1 Ltd.**Evaluation of various cheese whey treatment scenarios in single-chamber**
2 **microbial electrolysis cells for improved biohydrogen production**

3

4 Isaac Rivera[1], Péter Bakonyi[2], Manuel Alejandro Cuautle-Marín[1], Germán Buitrón[1,*]

5

6 [1]Laboratory for Research on Advanced Processes for Water Treatment, Instituto de
7 Ingeniería, Unidad Académica Juriquilla, Universidad Nacional Autónoma de
8 México, Blvd. Juriquilla 3001, Querétaro 76230, México

9

10 [2]Research Institute on Bioengineering, Membrane Technology and Energetics,
11 University of Pannonia, Egyetem ut 10, 8200 Veszprém, Hungary

12

13 * Corresponding Author: Germán Buitrón
14 Tel: +52 442 192 6165
15 Fax: +52 442 192 6185
16 E-mail: gbuitronm@ii.unam.mx

Abstract

In this study single-chamber microbial electrolysis cells (MECs) were applied to treat cheese whey (CW), an industrial by-product, and recover H_2 gas. Firstly, this substrate was fed directly to the MEC to get the initial feedback about its H_2 generation potential. The results indicated that the direct application of CW requires an adequate pH control to realize bioelectrohydrogenesis and avoid operational failure due to the loss of bioanode activity. In the second part of the study, the effluents of anaerobic (methanogenic) digester and hydrogenogenic (dark fermentative H_2-producing) reactor utilizing the CW were tested in the MEC process (representing the concept of a two-stage technology). It turned out that the residue of the methanogenic reactor – with its relatively lower carbohydrate- and higher volatile fatty acid contents – was more suitable to produce hydrogen bioelectrochemically. The MEC operated with the dark fermentation effluent, containing a high portion of carbohydrates and low amount of organic acids, produced significant amount of undesired methane simultaneously with H_2. Overall, the best MEC behavior was attained using the effluent of the methanogenic reactor and therefore, considering a two-stage system, methanogenesis is an advisable pretreatment step for the acidic CW to enhance the H_2 formation in complementary microbial electrohydrogenesis.

Keywords: microbial electrohydrogenesis; microbial electrolysis cell; cheese whey; methane; hydrogen, two-stage system

38 **1. Introduction**

39

40 The production of hydrogen via biological methods has undergone a
41 significant development in the recent decades. As a result, the contemporary
42 approaches emphasize the utilization of various by-products for simultaneous waste
43 treatment and bioenergy recuperation, providing maximal environmental benefits
44 (Kumar et al., 2015). Among the anaerobic bioprocesses, dark fermentation is
45 currently the most mature one to transform organic materials to sustainable energy
46 carrier, biohydrogen (Bakonyi et al., 2014a). Though this technology is attractive
47 from many aspects e.g. high production rates, flexibility of the microbial
48 communities to a wider range of complex feedstock, general robustness and ability
49 to work under non-sterile conditions, no need for sophisticated and costly bioreactor
50 design, the achievable H_2 yields due to the formation of metabolic side-products – in
51 particular volatile fatty acids, solvents e.g. ethanol – are quite limited
52 (Sivagurunathan et al., 2016). The effluent of dark fermentation (hydrogenogenic
53 reactor) is therefore rich in chemical energy, which should be utilized to maximize
54 the energy extracted from the substrates. This requires multi-stage processes, where
55 after the main technological step, complementary systems are installed to convert
56 the volatile fatty acids (VFAs) and other soluble metabolic products to various forms
57 of bioenergy e.g. CH_4 by anaerobic digestion (methanogenesis reactor),
58 bioelectricity in microbial fuel cells (MFCs), H_2 using microbial electrohydrogenesis
59 cells (MECs), etc. (Kumar et al., 2016).

60 MECs are devices with full of perspectives (Zhen et al., 2015, 2016a) and
61 have been proven to efficiently handle problematic feedstock i.e. wastewaters
62 (Cusick et al., 2010; Zhou et al., 2013), anaerobic sludge (Liu et al., 2012; Lu et al.,
63 2012) and fermentation effluents (Lalaurette et al., 2009; Lu et al., 2009; Rivera el
64 al., 2015; Wang et al., 2011). Bioelectrochemical systems, such as MECs are
65 powered by bacteria called exoelectrogens, which are capable of transferring
66 electrons (liberated from substrate oxidation) to external terminal electron acceptors
67 such as the anode under adequate anaerobic conditions (Kumar et al., 2017; Rago et
68 al., 2015). Basically, the exoelectrogens in MECs are able to acclimate to various
69 environmental conditions, among which the composition of the feed seems to have a
70 notable impact (Kadier et al., 2014; Pant et al., 2010; Sleutels et al., 2011). In fact,
71 raw materials having different characteristics can induce dynamic changes in the
72 anodic surface biofilm, hosting the communities of exoelectrogens and other sort of
73 microorganisms living by alternative metabolism i.e. fermentation and
74 methanogenesis. This association of diverse populations can be syntrophic (Gao et
75 al., 2014; Kiely et al., 2011; Lovley, 2006) but in many cases, a strong competition
76 for the substrates occurs that lowers the attractiveness of the bioelectrochemical
77 system (Koók et al., 2016; Ruiz et al., 2013). Hence, the origin and properties of the
78 substrates may eventually lead to distinct operational responses of the MECs.

79 In this study, we compared the performances of single-chamber microbial
80 electrolysis cells (i) first directly fed with raw cheese whey and then (ii) with the
81 effluents of methanogenic- and dark fermentative bioreactors treating this particular

82 residue of the dairy industry, which sector can reportedly provide good sources of
83 substrates for bioelectrochemical systems (Elakkiya and Matheswaran, 2013;
84 Mardanpour et al., 2012; Moreno et al., 2015; Rago et al., 2017). The primary
85 objective of the work was to determine the adequate strategy leading to better H_2
86 production in MEC and hence, the significance of the results is that it can guide how
87 the acidic cheese whey should be treated to accomplish its improved energetic
88 valorization using bioelectrohydrogenesis.
89
90 **2. Materials and methods**
91
92 **2.1. MEC operation**
93
94 One-chamber microbial electrolysis cells made of polyacrylate were used to
95 carry out the measurements employing graphite felt anode (60 cm^2 surface area,
96 Brunssen de Occidente S.A. de C.V., MEX) and Type 304 stainless steel mesh 60
97 cathode (71 cm^2 surface area, La Paloma Compañía de Metales S.A. de C.V., MEX)
98 with 4 cm electrode spacing. Titanium wire (Sigma-Aldrich Co, MO) was applied to
99 make the internal connections of the MEC, while copper wiring served for external
100 connections. The MEC bioanode was inoculated and colonized in preliminary in a
101 MFC. This MFC was operated using anaerobic sludge as inoculum source and 20
102 mM sodium acetate source in 48 hour cycles for about two weeks (until stable
103 current production had been observed), in accordance with our recently published

104 work (Rivera et al., 2015). When the voltage profile of the MFC could be
105 reproduced at least for 3 batch cycles, the anode was ready to be transferred to the
106 MEC.

107 The MECs in this work had 58 cm^3 headspace and 300 mL working volume.
108 In one series of the measurement, single-chamber MECs for treating complex, raw
109 cheese whey, which is a recognized by-product of the dairy industry (Moreno et al.,
110 2015; Rago et al., 2017) (collected from our industrial partner and stored at 4 ^{o}C
111 until use to limit changes of its composition over time) were tested. In this case, the
112 MEC working volume was composed of 225 mL raw cheese whey as substrate and
113 besides, only phosphate buffer 100 mM (5.3 g/L KH_2PO_4, 10.7 g/L K_2HPO_4),
114 without any nutrients added. The soluble initial COD of this sample was 19.9 g/L.

115 In another experimental set, effluents from continuous (i) anaerobic
116 (methanogenic) digester and (ii) dark fermentative (hydrogenogenic) bioreactors
117 treating the raw CW were employed in subsequent MECs, presenting the concept of
118 a multi-stage system. To explain these processes, **Fig. 1** can be consulted. In the
119 technological line of the methanogenic reactor, the CW (1:1 dilution with tap water)
120 entered first an acidogenic reactor where acetic acid production was promoted.
121 Afterwards, the effluent from acidogenic reactor (pH=5.5) was forwarded to a
122 neutralizer tank to raise the pH to neutral value by 1.5 M NaOH. Subsequently, this
123 stream was fed to the methanogenic reactor (pH=7.2) and last but not least, its
124 effluent was used as substrate for the MEC. In the case of the dark fermentation
125 reactor, the cheese whey was diluted 10:1 and fed directly to the bioreactor (pH =

4.5). After fermenting most of the carbohydrates in CW, the effluent from this process was fed to the MEC. In these measurements, the 300 mL MEC working volume contained 225 mL undiluted effluent and 75 mL phosphate buffer with the above mentioned composition. Before loading the effluents to the MEC, they were first centrifuged (10 min, 10000 rpm) and then membrane filtered (0.22 μm pore size) to get rid of the indigenous biomass.

The MECs in this study, regardless of the type of substrate, were allowed to run with 2 days long cycle times. Each experimental set was conducted in duplicates and the observed standard error was lower than 5 %. The initial pH in all cases was adjusted to 7 using 1 M HCl and NaOH. The MEC measurements started with high-purity (>99.99 vol.%) N_2 sparging to remove O_2 and maintain the anaerobic conditions thoroughly. The electric current was monitored via a 10 Ω external resistor connected in series with the cell. The voltage across this resistor was followed by a data recording card (USB 6008, National Instruments Inc. Austin, TX) in LabView 7 software. MEC temperature was kept at 32 °C by a water bath thermostat. Gas production was quantified using water displacement method by upturned measuring cylinders.

147 **2.2. Analytical methods**

148

149 H_2, CH_4 and CO_2 contents of the reactor headspace, volatile fatty acids
150 (VFAs) – acetic (HAc), butyric (HBu) and propionic (HPr) acids – and ethanol
151 (EtOH) were determined by gas chromatography as described earlier (Buitrón and
152 Carvajal, 2010). Chemical oxygen demand (COD) was analyzed by following the
153 Standard Methods (APHA, 1995). Total carbohydrates (T_{carb}) were measured as
154 described by Dubois et al. (1956), while lactic acid (HLa) (another VFA) was
155 analyzed in a DIONEX ICS-1500 ion chromatograph. Samples for liquid phase
156 analysis (in terms of VFA, EtOH and COD) were taken initially as well as at the end
157 of each MEC cycle (after 48 hours).

158

159 **2.3. Calculations**

160

161 MEC performance was assessed based on volumetric H_2 productivity
162 (HPR_V), cathodic hydrogen recovery (r_{cat}), energy yields relative to electrical (η_e)
163 and substrate (η_s) inputs and both (η_{e+S}) and Coulombic efficiency (E_c), according to
164 Eqs. 1-7:

165

166 $$HPR_V\ (L\ H_2/L\text{-}d) = \frac{Vh}{Vr \times t} \quad (1)$$

167

168 where V_h is the actual volume of H_2 formed (at STP conditions), while V_r and t are

169 assigned to MEC working volume and operational (cycle) time, respectively.

170

171 $$rcat\ (\%) = \frac{Nh}{Nce} \qquad (2)$$

172

173 where Nh is the moles of hydrogen actually produced and Nce represents the moles

174 of H_2 obtainable based on the measured current.

175

176 $$Nce = \frac{\int_{t=0}^{t} I(t)dt}{2F} \qquad (3)$$

177

178 where dt is the data recording time interval, 2 is a factor to convert moles of

179 electrons to moles of H_2 and F is the Faraday's constant (96 485 C/mol e^-).

180

181 $$\eta_e\ (\%) = \frac{Wh}{We}\ x\ 100 \qquad (4)$$

182

183 where Wh is the energy content of H_2 experimentally produced and We is the

184 electrical energy investment, calculated according to Logan et al. (2008).

185

186 $$\eta_s\,(\%) = \frac{Wh}{Ws} \times 100 \quad (5)$$

187

188 where Ws is the energy content of the substrate consumed, calculated according to

189 Logan et al. (2008).

190

191 $$\eta_{e+S}\,(\%) = \frac{Wh}{We+Ws} \times 100 \quad (6)$$

192

193 $$Ec\,(\%) = \frac{Nce}{Nth} \times 100 \quad (7)$$

194

195 where Nth is the moles of hydrogen maximally generated from the COD consumed,

196 calculated in accordance with Logan et al. (2008).

197 **3. Results and discussion**

198

199 **3.1. On the use of raw cheese whey for H_2 production in the MEC**

200

201 Cheese whey – in different forms i.e. powder and with various characteristics
202 – is a by-product generated at an industrial-scale and was shown to be feasible in
203 conventional dark fermentation process for H_2 production (Antonopoulou et al.,
204 2008; Davila-Vazques et al., 2009; Kargi et al., 2012). However, little attention has
205 been paid for its energetic valorization in bioelectrochemical systems so far as only
206 a limited number of papers investigated this possibility i.e. Moreno et al. (2015),
207 Rago et al. (2017) and Tremouli et al. (2013).

208 The results on the direct use of raw CW in the MEC process (**Fig. 2**) indicate
209 that the intensity gas production was quite high in the first 20 hours, after which a
210 plateau was reached. Moreover, it can also be seen in **Fig. 2** that the current density
211 had a declining tendency from the beginning off the experiments, meaning that the
212 electrogenic bacteria got inhibited and bioelectrochemical gas production decreased
213 proportionally. This assumes actually that after approx. the 10th hour of MEC
214 operation, the source of biological gas formation was almost exclusively the
215 classical fermentation pathways. Methane production was significant (45 vol.%),
216 more or less equal to that of H_2 (41 vol.%) and CO_2, constituted the rest of the
217 composition (14%). The appearance of methane may be related with the remarkable
218 carbohydrate content of the substrate (**Table 1**), which was previously found to be

219 responsible for boosted methanogenic activity in biological electrolysis cell (Rivera
220 et al., 2015). Besides, the fact that CH_4 could become a dominant gas is associated
221 with the properties of the anaerobic mixed culture that was originally employed to
222 colonize the MEC bioanode (Rivera et al., 2015).

223 The final pH of the MECs at the end of the 48 h cycle was 3.8. This can be
224 associated with the release of volatile fatty acids in considerable quantities during
225 carbohydrate degradation (**Table 1**). These compounds reduced the pH, which could
226 not apparently be compensated by the phosphate buffer. The accumulation of these
227 acidic components assumes that exoelectrogenic microorganisms (responsible for
228 VFA consumption) could not keep a pace with the VFA generation coming from the
229 metabolism of fermentative bacteria coexisting in the anodic biofilm. Probably, the
230 pH change from a value of 7 to 3.8 was too drastic, making the exoelectrogenic
231 microorganisms unable to properly acclimate to sudden acidification and causing in
232 the end the deterioration their exoelectrogenic activity. Previously, optimal pH range
233 for these strains was reported in the range of 6-9 (Patil et al., 2011). The hypothesis
234 concerning the negative impact of the pH drop is supported by the observations from
235 a consecutive MEC cycle (data not shown), where quasi no current production by
236 the microorganisms could be registered, thus it is implied that the biofilm was
237 seriously damaged. Overall, the fact that (i) only poor electric current was generated
238 and electrohydrogenesis came to an end quickly (**Fig. 2**) and (ii) the gas production
239 did not stop (but was rather continued by fermentation) led to the accumulation of
240 volatile fatty acids, which decreased pH and caused the loss of electrochemical

241 activity on the bioanode. However, understanding these complex phenomena will
242 require more experimentation and hence, elaborating the response of the MEC
243 bioanode community will definitely be an important aspect of our next study.

244 From the energetic aspects of MEC performance using raw CW, though
245 extremely high cathodic hydrogen recovery (r_{cat} = 263.7 %) and electricity input-
246 based energy recovery (η_e = 488.2 %) were attained, it may have been primarily
247 encountered due to the considerable fermentative reactions taking place in the MEC.
248 The calculation of the Coulombic efficiency (roughly 1 %) provides a good proof
249 for the weak bioelectrochemical phenomena to be taken into account. The low
250 Coulombic efficiency helps to deduce that electromicrobial H_2 production – due to
251 the quasi fully unexploited potential of the substrate via bioelectrocatalytic pathways
252 – remained negligible. These results suggest that preventive actions have to be taken
253 to keep the MEC system in good conditions for longer-terms in multiple cycles. For
254 example, on-line pH control or decreased organic loading rate (to avoid the
255 formation of VFAs in excessive quantities) can be proposed to prevent the
256 occurrence of unfavorably acidic conditions.

257 Alternatively, the raw cheese whey may be subjected to two-stage processes,
258 where it is first converted to energy carriers i.e. methane and hydrogen and
259 consecutively, the effluents of these reactors are used as input materials for
260 complementary H_2 production in the MEC system. This concept was further
261 investigated in this work and discussed in the next section. The experiences

262 regarding the conversion of raw cheese whey in the classical methanogenic and
263 hydrogenogenic reactors will be presented in another paper, here the focus is only on
264 the treatment of their effluents in the microbial electrolysis cells.

265

266 **3.2. Comparative evaluation of MEC performances operated with the effluents**
267 **of methanogenic and hydrogenogenic processes treating raw cheese whey**

268

269 The residual (soluble) by-products present in the effluent of anaerobic
270 reactors (i.e. methanogenic digester or H_2 fermenter) can be viewed as a good source
271 of chemical energy for electro-active strains working on the anode of microbial
272 electrohydrogenesis cells (Rózsenberszki et al., 2017, Zhen et al., 2016b). For
273 instance, typical compounds such as acetate, butyrate, propionate, lactate, etc. as
274 dead-end products cannot be further decomposed by fermentative H_2 producing
275 bacteria and therefore, multi-step, integrated systems e.g. those applying
276 bioelectrochemical systems as a complementary step are suggested to drive the
277 conversion towards better completeness and extract further amount of energy before
278 the effluent is finally discharged to the environment (Rózsenberszki et al., 2017).
279 In this work, two real effluents with initial characteristics listed in **Table 2**
280 were tested in a one chamber biocatalyzed electrolysis cell (i) to determine how the
281 MECs perform with VFA- or relatively carbohydrate-richer streams and
282 consequently (ii) to justify the adequate treatment (either methanogenesis or dark

283 fermentation) of cheese whey substrate before MECs are applied for additional H_2
284 recovery. As it can be seen in **Table 2**, although the two effluents were different
285 from an initial COD point of view, quite comparable removal efficiencies could be
286 obtained: 25.5 % and 24.3 % for the methanogenic and dark fermentation residue,
287 respectively. Nevertheless, according to **Fig. 3** it is clear that the methanogenic
288 effluent resulted in much higher cumulative gas production but the picture changes
289 significantly when it is normalized to the amount of COD actually removed (mg
290 ΔCOD). In this case, the MEC treating the spent media of the CH_4-producing reactor
291 achieved 0.11 mL gas/mg ΔCOD, while this value was 0.15 mL/mg ΔCOD for the
292 MEC operated using the dark fermentation effluent. Though the ΔCOD-based total
293 gas formation is 36 % higher for the dark fermentation effluent, it is worthy to take a
294 look at the compositions of the gases formed in the MECs. **Fig. 4** depicts the
295 average headspace gas quality at the end of the MEC cycles and it can be concluded
296 that in contrast with its methanogenic counterpart (where CH_4 percent was below
297 detection level), the dark fermentation effluent provoked remarkable methane
298 generation (43 vol. %), accompanied by lower H_2 percentage (32 vol.%). This, in the
299 end, caused a 62 % depression in the volumetric H_2 productivity (0.06 vs. 0.16 L
300 H_2/L-d). Since the MECs had bioanodes of identical initial characteristics (Rivera et
301 al., 2015), it seems to be a reasonable assumption that the dissimilar effluent
302 composition (higher VFA and lower carbohydrate content for the methanogenic and
303 the contrary for the dark fermentation residue, as seen in **Table 2**) was the
304 responsible factor for the different behaviors.

305 As mentioned above in Section 3.1, carbohydrates can likely enhance the
306 growth of non-electrochemically active microorganisms i.e. methanogens (Rivera et
307 al., 2015). Though the methane production could reportedly be a treat from acetate-
308 rich feedstock (Kumar et al., 2017), in this study, under the conditions tested with
309 the methanogenic effluent having remarkably higher acetate content, promoted CH_4
310 formation was not encountered, implying the primary involvement of carbohydrates
311 in this reaction.

312 Approaches with various degree of success have been proposed in the
313 literature to restrict the activity of these strains, such as pretreatment of the seed
314 inocula (Bakonyi et al., 2014b), application of antibiotics (Catal et al., 2015),
315 preliminary enrichment of the exoelectrogenic bacteria (Liu et al., 2008; Pierra et al.,
316 2015ab; Wang et al., 2010), reduced MEC cycle time (Rivera et al., 2015; Wang et
317 al., 2009), appropriate pH adjustment (Moreno et al., 2015) and operation with well-
318 regulated anode potential (Selembo et al., 2009). However, in some cases, the
319 methanogens can still survive (Escapa et al., 2013) and if they grow above a level to
320 tolerate, system re-start remains the only reasonable option (Nam et al., 2011).

321 Plotting the time profile of electric current produced by the bacteria for the
322 two series of experiments (**Fig. 5**) it can be inferred that it got stabilized at 0.13-0.15
323 mA cm^{-2} quite instantly and in return, the gas production started virtually having no
324 lag-phase (**Fig. 3**). On the other hand, the current with the spent media of the
325 methane reactor was growing rather slowly but gradually and after 20-25 hours it

326 exceeded 0.13-0.15 mA cm^{-2}. The highest, roughly mA cm^{-2} was registered in the
327 last phase of the MEC cycle. This better, peak electric current reflects the higher
328 activity of the exoelectrogenic strains in the bioanode, which contributed possibly to
329 achieve the enhanced HPRv with the methanogenic effluent. The final pH of the
330 MECs, in contrast with case of raw cheese whey evaluated in Section 3.1., did not
331 change significantly and was found in the 6.9-7.1 interval. The current densities
332 presented in **Fig. 3** were highly reproducible (on the grounds of less than 5 %
333 deviation in the results of duplicates), confirming that the behavior of the biofilm
334 was not affected and the bacteria were able to keep their activity for multiple cycles.

335 The comparison of the MEC performances from the point of view of
336 energetic process indicators is given in **Table 3**, where one can realize that the
337 MECs operated with the methanogenic effluent were far more attractive than with
338 the dark fermentation effluent. However, it is interesting to point to the fact that the
339 Coulombic efficiency in the latter MEC was over 90 %. Such high values are hardly
340 reported for bioelectrochemical systems unless the so-called H_2-recycling effect
341 plays a significant role in the single-chamber devices (Lalaurette et al., 2009;
342 Parameswaran et al., 2011; Ruiz et al., 2013; Ullery et al., 2013).

343 This means that the H_2 liberated at the cathode is partly uptaken by certain
344 members of the anodic biofilm to reconvert it to acetate via homoacetogenesis
345 (Saddy, 2013). This acetate is consecutively oxidized by the exoelectrogenic
346 bacteria that boosts current production (Dhar et al., 2015) or alternatively, the H_2 gas

347 can directly be used to generate bioelectricity (Montpart et al., 2014). In both cases,
348 higher E_c will be obtained at the expense of undesired H_2 consumption and hence
349 similar to methanogenesis, it is to avoid as much as possible i.e by constructing
350 systems where the anode and the cathode are spatially separated (Rago et al., 2017).

351

352 **4. Conclusions**

353

354 In this study it was demonstrated that microbial electrolysis cells can be
355 considered for the treatment of cheese whey to recover biohydrogen. In case cheese
356 whey is directly applied, strategies i.e. careful pH control seems to be necessary
357 otherwise the acidification will potentially inhibit the exoelectrogens. Nevertheless,
358 if cheese whey is converted in a two-step process (where complementary MEC
359 utilizes the effluents coming from methanogenesis or hydrogenesis treating the raw
360 cheese whey), H_2 gas can be gained with better success. Though the MECs operated
361 with either methanogenic effluent or dark fermentation effluents had similar organic
362 matter removal efficiencies, the latter system produced considerable amount of
363 methane, attributed possibly to the higher amounts of carbohydrates present. Thus, it
364 seems that anaerobic digestion rather than dark fermentation should be used as the
365 main technological step to valorize cheese whey and obtain a liquid residue that is
366 more suitable for auxiliary MEC process.

367

Acknowledgements

The financial support of Secretaría de Energía -Sustentabilidad Energética-CONACYT (Cluster Biocombustibles gaseosos project 247006) is gratefully acknowledged. Péter Bakonyi acknowledges the support received from National Research, Development and Innovation Office (Hungary) under grant number PD 115640. The technical assistance by Jaime Perez is appreciated.

References

1. Antonopoulou, G., Stamatelatou, K., Venetsaneas, N., Kornaros, M., Lyberatos, G., 2008. Biohydrogen and methane production from cheese whey in a two-stage anaerobic process. Ind. Eng. Chem. Res. 47, 5227-5233.
2. APHA, 1995. Standard methods for the examination of water and wastewater. 19th ed. New York, USA: American Public Health Association.
3. Bakonyi, P., Nemestóthy, N., Simon, V., Bélafi-Bakó, K., 2014a. Review on the start-up experiences continuous fermentative hydrogen producing bioreactors. Renew. Sustain. Energy Rev. 40, 806-813.
4. Bakonyi, P., Borza, B., Orlovits, K., Simon, V., Nemestóthy, N., Bélafi-Bakó, K., 2014b. Fermentative hydrogen production by conventionally and unconventionally heat pretreated seed cultures: A comparative assessment. Int. J. Hydrogen Energy 39, 5589-5596.

390 5. Buitrón, G., Carvajal, C., 2010. Biohydrogen production from Tequila
391 vinasses in an anaerobic sequencing batch reactor: effect of initial substrate
392 concentration, temperature and hydraulic retention time. Bioresour. Technol.
393 101, 9071-9077.
394 6. Catal, T., Lesnik, K.L., Liu, H., 2015. Suppression of methanogenesis for
395 hydrogen production in single-chamber microbial electrolysis cells using
396 various antibiotics. Bioresour. Technol. 187, 77-83.
397 7. Cusick, R.D., Kiely, P.D., Logan, B.E., 2010. A monetary comparison of
398 energy recovered from microbial fuel cells and microbial electrolysis cells
399 fed winery or domestic wastewaters. Int. J. Hydrogen Energy 35, 8855-8861.
400 8. Davila-Vazques, G., Cota-Navarro, C.B., Rosales-Colunga, L.M., de León-
401 Rodríguez, A., Razo-Flores, E., 2009. Continuous biohydrogen production
402 using cheese whey: Improving the hydrogen production rate. Int. J. Hydrogen
403 Energy 34, 4296-4304.
404 9. Dhar, B.R., Elbeshbishy, E., Hafez, H., Lee, H.S., 2015. Hydrogen
405 production from sugar beet juice using an integrated biohydrogen process of
406 dark fermentation and microbial electrolysis cell. Bioresour. Technol. 198,
407 223-230.
408 10. Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956.
409 Colorimetric method for determination of sugars and related substances.
410 Anal. Chem. 28, 350-356.

411 11. Elakkiya, E., Matheswaran, M., 2013. Comparison of anodic metabolisms in
412 bioelectricity production during treatment of dairy wastewater in microbial
413 fuel cell. Bioresour. Technol. 136, 407-412.
414 12. Escapa, A., Manuel, M.F., Morán, A., Gómez, X., Guiot, S.R., Tartakovsky,
415 B., 2009. Hydrogen production from glycerol in a membraneless microbial
416 electrolysis cell. Energy Fuels 23, 4612-4618.
417 13. Gao, Y., Ryu, H., Santo Domingo, J.W., Lee, H.S., 2014. Syntrophic
418 interactions between H_2 scavenging and anode-respiring bacteria can improve
419 current density in microbial electrochemical cells. Bioresour. Technol. 153,
420 245-253.
421 14. Kadier, A., Simayi, Y., Kalil, M.S., Abdeshahian, P., Hamid, A.A., 2014. A
422 review of the substrates used in microbial electrolysis cells (MECs) for
423 producing sustainable and clean hydrogen gas. Renew. Energy 71, 466-472.
424 15. Kargi, F., Eren, N.S., Ozmichi, S., 2012. Bio-hydrogen production from
425 cheese whey powder (CWP) solution: Comparison of thermophilic and
426 mesophilic dark fermentations. Int. J. Hydrogen Energy 37, 8338-8342.
427 16. Kiely, P.D., Regan, J.M., Logan, B.E., 2011. The electric picnic: synergistic
428 requirements for exoelectrogenic microbial communities. Curr. Opin.
429 Biotechnol. 22, 378-385.

430 17. Koók, L., Rózsenberszki, T., Nemestóthy, N., Bélafi-Bakó, K., Bakonyi, P.,
431 2016. Bioelectrochemical treatment of municipal waste liquor in microbial
432 fuel cells for energy valorization. J. Clean. Prod. 112, 4406-4412.

433 18. Kumar, G., Bakonyi, P., Zhen, G, Sivagurunathan, P., Koók, L., Kim, S.H., et
434 al., 2017. Microbial electrochemical systems for sustainable biohydrogen
435 production: Surveying the experiences from a start-up viewpoint. Renew.
436 Sustain. Energy Rev. 70, 589-597.

437 19. Kumar, G., Bakonyi, P., Kobayashi, T., Xu, K.Q., Sivagurunathan, P., Kim,
438 S.H., et al., 2016. Enhancement of biofuel production via microbial
439 augmentation: The case of dark fermentative hydrogen. Renew. Sustain.
440 Energy Rev. 57, 879-891.

441 20. Kumar, G., Bakonyi, P., Periyasamy, S., Kim, S.H., Nemestóthy, N., Bélafi-
442 Bakó, K., 2015. Lignocellulose biohydrogen: Practical challenges and recent
443 progress. Renew. Sustain. Energy Rev. 44, 728-737.

444 21. Lalaurette, E., Thammannagowda, S., Mohagheghi, A., Maness, P.C., Logan,
445 B.E., 2009. Hydrogen production from cellulose in a two-stage process
446 combining fermentation and electrohydrogenesis. Int. J. Hydrogen Energy 34,
447 6201-6210.

448 22. Liu, W., Huang, S., Zhou, A., Zhou, G., Ren, N., Wang, A., et al., 2012.
449 Hydrogen generation in microbial electrolysis cell feeding with fermentation
450 liquid of waste activated sludge. Int. J. Hydrogen Energy 37, 13859-13864.

451 23. Liu, Y., Harnisch, F., Fricke, K., Sietmann, R., Schröder, U., 2008.
452 Improvement of the anodic bioelectrocatalytic activity of mixed culture
453 biofilms by a simple consecutive electrochemical selection procedure.
454 Biosens. Bioelectron. 24, 1006-1011.
455 24. Logan, B.E., Call, D., Cheng, S., Hamelers, H.V.M., Sleutels, T.H.J.A.,
456 Jeremiasse, A.W., et al., 2008. Microbial electrolysis cells for high yield
457 hydrogen gas production from organic matter. Environ. Sci. Technol. 42,
458 8630-8640.
459 25. Lovley, D.R., 2006. Microbial fuel cells: novel microbial physiologies and
460 engineering approaches. Curr. Opin. Biotechnol. 17, 327-332.
461 26. Lu, L., Xing, D., Liu, B., Ren, N., 2012. Enhanced hydrogen production from
462 waste activated sludge by cascade utilization of organic matter in
463 microbialelectrolysis cells. Water Res. 46, 1015-1026.
464 27. Lu, L., Ren, N., Xing, D., Logan, B.E., 2009. Hydrogen production with
465 effluent from an ethanol–H_2-coproducing fermentation reactor using a single-
466 chamber microbial electrolysis cell. Biosens. Bioelectron. 24, 3055-3060.
467 28. Mardanpour, M.M., Esfahany, M.N., Behzad, T., Sedaqatvand, R., 2012.
468 Single chamber microbial fuel cell with spiral anode for dairy wastewater
469 treatment. Biosens. Bioelectron. 38, 264-269.
470 29. Montpart, N., Ribot-Llobet, E., Garlapati, V.K., Rago, L., Baeza, J.A.,
471 Guisasola, A., 2014. Methanol opportunities for electricity and hydrogen

472 production in bioelectrochemical systems. Int. J. Hydrogen Energy 39, 770-
473 777.

474 30. Moreno, R., Escapa, A., Cara, J., Carracedo, B., Gómez, X., 2015. A two-
475 stage process for hydrogen production from cheese whey: Integration of dark
476 fermentation and biocatalyzed electrolysis. Int. J. Hydrogen Energy 40, 168-
477 175.

478 31. Nam, J.Y., Tokash, J.C., Logan, B.E., 2011. Comparison of microbial
479 electrolysis cells operated with added voltage or by setting the anode
480 potential. Int. J. Hydrogen Energy 36, 10550-10556.

481 32. Pant, D., Van Bogaert, G., Diels, L., Vanbroekhoven, K., 2010. A review of
482 the substrates used in microbial fuel cells (MFCs) for sustainable energy
483 production. Bioresour. Technol. 101, 1533-1543.

484 33. Parameswaran, P., Torres, C.I., Lee, H.S., Rittmann, B.E., Krajmalnik-
485 Brown, R., 2011. Hydrogen consumption in microbial electrochemical
486 systems (MXCs): The role of homo-acetogenic bacteria. Bioresour. Technol.
487 102, 263-271.

488 34. Patil, S.A., Harnisch, F., Koch, C., Hübschmann, T., Fetzer, I., Carmona-
489 Martínez, A.A., et al., 2011. Electroactive mixed culture derived biofilms in
490 microbial bioelectrochemical systems: The role of pH on biofilm formation,
491 performance and composition. Bioresour. Technol. 102, 9683-9690.

492 35. Pierra, M., Carmona-Martínez, A.A., Trably, E., Godon, J.J., Bernet, N.,
493 2015a Microbial characterization of anode-respiring bacteria within biofilms
494 developed from cultures previously enriched in dissimilatory metal-reducing
495 bacteria. Bioresour. Technol. 195, 283-287.
496 36. Pierra, M., Carmona-Martínez, A.A., Trably, E., Godon, J.J., Bernet, N.,
497 2015b. Specific and efficient electrochemical selection of Geoalkalibacter
498 subterraneus and Desulfuromonas acetoxidans in high current-producing
499 biofilms. Bioelectrochemistry 106, 221-225.
500 37. Rago, L., Baeza, J.A., Guisasola, A., 2017. Bioelectrochemical hydrogen
501 production with cheese whey as sole substrate. J. Chem. Technol. Biotechnol.
502 92, 173-179.
503 38. Rago, L., Ruiz, Y., Baeza, J.A., Guisasola, A., Cortés, P., 2015. Microbial
504 community analysis in a long-term membrane-less microbial electrolysis cell
505 with hydrogen and methane production. Bioelectrochemistry 106, 359-368.
506 39. Rivera, I., Buitrón, G., Bakonyi, P., Nemestóthy, N., Bélafi-Bakó, K., 2015.
507 Hydrogen production in a microbial electrolysis cell fed with a dark
508 fermentation effluent. J. Appl. Electrochem. 45, 1223-1229.
509 40. Rózsenberszki, T., Koók, L., Bakonyi, P., Nemestóthy, N., Logrono, W.,
510 Pérez, M., et al., 2017. Municipal waste liquor treatment via
511 bioelectrochemical and fermentation (H_2 + CH_4) processes: Assessment of
512 various technological sequences. Chemosphere 171, 692-701.

513 41. Ruiz, Y., Baeza, J.A., Guisasola, A., 2013. Revealing the proliferation of
514 hydrogen scavengers in a single-chamber microbial electrolysis cell using
515 electron balances. Int. J. Hydrogen Energy 38, 15917-15927.
516 42. Saady, N.M.C., 2013. Homoacetogenesis during hydrogen production by
517 mixed cultures dark fermentation: Unresolved challenge. Int. J. Hydrogen
518 Energy 38, 13172-13191.
519 43. Selembo, P.A., Perez, J.M., Lloyd, W.A., Logan, B.E., 2009. High hydrogen
520 production from glycerol or glucose by electrohydrogenesis using microbial
521 electrolysis cells. Int. J. Hydrogen Energy 34, 5373-5381.
522 44. Sivagurunathan, P., Kumar, G., Bakonyi, P., Kim, S.H., Kobayashi, T., Xu,
523 K.Q., et al., 2016. A critical review on issues and overcoming strategies for
524 the enhancement of dark fermentative hydrogen production in continuous
525 systems. Int. J. Hydrogen Energy 41, 3820-3836.
526 45. Sleutels, T.H.J.A., Darus, L., Hamelers, H.V.M., Buisman, J.N., 2011. Effect
527 of operational parameters on Coulombic efficiency in bioelectrochemical
528 systems. Bioresour. Technol. 102, 11172-11176.
529 46. Tremouli, A., Antonopoulou, G., Bebelis, S., Lyberatos, G., 2013. Operation
530 and characterization of a microbial fuel cell fed with pretreated cheese whey
531 at different organic loadings. Bioresour. Technol. 131, 380-389.

532 47. Ullery, M.L., Logan, B.E., 2014. Comparison of complex effluent treatability
533 in different bench scale microbial electrolysis cells. Bioresour. Technol. 170,
534 530-537.

535 48. Wang, A., Sun, D., Cao, G., Wang, H., Ren, N., Wu, W.M., et al., 2011.
536 Integrated hydrogen production process from cellulose by combining dark
537 fermentation, microbial fuel cells, and a microbial electrolysis cell.
538 Bioresour. Technol. 102, 4137-4143.

539 49. Wang, A., Sun, D., Ren, N., Liu, C., Logan, B.E., Wu, W.M., 2010. A rapid
540 selection stragety fron an anodophilic consortium for microbial fuel cells.
541 Bioresour. Technol. 101, 5733-5735.

542 50. Wang, A., Liu, W., Cheng, S., Xing, D., Zhou, J., Logan, B.E., 2009. Source
543 of methane and methods to control its formation in single chamber microbial
544 electrolysis cell. Int. J. Hydrogen Energy 34, 3653-3658.

545 51. Zhen, G., Kobayashi, T., Lu, X., Kumar, G., Xu, K., 2016a. Biomethane
546 recovery from Egeria densa in a microbial electrolysis cell-assisted anaerobic
547 system: Performance and stability assessment. Chemosphere 149, 121-129.

548 52. Zhen, G., Kobayashi, T., Lu, X., Kumar, G., Hu, Y., Bakonyi, P., et al.,
549 2016b. Recovery of biohydrogen in a single-chamber microbial
550 electrohydrogenesis cell using liquid fraction of pressed municipal solid
551 waste (LPW) as substrate. Int. J. Hydrogen Energy 41, 17896-17906.

552 53. Zhen, G., Kobayashi, T., Lu, X., Xu, K., 2015. Understanding methane
553 bioelectrosynthesis from carbon dioxide in a two-chamber microbial
554 electrolysis cells (MECs) containing a carbon biocathode. Bioresour.
555 Technol. 186, 141-148.

556 54. Zhou, M., Wang, H., Hassett, D.J., Gu, T., 2013. Recent advances in
557 microbial fueld cells (MFCs) and microbial electrolysis cells (MECs) for
558 wastewater treatment, bioenergy and bioproducts. J. Chem. Technol.
559 Biotechnol. 88, 508-518.

560

561

562 **Figure Legend**

563

564 **Fig. 1** – Schematic figure of the treatment train for the three scenarios for cheese
565 whey treatment in MEC.

566

567 **Fig. 2** – The cumulative gas production (blue diamond) obtained with raw cheese
568 whey as substrate for H_2 production in MEC and registered current density (red
569 square) as a function of time.

570

571 **Fig. 3** – Progress curves presenting the gas production using the effluent of
572 methanogenic (red squares) and hydrogenogenic reactors (green triangles) treating
573 raw cheese whey as substrate.

574

575 **Fig. 4 –** (**A**) and (**B**) are headspace gas composition using the effluent of
576 methanogenic and hydrogenogenic reactors as substrates, respectively.

577

578 **Fig. 5** – The measured current densities in the MECs utilizing the effluent of
579 methanogenic (red) and hydrogenogenic reactors (blue) as substrates, respectively.

580

581 Table 1 – Initial and final liquid phase concentrations during raw cheese whey

582 treatment in MEC

583

	Concentration (mg/L)	
	Initial	Final
Total carbohydrates	17350	1440
Acetic acid	264	679
Propionic acid	18	39
Butyric acid	22	153
Lactic acid	BDL	1959
Ethanol	56	851

BDL: below detection level

584

585

586

587 Table 2 – Liquid phase analysis of MECs utilizing the effluents of anaerobic
588 digester (higher VFA, lower carbohydrate content) and dark fermentation reactor
589 (higher carbohydrate, lower VFA content)

590

MEC feedstock		COD (mg/L)	Tcarb (mg/L)	HAc (mg/L)	HPr (mg/L)	HBu (mg/L)	HLa (mg/L)	EtOH (mg/L)
Anaerobic digester effluent	Initial	4009	10	703	1697	140	271	BDL
	Final	2985	BDL	428	1399	121	30	BDL
Dark fermentation effluent	Initial	1624	87	176	424	35	98	BDL
	Final	1229	7	BDL	103	294	45	BDL

BDL: below detection level

591

592

593 Table 3 – Energetic performance of MEC treating different effluents

594

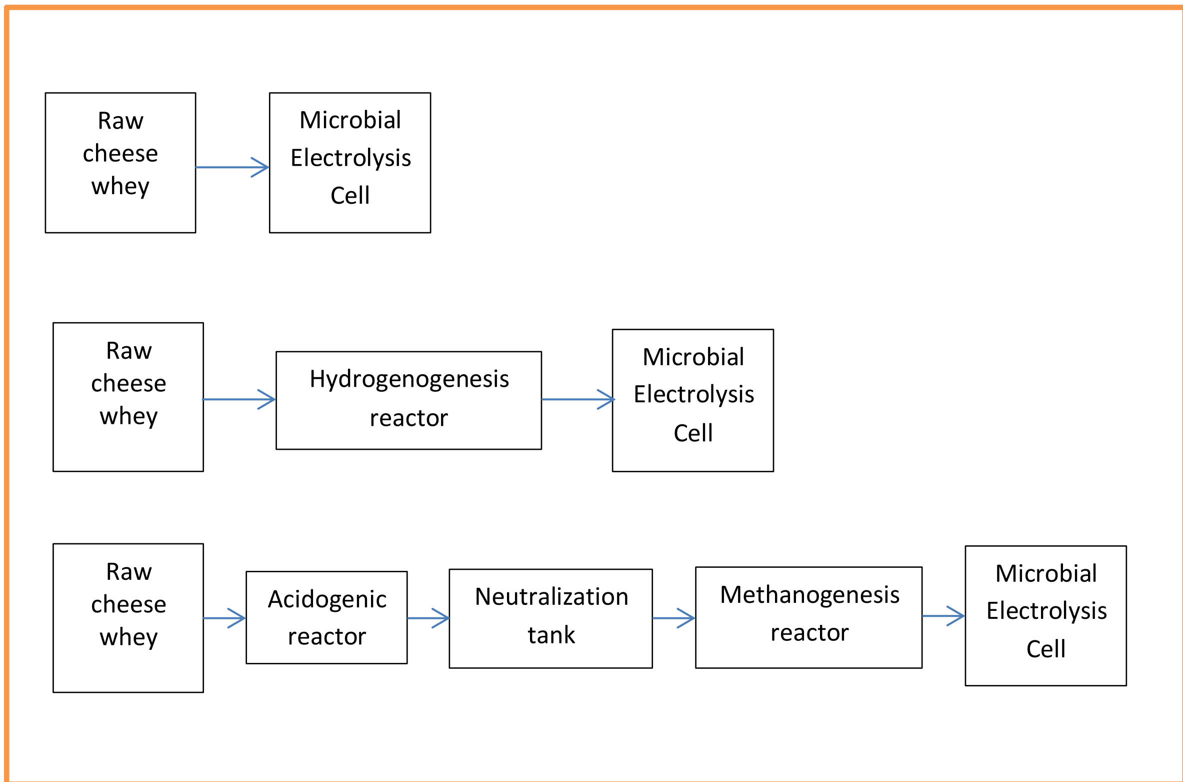
Source of effluent	r_{cat} (%)	η_e (%)	η_s (%)	η_{e+s} (%)	E_c (%)
Methanogenic reactor	63	116.6	25.3	20.8	31.8
Dark fermentative H_2 reactor	22	40.7	12.4	9.5	92.7

595

596

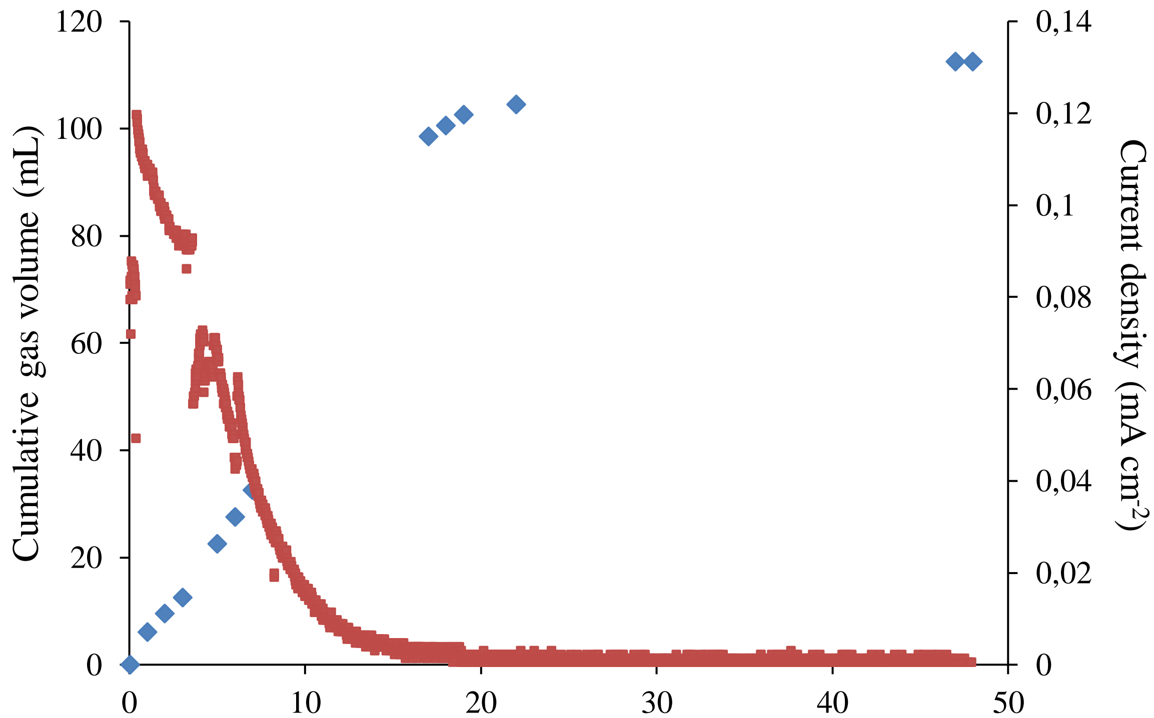
597 Fig. 1

598



599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623

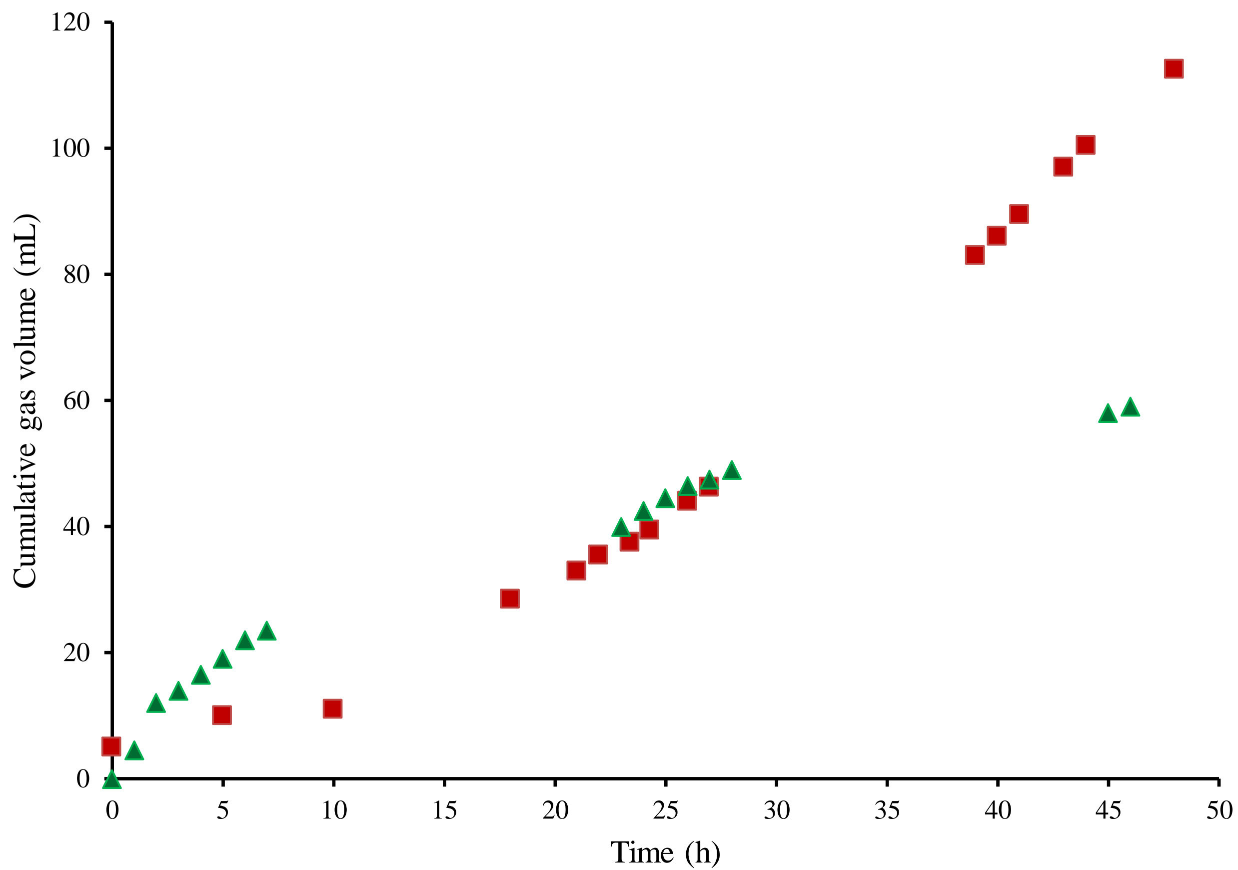
624 Fig. 2



625
626
627
628
629
630
631
632
633
634
635
636
637

638 Fig. 3

639



640

641

642

643

644

645

646

647

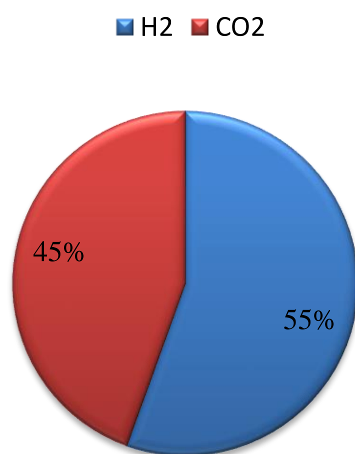
648

649

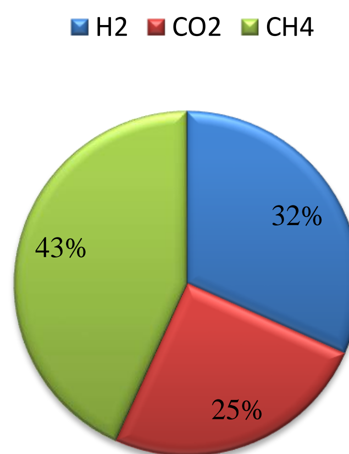
650 Fig. 4

651

A



B



652

653

654

655

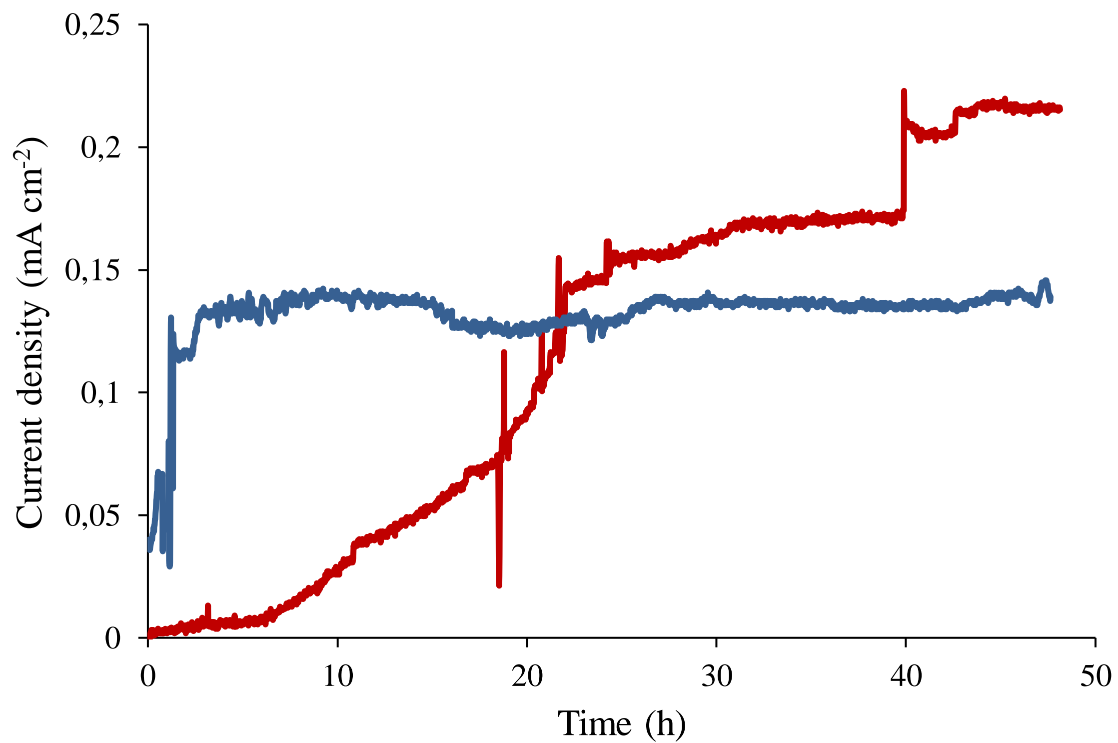
656

657

658

659

660 Fig. 5



661

662