

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

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17 **Abstract**

18

19 In this study single-chamber microbial electrolysis cells (MECs) were applied to  
20 treat cheese whey (CW), an industrial by-product, and recover H<sub>2</sub> gas. Firstly, this substrate  
21 was fed directly to the MEC to get the initial feedback about its H<sub>2</sub> generation potential.  
22 The results indicated that the direct application of CW requires an adequate pH control to  
23 realize bioelectrohydrogenesis and avoid operational failure due to the loss of bioanode  
24 activity. In the second part of the study, the effluents of anaerobic (methanogenic) digester  
25 and hydrogenogenic (dark fermentative H<sub>2</sub>-producing) reactor utilizing the CW were tested  
26 in the MEC process (representing the concept of a two-stage technology). It turned out that  
27 the residue of the methanogenic reactor – with its relatively lower carbohydrate- and higher  
28 volatile fatty acid contents – was more suitable to produce hydrogen bioelectrochemically.  
29 The MEC operated with the dark fermentation effluent, containing a high portion of  
30 carbohydrates and low amount of organic acids, produced significant amount of undesired  
31 methane simultaneously with H<sub>2</sub>. Overall, the best MEC behavior was attained using the  
32 effluent of the methanogenic reactor and therefore, considering a two-stage system,  
33 methanogenesis is an advisable pretreatment step for the acidic CW to enhance the H<sub>2</sub>  
34 formation in complementary microbial electrohydrogenesis.

35

36 **Keywords:** microbial electrohydrogenesis; microbial electrolysis cell; cheese whey;  
37 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<sub>2</sub> 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<sub>4</sub> by anaerobic digestion (methanogenesis reactor),  
58 bioelectricity in microbial fuel cells (MFCs), H<sub>2</sub> 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 et  
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<sub>2</sub>  
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<sup>2</sup> surface area,  
96 Brunssen de Occidente S.A. de C.V., MEX) and Type 304 stainless steel mesh 60  
97 cathode (71 cm<sup>2</sup> 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<sup>3</sup> 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 °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<sub>2</sub>PO<sub>4</sub>, 10.7 g/L K<sub>2</sub>HPO<sub>4</sub>),  
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 =

126 4.5). After fermenting most of the carbohydrates in CW, the effluent from this  
127 process was fed to the MEC. In these measurements, the 300 mL MEC working  
128 volume contained 225 mL undiluted effluent and 75 mL phosphate buffer with the  
129 above mentioned composition. Before loading the effluents to the MEC, they were  
130 first centrifuged (10 min, 10000 rpm) and then membrane filtered (0.22  $\mu\text{m}$  pore  
131 size) to get rid of the indigenous biomass.

132 The MECs in this study, regardless of the type of substrate, were allowed to  
133 run with 2 days long cycle times. Each experimental set was conducted in  
134 duplicates and the observed standard error was lower than 5 %. The initial pH in all  
135 cases was adjusted to 7 using 1 M HCl and NaOH. The MEC measurements started  
136 with high-purity (>99.99 vol.%)  $\text{N}_2$  sparging to remove  $\text{O}_2$  and maintain the  
137 anaerobic conditions thoroughly. The electric current was monitored via a 10  $\Omega$   
138 external resistor connected in series with the cell. The voltage across this resistor  
139 was followed by a data recording card (USB 6008, National Instruments Inc. Austin,  
140 TX) in LabView 7 software. MEC temperature was kept at 32  $^\circ\text{C}$  by a water bath  
141 thermostat. Gas production was quantified using water displacement method by  
142 upturned measuring cylinders.

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145

146

147 **2.2. Analytical methods**

148

149 H<sub>2</sub>, CH<sub>4</sub> and CO<sub>2</sub> 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<sub>carb</sub>) 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<sub>2</sub> productivity  
162 (HPR<sub>v</sub>), cathodic hydrogen recovery (r<sub>cat</sub>), energy yields relative to electrical (η<sub>e</sub>)  
163 and substrate (η<sub>s</sub>) inputs and both (η<sub>e+s</sub>) and Coulombic efficiency (E<sub>c</sub>), according to  
164 Eqs. 1-7:

165

166 
$$\text{HPR}_v \text{ (L H}_2\text{/L-d)} = \frac{V_h}{V_r \times t} \quad (1)$$

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

$$171 \quad r_{cat} (\%) = \frac{N_h}{N_{ce}} \quad (2)$$

172

173 where  $N_h$  is the moles of hydrogen actually produced and  $N_{ce}$  represents the moles  
174 of  $H_2$  obtainable based on the measured current.

175

$$176 \quad N_{ce} = \frac{\int_{t=0}^t I(t) dt}{2F} \quad (3)$$

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

$$181 \quad \eta_e (\%) = \frac{W_h}{W_e} \times 100 \quad (4)$$

182

183 where  $W_h$  is the energy content of  $H_2$  experimentally produced and  $W_e$  is the  
184 electrical energy investment, calculated according to [Logan et al. \(2008\)](#).

185

$$186 \quad \eta_s(\%) = \frac{W_h}{W_s} \times 100 \quad (5)$$

187

188 where  $W_s$  is the energy content of the substrate consumed, calculated according to  
189 [Logan et al. \(2008\)](#).

190

$$191 \quad \eta_{e+s}(\%) = \frac{W_h}{W_e+W_s} \times 100 \quad (6)$$

192

$$193 \quad E_c(\%) = \frac{N_{ce}}{N_{th}} \times 100 \quad (7)$$

194

195 where  $N_{th}$  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<sub>2</sub> 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<sub>2</sub> 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 10<sup>th</sup> 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<sub>2</sub> (41 vol.%) and CO<sub>2</sub>, 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<sub>4</sub> 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 ( $r_{\text{cat}} = 263.7 \%$ ) and electricity input-  
246 based energy recovery ( $\eta_e = 488.2 \%$ ) 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  $\text{H}_2$  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  $\text{H}_2$  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<sub>2</sub> 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 ([Rózsensberszki 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<sub>2</sub> 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 ([Rózsensberszki 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<sub>2</sub>  
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  $\Delta$ COD). In this case, the MEC treating the spent media of the CH<sub>4</sub>-producing reactor  
291 achieved 0.11 mL gas/mg  $\Delta$ COD, while this value was 0.15 mL/mg  $\Delta$ COD for the  
292 MEC operated using the dark fermentation effluent. Though the  $\Delta$ COD-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<sub>4</sub> percent was below  
297 detection level), the dark fermentation effluent provoked remarkable methane  
298 generation (43 vol. %), accompanied by lower H<sub>2</sub> percentage (32 vol.%). This, in the  
299 end, caused a 62 % depression in the volumetric H<sub>2</sub> productivity (0.06 vs. 0.16 L  
300 H<sub>2</sub>/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<sub>4</sub>  
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<sup>-2</sup> 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<sup>-2</sup>. The highest, roughly mA cm<sup>-2</sup> 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 HPR<sub>v</sub> 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<sub>2</sub>-recycling effect  
341 plays a significant role in the single-chamber devices ([Lalurette et al., 2009](#);  
342 [Parameswaran et al., 2011](#); [Ruiz et al., 2013](#); [Ullery et al., 2013](#)).

343         This means that the H<sub>2</sub> 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<sub>2</sub> 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

## 368 **Acknowledgements**

369

370 The financial support of Secretaría de Energía -Sustentabilidad Energética-  
371 CONACYT (Cluster Biocombustibles gaseosos project 247006) is gratefully  
372 acknowledged. Péter Bakonyi acknowledges the support received from National  
373 Research, Development and Innovation Office (Hungary) under grant number PD  
374 115640. The technical assistance by Jaime Perez is appreciated.

375

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## Figure Legend

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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<sub>2</sub> 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

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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)

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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

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593 Table 3 – Energetic performance of MEC treating different effluents

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Source of effluent	$r_{\text{cat}}$ (%)	$\eta_e$ (%)	$\eta_s$ (%)	$\eta_{e+s}$ (%)	$E_c$ (%)
Methanogenic reactor	63	116.6	25.3	20.8	31.8
Dark fermentative H <sub>2</sub> reactor	22	40.7	12.4	9.5	92.7

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597 Fig. 1

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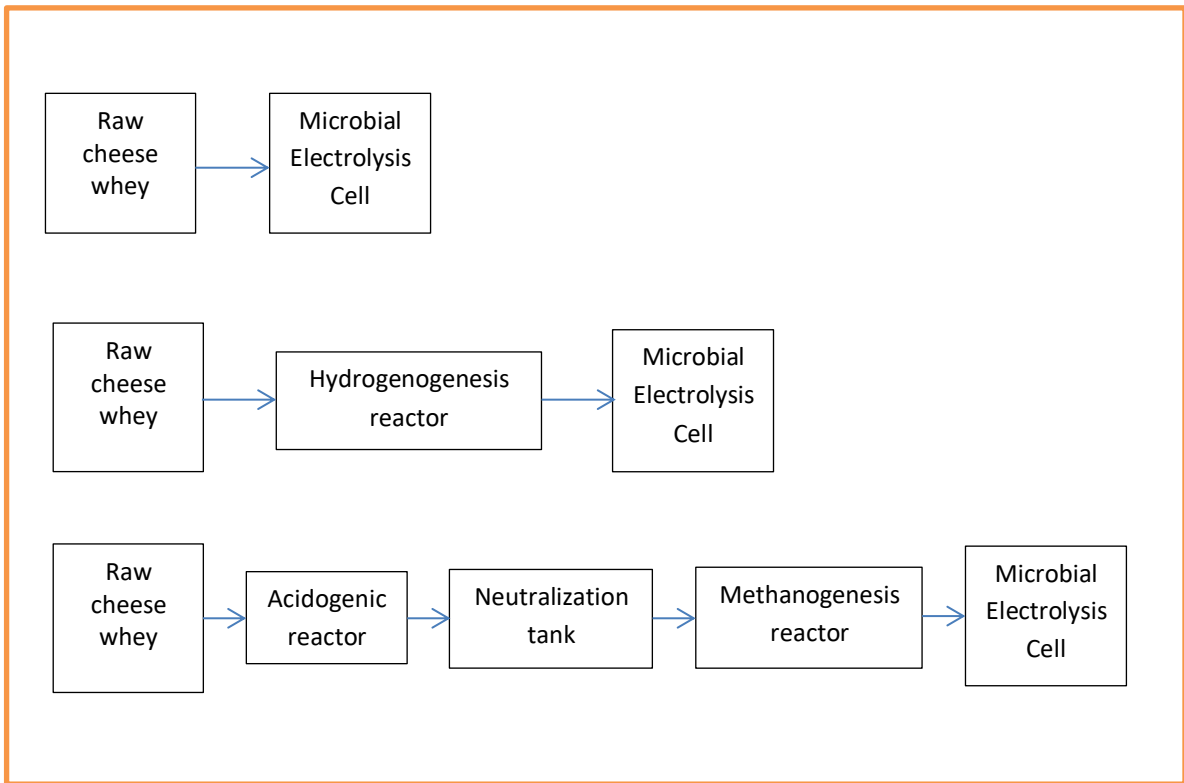
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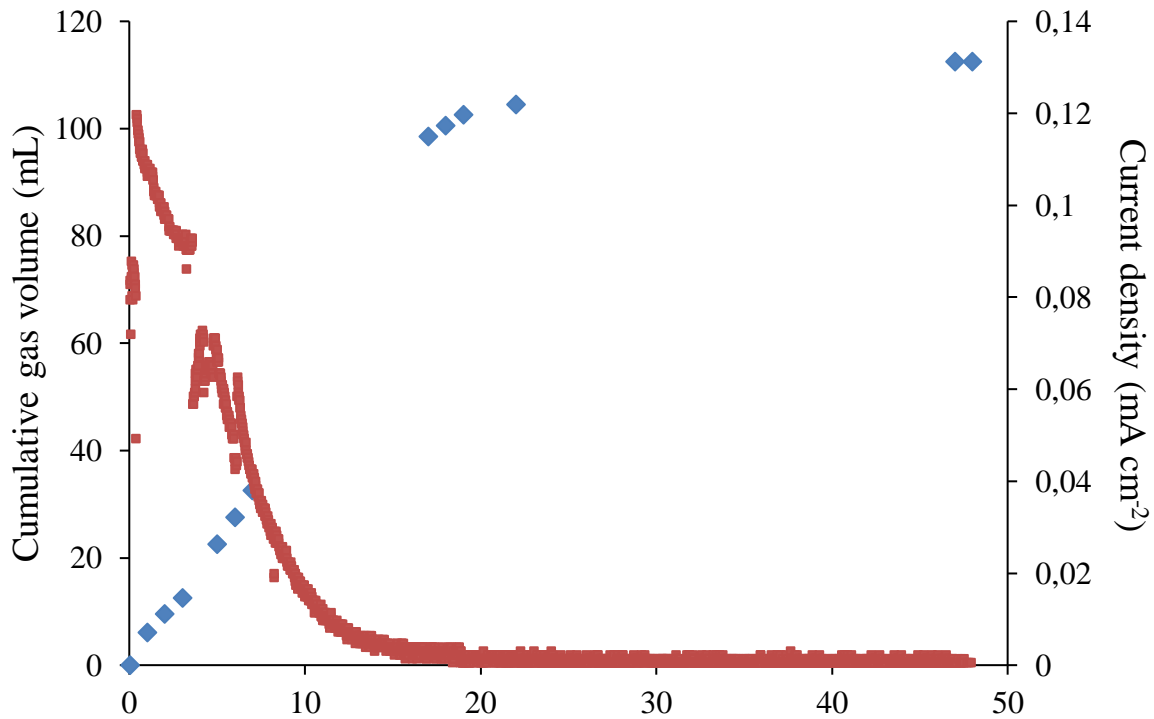
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624 Fig. 2



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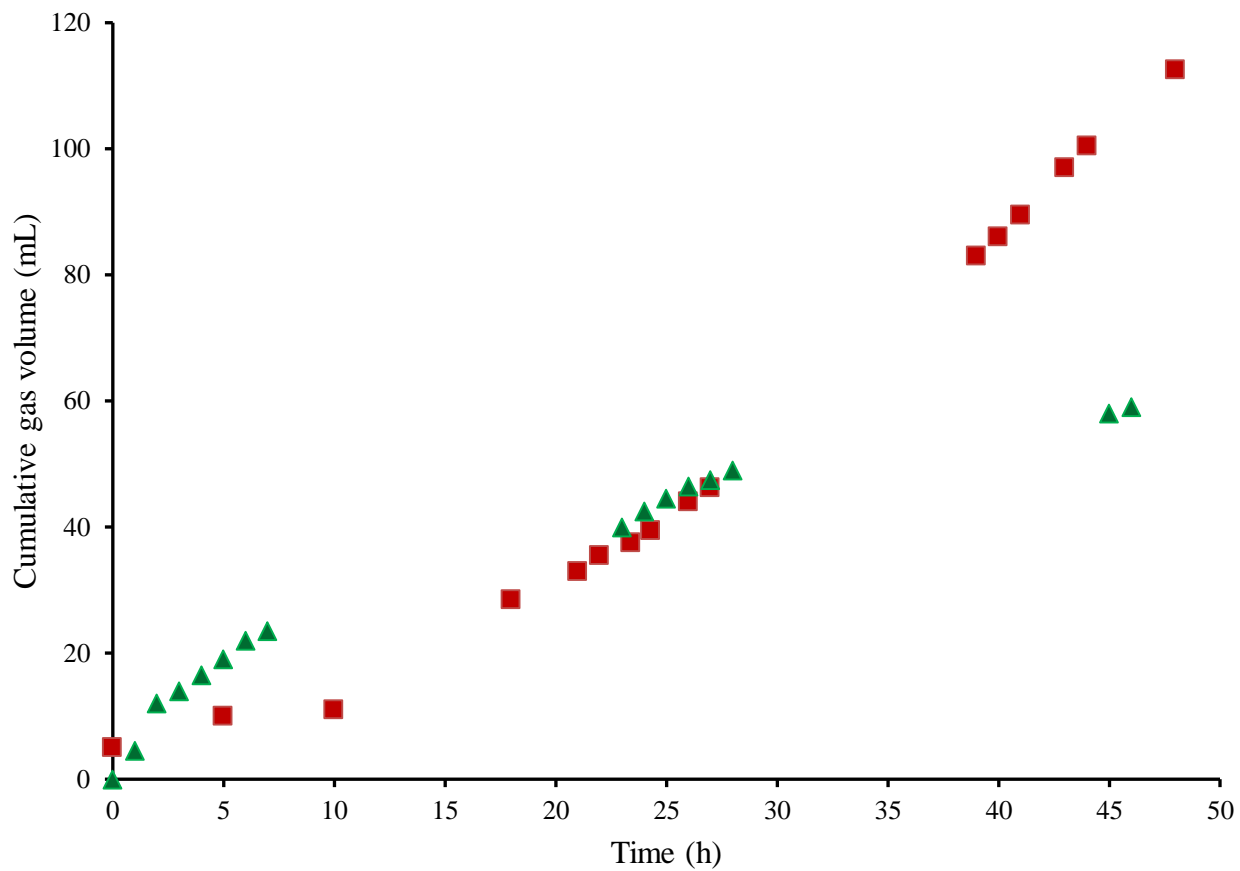
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638 Fig. 3

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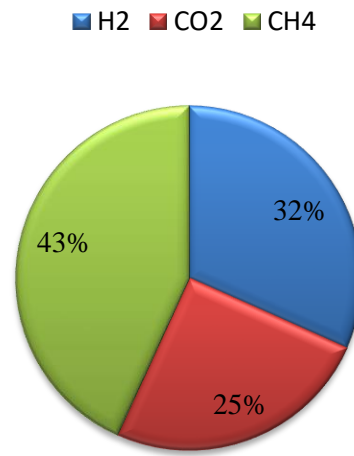
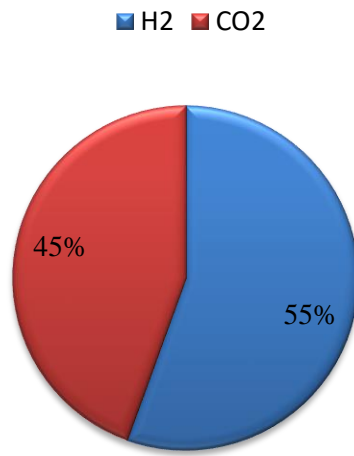
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650 Fig. 4

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**A**

**B**



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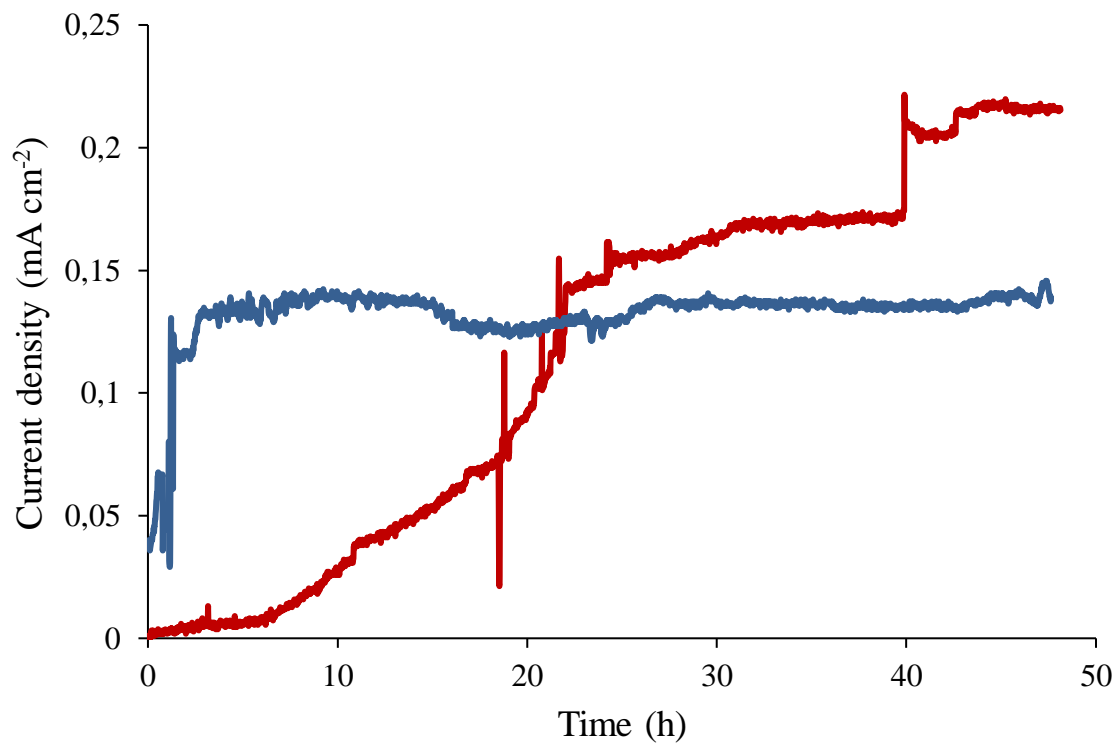
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660 Fig. 5



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