sucrose.

271
272 *e)microwave*

273 Guo et al. [82] prepared inoculum by heating the sample in a microwave reactor powered at
274 560 W for 2 min. and later obtained a yield of 11.04 ml H₂ g⁻¹COD [82]. Kotay and Das [40]
275 used microwaves with power 600 W for 2 min. obtaining a yield of 8 ml H₂ g⁻¹ COD.

276
277 *f) ultrasonication*

278 According to Hsia et al.[83] ultrasound pretreatment stresses with thermal and nonthermal
279 (mechanical) and empty-cell effects like radiation pressure, radiation force, acoustic torque,
280 acoustic streaming effect and cavitation. Thermal effect refers to micromassage causing
281 tissues to generate ultrasonic efficacy and thus produce additional heat energy and also refers
282 to the increased heat production by the biomass organisms after eating, owing to the
283 metabolic energy cost of digestion [83]. Kim et al. [77] applied ultrasonication with
284 a frequency of 42 kHz from 10 to 120 min. to a sample of inoculum. Approximately 18.4% of
285 COD was converted to hydrogen. The effect of ultrasonic pretreatment was increased by
286 earlier thermal pretreatment in (121°C at pressure 1.5 atm for 30 min. and addition of 7g/l
287 NaOH); 19.4% COD conversion was achieved [77]. Kotay and Das [40] used sonification at
288 frequency 20 kHz and power 140W, interacting at 2 mm depth, under temperature 25°C. Hsia
289 et al [83] obtained hydrogen production rate of 271 ml of H₂/h at 4 J energy for 15 minutes at
290 frequency 0.5 MHz at starch concentration 30 g/l.

291
292 *g) chemical supplementation*

293 Ghimire et al. [22] and Zhu and Béland [55] proposed using BESA (sodium 2-bromoethane-
294 sulfonic acid) method for inoculums pretreatment. BESA was added to kill methanogenic
295 bacteria after pasteurization at 120°C for 30 min. [14]. The methanogenesis of 0.1 g cells of
296 bacteria can be blocked by 0.01 mol of BESA. Zhu and Béland [55] proposed also an

iodopropane method in which diluted iodopropane in ethanol is added to sample with inoculum at room temperature for 30 min.

Chaganti et al. [66] proposed to add linoleic acid to inoculum. The inoculum was a mixed culture consisting: 26% Archea, 10% Bacteroidaceae, 12% Bacillaceae, 33% Clostridiaceae, 6% Enterobacteriaceae, 6% Geobacteriaceae and 5% Methylobacteriaceae. The inoculum was treated with 2000 mg of linoleic acid and then left in 37°C for 24 h. Then, inoculum was aligned to pH=5.5 using 1M HCl and 1M NaOH solutions. The obtained yield was 3.48 mol H₂ mol⁻¹ glucose. According to Hu and Chen [33] chloroform pretreatment could be an efficient method for sludge in granular form; however according to Wang and Wan [54] it is less efficient than alkaline, aeration, and the thermal method. In case of culture of Clostridium, hydrogen production is more resistant to chloroform presence of higher concentration than methane production in both granule and sewage sludge. Therefore, in Clostridium culture the methane production can be blocked by adding chloroform in the range from 0.05-2.5% [33]. The addition of chloroform above 2.5 blocks both hydrogen and methane fermentative production [31]. The optimal amount is 0.1% of chloroform; hydrogen production was 180ml H₂ g⁻¹ glucose in hydraulic retention time of 3 days [54]. The pretreatment method proposed by Nath et al. [84] based on keeping the sample in solution 1% v/v chloroform for 24 h at temperature 25°C.

h) Centrifuging

These methods apply centrifuging wash-out of bacteria cells as stress condition. Yokoyama et al. [85] used centrifuging of frequency 1500 rpm for 15 minutes for pretreatment of inoculum from cow waste slurry before applying thermophilic conditions in a batch system. Cigneroz-Perez et al. [86] performed the pretreatment using frequency 14000 rpm for 15 minutes. A pretreatment method at frequency 100 rpm for 30 minutes was applied in the case of wheat waste or powder [87–92]. In case of cheese whey powder Kargi et al. [93] used frequency 8000 rpm for 30 minutes. Yokoyama et al. [85] obtained the highest hydrogen production of 392 ml H₂ per litre of slurry. Perez-Pimienta et al. [69] obtained 176 ml H₂ per litre of organic wastes. Kargi et al. [93] obtained hydrogen productivity of 142 ml H₂ from one litre of cheese whey powder. In the case of wheat waste, hydrogen production was 77.375 ml per litre of wheat waste [95] and 223 ml H₂ from g of starch (from wheat powder) [96].

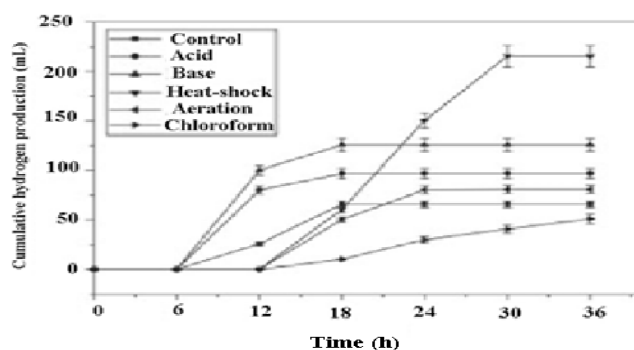


Fig. 1. Effect of pretreatment method of inoculum on cumulative hydrogen production[54]

331 *i) Comparison of methods*

1 332 According to Wang and Wan[54] selection of the most efficient method of pretreatment for
2 333 hydrogen production depends on projected time of the preparation of inoculum. The
3 334 cumulative hydrogen production rate as a function of pretreatment method duration is
4 335 presented in Fig. 1.
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7 336 As can be seen the most efficient method for the time lag between 6-22.6 h is the base
8 337 pretreatment method. For process longer than 22.6 h the heat shock method is the most useful.
9 338 Less efficient is the acid method. The heat shock method was less efficient than base
10 339 pretreatment in pH~10.0 for 30 min or aerobic method. In the case of applying secondary
11 340 batch recultivation basal pretreatment was the most efficient leading to 6.12 mol H₂ mol⁻¹
12 341 sucrose. During alkaline pretreatment, fewer metabolites are produced than in a heat shock. In
13 342 the case of mixed culture of *Enterobacter cloacae* IIT-BT 08, *Citrobacter freundii* IIT-BT
14 343 L139 and *Bacillus coagulans* IIT-BT S1 (ratio 1:1:1) according to Kotay and Das [97] the
15 344 most efficient pretreatments were: heat shock, microwave and base pretreatment. The heat
16 345 shock pretreatment leads to a yield of 14 ml H₂ g⁻¹ COD, while microwave and base
17 346 pretreatment resulted in 8 ml H₂ g⁻¹COD. Ultrasonication and acid pretreatment were also
18 347 quite efficient methods; in both cases yields were ca. 7 ml H₂ g⁻¹ COD.
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23 348 According to Zhu and Béland [55] the most efficient pretreatment method with one batch
24 349 cultivation is that which applies iodopropane and BESA.
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27 351 **3. Reactors used in dark fermentation.**

28 352 Reactor design is an important factor to ensure the process of dark fermentation can be
29 353 controlled, by selection of process temperature, mixing speed, the surface of reactions and pH
30 354 of the medium. A proper selection of reactor type enables the maintenance of suitable
31 355 conditions for efficient production of hydrogen.
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35 356 Reactors differ by type of the process (continuous, batch, -semi-continuous) and its phase
36 357 multiplicity e.g. two-phase and multiphase reactors. The bioreactors can be singular or work
37 358 in parallel or in series. Reactors in series allow high conversion of the substrate to be
38 359 obtained, while the singular ones are cheaper and simpler in form. The continuous reactors
39 360 include CSTR (continous stirred tank reactor), ANABR (Anaerobic Baffled Reactor), UASB
40 361 (Upflow Anaerobic Sludge Blanket reactor), fluidized bed reactor, packed-bed reactor, and
41 362 fixed-bed reactor [98]. The batch type includes: vials, fermenters and leaching-bed reactors
42 363 [99,100]. A chemostat is an example of a semi-continuous type reactor [101,102]. Reactor are
43 364 described and then compared in table 3.
44
45
46

47 365 a) *Batch type*

48 366 Batch type reactors are the primary option in most experiments of dark fermentation [5]. **An
49 367 advantage of the batch process is its simplicity, high conversion–level of substrate and
50 368 small pretreatment requirements.** A disadvantage of batch type reactors is generally low
51 369 production rate. The most common material for construction of a batch type reactor is glass
52 370 [103,104] A batch reactor for an anaerobic process is called ABR (Anaerobic Batch Reactor)
53 371 or ASBR (Anaerobic Sequencing Batch Reactor) [72]. A specific kind of batch process were
54 372 performed in serum bottles [37, 57] used for testing inoculums growth under different
55 373 conditions. Logan et al. [105] obtained high level (23%) of conversions of glucose and
56 374 sucrose into hydrogen, lower level (15%) from molasses, (0.5%) from lactate and (0.075%)
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65

375 from cellulose. Shin and Han [106] designed a special type of batch reactors in a series
1 376 (leaching bed reactors in rotation mode) for hydrogen production from food waste. The
2 377 hydrogen yield of the whole series was 310 ml H₂ g⁻¹ food waste. The hydrogen production
3 378 rate was 1321.6 ml H₂ l⁻¹h⁻¹. In the case of glucose the yield was 1.04 mol H₂ g⁻¹ glucose [72].

6 379 *b) Semi-continuous reactors*

7
8 380 The semi-continuous process can be used for preparation of bacterial cultures for continuous
9 381 process that prevents wash-out in a continuous stage. The semi-continuous part includes
10 382 intervals of feeding and digestion [14,107,108]. Generally hydrogen yield is lower than batch
11 383 process but this type allows for better reaction control and is also used as preparation of
12 384 bacteria in continuous flow [109]. The semi-continuous reactors are semi-continuous drummer
13 385 and chemostat. Oh et al. [59] applied in chemostat mixed cultures of *Clostridium acidisoli*
14 386 CAC237756, *Linmingia china* AF481148, and *Cytophaga* sp. MDA2507AF238333
15 387 (*Flexibacteraceae*). The concentration of hydrogen in off-gas was 57-60%. In the feed was
16 388 glucose of concentration 10 000 mg l⁻¹. The conversion of glucose to hydrogen for HRT 5 h
17 389 was 20% at pH =5.5 [59].

21 390 Semi-fed process for swine manure fermentation was performed in 8 l tank with hot plate
22 391 stirrer with 200 rpm [58]. Chen et al. [110] used the same type (4 l) reactor for investigation
23 392 of culture kinetics. The semi –continuous mode can be used as the start-up stage of
24 393 continuous processes like in Chen and Lin [111]. The semi-continuous process can be used
25 394 for preparation of bacterial cultures for continuous process that prevents wash-out in a
26 395 continuous stage. The semi-continuous part includes intervals for feeding and digestion.

30 396 *c) Continuous process Continuous stirred tank reactors (CSTR's)*

31
32 397 Advantages of CSTR are: high mass transfer due to mixing, simplicity of construction and
33 398 operation. The CSTR process is limited by: low biomass concentration, cell retention in low
34 399 dilution rate and risk of washout of cells at a high dilution rate [112]. Continuous stirred tank
35 400 reactors (CSTR) are often used to investigate the influence of process conditions in the case of
36 401 continuous process in lab scale like in Kim et al. [40, 62]. Ren et al. [46] developed a pilot
37 402 plant based on CSTR reactors, where the hydrogen production rate from molasses was 201.4
38 403 ml H₂ l⁻¹molasses/h . A continuous process was designed also for fermentation of a xylose and
39 404 glucose mixture by Taguchi et al. [37]. The continuous process provided a higher hydrogen
40 405 yield than in the case of the batch process. In the case of pure xylose, Taguchi et al. [37]
41 406 obtained a higher hydrogen production rate in continuous than in batch mode, while hydrogen
42 407 yield was lower. Yokoi et al. [41] used the continuous dark fermentation stage during the
43 408 hybrid process of dark and photofermentation. Inoculum is often prepared in a batch mode
44 409 reactor and then transferred into a continuous mode reactor.

49 410
50 411 The hydrogen yield from continuous fermentation of starch using mixed cultures of
51 412 *Clostridium butyricum* and *Enterobacter aerogenes* HO-39 was 2.9 mole of hydrogen per
52 413 mole of glucose [80]. Hussy et al. [68] obtained a hydrogen yield of 1.3 mole of hydrogen per
53 414 mole of glucose in the CSTR process of mixed cultures of *Clostridium butyricum* and
54 415 *Enterobacter aerogenes* [68]. Besides, a high organic load-rate cannot be used [28]. Therefore,
55 416 Wu et al. [114] modified CSTR by inserting anaerobic sludge immobilized by seeding with
56 417 silicone. Such seeding enables granulation of sludge and increased biomass concentration to
57 418 35 g of biomass/l to be obtained. The highest hydrogen yield for *Clostridium pasteurianum*

419 was 1.93 mole of hydrogen/mol of hexose or 3.5 mol H₂/mol sucrose. The hydrogen
 420 production rate was 115.1 ml H₂ h⁻¹ l⁻¹ or 0.61 mol H₂ h⁻¹ l⁻¹ for initial concentration 40 g COD
 421 l⁻¹ [114]. At an initial substrate concentration of 30 g COD l⁻¹ the hydrogen production rate
 422 was 14.5 ml H₂ h⁻¹ l⁻¹ [114]. The specific hydrogen production rate was 0.439 l H₂ h⁻¹ g⁻¹
 423 biomass [64]. The scheme is shown in Fig. 2.

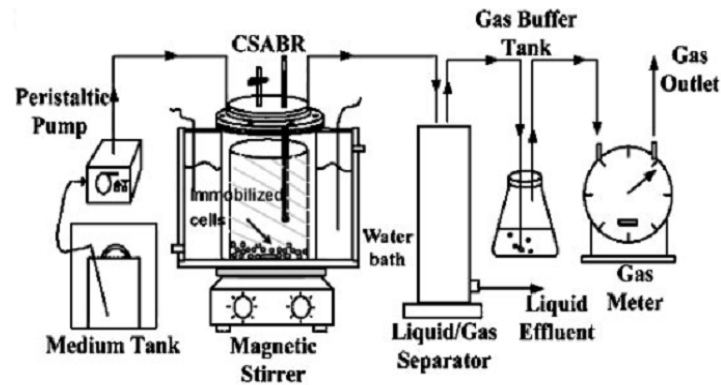


Fig. 2. Scheme of continuous stirred anaerobic bioreactor, after [64]

425 Wu et al. [115] used a CSTR reactor, while Ghimire et al. [22] a UASB reactor, as incubator
 426 for 8 h HRT of sludge for fluid-bed reactor. According to Hawkes et al. [36] optimal
 427 conditions for the continuous process of simple substrates are: pH= 5.5, working temperature
 428 30°C and HRT 8 h – 12 h.

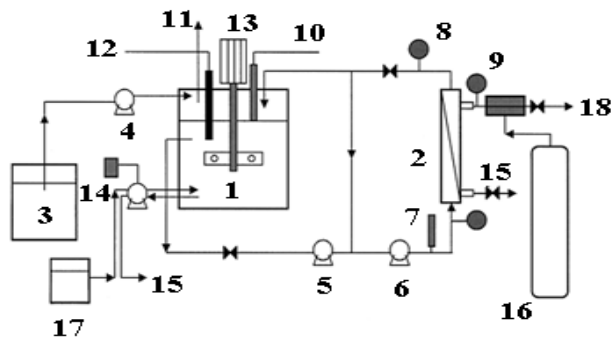


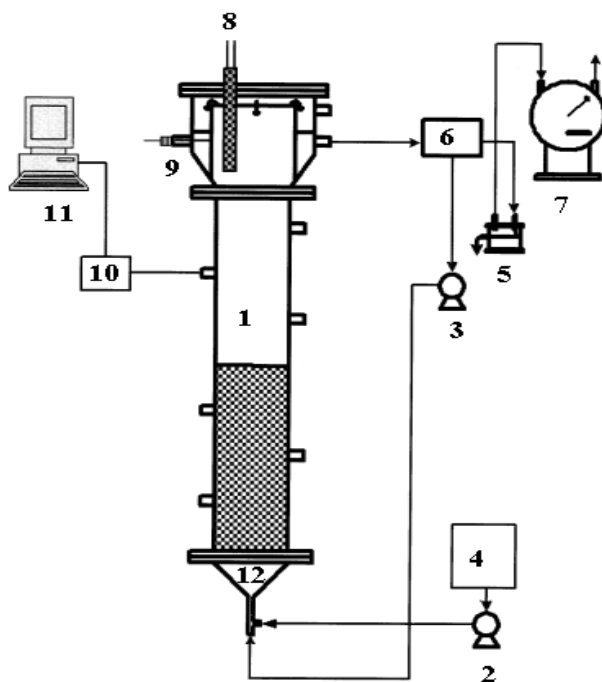
Fig. 3 Diagram of membrane bioreactor for hydrogen production. 1 - anaerobic reactor;
 2 - cross-flow membrane; 3 – influent purged with nitrogen; 4 - feed pump; 5 - recirculation
 6 – high recirculation pump; 7 - flow meter; 8 - manometer measuring pressure at
 inlet, outlet and permeate side; 9 – backpulsing every 10 s to avoid fouling; 10 - level
 controller; 11 - gas monitor; 12 - pH controller; 13 - motor; 14 - timer; 15 - waste;
 16 - nitrogen gas; 17 - medium w/o organics; 18 - effluent after [59].

429 d) *Membrane bioreactor*

1
2 430 Membrane reactors increase conversion by separation of products but only in short detention
3 431 times not longer than 3.3 h HRT. In this condition internal fouling is minimal[59].
4 432 A membrane bioreactor is a semi-continuous bioreactor with applied cross-flow membrane
5 433 module [59] - see Fig. 3. A ceramic-alumina membrane module of tubular type keeps the
6 434 biomass in the reactor [35]. The applied membrane facilitates increasing conversion of
7 435 glucose to hydrogen from 20% to 38% [59]. The dilution rate was decreased while sludge
8 436 retention time was increased. KOH was used as a pH controller. The recycle-loop flow-rate
9 437 was 378 l h⁻¹ with cross-flow velocity 2.8 m/s [59]. Before process membranes were cleaned
10 438 by rinsing with 1% nitric acid for 2 h, 2% of NaOCl with water for 2 h. Pore sizes of
11 439 membrane were 0.2 μm, 0.5 μm and 0.9 μm [35]. Membrane surface was 55 cm²[35].

15 440 e) *Fluidized bed reactor*

16
17 441 Fluidized bed reactor allows the use of high volume fraction of biomass without risk of
18 442 attrition like in CSTR. Besides, bubble column like reactor allows for high mass transfer. Wu
19 443 et al. [65, 67] applied fluidized bed reactors with acrylic latex plus silicone to immobilize
20 444 sludge, see Fig. 4. The bed of sludge consisted of particles of 3.0-4.0 mm formed from a
21 445 CaCl₂ mixture of alginate sodium with 75% (v/v) acrylic latex/silicon. Additionally, seed
22 446 from municipal sludge was supported by alginate gel. The use of sewage sludge as a seed in
23 447 fluidized bed and optimal concentration of sucrose 17.8 g l⁻¹, provided a hydrogen yield of
24 448 1.34 mol H₂ mol⁻¹hexose[116]. Relevant parts of bed reactors (fluidized, packed or fixed) are
25 449 carrier materials that keeps bacteria in the form of biofilms. According to Barca et al.[98]
26 450 carrier material diameters range from 0.2 and 4 mm, and their density between 1.05 and 1.50
27 451 g/cm³.



58 Fig. 4. Fluidized bed column of diameter 8 cm, static bed height 40 cm and column
59 height 120 cm, total working volume 10l (1); 2 - pump for substrate, 3 - recycle pump,

4 - substrate tank, 5 - gas-liquid separator, 6 - buffer tank, 7 - gas meter, 8 - heating coil, 9 - thermal couple, 10 - PF acquisition, 11 - PC analysis system, 12 - liquid distributor[116].

f) *Packed-bed reactor.*

Packed-bed keeps biomass in the reactor, preventing its washout like in CSTR. An example of a scheme of setup with packed-bed reactor is shown in Fig. 5. Various packing materials were used: glass beads, activated carbon, ceramic fittings and polymeric (for example polyethylene)[67,98].

In the case of these reactors Barca et al. [98] report that carrier material diameters are from 1.5mm to 25 mm and density from less than 0.5 up to 2 g/cm³. The ratio between carrier particle diameter and reactor vessel diameter should be less than 0.1 [98,117]. Intra particle porosity (an important parameter of the reactors) depends on the material. Barca et al. [98] pointed out that increasing of porosity results in increased adhesion of hydrogen, while more rough surface better protects biofilms from shear stresses. When activated, carbon porosity is in the range 1100–1350 m²/g and for polyethylene pellets 12 cm²/g [118,119]. The plugging of the packing by substrates can be prevented by designing proper configuration of packing. According to Kumar and Das [120] in dark fermentation the most efficient configuration of packing material (from among tubular, rhomboidal or tapered) is rhomboidal. The packing material was designed using a lignocellulosic matrix. At the optimal glucose concentration of 10 g l⁻¹: hydrogen yield was 2.04 mol H₂ mol⁻¹ glucose, the specific hydrogen production rate was 6.85 l H₂ h⁻¹ g⁻¹ biomass and volumetric hydrogen rate was 1.85 l H₂ h⁻¹ l⁻¹ substrate. The highest hydrogen production rate was 75.6 mmol H₂ l⁻¹h⁻¹.

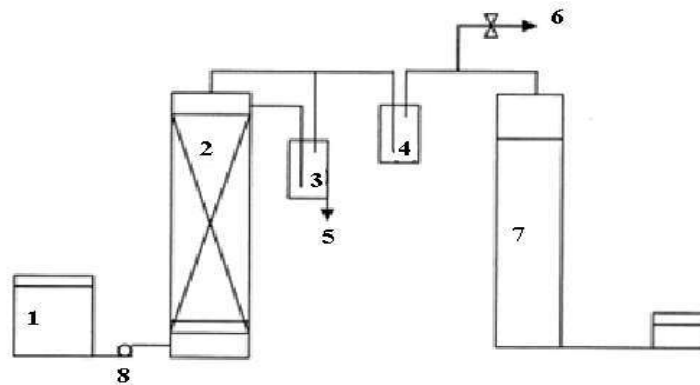


Fig. 5 Scheme of the diagram with packed-bed reactor with rhomboidal packing material: 1 - feed tank, 2 - packed bed reactor, 3 - liquid trap, 4 - CO₂ absorber, 5 - effluent, 7 - gas collector, 8 - peristaltic pumps [67]

Palazzi et al. [67] applied a packed column for dark fermentation of starch with *Enterobacter aerogenes*. Packing to immobilize bacteria was composed of spongy particles and glass beads. Another form of packing was composed of coir magnetite nanoparticles [121,122]. Sponge particles of dimensions 5×5×2 mm³ were obtained after sterilization of sponge. Applying glass beads of diameter 7 mm with spongy particles lowered uniform residence time

476 distribution in the bed. If the flow rate was low ($4 \text{ cm}^3\text{h}^{-1}$) and residence time was high
1 477 (100 h), the reaction was shifted towards butane-2,3-diol, and the hydrogen production rate
2 478 was $1.3 \text{ mmol H}_2 \text{ h}^{-1}$. Selectivity of hydrogen was increased when the flow rate was
3 479 increasing, from 4 to $40 \text{ cm}^3\text{h}^{-1}$, and residence time decreased, e.g. $\sim 10 \text{ h}$. The hydrogen
4 480 production rate at flow rate $40 \text{ cm}^3\text{h}^{-1}$ was found to be $4.06 \text{ mmol H}_2 \text{ h}^{-1}$, but its yield
5 481 decreased from $3.02 \text{ mmol H}_2 \text{ mol}^{-1}\text{glucose}$ to $1.54 \text{ mmol H}_2 \text{ mol}^{-1}\text{glucose}$ [67]. Barca et al.
6 482 [123] used glass beads of 4mm in diameter and porosity 0.38 as packing for biofilm of
7 483 *Clostridium acetylobutylicum* and *Desulfibrio vulgaris*. They obtained a hydrogen yield 1.34
8 484 $\text{mol H}_2/\text{mol glucose}$ and hydrogen production $0.097 \text{ l H}_2 / \text{h l glucose}$.

12 485 *g) Fixed-bed reactor*

14 486 In a fixed-bed reactor a biofilm typically replaces the catalytic layer. The advantages are
15 487 generally lower pressure drop together with simple and robust construction. Other advantages
16 488 mentioned by Contreras–Davila et al. [124] include higher magnitudes of volumetric mass
17 489 transfer coefficients. The disadvantage of fixed-bed reactor is limited surface area [125].

20 490 Chang et al.[126] designed fixed bed reactors with support matrices, such as expanded clay
21 491 and activated carbon. Anzolar-Rojas et al. [127] used as a support recycled polyethylene
22 492 cylinder-shaped particles of diameters from 7.1mm and 17.5mm and length 30mm. For
23 493 expanded clay working volume 0.3 l, hydrogen volumetric production rate was $0.415 \text{ l H}_2 \text{ h}^{-1}$
24 494 l^{-1} sucrose at 20 g COD l^{-1} , specific hydrogen production rate was $0.0965 \text{ l H}_2 \text{ h}^{-1}\text{g}^{-1}$ biomass
25 495 [110].

29 496 Wu et al. [116] designed a fixed bed reactor for dark fermentation from sucrose, glucose, and
30 497 fructose. Polyethylene octane elastomer was used as a sludge and catalyst. The hydrogen
31 498 production rate increased by increasing up-flow velocity to 0.91 cm s^{-1} [116]. Gomes et al.
32 499 [128] designed a multiple-tube fixed bed reactor with PVC tube avoiding washing out. The
33 500 hydrogen production rate was $0,061 \text{ l H}_2 \text{ h}^{-1}$ [128].

36 501 *(h) Upflow Anaerobic Sludge Blanket (UASB) reactor*

38 502 UASB reactors are characterized by conversion of biomass in large quantities and using high
39 503 organic load. However, according to Lee et al. [129] the main disadvantage of the UASB
40 504 reactor is its sensitivity to the channeling effect causing loss of contact with the substrate and
41 505 the bed. The reactor with mixing of 120 rpm was used by Mu et al. [60].

44 506 The expanded granular sludge bed (EGSB) reactor is a special type of UASB. EGSB is
45 507 characterized by a larger height to diameter ratio than that of UASB and so recirculation of
46 508 effluent velocity causing higher up-flow velocity [129]. Hernandez et al.[130] used as a
47 509 support material recycled tire rubber. Kisieleska et al. [131] obtained from whey permeate
48 510 $0.29 \text{ l H}_2/\text{h}$ and yield $4.55 \text{ mol H}_2/\text{kg COD}$. Sui et al. [132] used SiC support obtaining
49 511 hydrogen production rate $0.22 \text{ l H}_2 / \text{h}$ and hydrogen yield $0.93 \text{ mol H}_2/\text{mol of glucose}$. Rosa et
50 512 al.[133] from cheese whey obtained $1.33 \text{ mol mol H}_2/\text{mol of lactose}$ and hydrogen production
51 513 rate $0.51 \text{ l H}_2/\text{h}$.

55 514 *(i) Carrier-induced granular sludge bed (CIGSB)*

57 515 Lee et al. [129,134] designed a CIGSB reactor **to improve mixing properties** by applying
58 516 a different variation of agitation system (physical, mechanical) and a different height to
59 517 diameter ratio to the reactor. Comparing height to diameter ratios of 4, 8 and 12, the ratio 8

518 results in the highest hydrogen generation from sucrose in wastewater. The hydrogen
 519 production rate was 9.3 l H₂ h⁻¹ l⁻¹ of sucrose and a maximum H₂ yield of 4.02 mol H₂ mol⁻¹
 520 substrate.

521 Table 3 Comparison of techno-economical aspects of reactors

Reactors	Technical aspects	Max. yield	Economical aspects	Ref.
Batch	Simple and facile system operation, high yield	4708 ml H ₂ /g glucose	The hydrogen production potential is too low for industrial scale	[135]
Semi-continuous	Necessary bacteria preparation for continuous regime	460 ml H ₂ /g glucose	The hydrogen production potential is too low for industrial scale	[136]
CSTR	High risk of bacteria washout; mixing allows intimate contact between substrate and biomass; efficient pH and temperature control	63 ml H ₂ /g glucose	The simple form, cheap	[137, 138]
Membrane	HRT shorter than 3.3 h; much lower conversion than in CSTR	116 ml H ₂ /g glucose	Costly membrane exchange due to possible fouling.	[59]
Fluidized-bed	Risk of biomass over-accumulation	12 ml H ₂ /g glucose	High complexity of the system and the high energy costs	[6]
Packed-bed	Risk of biomass over-accumulation; limited mixing	125 ml H ₂ /g substrate	Complexity is less than in fluidized bed but still high	[123, 139]
Fixed-bed	Risk of biomass over-accumulation	11 ml H ₂ /g sucrose	High pressure drop can make process hard to implement	[140]
UASB	High treatment efficiency, low and stable HRT; no granulation of biomass is observed; more tolerant to fluctuation of process parameters than CSTR	263 ml H ₂ /g glucose	High pressure drop can make process hard to implement; long term production stability possible	[114, 141–143]
CIGSB	Poor efficiency of mass transfer, risk of bacteria washout.	179 ml H ₂ /g sucrose	High pressure drop can make process hard to implement	[134]

522
 523 *(j) Multi –stage process*

524 In the case of two-stage dark-fermentation process, the feed preparation and hydrolysis are
 525 kept as a continuous process e.g. in a continuous stirred tank reactor but the second stage
 526 proceeds e.g. in the batch periodic anaerobic baffled reactor (PABR) [144]. At the second
 527 stage sugars are converted to hydrogen, carbon dioxide and organic acids. The hydrogen
 528 production rate was 7.53 l H₂ per day with removal of 95% of COD (cheese whey) [144].

529 Another type of hybrid batch and continuous stirred tank reactor with sludge was used by Wu
 530 and Chang [72]. Sludge was immobilized by cells from activated carbon and PMMA
 531 (Poly(methylmethacrylate)). The substrate of hydrogen was sucrose from wastewater. 90% of
 532 sucrose was converted [72]. The maximum production rate was 1800.4 ml H₂ l⁻¹ h⁻¹ and
 533 maximum H₂ yield 2.25 mol H₂ mol⁻¹ substrate [72].

534 **4. Influence of process parameters on hydrogen yield in dark fermentation**

535 In order to optimize the process of dark fermentation (in relation to the highest hydrogen
 536 production rate) one should aim at: increasing efficiency of the Fe-hydrogenase and usually

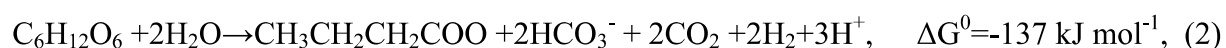
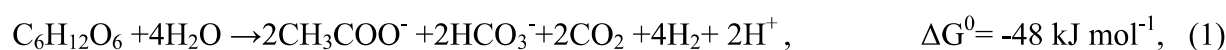
537 inhibiting of the NiFe-hydrogenase as well as obtaining optimal conditions for bacterial
538 culture growth.

539 It is well known that the efficiency of dark fermentation is influenced by process parameters
540 such as: feed type, temperature, partial pressure, pH and presence of metal ions. The
541 importance of a particular factor depends on the reactor type and feed.

542 **4.1. Feed type**

543 According to Hallenbeck [145] and Gomez et al. [6] theoretical maximum yield of hydrogen
544 from hexoses (including glucose) in dark fermentation is 32%. Similar yields were obtained
545 for hydrogen production from pentoses (30-33%) [27]. In the case of glycerol the hydrogen
546 yield in dark fermentation is 38% [25].

547 Bartacek et al. [28] and Woodward et al. [146] pointed out that there are three
548 thermodynamically possible dark fermentation pathways from hexoses: acetate equation (1),
549 butyrate equation (2) and acetate-ethanol equation (3):



553 The acetate pathway is the one with the highest theoretical hydrogen yield: 4 moles of H₂
554 from mole of hexose. The most efficient way according to ref. [24, 122] is the acetate
555 pathway (1) but the most probable is the butyrate fermentation (2). Alkaline pretreatment
556 leads most often towards acetate fermentation.

557 The process which could theoretically yield 12 moles of hydrogen from 1 mole of glucose:



559 is thermodynamically impossible due to positive value of Gibbs free energy.

560 In the case of pentoses, the reaction proceeds according to scheme (5) [27]



562 According to Gomez et al. [79] low loading rate and mixed reaction schemes are leading to
563 stable dark fermentation.

564 **4.2. Optimal temperature**

565 The optimum temperature for the process depends on the feed type and bacterial inoculum.
566 In the case of hydrogen generation from crop residues hydrogen yields under thermophilic
567 conditions (T = 70°C) are higher than under mesophilic conditions (T = 37°C) [6, 76]. On the
568 other hand, Azbar et al. [53] compared hydrogen generation from cheese whey: under
569 thermophilic conditions conversion of whey to hydrogen at T = 55°C was lower than in the
570 case of mesophilic at 35°C.

571 Zhang and Shen [147] studied mixed culture bacteria with *Clostridium pasteurianum* as
572 methanogenic bacteria and hydrogen generation was stopped when the temperature reached
573 45°C. Mixing mesophilic culture with other bacteria does not shift optimum temperature;

570 35°C was still the optimum temperature for hydrogen production [147]. In the case of
1 571 mesophilic *Enterobacter aerogenes* strain HO-39 in batch process optimum temperature was
2 572 37°C while in a continuous process 35°C [42]. According to Hawkes et al. [36] optimal
3
4 573 temperature for a butyrate type hydrogen-production was 30°C.

574 **4.3 Partial pressure**

575 According to Hallenbeck [148] there is no unique answer as to whether the partial
576 pressure of hydrogen should be close to 0 or be increased. Kramer and Bagley [81] considered
577 that increase of hydrogen yield of more than 2 mol H₂ mol⁻¹ glucose was achieved by
578 lowering partial pressure. Kim et al. [69] obtained a yield of 1.68 mol H₂ mole⁻¹ glucose by
579 lowering partial pressure. Mandal et al. [47] by lowering partial pressure from 760 mm Hg to
580 380 mm Hg increased yield from 1.9 mol H₂ mol⁻¹ glucose up to 3.9 mol H₂ mol⁻¹ glucose.

581 Lowering of partial pressure is obtained usually by gas sparging. Contrary to [40, 79] Mizuno
582 et al. [113] for mixed culture of clostridium increased hydrogen yield from 0.85 to 1.43 mol
583 H₂ mol⁻¹ glucose by applying nitrogen sparging in a continuous process. In the case of argon
584 sparging in a batch type process with *Enterobacter aerogenes*, hydrogen yield increased from
585 0.52 to 1.52 mol H₂/mol glucose [34, 50, 62]. Results of Mizuno et al. [113] are similar to
586 results for continuous process obtained by Hussy et al. [150]. According to Hussy et al. [150]
587 lowered partial pressure of hydrogen by the nitrogen sparging in continuous reactor, reduced
588 the hydrogen concentration in the outflow from 50% to 7%. The decrease of off-gas
589 concentration resulted in stable hydrogen yield of 1.9 mol H₂ mol⁻¹ hexose in a 18 days
590 period.

591 **4.4. C/N ratio**

592 Low C/N ratio (carbon to nitrogen ratio) is considered to inhibit dark fermentation [93]. The
593 optimum value C/N ratio depends on the raw material, type of process and bacteria. The
594 optimal C/N ratio should be high; for cheese whey is in the range of 30-40:1. Anzola-Rojas et
595 al. [127] determined in the case of the wastewater optimum C/N ratio to be 137:1 and
596 obtained hydrogen yield 3.5 mol H₂/mol sucrose. Argun et al. [89] determined for wheat
597 powder fermentation optimal C/N ratio ~200:1.

598 **4.5. Metal, phosphate ions**

599 According to Wang and Wan [151] one of the most important for the process efficiency is
600 Fe²⁺ ions. Iron ions are part of the Fe-hydrogenase enzyme that activates hydrogen generation
601 in the anaerobic bacteria. The optimum concentration of iron ions is still not known [151].
602 Iron ions optimum concentration decreases with rise in temperature. Nath et al. [84] and
603 Wang and Wan [28, 32] used FeSO₄·7H₂O solution as a source of iron ions while others
604 preferred FeCl₂ [83].

605 According to Zhang et al. [147] the optimum iron concentration depends on the bacteria type
606 and temperature. In the case of a mixed culture of *Clostridium pasteurianum* and starch as
607 substrate, the optimum iron concentration was 800 mg l⁻¹ at temperature T=25°C and resulted
608 in a hydrogen yield of 356 ml H₂/l starch; for T= 35°C the optimum iron concentration was
609 200 mg l⁻¹ (hydrogen yield 377 ml H₂ l⁻¹) and for T= 40°C the optimum iron concentration
610 was 25 mg l⁻¹ (hydrogen yield 351.1 ml H₂ l⁻¹). The concentration of iron ions influenced
611 duration of fermentation: at iron ions concentration between 25-100 mg l⁻¹ fermentation lasted

60 h, in range of 100 to 1600 mg l⁻¹ the time decreased to 48 h [65]. Moreover, Zhang et al. [147] show that an iron concentration above 100 mg l⁻¹ for *Clostridium pasteurianum* improved hydrogen yield from starch but it shortened the hydrogenase activity. In the case of cheese whey iron concentration in the range of 50-150 mg l⁻¹ improves the generation of hydrogen [152]. According to Nath et al. [84], in the case of *Enterobacter cloacae* at temperature 37°C the optimum iron ion concentration was 20 mg l⁻¹ (hydrogen yield was 3.31 mol H₂ mol⁻¹ glucose).

Wang and Wan[52] considered that for Ni²⁺ ion optimum concentration is 0.1 mg l⁻¹, which led to a yield of 232 mg H₂ g⁻¹ glucose. As the source of Ni²⁺ ion NiCl₂ was proposed [29].

Azbar et al. [152] investigated the influence of various metal-ions concentrations on dark fermentation yield from cheese whey. Optimal metal salt concentrations were proposed: ZnCl₂ (1.25–2.5 mg/l); CaCl₂ (250–500 mg/l); MgCl₂ (50–100 mg/l); MnCl₂ (2.5–5 mg/l) and FeCl₂ (50–100 mg/l) leading to hydrogen yield 3.5 mol H₂ mol⁻¹ lactose [152].

According to Kothari et al. [99], Fang et al. [153] fragility of the bacteria increases in order with copper, zinc and nickel, cadmium, chromium and lead, respectively. However, according to Wang and Wan [151] after Shei and Lin [154] considered for sludge wastes in order zinc, copper and chromium. Phosphate ions are both good nutrition and buffer components [99]. According to Argun et al [89] optimum ratio of C/P is 2000:1.

4.6. pH and Oxidation-Reduction Potential (ORP)

The value of optimum pH depends upon the substrate and bacterial culture. The influence of pH control and dilution rate in the range 0.4 to 1.0 h⁻¹ on glucose and xylose fermentation using *Clostridium* sp was investigated by Taguchi et al. [37]. The hydrogen production rate of fermentation increased with dilution rate under controlled pH = 6.0 conditions. The highest hydrogen production rate registered was 21.03 mmol H₂ h⁻¹l⁻¹ xylose for a dilution rate of 0.96 h⁻¹. Without Ph control, the maximal hydrogen production rate was 16 mmol H₂/h l xylose when the dilution rate was 1.03 h⁻¹ [37]. The pH value influences the yield more than does it dilution rate, so for any dilution rate choice of optimal pH value of solution is essential.

The highest yield with pH control was 2.06 mol H₂ mol⁻¹xylose at dilution rate 0.21 h⁻¹ and 2.15 mol H₂ mol⁻¹glucose at dilution rate 0.19 h⁻¹ [37]. However, the role of pH control is not clear always as in the case of uncontrolled (freely evolving) pH the highest achieved yield was smaller in relation to pH controlled conditions (1.82 mol H₂ mol⁻¹xylose) at dilution rate 0.22 h⁻¹ and larger (2.36 mol H₂ mol⁻¹glucose) at dilution rate 0.18 h⁻¹ [37]. Xu et al. [155] recommended an acetate buffer of 110mM to 250mM as an efficient method for adjustment of pH level.

In order to control pH during dark fermentation with *Clostridium* sp, Zhu et al. [58] used solutions of 1.0 M of NaOH and 1.0 M HCl. Generally, optimum pH for hydrogen production by mesophilic bacteria lies in the range 5.5-6.5 while thermophilic bacteria like *Clostridium thermopalmarium* or *Thermatoga neapolitana* prefer pH in the range of 6.9-7.2 [22].

Kargi et al.[93] analyzed time evolution of pH and oxidation-reduction potential (ORP) for initial total lactose concentration 20 g l⁻¹ - see Fig. 6. The data provide information on optimal pH value i.e. 5.5-6.0, for cheese whey powder fermentation under thermophilic condition. The

654 pH changes influence ORP value, which vary in the range from 53mV to -200 mV. According
 655 to Kargi et al. [93] the optimum ORP value is in the range -50 mV to -200 mV.

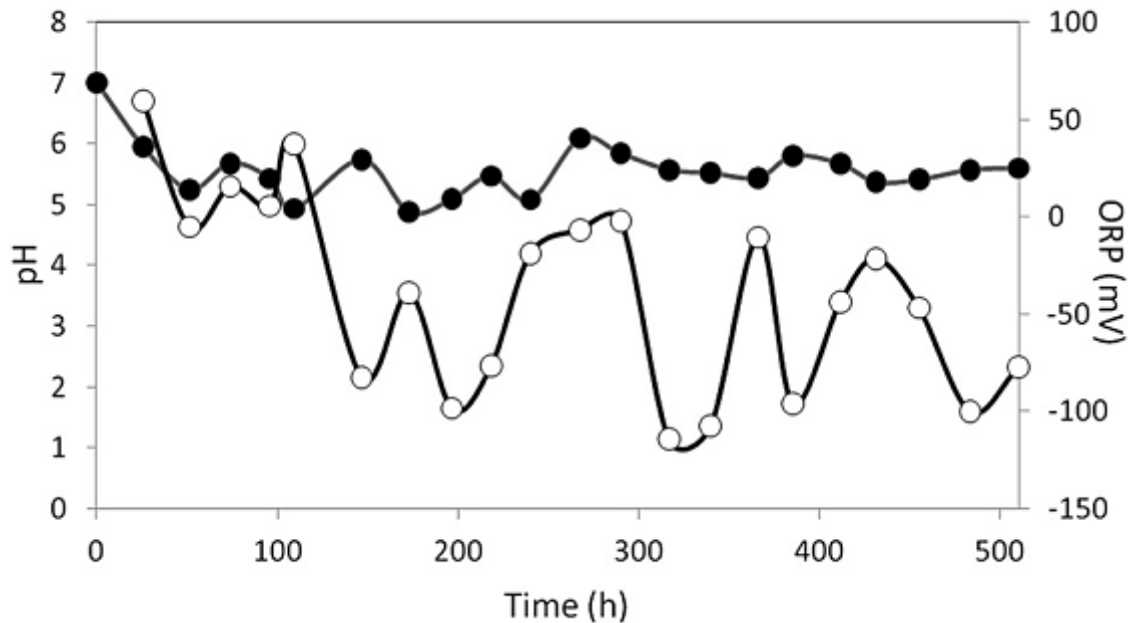


Fig. 6. Time evolution of pH (black dots) and ORP (open circles) values for substrate with initial total sugar from whey (mainly lactose) concentration 20 g l⁻¹[93]

656

657 Zhang and Shen [65] obtained an optimum pH range of 7.0-8.0 for dark fermentation of starch
 658 by *Clostridium pasteurianum*. Zhu et al. [58] determined optimum pH=5.0 for fermentation of
 659 pig manure by *Clostridium* sp. According to Yokoi et al. [42] optimum pH is in the range 6.0-
 660 7.0 for fermentation caused by *Enterobacter aerogenes* strain HO-39.

661 The value of pH can influence the mechanism of bacteria growth. At pH between 3.3-4.0.
 662 *Enterobacter* bacteria grow aerobically while above this range anaerobically [156]. Hussy et
 663 al. [68] claims that the pH in the range from 4.5 to 5.2 is undesirable for fermentation of
 664 sewage sludge from wheat flour industry. In this range of pH hydrogen can be consumed by
 665 homoacetogenesis and propionate producing processes [68]. Optimum pH for *Enterobacter*
 666 *cloacae* DM11 is 6.5 [84]. According to Vijayaraghavan and Ahmad [157] optimal hydrogen
 667 yield was 4708 ml H₂ l⁻¹ for palm oil mill waste fermentation achieved at pH 5.0. According
 668 to Ren et al. [45] pH below 4.5 leads during hydrogen fermentation to ethanol production.

669 4.7 Hydraulic retention time (HRT)

670 According to Zhu et al. [72] hydraulic retention time plays a significant role in the case of
 671 the semi-continuous fermenter. A change of HRT can cause a variation of hydrogen
 672 concentration in the gas outflow - Fig. 7.A. The dependence of biogas production on pH and
 673 HRT is shown in Fig. 7.B.

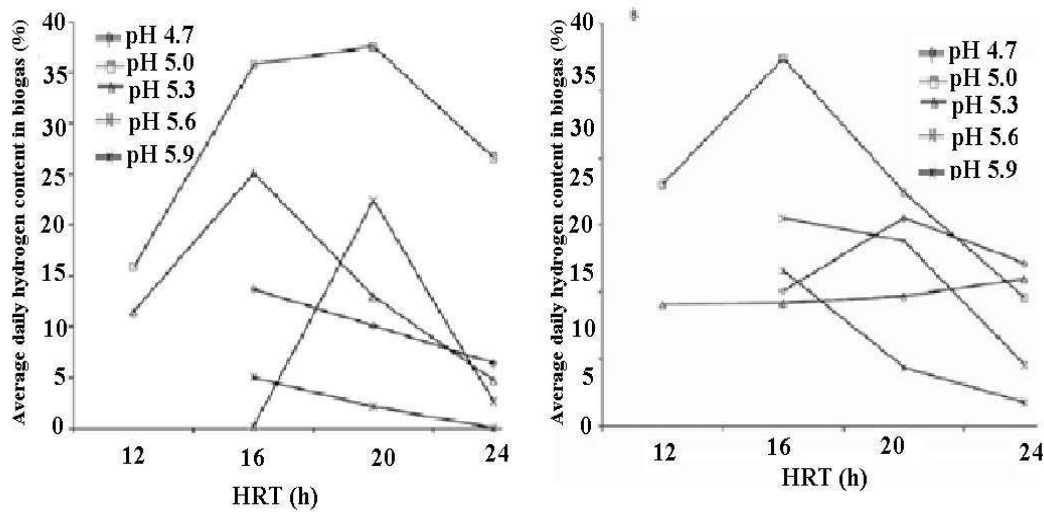


Fig. 7. Influence of change HRT, and pH to hydrogen content (A), biogas production B after Zhu et al. [58].

674

As it is shown in Fig. 7 A the highest concentration of hydrogen in biogas was observed for HRT in the range 16-20 h at 35.7-37% [58]. Maximal gas production of 27 l/day was registered at pH 5. Optimal hydraulic retention time for hybrid reactor series of Wu and Chang [74] was in the range 4-8 [h].

According to Lee et al. [134] reduction of hydraulic reduction time increases hydrogen production rate from sucrose independently from reactor's height to diameter ratio. Chen et al. [111] decreased hydrogen production rate from sucrose from $0.094 \text{ mol H}_2 \cdot \text{h}^{-1}$ to $0.032 \text{ mol H}_2 \cdot \text{h}^{-1}$ by increasing HRT from 6 to 13.3 h.

According to Xing et al. [158] low HRT is desired for hydrogen production especially in CSTR. Low HRT enables the removal of methanogenic bacteria from sludge due to its short specific growth time. The HRT value correlates with dilution rate. According to Chen et al. [111] dilution rate should be in the range of 0.075 h^{-1} to 0.167 h^{-1} for efficient hydrogen production in CSTR. Chen et al [110] decreased HRT from 13 h to 3.3 h improving hydrogen gas production from 4.9 to $26.9 \text{ l H}_2 \text{ l}^{-1}$ of sucrose. However, a decrease of HRT to values lower than 3.3 h lowered the hydrogen production [110].

Wu et al. [159] studying continuous anaerobic process determined HRT to be 0.5 h for the semi-continuous reactor with sewage sludge. However, the optimum HRT value was 6 h when $\text{pH} = 5.7$. Oh et al [55] and Logan et al. [101] have reduced HRT from 5h to 3.3h in a membrane reactor; this led to increased conversion of glucose from 90% to 98%. The decrease of HRT can lead to lower pH [158]. The dependence results from an increased accumulation of volatile fatty acids. However, pH can be adjusted by 0.1 M NaOH solution like in Chen and Lin [110]. In the case of the membrane reactor, the retention time can be divided into sludge retention time and hydraulic retention time. The increase of sludge retention time from 3.3 h to 12 h improved efficiency of glucose to hydrogen conversion from 22 to 25% [160].

700 In the case of UASB reactor decrease of HRT from 24 to 2 h lead to lower hydrogen yield
701 from 2.14 mol H₂ mol⁻¹glucose to 1.83 mol H₂ mol⁻¹glucose. However, the reduction of HRT
702 increased glucose conversion to 85% [160].

703 In the fluidized bed reactor using sucrose as substrate optimal HRT was 2 h with hydrogen
704 yield 1.34 mol H₂ mol⁻¹ hexose [161].

705 In the case of packed-bed reactors, optimal HRT depends on carrier and immobilized bacteria
706 cultures. The HRT value changes from 0.5 h when sludge is packed with activated carbon to
707 HRT =10 h when the carrier is from glass/spongy beads [116]. For a packed-bed with
708 *Enterobacter aerogenes* HU 101, optimal HRT was 0.67 h, while for the mutant bacteria AY-2
709 it was 0.55 h [162]; at the same time the hydrogen production from sucrose changed from 31
710 to 58 mmol H₂ l⁻¹h⁻¹ for the mutant.

711 For fixed bed reactor optimal HRT also depends on the material of matrices that immobilized
712 the bed. In the case of support from activated carbon optimal HRT was 1 h while for
713 expanded clay it was 2 h [126].

714 **5. Separation methods of hydrogen from the dark fermentation products**

715 The most common conventional method for hydrogen separation is pressure swing adsorption
716 (PSA). It depends on an adsorbent bed that captures the impurities in the waste gas stream
717 under high pressure while the impurities release at low pressure. Multiple beds are used
718 simultaneously, in order that a continuous separation of hydrogen can accrue up to a purity of
719 99.9%. Another method is temperature swing adsorption (TSA), which is different from PSA
720 and based on adsorption under high temperature. This method is not widely used due to the
721 need of a relatively long process of heating and cooling of sorbents. Another new process is
722 electrical swing adsorption based on the use of electric field to drive hydrogen separation
723 from the gases mixture. In this process, the switching between adsorption and desorption
724 works like on/off switching, which reduces the need to transport or heat sorbent materials.
725 The cryogenic process was also used to purify hydrogen, but it needs very low temperatures,
726 therefore, they are relatively expensive [163].

727 **5.1. Hydrogen separation using membranes.**

728 The mechanism of membrane separation is based on selective penetration of hydrogen
729 through the membrane, with selectivity depending on the membrane properties. The partial
730 pressure of hydrogen in the feed stream is the driving force for permeation, which is balanced
731 by the partial pressure of hydrogen in the permeate stream.

732 The selectivity of hydrogen penetration through the membrane depends on the membrane
733 material. There are two types of membrane: organic (polymer or carbon) and inorganic
734 (metallic or ceramic). The purity of hydrogen reached 99.99% through dense metallic
735 membranes, especially through Pd and its alloys, but there are limitations for metallic
736 membranes due to: (i) poisoning effect of hydrogen sulfide (H₂S) and other feedstocks have
737 on the hydrogen transport mechanism, (ii) high cost for the preparation of Pd membranes and
738 (iii) mechanical stability [164]. The key advantages of polymer membranes are their ability to
739 withstand high pressure drops and their low cost. Therefore, the separation of H₂ by
740 polymeric membranes has become an attractive technology.

741 The transport of hydrogen through dense membranes occurs via the diffusion mechanism,
742 comprised of three main steps:

- 743 1. sorption of the gaseous penetrants at the upstream side of the membrane,

- 745 2. diffusion of the penetrants across the membrane,
 1 746 3. desorption of the penetrants at the downstream side of the membrane [165,166].

2 747 The mechanism is driven by a difference in the thermodynamic activities existing at the
 3 748 upstream and downstream faces of the membrane as well as the interaction between the
 4 749 molecules that constitute the membrane material and permeating molecules.
 5 750

7 751 **5.2. Selection of polymeric membrane materials**

8 752 A membrane separation of hydrogen from various mixtures of gases (including those
 9 753 generated during dark fermentation process) can provide the best performance depending on
 10 754 the membrane polymeric material. The polymers which are used for membrane preparation
 11 755 may be both glassy and rubbery polymers [167]. Usually, when rubbery polymers are used for
 12 756 membrane preparation high permeability with a relatively low selectivity results. When glassy
 13 757 polymers are used for membrane preparation, this leads to high selectivity and lower
 14 758 permeability with high product purity of membranes [168]. Examples of polymers that can be
 15 759 used to prepare membranes for gas separation are shown in Table 4.
 16 760

17 761 Table 4. Polymeric materials and their characteristics


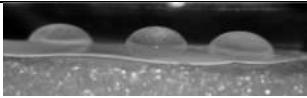
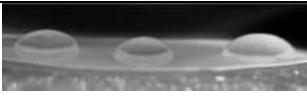

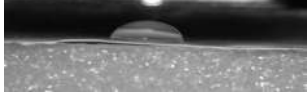
<i>Polymers Materials</i>	<i>Glass transition temperature (T_g, K)</i>	<i>Density g/cm³</i>	<i>Ideal Selectivity of H₂/N₂</i>	<i>Ideal selectivity Of H₂/CO₂</i>	<i>References</i>
Cellulose acetate (CA)	243	1.3	12.52	0.4	[169]
Poly(vinylidene fluoride) (PVDF)	238	1.75	3.42	2	[170]
Polydimethylsiloxane (PDMS)	150	0.97	2.2	0.2	[170]
Polysulfone (PSU)	459	1.24	56	2.5	[166]
Poly(ether sulfone) (PESU)	498	1.37	69.5	2.7	[171]
Poly(phenylene oxide) (PPO)	483	1.06	29.7	1.5	[170]
Polyimide (PI); Matrimid	502	1.24	97	3.9	[172]

50 762 **5.3. Implementation of nano-materials in gas separation-membranes**

51 763 The asymmetric PES/Mn(acac)₃ blend membranes were successfully fabricated by the
 52 764 phase inversion method and by application of the metalorganic compound Mn(acac)₃ in the
 53 765 polymer solution mixture. The addition of Mn(acac)₃ resulted in a reduction in pore size,
 54 766 porosity and low contact angle due to an improvement in hydrophilicity in relation to bare
 55 767 PES. The tensile strength of the prepared membrane was 57.8 kg/cm² with an elongation of
 56 768 6.2% [173].
 57 769

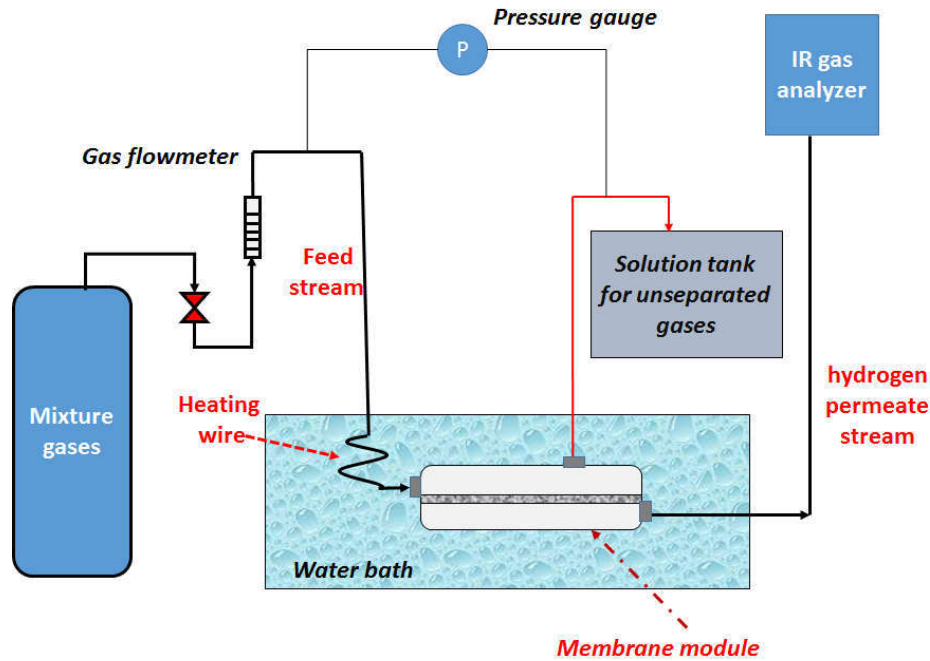
Nanoparticle materials such as titanium oxide were used to produce a PES/TiO₂NTs blend membrane. This membrane was used to separate water vapour, where the selectivity of water vapour reached up to 100% and the average flux of 18.2 kg/m² h. A small addition of titanium oxide nanotubes decreased the contact angle as shown in Table 5 [174].

Table 5. Membrane porosity and contact angle of bare PES and PES/TiO₂NTs blend membranes

TiO ₂ NTs %	Porosity (%)	Contact angle	Membrane wettability photos
0.18%	61	75°	
0.35%	73	65°	
0.53%	79.4	55°	
0.85%	91	45°	
bare PES	27.7	85°	

5.4. Hydrogen separation system using membrane technique

The design of the feed part of the apparatus enables pure and mixed gas permeation experiments, at predetermined gas concentrations and flow rates [175] - see Fig. 8. The required concentration of gases in the mixture is obtained using mass flow controllers. The gas mixture passes through the polymeric membrane film sandwiched in the membrane test module, which has three openings: for feed, retentate and permeate flux. The last leaves the system via the backpressure regulator to control the total pressure on the feed side of the membrane. The membrane system is located in a water bath to facilitate studies of the effect of temperature and to indicate possible gas leakage. The gases are tested using an IR or GC analyzer.



787
788 Fig. 8. System for hydrogen separation by membrane

789 **5.5 Membrane Modules - Efficiency and Cost Saving**

790 A membrane separation is one of the commercially available separation methods that can be
791 used to minimize wastes, equipment requirements and to improve product quality. The
792 membrane systems are particularly effective for recovery and reuse of liquids, gases and
793 solids. The use of a membrane system can lead to reduction of cost and industry
794 environmental-impact as well as to increased competitiveness [176].

795 Membranes can be found in four main configurations: tubular, spiral wound, plate-and-frame,
796 and hollow fibre. The simplest module is the plate-and-frame setup which resembles
797 conventional filtration; it consists of a flat sheet membrane in addition to spacers between the
798 membranes to prevent dynamic adhesion between them. The tubular membrane configuration
799 consists of a multi-tubes set, where the feed is pushed around tubes, while the product is
800 permeating inside the tube. The spiral wound element is the most widely used configuration in
801 the market for commercial applications such as nanofiltration and reverse osmosis
802 application. This module is basically a flat sheet membrane but it is wrapped around a
803 particular tube [176]. The feed flows through the membrane and the permeate is collected on
804 the other side of the membrane then twists or spirals into the centre of the tube. The hollow-
805 fibre membrane module composed of a bundle of hollow fibres is placed in a certain
806 container; the feed is pushed under pressure to pass through the fibre and the permeate is
807 collected at the end of the bundle.

808 The characteristics of modules for gas separation depend largely on membrane material and
809 structure [176]. An industrially useful membrane module must exhibit several properties such
810 as high separation efficiency, high permeation flux, good mechanical stability, tolerance to
811 temperature variation, fouling resistance and low costs. The operating units must contain
812 a number of modules to provide the required membrane surface area for effective separation.
813 Each module must have an inlet and an outlet plus a permeate port and a cleaning system if
814 appropriate. Modules may be arranged in either series or parallel units depending on the
815 applications [176,177]. Table (6) illustrates the typical characteristics of membrane modules.

816 For example, the membrane CO₂-separation-technique has lately attracted considerable
 1 817 attention owing to the new regulations on carbon dioxide emissions. These resulted in rapid
 2 818 development of CO₂ capture technologies to be used in existing and new power plants with
 3 819 the goal of achieving 90% CO₂ capture. The aim was for a limited increase of electricity costs
 4 820 - no more than 35% [178]. The power and hydrogen production units as well as heating
 5 821 systems, especially in the steel and cement industries, etc. are examples where carbon dioxide
 6 822 is produced in huge amounts. The use of membrane modules for gas separation in these
 7 823 industries can reduce the cost of the procedure [179].

824 Table 6: Typical characteristics of membrane modules

Membrane module	Packing density m ² /m ³	Common application	Relative investment cost
Hollow fibre	600-1200	ultrafiltration, gas separation	medium
Spiral-wound	300-1000	ultrafiltration, nanofiltration, reverse osmosis, pervaporation, gas separation	low
Tubular	<100	filtration of high solids content streams	very high
Plate-and-frame	100-600	reverse osmosis, pervaporation, gas separation	high

825 826 **5.6. Membrane fouling**

827 In spite of the merits of membrane-based separation process, fouling is a major and serious
 828 problem limiting the use of membranes in a wide range of applications [176]. Fouling refers
 829 to irreversible precipitation of organic or inorganic materials, suspended particles or bacteria
 830 on the surface of the membrane and in its pores, which results in a decline of the permeate
 831 flux and an increase in hydraulic resistances [176]. There are four different types of fouling:
 832 organic, scaling, biofouling and colloidal fouling. Organic fouling results from organic
 833 compounds such as hydrocarbons, humic or fulvic acids, etc., which coat the surface of the
 834 membrane or clogs the pores of the top membrane layer [176]. Scaling fouling comes from a
 835 precipitation of salts or inorganic compounds on the membrane surface. When the
 836 concentration of an inorganic compound exceeds its solubility limit it is deposited on the
 837 surface of the membrane. The scaling results in a higher pressure drop across the filtration
 838 system, with a decline of separation efficiency (and permeate flux) leading to low quality of
 839 the product. Calcium carbonate (CaCO₃) is an example of a common scale formed on a RO
 840 membrane. Microbial contamination of feed leads to bacterial adhesion on the membrane
 841 surface and creation of biofilm called biofouling which increases the hydrolytic resistance to
 842 penetration of permeate through a membrane. Colloidal fouling results from insoluble
 843 particles such as silica or clay, gathering on the membrane surface. The fouling limitation and
 844 control can be achieved by some pre-treatment procedure, using anti-scaling materials or an
 845 addition of disinfectants [176].

846 For dense membranes which are used in gas separation, two types of fouling can be identified:
 847 surface blocking and particle blocking. In the case of surface blocking, some of the membrane

848 area is not able to transport any mass and, therefore, the active membrane area is reduced.
1 849 That can also lead to surface blinding. The flux through the partly blocked membrane can be
2 850 described as a function of the theoretical flux through the unblocked membrane by:

$$5 \quad 851 \quad J(t) = J_0 * \frac{(A_t - A_{bl}(t))}{A_t} \quad (6)$$

7 852 where J_0 is the flux through the unblocked membrane, A_t is the total membrane area, A_{bl} is
8 853 a blocked area of membrane and t is time. In the case of particle blocking there are two
9 854 contributory effects: a reduction of active membrane-area and an increase of the diffusion
10 855 path through the membrane [180].

13 856 During gas separation the membrane fouling is not caused only by the wetting but also by
14 857 synergetic effects of water vapour with gaseous components: SO_x, NO_x or fine particles in
15 858 the flue gas. For example, a membrane performance suffers a notable reduction after exposure
16 859 to SO₃ and particles in a wet gas stream. The effects of SO₃ and particles is irreversible,
17 860 whereas the fouling by SO₂ and NO₂ can be reversed by N₂ sweeping. The investigations of
18 861 membrane modules, used for gas separation, confirm that they are polluted by gaseous and
19 862 particle impurities to various extents. However, during the CO₂ separation from flue gas
20 863 containing fine particles, the pores of the membrane are blocked and the surface is covered by
21 864 a particle layer. These fine particles occupy a significant area and lead to increased mass-
22 865 transfer resistance and reduced membrane performance [177,181].

27 866 To overcome these problems, a number of methods have been developed by designing special
28 867 modules, several pretreatment processes and membrane modification. The most effective and
29 868 relatively low-cost method is membrane surface treatment or modification. It is widely known
30 869 that hydrophilicity, charge and roughness of the membrane surface greatly influence
31 870 membrane fouling. So, much effort has been expended by many researchers to modify and
32 871 enhance surface hydrophilicity of membranes. These methods included blending with
33 872 hydrophilic polymers, decrease of physical adsorption, surface coating, grafting and plasma
34 873 polymerization, which resulted in extended membrane lifetime [176]. The membrane
35 874 modification techniques include [176–181]:

- 39 875 • the bulk modification of the membrane matrix, such as blending and copolymerization
- 40 876 and
- 41 877 • the modification of the membrane surface, such as grafting of certain hydrophilic
- 42 878 monomers at the surface or chemical treatment to introduce polar groups on the
- 43 879 membrane surface or coating with hydrophilic materials.

47 880 **6. Summary**

48 881 Dark fermentation is a process of microbial anaerobic conversion of simple organic
49 882 carbohydrates or glycerol molecules into short-chain organic acids, carbon dioxide and, most
50 883 importantly, hydrogen molecules. The standard dark fermentation as a direct source of
51 884 hydrogen processes glucose, while extended dark fermentation can make use of any simple
52 885 organic compounds or mixture of organic compounds.

54 886 The bacteria used to produce hydrogen need to possess special enzymes - hydrogenases. The
55 887 most efficient hydrogen production occurs with strict bacteria, including clostridium. Other
56 888 bacteria, called facultative, produce hydrogen less efficiently but they are more oxygen
57 889 resistant. Facultative bacteria include: Enterobacters, Baccilus and Citrobacters.

890 Particularly if they are stressed, the bacteria activate hydrogenase, in defence as an emergency
1 891 action. Bacteria need to retain carbon that will be used to rebuild bacterial structure. It can be
2 892 illustrated in the case of clostridium perfringens, at so called gas gangrene or clostridial
3 893 myonecrosis stage. Bacteria produce hydrogen as metabolite because they are on alert and
4 894 retain carbon for possible rebuilding after contact with victims' defense systems like
5 895 antibodies (leucocytes - a stress agent for bacteria). Therefore, for efficient hydrogen
6 896 production bacteria need to be pretreated using one or more of the following stress factors:

8 897 physical: ultrasonication, microwave, shaking (centrifuging), heat (heat-shock or
9 898 thawing), or

11 899 chemical: pH change (acid/base pretreatment; for example linoleic acid, chloroform).

13 900 The yield of hydrogen production varies in different reactors. These are special vessels
14 901 designed to provide optimal conditions for bacteria multiplication and substrate distribution in
15 902 order to enable efficient hydrogen production. Batch type reactors are the primary option due
16 903 to their simplicity, high conversion-level of substrate and small pretreatment requirements.
17 904 A disadvantage of batch type reactors is their generally low production rate. The semi-
18 905 continuous process can be used for preparation of bacterial cultures for continuous process
19 906 that prevents wash-out in a continuous stage. Advantages of CSTR reactors are: high mass
20 907 transfer due to mixing, simplicity of construction and operation. The CSTR process is limited
21 908 by low biomass concentration, cell retention in low dilution rate and risk of washout of cells
22 909 at a high dilution rate. Membrane reactors increase conversion by separation of products from
23 910 substrates. Fluidized bed reactors allow for use of high volume fraction of biomass without
24 911 the risk of attrition which occurs with CSTR. Besides, bubble column like reactor allows for
25 912 high mass transfer. Packed-bed keeps biomass in the reactor, preventing its washout like in
26 913 CSTR. In the fixed-bed reactor a biofilm typically replaces the catalytic layer (fixed bed) in
27 914 reactors. The advantages are generally low pressure drop, and the simple and robust
28 915 construction. Another advantage is the high magnitude of volumetric mass transfer
29 916 coefficient. The disadvantage of the fixed-bed reactor is its limited surface area. UASB
30 917 reactors are characterized by conversion of biomass in large quantity and using high organic
31 918 load. The main disadvantage of the UASB reactor is its sensitivity to the channeling effect
32 919 causing loss of contact with the substrate and the bed. The CIGSB reactor allows
33 920 improvement of the mixing properties by applying different variations of agitation systems
34 921 (physical, mechanical) and different height to diameter ratio of the reactor. In the case of a
35 922 multi-stage process, the feed preparation and hydrolysis are kept under different conditions
36 923 from the main process.

43 924 The relevant parameters for the dark fermentation process are: feed, temperature, partial
44 925 pressure, C/N ratio, pH and ORP, metal ion concentration and HRT. The most important
45 926 metal ion seems to be the iron ion because it is the necessary element of most hydrogenase
46 927 enzymes. Other metal ions mentioned in the literature are Ni^{2+} , Mg^{2+} , Ca^{2+} , Mn^{2+} , Zn^{2+} , and
47 928 Co^{2+} . Optimum values of parameters depend on the bacteria genus and substrate type.

49 929 Different polymeric membranes were discussed and a membrane hydrogen separation system
50 930 was designed. Implementation of nanoparticles to the membrane preparation process is
51 931 described. It was found that a small addition of titanium oxide nanotubes decreased the
52 932 contact angle.

55 933

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5 939

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