

## Letter

## Active H<sub>2</sub> Harvesting Prevents Methanogenesis in Microbial Electrolysis Cells

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1           **Active H<sub>2</sub> Harvesting Prevents Methanogenesis in Microbial**  
2   **Electrolysis Cells**

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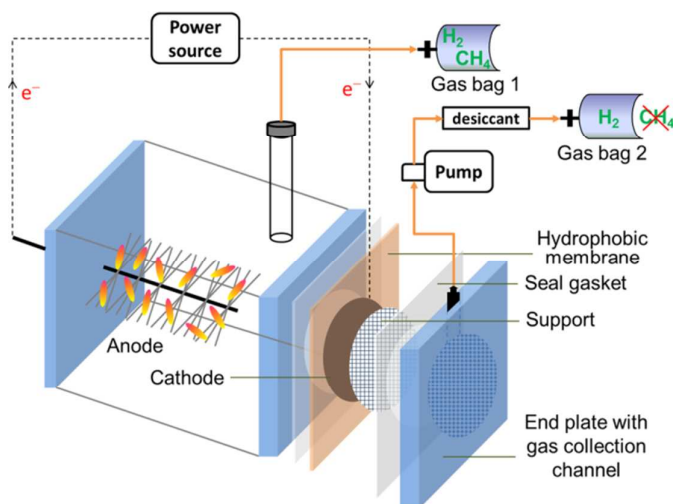
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30 **ABSTRACT**

31 Undesired H<sub>2</sub> sinks including methanogenesis are a serious issue faced by microbial  
32 electrolysis cells (MECs) for high rate H<sub>2</sub> production. Different from current top  
33 down approaches on methanogenesis inhibition that showed limited success, this  
34 study found active harvesting can eliminate the source (H<sub>2</sub>) from all H<sub>2</sub> consumption  
35 mechanisms via rapid H<sub>2</sub> extraction using a gas-permeable hydrophobic membrane  
36 and vacuum. The active harvesting completely prevented CH<sub>4</sub> production and led to  
37 much higher H<sub>2</sub> yields than the control using traditional spontaneous release  
38 (2.62-3.39 vs. 0.79 mol H<sub>2</sub>/mol acetate). In addition, existing CH<sub>4</sub> production in the  
39 control MEC was stopped once switched to active H<sub>2</sub> harvesting. Active harvesting  
40 also increased current density by 36%, which increased operation efficiency and  
41 facilitated organic removal. Energy quantification shows the process was  
42 energy-positive, as the produced H<sub>2</sub> energy in active harvesting was 220 ± 10% than  
43 external energy consumption, and high purity of H<sub>2</sub> can be obtained.

44



45

46 **Table of Contents Graphic**

47 **INTRODUCTION**

48 Energy and resource recovery has become the best practice of the wastewater  
49 treatment industry, and hydrogen gas ( $H_2$ ) is among the most desired products due to  
50 its high energy density, potential for detoxifying water pollutants, and great value to  
51 different industries.<sup>1-3</sup> Among the technologies that can generate  $H_2$  during  
52 wastewater treatment, microbial electrolysis cells (MECs) showed superior  
53 performance than fermentation or photo-biological processes<sup>4,5</sup>, and it was identified  
54 by the US Department of Energy (DOE) as a key technology to meet the cost goals of  
55 \$2-4/gge (gasoline gallon equivalent)  $H_2$  from renewable biomass in nearer term.

56 MECs employ exoelectrogenic microbes at the anode to convert biodegradable  
57 substrates into electrons, which are then transferred to the cathode to reduce  $H^+$  for  $H_2$   
58 evolution under a small voltage of 0.2~1.0 V.<sup>5</sup> The produced hydrogen energy derives  
59 from the chemical energy stored in the substrates, so MECs can generate more energy  
60 than the external energy input. MECs have been shown with high  $H_2$  yield (up to 11  
61 mole  $H_2$  per mole hexose), high production rate (up to 50  $m^3 H_2/m^3$  reactor/day),  
62 low-temperature operation, and diverse substrate utilization,<sup>4,6,7</sup> and the technology  
63 has been scaled up to cubic meter scale.<sup>8</sup>

64 However, undesired  $H_2$  sinks such as hydrogenotrophic methanogenesis remain a  
65 serious challenge for all MECs.<sup>9-11</sup> The dominance of hydrogenotrophic methanogens  
66 in MECs has been confirmed by our previous research<sup>10-12</sup> and multiple groups using  
67 different substrates in MECs<sup>9,13,14</sup> and even in microbial fuel cells.<sup>15</sup> For example,  
68 like many previous studies, the first MEC pilot study using real wastewater showed

69 the production of CH<sub>4</sub> instead of H<sub>2</sub> after a period of operation.<sup>8</sup> While acetoclastic  
70 methanogens were generally outcompeted by exoelectrogenic microbes in MECs,  
71 hydrogenotrophic methanogenesis can scavenge H<sub>2</sub> at nanomolar level and produce 1  
72 mole CH<sub>4</sub> from 4 moles of H<sub>2</sub> ( $4\text{H}_2 + \text{CO}_2 = \text{CH}_4 + 2\text{H}_2\text{O}$ ).<sup>12</sup> Many different  
73 approaches have been used to reduce methanogenesis in MECs, including chemical  
74 inhibitors,<sup>16, 17</sup> exposure to O<sub>2</sub>,<sup>18, 19</sup> reduction of hydraulic retention time,<sup>18, 20, 21</sup> low  
75 pH operation,<sup>16, 19</sup> temperature control,<sup>7, 10, 16</sup> carbonate limitation,<sup>20</sup> and ultraviolet  
76 irradiation<sup>22</sup>, but most of them observed limited success and many of them  
77 encountered other problems, such as damage of exoelectrogens, high cost, and only  
78 short-term success. A recent review summarized and discussed in detail the  
79 advantages and challenges of these methods.<sup>4</sup>

80 Furthermore, these strategies only repressed methanogenesis but overlooked other  
81 routes of H<sub>2</sub> re-consumption. Exoelectrogens can directly oxidize H<sub>2</sub> as the electron  
82 donor, and they can also utilize the acetate produced via homoacetogenesis ( $2\text{CO}_2 +$   
83  $4\text{H}_2 \rightarrow \text{CH}_3\text{COOH} + 2\text{H}_2\text{O}$ ). Both routes lead to the re-generation of current and then  
84 again H<sub>2</sub> production at the cathode, which is called H<sub>2</sub>-recycling.<sup>9</sup> Although  
85 H<sub>2</sub>-recycling between the anode and cathode may not result in significant H<sub>2</sub> loss, it  
86 increases the electrode overpotential loss and duration of operation cycle and  
87 therefore low H<sub>2</sub> recovery.<sup>9, 23, 24</sup> Standalone methanogenesis inhibition can boost the  
88 growth of homoacetogens and increase the H<sub>2</sub>-recycling loss.<sup>25</sup> As explained by Lee,  
89 et al., for these reasons the rapid separation of the H<sub>2</sub> product from the MEC reactor  
90 becomes crucial to minimize H<sub>2</sub> diffusion towards the anode.<sup>23</sup> The development of

91 new reactor configuration or harvesting method represents new approaches to reduce  
92 H<sub>2</sub> sinks and energy loss in MECs.

93 This study employs a new and different approach for preventing the undesired H<sub>2</sub>  
94 sinks in MECs. Rather than doing the top down inhibition, we rapidly harvest H<sub>2</sub> by  
95 removing it from the reactor. This approach eliminates the source (H<sub>2</sub>) from all  
96 potential H<sub>2</sub> consumption mechanisms and also increases H<sub>2</sub> production rates. We  
97 tested this hypothesis by using a gas-permeable hydrophobic membrane, which allows  
98 a timely gas separation from the solution via low pressure vacuum. The  
99 methanogenesis inhibition and H<sub>2</sub> recovery in this active harvesting system was  
100 compared with control MECs using traditional spontaneous gas releases. The H<sub>2</sub>  
101 yields, current densities, H<sub>2</sub> production rates and energy efficiencies were also  
102 examined for both systems.

103

## 104 **MATERIALS AND METHODS**

### 105 **Reactor setup**

106 Figure S1 (Supporting Information) shows the MEC setup for H<sub>2</sub> production and rapid  
107 harvesting. Each traditional cubic MEC has a cylinder chamber (3 cm x 4 cm) with a  
108 working volume of 25 mL.<sup>12</sup> Carbon brush anode (D=2 cm, L=2.5 cm) and carbon  
109 cloth cathode (effective projected area = 7 cm<sup>2</sup>) coated with 0.5 mg/cm<sup>2</sup> Pt catalyst  
110 were mounted on different ends of the MEC. Hydrophobic PTFE membrane (16 cm<sup>2</sup>,  
111 0.22 μm pore size, 160-220 μm thickness, Membrane Solutions, LLC.) was covered  
112 on the cathode for H<sub>2</sub> separation. An end plate carved with gas collection channels

113 was pressed against the membrane, and a port connected with all channels was joined  
114 to a vacuum pump (BT100-1L, Langer Instruments) for gas harvesting into a gas  
115 collection bag (Cali-5-Bond, Calibrated Instruments Inc.) after passing through a  
116 desiccator. Nylon mesh was used as the support layer to prevent membrane  
117 deformation. The whole reactor was tightly sealed using epoxy. Gas was also  
118 passively collected by its spontaneous release to a glass tube (15 mL), which was  
119 mounted above the cathode and connected with another gas bag (bag 1).

120

### 121 **MEC operation**

122 Two acetate-fed MFC anodes with almost identical performance (Figure S2) were  
123 selected and transferred to two MECs (MEC1 and MEC2). During the first 47 batch  
124 cycles (36–39 days), MEC1 was operated in active H<sub>2</sub> harvesting mode (vacuum) and  
125 MEC2 was in passive harvesting mode (spontaneous release). Then their harvesting  
126 modes were switched and operated for another 13 batch cycles (12 days). To further  
127 evaluate the repeatability and efficacy of active H<sub>2</sub> harvesting, two additional MECs  
128 were operated in parallel with spontaneous gas release till they showed same level of  
129 methanogenesis. After that, one reactor (MEC3) was converted to active H<sub>2</sub>  
130 harvesting mode and the other reactor (MEC4) kept the same H<sub>2</sub> releasing protocol.  
131 Their performances were compared side-by-side. All MECs were fed with 1 g/L  
132 sodium acetate in 50 mM phosphate buffer solution, and were operated under an  
133 applied voltage of 0.6 V and the temperature of 25 ± 1 °C.

134

### 135 **Analyses and calculations**

136 Gas, current and chemical analyses are provided in the Supporting Information. The  
 137 energy content of the gas was calculated as:

$$138 \quad W_{\text{gas}} \text{ (J)} = n_{\text{H}_2} \Delta H_{\text{H}_2} + n_{\text{CH}_4} \Delta H_{\text{CH}_4} \quad (1)$$

139 Where  $n_{\text{H}_2}$  and  $n_{\text{CH}_4}$  are the moles of H<sub>2</sub> and CH<sub>4</sub>, respectively.  $\Delta H_{\text{H}_2}$  (285,830  
 140 J/mol) and  $\Delta H_{\text{CH}_4}$  (891,000 J/mol) are the energy content of H<sub>2</sub> and CH<sub>4</sub>,  
 141 respectively.<sup>26</sup> System energy consumption by the MEC from the power source and  
 142 vacuum pump was calculated using<sup>2, 26</sup>

$$143 \quad W \text{ (J)} = \sum_1^n (IE_{\text{ap}} \Delta t_{(s)} - I^2 R_{\text{ex}} \Delta t_{(s)}) + Q \gamma E t_{(h)} 3600 \quad (2)$$

144 Where  $I$  is the current (A),  $E_{\text{ap}}$  is the applied voltage (0.6 V),  $\Delta t_{(s)}$  is the data  
 145 sampling increment (600 s),  $R_{\text{ex}} = 10 \ \Omega$ ,  $Q$  (m<sup>3</sup>/s) is the maximum H<sub>2</sub> flow rate  
 146 through membrane,  $\gamma$  is 0.8016 N/m<sup>3</sup> for H<sub>2</sub>,  $E$  is the maximum head loss of 10.34 m  
 147 (1 atm) due to transmembrane pressure (TMP), and  $t_{(h)}$  (hour) is the reaction time of  
 148 one batch cycle. The energy efficiency  $\eta_E$  (%) is the ratio of the energy content in the  
 149 gas to the energy consumption of system:

$$150 \quad \eta = \frac{W_{\text{gas}}}{W} \quad (3)$$

151

## 152 **RESULTS AND DISCUSSION**

### 153 **Rapid H<sub>2</sub> harvesting prevented methanogenesis in the MEC**

154 MEC1 was firstly operated for 47 batch cycles (36 days) under active H<sub>2</sub> harvesting  
 155 condition, in which vacuum was applied to extract H<sub>2</sub> through a gas-permeable



156 hydrophobic membrane (Figure S1, Figure 1). No CH<sub>4</sub> was detected during this whole  
157 period, and stable H<sub>2</sub> was produced. High H<sub>2</sub> yields of 2.62–3.39 mol H<sub>2</sub>/mol acetate  
158 or H<sub>2</sub> recoveries of 66–85% were obtained during this operation<sup>4</sup> (Figure 1). In  
159 contrast, CH<sub>4</sub> started to accumulate after 12 days in MEC2, which was operated under  
160 traditional condition where gas was spontaneously released into the headspace. In the  
161 meantime, H<sub>2</sub> yield decreased from 2.82 mol H<sub>2</sub>/mol acetate (14<sup>th</sup> batch cycle) to 0.79  
162 mol H<sub>2</sub>/mol acetate (47<sup>th</sup> batch cycle). Although H<sub>2</sub> has a low solubility (0.0016 g  
163 H<sub>2</sub>/kg water at 293 K),<sup>27</sup> its uptake by methanogens on the anode will create a  
164 concentration gradient to facilitate diffusion of H<sub>2</sub> towards the anode, especially under  
165 low H<sub>2</sub> production rate. If we assume that no H<sub>2</sub> was consumed by methanogenesis,  
166 the total H<sub>2</sub> yield during the first 47 batch cycles is calculated to be 2.52–3.40 mol  
167 H<sub>2</sub>/mol acetate by using a conversion factor of 4 mol H<sub>2</sub>/mol CH<sub>4</sub>. This range is  
168 exactly the same as the H<sub>2</sub> yield obtained in MEC1, indicating no methanogenesis  
169 occurred in MEC1.

170 To further prove that the methanogenesis inhibition was due to rapid H<sub>2</sub>  
171 harvesting, we reversed H<sub>2</sub> harvesting methods for the two MECs after 47<sup>th</sup> batch  
172 cycles. Methanogenesis occurred rapidly in MEC1 when the vacuum was removed,  
173 and H<sub>2</sub> yield dropped from 2.77 to 1.43 mol H<sub>2</sub>/mol acetate after another 13 batch  
174 cycles (12 days) (Figure 1). Meanwhile, around half of the H<sub>2</sub> were converted to CH<sub>4</sub>.  
175 On the contrary, CH<sub>4</sub> production in MEC2 stopped only after another 2 batch cycles  
176 when vacuum was applied to actively extract H<sub>2</sub>. H<sub>2</sub> yield rapidly recovered from 0.79  
177 to a maximum of 2.94 mol H<sub>2</sub>/mol acetate. In addition, the total yield in both reactors

178 kept stable, suggesting no other losses were unaccounted for. These findings were  
179 further supported by the side-by-side comparison of parallel reactors MEC3 and  
180 MEC4. Figure S3 shows that initially both reactors showed similar levels of  
181 methanogenesis during passive harvesting, but dramatic difference was observed  
182 when MEC3 was switched to active harvesting. While a similar level of CH<sub>4</sub> was  
183 maintained in the MEC4 control with passive H<sub>2</sub> release, CH<sub>4</sub> production was  
184 effectively inhibited after MEC3 was switched to active H<sub>2</sub> harvesting for 2 batch  
185 cycles (Figure S3). All these results support the hypothesis that rapid harvesting of H<sub>2</sub>  
186 can completely inhibit CH<sub>4</sub> production in MEC. Rather than the direct suppression of  
187 methanogenesis, this source deprivation strategy proved to be very effective.

188

### 189 **Rapid H<sub>2</sub> harvesting facilitated current production and H<sub>2</sub> rate**

190 The volumetric current density in MECs has shown a strong positive correlation with  
191 H<sub>2</sub> production rate.<sup>2</sup> This is very important because higher rate can significantly  
192 reduce capital costs. For example, the capital cost of MEC is calculated more than 30  
193 times than a conventional electrolyzer due to MEC's low H<sub>2</sub> rate, although both  
194 technologies use similar electrode materials and configurations.<sup>28, 29</sup> It is interesting  
195 that higher current density was obtained during active H<sub>2</sub> harvesting than spontaneous  
196 gas collection. The average current density decreased from  $177 \pm 9$  to  $157 \pm 6$  A/m<sup>3</sup> in  
197 MEC1 when the H<sub>2</sub> harvesting switched from active extraction to passive collection  
198 (Figure 2A). In MEC2 where H<sub>2</sub> was passively harvested, the current density  
199 decreased from 191 to 125 A/m<sup>3</sup> on day 39. However, when switch to active

200 harvesting current density increased rapidly by 36% to an average of  $170 \pm 3 \text{ A/m}^3$ . It  
201 is believed that active harvesting reduced fine  $\text{H}_2$  bubble entrapment on the cathode,  
202 which has been found in most high surface area cathodes under spontaneous  $\text{H}_2$   
203 release condition.<sup>30,31</sup> This equivalently increased the effective electrode surface area.  
204 Moreover, higher current represents faster substrate degradation and therefore more  
205 efficient wastewater treatment. In this case, active  $\text{H}_2$  extraction reduced duration of  
206 47 batch cycles from 39 to 36 days compared with passive  $\text{H}_2$  release, and high COD  
207 removal (> 90%) was consistently achieved in each cycle. Such increased efficiency  
208 will also reduce the energy input and operation costs. The average  $\text{H}_2$  production rate  
209 was  $1.58 \pm 0.15 \text{ m}^3/\text{m}^3/\text{d}$  for MEC1 and MEC2 under active  $\text{H}_2$  harvesting, which was  
210 216% higher than that of  $0.5 \pm 0.26 \text{ m}^3/\text{m}^3/\text{d}$  in passive  $\text{H}_2$  release (Figure 2B). Even  
211 more, it can be seen the  $\text{H}_2$  rate can be very stable in active harvesting, which is  
212 desired for scaled applications. This positive effect of active  $\text{H}_2$  harvesting on current  
213 production was also confirmed in further replicate experiments (MEC3 and MEC4)  
214 (Figure S3).

215

#### 216 **Active $\text{H}_2$ harvesting consumed low energy**

217 Many MEC studies demonstrated the potential of energy-positive operations, which  
218 means the energy value of the produced  $\text{H}_2$  is higher than the energy input supplied by  
219 the external power source. The extra energy comes from the chemical energy  
220 embedded in the substrates. In this study, additional energy is consumed by the  
221 vacuum, so it is important to understand the updated energy balance. Based on the

222 aforementioned calculations, the energy efficiency was defined as the ratio of  
223 produced energy to the energy input. The energy efficiency ranged from 168% to 250%  
224 with an average of  $215 \pm 15\%$  for both reactors (Figure 2C). During active H<sub>2</sub>  
225 harvesting in MEC1, the energy efficiency was  $220 \pm 10\%$  with H<sub>2</sub> as the only  
226 product. This indicates that active H<sub>2</sub> harvesting operation can still be energy-positive  
227 and in fact may generate twice amount of the energy than the energy consumed. Due  
228 to the low transmembrane pressure (TMP) needed (a maximum of 1 atm) and low  
229 molecular weight of gas compared to liquid filtration using hydrophilic membranes,  
230 the energy consumed by vacuum of H<sub>2</sub> is only  $2-3 \times 10^{-4}$  J for each batch cycle, which  
231 is orders of magnitude lower than electrical energy input to MEC for H<sub>2</sub> production  
232 (100–150 J) and energy content of produced H<sub>2</sub> (around 300 J) (Figure S4).

233

### 234 **Outlook**

235 The new approach of H<sub>2</sub> harvesting demonstrates good potential to increase H<sub>2</sub>  
236 recovery and prevent methanogenesis in MECs, but more work is needed to further  
237 understanding and development. No obvious membrane fouling was observed during  
238 51 days of operation attribute to the hydrophobic feature of membrane that only  
239 allows gas to permeate with low TMP. This feature leads to reduced biofilm growth  
240 and foulant accumulation on membrane surface compared to hydrophilic membranes  
241 targeting effluent quality. However, long term performance does need to be monitored  
242 to understand the fouling behavior and methanogenesis prevention. Another aspect  
243 needs further study is the purity of H<sub>2</sub>. No CO<sub>2</sub> was detected in this study during

244 active gas harvesting due to its absorption by alkaline desiccant. Other biogas such as  
245 H<sub>2</sub>S could be produced when real wastewater is used, which is a common possibility  
246 for any bio-H<sub>2</sub> technology. However, existing gas purification system used in  
247 separation industry should be applicable in this system for H<sub>2</sub> purification. While this  
248 study demonstrates the proof-of-concept using acetate substrate, further studies are  
249 needed to characterize and optimize system's performance using real wastewater.  
250 Wastewater contains mixed organic substrates that lead to different reactions  
251 involving complex microbial communities, therefore the quantity and quality of gas  
252 production can vary significantly. In addition, biofouling and scaling can become  
253 significant due to excessive growth of microorganisms and pH and conductivity  
254 change. These conditions need to be investigated to optimize operations such as  
255 hydraulic retention time (HRT), temperature and vacuum pressure. Further  
256 development on membrane that is selective for gas separation is also needed. Because  
257 active harvesting doesn't kill methanogens, temporary shutdown of vacuum will lead  
258 to recovery of CH<sub>4</sub> production, therefore this method can be used in conjunction with  
259 other methanogenesis inhibition measures.

260

### 261 **Acknowledgements**

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263 CEBT-1510682.

### 264 **Supporting Information Available**

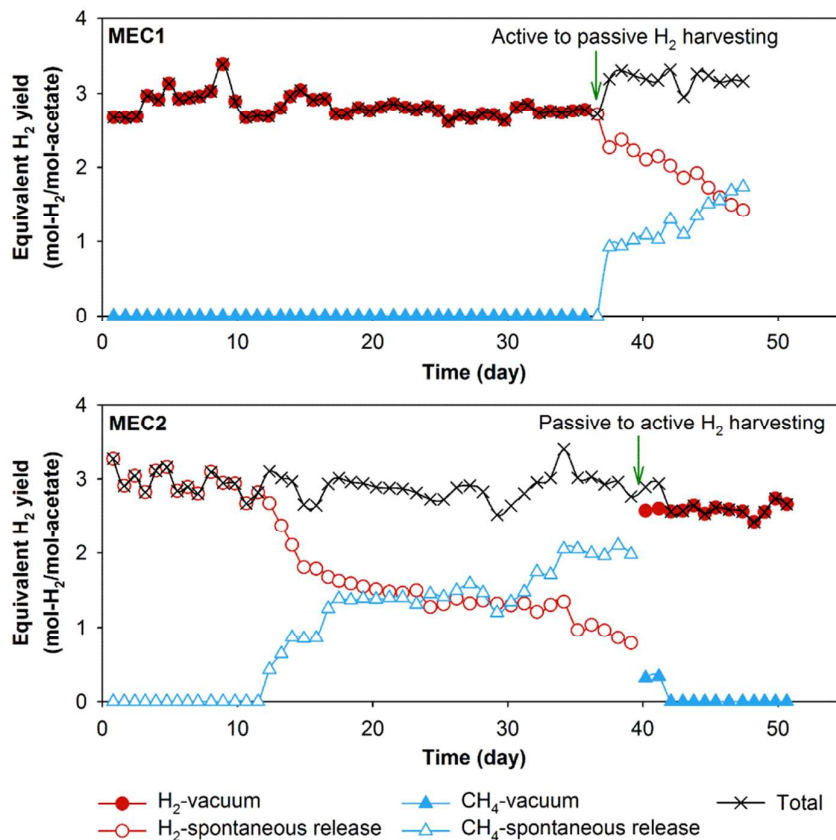
265 Additional tables and figures are included in the Supporting Information. This

266 information is available free of charge via the Internet at <http://pubs.acs.org/>.

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270

271 **Figure 1.** H<sub>2</sub> yield and CH<sub>4</sub> yield (represented by an equivalent H<sub>2</sub> yield) in MECs

272 during different stages of gas harvesting (active vacuum extraction or spontaneous

273 release). The CH<sub>4</sub> yield (mol CH<sub>4</sub>/mol acetate) was converted to an equivalent H<sub>2</sub>

274 yield (mol H<sub>2</sub>/mol acetate) using a conversion factor of 4 mol H<sub>2</sub>/mol CH<sub>4</sub>. The “total”

275 yield is a sum of equivalent H<sub>2</sub> yield from H<sub>2</sub> and CH<sub>4</sub>.

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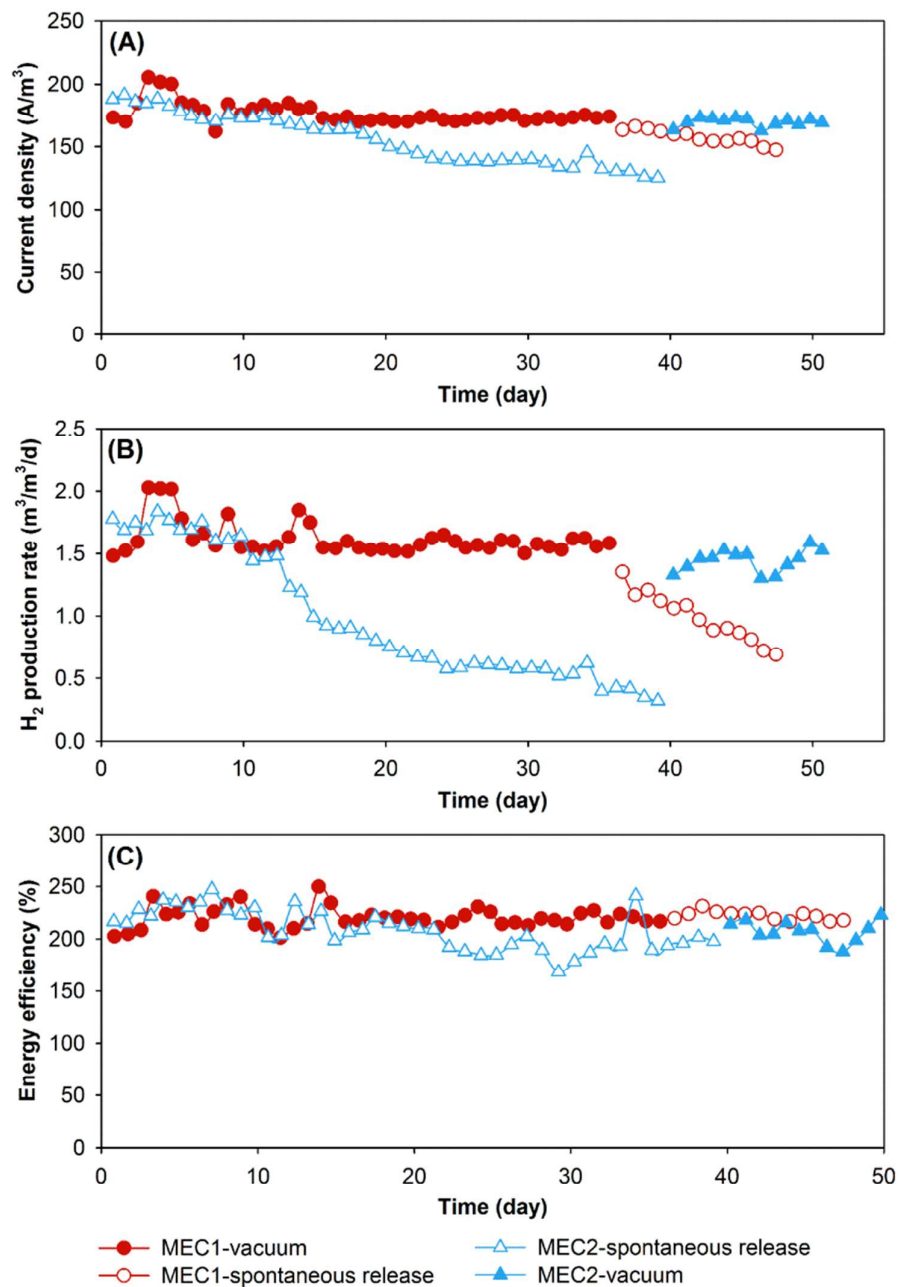
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288 **Figure 2.** (A) Current density and (B) H<sub>2</sub> production rate normalized to reactor

289 working volume of 25 mL, and (C) energy efficiency in the two MECs during

290 different stages of gas harvesting (active extraction or spontaneous release).

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