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1 **Coupling electric energy and biogas production in anaerobic digesters -**
2 **impacts on the microbiome**

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17

18 **Abstract**

19 The combination of anaerobic digestion (AD) and microbial electrochemical technologies
20 provides the opportunity to efficiently produce methane and electric energy from complex
21 biomass. Enhanced methane production and system stability are reported but the causes
22 (electrolysis or microbial-electrochemical interaction) less understood.

23 Using the model substrate corn silage it is demonstrated that, for conditions allowing
24 microbiome growth and adaptation, the methane yield of combined reactors remains constant
25 ($216 (\pm 29) \text{ mL g}_{\text{odm}}^{-1}$) while the second product, electrons ($q = 14.4 (\pm 0.8) \text{ kC}$, $j_{\text{max}} = 1.34 \text{ mA}$
26 cm^{-2} geometric current density), is generated. The combined strategy allowed an up to 27%
27 increase in total yield while the reactor community and its dynamics over time were not
28 affected. A typical AD composition with *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and
29 *Synergistetes* (bacteria) as well as *Methanosarcina*, *Methanoculleus* and *Methanobacterium*
30 (archaea) was found in the bulk liquid. Specific enrichments of *Geobacter* (anode) and
31 *Methanobacterium* (cathode) were of functional relevance.

32

33

34 **Keywords:** microbiome resource management, bioelectrochemical system, biogas, anaerobic
35 digestion, microbial community, mixed culture biotechnology

36

37 1 Introduction

38 Anaerobic digestion (AD) is a widely applied technology allowing turning biomass to
39 methane that is subsequently most often exploited by combustion. In Germany about 7700
40 biogas plants are installed ¹, most of them in an agricultural setting while internationally AD
41 is more relevant for treating slurries and concentrated industrial or domestic wastewaters with
42 low solid content ². During anaerobic digestion complex organic substrates are degraded by
43 primary and secondary fermenting bacteria to small organic acids, which are then transformed
44 by methanogenic archaea to methane and carbon dioxide. Imbalances between the trophic
45 levels of the reactor microbiome often result in accumulation of organic acids, which leads to
46 process inhibition and failure ³. Microbial fuel cells (MFCs), being the archetype of microbial
47 bioelectrochemical systems (BES), are considered an alternative microbial electrochemical
48 technology (MET) ⁴ for converting biomass to electricity. The core of every BES is the
49 interaction of electroactive microorganism and the electrode, which directly links the
50 microbial and electrochemical activity ⁵⁻⁷. At microbial fuel cell anodes, the electroactive
51 microorganisms oxidize their substrate molecules and thus generate electricity. Important,
52 however, is that most known electroactive microorganisms can only utilize small organic
53 molecules (e.g. acetate and lactate) and rely on the pre-digestion by fermenting bacteria (see
54 e.g. ⁸) for utilizing complex biomasses. Thus an integrated exploitation of complex biomass
55 by AD (to CH₄) and MFCs (to electricity) is appealing, as it shall allow a complete substrate
56 digestion based on flexible product utilization on different trophic levels. This provides the
57 opportunity of process management not only in terms of desired products (steering between
58 CH₄ and electric energy gain), but also regarding efficient consumption of substrates,
59 intermediates or undesired side products.

60 The particular combination of anaerobic digestion with microbial electrochemical
61 technologies, sometimes denominated as “eAD”, was introduced in the recent years. Thereby,

62 enhanced methane production as well as higher system stability was proposed in comparison
63 to “conventional” AD⁹⁻¹¹. An overview on eAD -studies and their main results is given in
64 Table 1. Obviously, the reactor type, substrate, microbial source and electrochemical
65 operation conditions differed considerably not allowing any systematic assessment. Most
66 importantly, in all types of setups electric energy was invested, whereas we show here that
67 additional electric energy can potentially be gained. Further, it is most important to clarify
68 whether the (sometimes) reported increased biogas production after investment of electricity
69 was caused by electrolysis related effects or by direct electrochemical interaction of
70 microorganisms with the electrodes.

71 In this study the effect of an electrochemical setup on anaerobic digestion in eAD reactors is
72 examined. The system performance and reactor microbiome in eAD reactors was investigated
73 using an automated biomethane potential test system¹² combined with a potentiostatically
74 controlled (providing a constant electrode potential) three-electrode setup. Single chamber
75 eAD reactors (where the microbiome faces the anode and the cathode) as well as dual-
76 chamber eAD reactors (where the microbiome faces only the anode) were investigated at
77 potentials being low enough to avoid water electrolysis. These were benchmarked on
78 “conventional” AD reactors as well as to eAD reactors facing water electrolysis. Process
79 parameters (current production, volatile fatty acids concentration, methane production, pH)
80 were frequently recorded, the reactor microbiome was monitored over time and the
81 community composition (bacteria and archaea in the bulk liquid as well as on the electrodes)
82 was determined at the end of each experiment.

83 **2 Material and Methods**

84 **2.1 General conditions**

85 All experiments were conducted under anoxic conditions at 37°C. All chemicals were of
86 analytical or biochemical grade. If not stated otherwise, all potentials provided in this article

87 refer to the Ag/AgCl reference electrode (sat. KCl, 0.195 V vs. SHE (standard hydrogen
88 electrode)).

89 **2.2 Reactor setup**

90 A modified Automatic Methane Potential Test System (AMPTS, Bioprocess Control AB,
91 Sweden¹²) allowing up to 15 parallel batch experiments was used. It consists of a temperature
92 controlled incubation unit (37°C) hosting up to 15 tailor-made glass reactors (see
93 Supplementary Figure S1). Figure 1 shows the dual-chamber (DC) setup, for the single-
94 chamber (SC) setup the counter electrode shielding and membrane were removed and the
95 counter electrode fully immersed. All setups allowed the introduction of electrodes and
96 provided further sampling ports. The stirring was either performed with the AMPTS stirrers
97 (slow rotating agitator from top) for setting I or with magnetic stirrer bars (bottom of reactors,
98 120 rpm). In the latter case, the experiments were performed within a temperature controlled
99 incubation chamber (37°C). The change of the steering system was necessary dependent on
100 the seeding sludge (see below) to avoid settling of substrate particles and ensure homogenous
101 mixing of the reactor content in experimental setting II and III, respectively (see Table 2 for
102 details). The standard reactor liquid volume was 400 mL (details see section 2.4). For gas
103 quantification a CO₂ fixing unit and gas volume measuring device with 15 channels was
104 connected and operated according to the provider's regulations. The produced gas from the
105 reactors passed the CO₂ fixing bottles that contained each 80 mL 3 M NaOH and
106 thymolphthaleine pH-indicator (0.002%). The remaining gas is supposed to be methane and
107 was quantified by the gas volume measuring device, based on liquid displacement and
108 buoyancy, interfaced to automated data acquisition for each channel including pressure and
109 temperature compensation.

110 2.3 Electrochemical setup

111 Each reactor contained a three electrode arrangement consisting of a graphite rod working
112 electrode (projected surface area; 16.2 cm², CP-Graphite GmbH, Germany), a Ag/AgCl
113 reference electrode (sat. KCl, SE11, Sensortechnik Meinsberg, Germany, 0.195 V vs. SHE)
114 and a graphite rod serving counter electrode (projected surface area; 19.6 cm², CP-Graphite
115 GmbH, Germany). The three electrodes were either arranged as single-chamber (SC) setup,
116 where the reactor microbiome was facing the working and the counter electrode, or the
117 counter electrode was separated using a cation exchange membrane (fumasep FKE, FuMA-
118 Tech, Germany), denominated as dual-chamber (DC) setups, here the reactor microbiome
119 faces only the working electrode. The latter setup equals the anode chamber of a microbial
120 fuel cell. Thus the anode based effects can be individually studied using DC, while in the SC
121 setup both electrodes can functionally contribute. As AD control reactors served SC setups
122 without any potential applied to the working electrodes (open circuit conditions (OCP)).
123 The experiments were carried out under potentiostatic or galvanostatic control using a
124 potentiostat (MPG-2, BioLogic Science Instruments, France) equipped with 16 independent
125 channels. Current production, *i*, was monitored with chronoamperometry and recorded every
126 5 min. The current density is calculated per projected surface area and denominated as
127 “geometric current density”, *j* (see also ¹³, volumetric current density refers to the liquid
128 reactor volume.

129 2.4 Seeding sludge and substrate

130 According to the guidelines by the Association of German Engineers (VDI 4630), the seeding
131 sludge, *i.e.* inoculum, for all reactors was an anaerobic digestion sludge mixture consisting of
132 wastewater sludge and sludge from a biogas plant and pre-incubated without any substrate. It
133 was sieved (pore size 1 mm) and diluted with mineral salt and buffer solution containing in g
134 L⁻¹ NaHCO₃ 1.36, KHCO₃ 1.74, NH₄Cl 0.31, KCl 0.13, as well as trace metal and vitamin

135 solution (according to ^{9,14} allowing routine sampling with a 0.9 x 70 mm syringe. The
136 inoculum was either 50% or 5% (vol/vol) of the 400 mL reactor content (final pH 7.7-7.8).
137 The only organic carbon and energy source was 2.5 g dried ground corn silage (1mm (MF
138 10.1, IKA[®]-Werke GmbH & Co. KGA GmbH, Staufen, Germany) with 874 g organic dry
139 matter (g_{odm}) per kg of fresh mass (for odm determination see Supplementary Methods). It
140 was added to each individual reactor immediately before the start of the experiment.

141 **2.5 Analytical methods**

142 Regularly (every 2-3 days), all reactors were sampled (3 mL) with a syringe for analytical and
143 microbiological analysis. For the sampling procedure, the gas tubes were closed and 3 mL
144 nitrogen gas added for volume adjustment. The pH was determined with a pH-meter (H138
145 miniLab[™] Elite (HACH-Lange, Germany)) that was calibrated on the daily basis. In case of a
146 pH drop in the medium below pH = 6.4 sodium carbonate (1 g per reactor) was added for
147 adjustment. Volatile fatty acids (VFA) concentrations were determined using HPLC (details
148 Supplementary Methods).

149 Methane production was monitored online with a tailor made AMPTS (see 2.2.). Due to the
150 specific setup higher standard deviations, of in average ~10%, compared to conventional
151 AMPTS, were achieved. All values for methane production are given in $mL_{NORM} CH_4$ per
152 gram odm ($mL CH_4 g_{odm}^{-1}$). Norm conditions refer to the dry gas at 101.325 kPa and 273.15
153 K.

154 The methane production potential of the seeding sludge without substrate addition for all
155 settings was determined. It was 23 (± 4) $mL CH_4$ per setup in setting I, here being subtracted,
156 and $< 8 mL CH_4$ per setup for setting II and thus below one tipping unit of the gas counter
157 (Supplementary Table S1). For some reactors in setting III regular gas sampling in the
158 headspace and GC analysis (Micro GC CP 2002 P, Chrompack, with Molsieve 5A PLOT and
159 Haye Sep A) was performed.

160 2.6 Experimental conditions

161 Three different conditions, denominated further as settings I, II and III were investigated.
162 Each experiment was performed in minimum as independent biological triplicate in parallel
163 (if not stated otherwise, see setting III) and up to 15 reactors were run per installation – see
164 Table 2 for an overview.

165 2.6.1 Setting I: Validation of standard setup for anaerobic digestion batch tests

166 The first set of experiments was performed with minor dilution of the seeding sludge (50%
167 (vol/vol) of the total reactor content), adapted from a standard setup for methane production
168 potential tests in anaerobic digestion (VDI 4630). Five electrochemical setups were applied
169 (Table 2): Three reactors were run at a constant potential of -0.2 V at the working electrode
170 using single-chamber (SC) setup, denominated as SC_{-0.2V}, and three reactors with the same
171 potential as dual-chamber (DC) setup (DC_{-0.2V}). Another set of reactors was run at a potential
172 of +0.2 V in SC and DC setup, respectively (SC_{+0.2V}, DC_{+0.2V}). The chronoamperometric
173 measurements were intermitted by cyclic voltammetry (CV) measurements every 24 hours.
174 Further, eight reactors were run as “conventional” AD reactors at open circuit potential (OCP,
175 no potential applied), with the OCP-measurements being intermitted for regular CV
176 measurements. The CV measurements were performed in the potential range of -0.5 to 0.3 V
177 with a scan rate of 1 mV s⁻¹, three cycles were recorded and only the third cycle analyzed.

178 2.6.2 Setting II: Electrochemical stimulation under biomass growth conditions

179 By applying the standard setup for anaerobic digestion batch tests (setting I) a relatively low
180 amount of carbon and energy substrate (corn silage) is provided per microorganisms in the
181 inoculum, therefore, only minor or even no growth is expected. To monitor the effect of
182 electrochemical stimulation on the microbial community under actively growing conditions
183 the experiments were adapted: the seeding sludge was further diluted ((5% (vol/vol)) and an

184 at identical organic substrate load (2.5 g dried ground corn silage) per reactor as in setting I
185 was added.

186 Whereas the chronoamperometric and AD control conditions were used for setting II as
187 described for setting I, the scan rate for the CV measurements was adapted: first two CV
188 measurements were performed at 2 mV s^{-1} (only 2nd cycle analyzed) and one further CV at 0.5
189 mV s^{-1} .

190 2.6.3 Setting III: Electrolysis conditions

191 The third set of experiments was performed to create a link to previous publications
192 performed under galvanostatic conditions resp. constant electrolysis (see also 3.1.3). Using
193 biomass growth conditions (setting II) two SC reactors were set to a constant current of -1.2
194 mA (geometric current density of -0.074 mA cm^2 , volumetric current density of -3 mA L^{-1})
195 denominated as $\text{SC}_{-1.2\text{mA}}$. This value was chosen in accordance to previous studies operating
196 at a current range of -40 to -180 mA for a 24 L reactor¹⁰, thus equalling -2.9 mA L^{-1} on
197 average. For direct comparability of their performance, three DC reactors were run at a
198 potential of $+0.2 \text{ V}$ ($\text{DC}_{+0.2\text{V}}$) and three AD control reactors under OCP conditions in parallel
199 according to setting II (Table 2). CV measurements for all reactors were performed as
200 described for setting II.

201 For these experiments the AMPTS gas measuring device was not suitable, as in addition to
202 methane, also hydrogen (produced at the working electrode) and oxygen (produced at the
203 counter electrode) are supposed to enter the gas volume measuring device. Instead, GC
204 measurements for determining the gas composition in the headspace of the reactors were
205 performed regularly (see 2.5).

206 2.7 Microbiological analysis

207 Microbial community analysis can be performed on different levels and entities. Within this
208 study the microbiome has been described by (1) its structure and structural variation using the
209 single cell based method flow cytometry and cytometric fingerprinting and by (2) its
210 composition on a phylogenetic level using the DNA based fingerprint method T-RFLP.

211 2.7.1 Flow cytometry

212 Every cell has individual characteristics based on cell morphology and DNA content. Both
213 can be measured using e.g. the cell size related forward scatter signal (FSC) as well as the
214 DNA content after staining using the DNA specific fluorescent dye DAPI (4',6-diamidino-2-
215 phenylindole). Performing these measurements for diverse microbial communities sample
216 specific cytometric fingerprints are recorded within minutes for every sample and reflect the
217 specific structure of the microbial community. Changes in the community structure (resulting
218 from changes in the presence of cells and their activity) are reflected by changes in the
219 cytometric fingerprint. Regularly, the bulk liquid of the reactors was sampled for the reactor
220 community. The electrode biofilms (if present) were additionally sampled at the end of the
221 experiments. The sample fixation, staining procedure, cytometric measurements and data
222 analysis were performed according to ^{15, 16}. In short, the samples were fixated in 2%
223 paraformaldehyde solution, washed with phosphate buffer and finally stained with DAPI
224 applying a two-step procedure. First, the cells were incubated with solution A (2.1 g citric
225 acid and 0.5 g Tween 20 in 100 mL bidistilled water) for 20 min and then washed and
226 incubated in solution B (0.68 μ M DAPI (Sigma-Aldrich, Germany), 400 mM Na₂HPO₄, pH
227 7.0) for 3 h in the dark at 20°C. The cytometric measurements were performed with a MoFlo
228 cell sorter (DakoCytomation, USA) which is equipped with a blue (488 nm) and a UV (355
229 nm) laser. Excitation with the blue laser was used to analyze the forward and sideward scatter,
230 and the UV laser for the UV induced DAPI-DNA fluorescence. Fluorescent beads (yellow-

231 green fluorescent beads: 2 μm , FluoSpheres 505/515, F-8827, crimson fluorescent beads: 1
232 μm , FluoSpheres 625/645, F-8816, Molecular Probes Eugene, Oregon, USA, Fluoresbrite BB
233 Carboxylate microspheres, 0.5 μm , Polyscience, USA) were used to ensure instrumental
234 alignment. The cytometric data files were uploaded to the Flow Repository: *to be added*

235 2.7.2 DNA extraction, T-RFLP, sequencing

236 In addition to the community dynamics in the course of the experiment, the microbial
237 community composition of the reactor community and the electrode biofilms were determined
238 on DNA level using T-RFLP at the end of the experiments. In addition, a clone library was
239 constructed from a $\text{SD}_{+0.2\text{V}}$ reactor sample that showed an even distribution of the major
240 terminal restriction fragments (T-RFs) in the T-RFLP analysis.

241 DNA extraction was performed with the NucleoSpin Soil[®] kit (Macherey-Nagel) following
242 the manufacturer's instruction (lysis buffer 2 for biofilms, lysis buffer 1 for reactor content,
243 sample lysis with FastPrep[®] (Thermo Fisher Scientific) speed 4 for 20 s). The final elution
244 step was performed with 50 μL elution buffer and yielded up to 110 $\text{ng } \mu\text{L}^{-1}$ genomic DNA
245 for reactor content and up to 325 $\text{ng } \mu\text{L}^{-1}$ for biofilms.

246 PCR was performed with the primer set UniBac27f and Univ1492r for amplifying the partial
247 sequence of the 16S rRNA gene of bacteria¹⁷ and the primer set mlas und mcrA_rev for
248 amplification of the archaeal mcrA gene (subunit A of methyl coenzyme M reductase)¹⁸. T-
249 RFLP analysis, cloning and sequencing were performed according to standard procedures
250 (further details Supplementary Methods).

251 2.8 Electron balances

252 The complete aerobic oxidation of the substrate corn silage, $\text{C}_{22}\text{H}_{36}\text{O}_{18}$ (details Supplementary
253 Table S2), can be described as $\text{C}_{22}\text{H}_{36}\text{O}_{18} + 22 \text{O}_2 \rightarrow 22 \text{CO}_2 + 18 \text{H}_2\text{O}$. The average
254 oxidation number of the carbon in the substrate corn silage, $\text{C}_{22}\text{H}_{36}\text{O}_{18}$, is 0 and +4 in the

255 combustion product CO_2 , thus, 88 moles of electrons are released per mol of substrate.
256 Further, 2.19 g_{odm} of substrate were provided to each reactor which equals 1.77 g of
257 fermentable dry matter¹⁹. Based on this consideration and a molecular weight of 588 g mol^{-1}
258 for $\text{C}_{22}\text{H}_{36}\text{O}_{18}$, 0.003 mol fermentable substrate was given per reactor.

259 As an eAD is considered being a combination of anaerobic digestion and a bioelectrochemical
260 system, the electrons of the substrate oxidation can either be transferred to methane (case I) or
261 to the anode as terminal electron acceptor (case II), see also Figure 1.

262 Case I: Considering the complete anaerobic digestion of the substrate to CH_4 and CO_2
263 according to²⁰ and a molar volume of methane of 22.4 L mol^{-1} (0°C) a total of 739 mL
264 methane per reactor can be expected at maximum, equaling to $337 \text{ mL g}_{\text{odm}}^{-1}$. The efficiency
265 of the anaerobic digestion process in terms of methane yield (Y_{CH_4}) can be calculated as the
266 ratio of measured and maximum methane production.

267 Case II: Based on Faraday's law, a total electric charge, q , of 25.5 kC ($q = 0.003 \text{ mol} \times 88 \times$
268 96485 C mol^{-1}) can be generated per reactor for the complete oxidation of the substrate,
269 equaling to $11.6 \text{ kC g}_{\text{odm}}^{-1}$. The yield of the electrochemical process, i.e. coulombic
270 efficiency, CE , is then calculated as percentage of measured charge compared to the
271 theoretical maximum value.

272 Consequently, the yield using eAD is calculated by summing up Y_{CH_4} and CE and reaches
273 100% for the complete anaerobic oxidation of the degradable substrate to methane or electric
274 energy, respectively. This definition applies strictly for the DC setups, equaling MFCs, but for
275 the SC setups the "recycling" of electrons from the unshielded cathode in the reactor
276 compartment resulting in seemingly higher methane formation has to be considered (see also
277 3.4).

278 **3 Results and Discussion**

279 Three experimental settings aiming at different eAD conditions were investigated on process
280 performance and microbial community dynamics using a standardized seeding sludge as
281 inoculum and identical substrate (see Table 2 for a summary). Generally for eAD experiments
282 single chamber (SC) reactors and double chamber (DC) reactors were used. In DC reactors
283 the microbiome faces only the working electrode (anode) and thus mimics a typical anode
284 half-cell; in SC reactors the microbiome faces working electrode (anode) and counter
285 electrode (cathode), as in a microbial electrolysis cell. Two working electrode potentials,
286 being typical for anode half cells (-0.2 V vs. A/ AgCl and +0.2 V vs. Ag/ AgCl), were applied
287 in order to study different driving forces, resp. potential terminal electron acceptors on the
288 eAD process. Furthermore, AD reactors hosting electrodes, but without applying a potential,
289 *i.e.* open circuit potential (OCP), were used as benchmark.

290 **3.1 Process characteristics: Combining current and methane production**

291 3.1.1 System validation

292 First, the setups were validated using AD standard conditions for testing the methane
293 production potential (setting I). The major methane formation for all reactors occurred within
294 the first few days of the experiment and the average production for all setups was 325 (± 25)
295 mL CH₄ g_{odm}⁻¹ being in good accordance with the expected value²¹ (see Supplementary Table
296 S1 for details). Metabolite analysis revealed that acetate and propionate were only detected at
297 the first sampling point (day 3) and the pH was not affected. An oxidative, *i.e.* positive,
298 current flow was shown by all eAD setups with maximum current densities between 1.2 and 6
299 $\mu\text{A cm}^{-2}$. With decreasing methane production also the current density declined and stabilized
300 in all reactors below 0.004 $\mu\text{A cm}^{-2}$. Noteworthy, spiking the reactor with acetate resulted in
301 an immediate oxidative current flow (details Supplementary Figure S2).

302 As expected, a very fast substrate turnover took place applying setting I. The fast turnover is
303 intended by this type of experiment designed for testing the methane production potential of
304 substrates, *i.e.* the maximum methane yield per g of organic dry matter. Microbial growth and
305 thus microbiome shifts were marginal. As consequence the current production of the not
306 growing cells was very low indicating a major conversion of the substrate to methane (see 3.2
307 Yield and electron balances). In accordance, only a very thin layer of attached biomass was
308 found at the anodes (further details in section 3.3 Microbiome structure and composition).

309 3.1.2 Biomass growth conditions

310 For understanding the impact of the electrochemical setting on the microbiome, methane
311 production potential and current production, the experiments were adapted (setting II). The
312 same reactor designs as used for setting I, with the only exception that lower biomass seeding
313 of only 5% was used. The methane production and VFA accumulation started slower and
314 lasted longer than in setting I, indicating an overall slower substrate turnover. As a
315 consequence of the higher substrate availability per cell, actively metabolising cells were able
316 to reproduce and thus an overall shift of the reactor microbiome took place (details section
317 3.3). The methane production depleted after 12 to 18 days at an overall average of 216 (± 29)
318 mL CH₄ g_{odm}⁻¹ for all settings without significant differences between the eAD and AD
319 reactors (for details see Supplementary Table S1).

320 Figure 2 shows the course of the VFA concentrations, methane formation and current
321 production exemplarily for an SC_{+0.2V} reactor applying setting II. All processes started within
322 four days and the successful substrate degradation is reflected by accumulation of volatile
323 fatty acids which are then degraded until day 20 of the experiment. This is in accordance with
324 the methane production curve, which reaches its plateau at day 16 indicating no further
325 methanogenesis. The oxidative current production at the anodes showed a similar
326 development as the daily methane production with a rapid start within the first four days, a

327 nearly constant current density between day four and 11 and a sharp decline afterwards.
328 Interestingly, the VFA concentrations are in line with the current production, *i.e.* after
329 degradation of free acetate, propionate and butyrate on the reactor liquid the current density
330 declines.

331 Considering all reactors with setting II (Figure 3), acetate, propionate, and butyrate were
332 detected in the bulk liquid after three days but in significantly different amounts. The lowest
333 maximum concentrations for acetate were present in the SC reactors (acetate: 970 (± 85) mg L⁻¹
334 SC_{-0.2V} and 1054 (± 108) mg L⁻¹ SC_{+0.2V}, both at day 3), while the AD control reactors
335 reached the higher concentrations of VFAs with 1620 (± 35) mg L⁻¹ acetate at day seven. For
336 propionate and butyrate the peak concentrations showed no significant differences, but the
337 degradation rate was higher for the SC setups, with the highest rate in the SC_{-0.2V} reactors.
338 Finally, all VFAs were degraded after 20 days. This is in accordance with the by then
339 stationary methane production curves.

340 All eAD reactors were characterized by oxidative current production starting after 2 days (-
341 0.2 V) and after 3 days (+0.2 V) independently of the SC or DC setup (see Supplementary
342 Figure S3 for all chronoamperograms as well as Supplementary Figure S4 for representative
343 CV measurements). Peak values of current production in the SC setups reached $j_{max} = 1.34$
344 mA cm⁻² geometric current density (SC_{-0.2V}, day 3) and $j_{max} = 1.18$ mA cm⁻² (SC_{+0.2V}, day 4).
345 This equals to volumetric peak current densities up to 54.3 mA L_R⁻¹ (SC_{-0.2V}, day 3). The peak
346 current production in the DC reactors was about 22% (DC_{-0.2V}) and 30% (DC_{+0.2V}) lower than
347 in the respective SC reactors, which can be assigned to separation of the anode and cathode
348 chamber (see section 3.4 for detailed discussion). The course of the current production
349 correlated with the acetate concentrations in the reactors, this is not as pronounced for the
350 other VFAs. This finding as well as the CV results (Supplementary Figure S4) already
351 indicates a dominance of *Geobacteraceae* for the microbial electrocatalysis in the anode
352 biofilms.

353 The described combined methane production and electric current flow in setting II can either
354 be the result of an adaptation of the reactor community to the electrochemical stimulation or
355 be related to the formation of a biofilm on the electrodes which additionally contributes to the
356 anaerobic digestion process. Both options and their functional implications will be discussed
357 in section 3.3.

358
359 As Figure 3 shows, pH decreased below pH 7 at day 2 and was adjusted one time for all
360 reactors (indicated in the Figure). Over the time course of the experiment, the DC reactors had
361 considerable lower pH values compared to SC and AD control setup (Figure 3). This pH-shift
362 between anode and cathode compartment is caused by charge balancing ion transport not
363 being based on H^+/OH^- , due to the membrane performance as described earlier^{22,23}, resulting
364 in a proton accumulation in the anode chamber. As a pH-value below 7.0 is seen as critical for
365 the anaerobic digestion process²⁴, there might have been some inhibitory effects in the DC
366 reactors leading to reduced VFA degradation. The pH change was not found in the previous
367 set of experiments (setting I) as the total charge produced was lower and the higher amount of
368 seeding sludge led to a higher buffer capacity.

369 These results clearly show that for conditions allowing microbial growth (*i.e.* setting II) a
370 combined substrate exploitation for methane and electricity production takes place. This is
371 reflected in an adaptation of the microbial community and leads to a general conceptual
372 model of substrate utilization (section 3.4).

373 3.1.3 Electrolysis conditions

374 In previous studies (see Table 1) voltages (of the electrochemical cell) or constant currents
375 were applied to eAD setups facilitating water electrolysis. Water electrolysis results in
376 hydrogen production at the cathode and oxygen production at the anode. These reactions can
377 therewith improve the AD process (among others increased methane production, see Table 1)

378 by i) abiotic electro-hydrolysis of the substrate, ii) support of microbial substrate hydrolysis
379 based on micro-aerobic conditions and iii) hydrogenotrophic methane formation.
380 Consequently, to investigate, if the positive effects of electrochemical stimulation on
381 anaerobic digestion described (Table 1) result from electrochemically stimulated microbial
382 activity (as found in the current study) or is rather based on abiotic substrate electro-
383 hydrolysis an additional set of experiments was performed (setting III). According to the
384 conditions in a previous study¹⁰ two SC reactors were set to a constant current of -1.2 mA
385 ($SC_{-1.2mA}$, equal to $54 \text{ mmol H}_2 \text{ d}^{-1}$ at the working electrode) and three $DC_{+0.2V}$ reactors as well
386 as three AD control reactors were run in parallel.

387 When using the constant electrolysis current ($SC_{-1.2mA}$) the anaerobic digestion process was
388 delayed in comparison to $DC_{+0.2V}$ and AD control reactors run in parallel. While similar peak
389 concentrations of acetate and propionate were found in all reactors (acetate: $1802 (\pm 167) \text{ mg}$
390 L^{-1} $DC_{+0.2V}$ (day 7), $1749 (\pm 76) \text{ mg L}^{-1}$ OCP (day 7), $1940 (\pm 114) \text{ mg L}^{-1}$ $SC_{-1.2mA}$ (day 15);
391 propionate: $415 (\pm 60) \text{ mg L}^{-1}$ $DC_{+0.2V}$ (day 15), $424 (\pm 101) \text{ mg L}^{-1}$ OCP (day 15), $390 (\pm 25)$
392 mg L^{-1} $SC_{-1.2mA}$ (day 21)), their complete degradation was only achieved for the $DC_{+0.2V}$
393 reactors and the AD control reactors. Significant amounts were accumulated at day 23 in the
394 electrolysis reactors $SC_{-1.2mA}$ ($780 (\pm 56) \text{ mg L}^{-1}$ acetate, $358 (\pm 44) \text{ mg L}^{-1}$ propionate). For
395 butyrate higher concentrations of up to $504 (\pm 17) \text{ mg L}^{-1}$ (day 3) were measured compared to
396 the other setups ($427 (\pm 24) \text{ mg L}^{-1}$ $DC_{+0.2V}$ (day 7), $295 (\pm 47) \text{ mg L}^{-1}$ OCP (day 4) but found
397 degraded by the end of the experiment.

398 Analysis of gas composition in the headspace revealed a delay in methane production of
399 electrolysis reactors. They differed in gas composition during the first days of the experiment:
400 hydrogen ($9(\pm 4)\%$) was found in the continuous electrolysis reactors $SC_{-1.2mA}$ at day 2 but
401 depleted at the following sampling points, meaning that microorganisms metabolised 1.4 L H_2
402 and 0.7 L O_2 produced daily by electrolysis. The maximum relative methane concentration in

403 the headspace of the reactors was comparable for all setups in setting III (DC_{+0.2V}, 58 (±14)%
404 day 17; SC_{-1.2mA}, 59 (±9)% day 21; AD control (OCP), 57 (±29)% day 21).

405 In conclusion, no positive effect of electrolysis on the biogas production process performance
406 was found. This is in contrast to earlier studies^{9, 10, 25, 26}. For the reported differences the
407 increased substrate availability after electrolysis might be one reason, as better process
408 performance may also result from (abiotic) substrate disintegration by radicals formed during
409 electrolysis. This cannot be accounted for in the current study, as carbohydrates in corn silage
410 are already well available, but may play a bigger role for more complex substrates like
411 manure^{10, 25} or lignin rich compounds²⁷. However, positive effects of electrolysis on AD
412 were also described for synthetic wastewater fed reactors⁹. Thus, the substrate disintegration
413 cannot be the only positive effect. However, also different experimental setups (batch and fed-
414 batch experiments²⁶ vs. continuous reactors²⁸, electrode material⁹ and biomass retention¹¹)
415 will play a role and hamper a systematic comparison (Table 1).

416 3.2 Yield and electron balances

417 Considering that all electrons of the complete oxidation of the substrate (corn silage) would
418 be used for methane formation 337 mL g_{odm}⁻¹ would be expected (see 2.8). For standard AD
419 conditions (setting I) an average methane production of 325 mL g_{odm}⁻¹, representing a
420 methane yield (Y_{CH_4}) of 96%, and thus an eAD overall performance efficiency of 96%, was
421 found, being in good accordance with literature²¹. For biomass growth conditions (setting II),
422 however, the total methane production was independent of the reactor type (on average 216
423 mL g_{odm}⁻¹), equaling a Y_{CH_4} of 64%. It can be assumed that under the biomass growth
424 conditions, provided by setting II, a higher amount of carbon from the substrate was stored in
425 biomass instead of being converted to methane which is causing the lower methane yield in
426 comparison to setting I.

427 Most interestingly, whereas for standard AD (setting I) conditions the electric current
428 production was negligible, for growth conditions (setting II) the electric current production
429 had a significant contribution to the eAD yield independent of the applied working electrode
430 potential. In detail, the total charge transfer, q , per batch in setting II was higher for the SC
431 reactors ($q_{SC-0.2V} = 14.4 (\pm 0.8)$ kC, $q_{SC+0.2V} = 11.3 (\pm 0.8)$ kC) than for the DC reactors ($q_{DC-0.2V}$
432 $= 5.3 (\pm 1.2)$ kC, $q_{DC+0.2V} = 4.9 (\pm 2.2)$ kC) meaning that a higher share of available electrons
433 was transferred to the electrode, leading to coulombic efficiencies of $CE_{SC-0.2V} = 56.5$
434 $(\pm 3.1)\%$, $CE_{SC+0.2V} = 44.3 (\pm 3.0)\%$, $CE_{DC-0.2V} = 20.8 (\pm 4.6)\%$, $CE_{DC+0.2V} = 19.2 (\pm 8.6)\%$.
435 Consequently, the overall eAD yield, calculated by summing up the Y_{CH_4} and CE for each
436 setup, shows that the eAD yield was clearly higher (SC_{-0.2V} (123%), SC_{+0.2V} (118%), DC_{-0.2V}
437 (82%), DC_{+0.2V} (81%)) than the anaerobic digestion process alone (OCP, 64% setting II). For
438 the latter the productivity is restricted to methane production and no additional value can be
439 obtained. On the first sight the yield of the SC reactors exceeded 100%. This can be explained
440 by the unshielded counter electrode, serving as cathode to the microbiome, in the SC setups in
441 contrast to the DC setups. The electrons that contributed to the increased coulombic efficiency
442 are reintroduced into the reactor and can be “recycled” by the microorganisms for methane
443 production (details section 3.3). Thus, a yield exceeding 100% is certainly a mathematical
444 artifact, owing to the underlying concept (see 2.8.). However, as discussed below this
445 pathway may provide an opportunity for process management. The methane productivity of
446 the DC reactors was below the SC reactors. One reason for that could be a reduced microbial
447 activity in the DC reactors, due to lower pH, caused by the volatile fatty acid production
448 resulting also in lower peak currents. In addition, SC reactors showed biofilm formation at the
449 cathode, which cannot take place in the DC reactors. The cathode biofilm consisted of
450 hydrogenotrophic methanogenic archaea that obviously contributed to the methane
451 production. This formation of an alternative loop for substrate utilization will be further
452 discussed in the section 3.3 Microbiome structure and composition.

453 3.3 Microbiome structure and composition

454 To analyze the microbiome structure and function, the microbial communities of the reactors
455 were sampled regularly (i.e. simultaneously with the process parameters) and the electrode
456 biofilms at the end of each experiment.

457 3.3.1 Reactor community

458 The community structure was monitored over time using flow cytometry (FCM). In general,
459 flow cytometry revealed a diverse microbial community being typical for AD reactors ¹⁵
460 (exemplary cytometric fingerprint Supplementary Figure S5). The microbial seeding
461 community (inoculum) was found to differ from all other samples in setting II and III
462 regarding its cytometric fingerprint, i.e. community structure, as well as number of stainable
463 cells. This is in line with expectations, as the microbial communities started immediately to
464 utilize the substrate and its degradation products. As a result, general growth (higher number
465 of stainable cells) but also a shift of the microbiome to its new habitat and the provided
466 substrate was reflected in the community structure. In setting I after an initial community shift
467 the community structure was found stable for all reactor setups in accordance with the main
468 methane and VFA production as well as their utilization in the first few days. In contrast, the
469 microbial communities in settings II and III were performed under microbial growth
470 conditions (5% seeding sludge) thus showing stronger structural variation over the complete
471 course of the experiment (Figure 4 A). Importantly, the variation over time was similar for all
472 reactors and respective triplicates of setting II (Figure 4 A). This indicates that most
473 organisms in the microbial community, in the setup under study, were not immediately
474 affected by the applied electrochemical conditions or the additional substrate utilization by the
475 electrode associated communities (see below).

476 In addition to the time resolved monitoring the bacterial community composition was
477 determined at the end point of the experiments (setting II and III) using T-RFLP and analysis

478 of a clone library. The reactor microbiome was found to be a typical diverse anaerobic
479 digestion community with *Firmicutes* contributing as the major bacterial phylum
480 accompanied by *Bacteroidetes*, *Proteobacteria*, and *Synergistetes* (Figure 4 B and details in
481 Supplementary Results). The T-RFLP analysis confirmed that the reactor communities
482 differed from the seeding community. The reactor communities themselves were similar
483 towards presence of T-RFs but differed in the individual T-RF's contribution in the different
484 samples (Figure 4 B and Supplementary Results). Grouping T-RFs based on the eAD setups
485 allowed a certain differentiation, but the differences were relatively small and mostly not
486 significant (Supplementary Figure S6). The methanogenic archaea in the reactor community
487 were also investigated with T-RFLP at the end point of the experiments (setting II and III,
488 details Supplementary Figure S7). The highest abundance showed *Methanosarcina*, a
489 mixotrophic genus, while all other found groups (*Methanoculleus* spp. and three groups of
490 *Methanobacterium* spp.) are hydrogenotrophic. Therefore, acetate and hydrogen were
491 probably both used as substrates for the methanogenic archaea in the reactor community.

492 3.3.2 Electrode associated communities

493 The biofilms were sampled at the end of the experiments and no biomass was found on the
494 counter electrodes (cathodes) of the DC setups and a very thin biofilm was present at some
495 working electrodes (anodes) of the AD control reactors (these were operated at OCP, but we
496 assume that these are mainly formed due to the presence of an electrode potential during daily
497 CV measurements). In contrast to the working electrodes in setting I, which showed only a
498 small amount of adherent biomass from the reactor community, there was a thick biofilm
499 formed on the working electrodes of the SC_{-0.2V}, SC_{+0.2V}, DC_{-0.2V} and DC_{+0.2V} setups for
500 setting II. Similar to the description in ²⁹ two different biofilm layers were found: a reddish
501 biofilm layer closer to the electrode surface and covered by a darker brownish one
502 (Supplementary Figure S5).

503 FCM revealed that the microbial community structure was very similar for all working
504 electrodes in setting II (independent of the applied potential +0.2 V or -0.2 V) and differed
505 clearly from the reactor community (Supplementary Figure S5). The biofilms were dominated
506 by one phylotype, which can be derived from the characteristic cell cycle related distribution
507 in the cytometric histograms¹⁴. DNA based analyses supported these results, as dominance of
508 a single T-RF (240 bp) with a contribution of 83 to 95% of the total T-RF area was found.
509 Sequencing assigned this T-RF to a *Geobacter* sp. and BLAST search (15/01/27) revealed
510 highest similarity (99% identity with 100% query coverage) with the GenBank entry
511 NR_126282.1, a novel isolate *Geobacter anodereducens* strain SD-1³⁰. For *Geobacter*
512 *sulfurreducens* strain PCA (NCBI Reference Sequence: NR_075009.1) the identity was 97%.
513 This finding is in accordance with many previous studies that also found a strong anodic
514 enrichment of *Geobacter* spp. using similar electrode potentials^{31,32}.
515 The counter electrodes, i.e. cathodes, operated at -1.4 V vs. Ag/ AgCl maximum negative
516 voltage, in the SC reactors were also covered by a biofilm at the end of the experiment. Flow
517 cytometric analysis showed that the microbial community structure was clearly different from
518 the anode biofilms as well as from the reactor community. DNA analysis revealed that the
519 cathode biofilm was dominated by one group of *Methanobacterium* spp. and fluorescence
520 microscopy showed a biofilm consisting of bright autofluorescent cells, which is typical for
521 methanogenic archaea (Supplementary Figure S8). We therefore conclude that a specific
522 enrichment of archaea on the counter electrode took place (see also discussion below). They
523 are supposed to either use hydrogen produced at the cathode or directly take up electrons by
524 extracellular electron transfer^{2, 33-35}.

525 **3.4 Increasing the overall performance by division of labour in the reactor** 526 **microbiome**

527 It was shown, for conditions allowing microbiome adaptation to new substrates (setting II)
528 that the methane production remains constant for eAD setup and AD setup, but in addition the
529 electron yield of the eAD setup could be utilized for electric energy generation. This increased
530 turnover of substrate into useful products was achieved with an overall eAD yield of 123% in
531 the SC setup and 82% for the DC setup. It has to be stressed that the electric current in the DC
532 setup, equaling the anode compartment of a MFC with an oxygen reduction cathode, is
533 directly exploitable. For the SC setup, being related to a microbial electrolysis cell, additional
534 energy input is necessary, but this approach does allow a better steering of the processes and
535 energy fluxes. Independent from the electric current production, the microbial community
536 structure and composition in the reactor liquid remained unaffected. Thus, the generation of
537 the second product, electrons, can only be explained by the formation of a functional anodic
538 biofilm at the working electrodes (both SC and DC setup) and a cathodic biofilm at the
539 counter electrode for SC setups leading to a functional and spatial division of labour in the
540 microbiome. The overall concept is depicted in Figure 5. The substrate is not only utilized
541 regarding carbon (which can also be stored as biomass and thus not converted to a chemical
542 product) but also regarding the electrons for anode respiration. Whereas in AD reactors only
543 the methane production process in the bulk liquid takes place, additional microbial
544 transformations can take place at the electrode(s) for eAD reactors utilizing excess substrates
545 or substrates unsuitable for the methanogenic community without affecting the methane
546 production. In the DC setup the functional contribution is restricted to the working electrode
547 biofilm performing anode respiration and current production as the counter electrode is
548 shielded from the reactor community. In the SC reactors, showing best overall yield,
549 extensive biofilms were found at working and counter electrodes. This finding suggests the
550 formation of an alternative loop for metabolite utilization (Figure 5) allowing the additional

551 valorisation pathway of acetate conversion to electrons, CO₂ and protons on the anode
552 (working electrode) and methane production from electrons, protons and CO₂ on the cathode
553 (counter electrode) as follows:

554 At the anode the *Geobacteraceae* dominated biofilm utilizes (excess) acetate, accumulating in
555 the reactor liquid, for current production while competing with syntrophic acetate oxidizing
556 bacteria and acetotrophic methanogens of the reactor community. As only a low number of
557 other species was found in the anode biofilm little, if any, utilization of other substrates is
558 expected as long as acetate is available. Noteworthy, the acetate oxidation at the anode does
559 not hamper the functional groups in the reactor liquid under the applied conditions. Further
560 investigations with more sophisticated methods like carbon tracer experiments could help to
561 quantify the differential metabolic contribution of the subcommunities under varying
562 conditions. In the SC reactors, where the microbiome faces both electrodes, protons and CO₂
563 can additionally be utilized by the cathodic biofilm of hydrogenotrophic methanogenic
564 archaea at the counter electrodes. The formation of methanogenic biofilms on biocathodes
565 was reported before ³⁵⁻³⁷. In these studies also *Methanobacterium spp.* (*Methanobacterium*
566 *palustre* and *Methanobacterium aarhusense*) and *Methanococcus maripaludis* were the major
567 involved organisms. The presence of two functionally distinct biofilms at anode and cathode
568 did not significantly affect the microbial community structure of the reactor community and
569 its development over time (for the used electrode surface to reactor volume ratio). On DNA
570 level, only the contribution of hydrogenotrophic archaea to the reactor community was higher
571 in the SC reactors compared to DC and AD control.

572 The data strongly suggests the presence of an alternative anode-based loop for acetate
573 utilization that does not interfere with the reactor community, but has the potential to yield
574 electric energy as additional product. The reaction of the cathode biofilm (in the SC-setup)
575 may additionally contribute to the methane production. Furthermore, the anodic oxidation
576 may also buffer against acidification (accumulation of acetate, pH decrease) in the reactor.

577 This is in line with the finding of ¹¹ that colonized electrodes can have a stabilizing effect for
578 the anaerobic digestion process although these authors conclude biomass retention being the
579 key-effect, whereas here the specific functional enrichment is sought to account for it. The
580 advantage of an alternative loop for substrate utilization may also apply to biogas processes
581 that suffer inhibition due to e.g. high ammonium loadings ^{38,39}. Here the spatial and functional
582 labour division can support a more flexible process. So far, the principle of spatial and
583 functional labour division was only demonstrated using batch experiments. But the
584 development of similar interactions is supposed to remain also in a continuous process
585 including system scale up.

586 **4 Conclusions**

587 We have investigated the combination of anaerobic digestion and microbial electrochemical
588 technologies and found that this strategy allowed an up to 27% increase in total yield. This is
589 achieved by the functional contribution of electroactive biofilms at the electrodes showing
590 specific enrichments, while the reactor community kept its composition and functionality. The
591 general concept can be transferred to related MET processes for increasing substrate
592 utilization efficiencies, side product valorization and process stabilisation and, therewith, lead
593 to a sustainable production of energy and commodities.

594 **5 Acknowledgements**

595 We thank Franciska Hedrich for very valuable technical assistance in molecular biology
596 analysis and Birke Brumme for GC measurements. F.H. acknowledges support by the BMBF
597 (Research Award “Next generation biotechnological processes - Biotechnology 2020+”) and
598 the Helmholtz-Association (Young Investigators Group). S.M. acknowledges support by the
599 European Fond for Regional Development (EFRE) and Sächsische AufbauBank (SAB). This

600 work was supported by the Helmholtz Association within the Research Programme
601 Renewable Energies.

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674

675 **7 Figure Captions**

676 **Figure 1: Experimental setup:**

677 (A) Schematic illustration of dual-chamber (DC) eAD reactor. A tailor-made glass reactor
678 was modified to introduce working electrode (anode), Ag/AgCl reference electrode with
679 luggin capillary and shielded counter electrode (cathode) – components not drawn to scale
680 (details see also section 2.2). (B) Reaction equations for methane and current production and
681 theoretical maximum product yields for the present setup (details section 2.8).

682

683 **Figure 2: Exemplary course of process parameters of a $SC_{+0.2V}$ eAD-reactor:**

684 The geometric current density j was monitored continuously (chronoamperometry) and is
685 given as solid black line. Volatile fatty acid concentrations, c_{OA} , were measured regularly in
686 reactor samples: acetate (green rectangle), propionate (circle) and butyrate (triangle). The
687 accumulated methane production on the daily basis is given with black rectangles.

688

689 **Figure 3: Organic acids production:**

690 Time-course of volatile fatty acids concentrations (A-C) and pH (D) for growth conditions
691 (setting II) in different reactor setups and applied potentials. The pH was adjusted after
692 measurement at day 2 (*).

693

694 **Figure 4: Microbial community analysis:**

695 (A) Time resolved analysis of the microbial community in setting II: The microbial
696 community structure of the reactor communities was monitored with flow cytometry, the
697 derived results were arranged regarding their similarity (non-metric multidimensional scaling,
698 NMDS) for $SC_{-0.2V}$, $SC_{+0.2V}$, $DC_{-0.2V}$, $DC_{+0.2V}$ and AD control (OCP) setup. The reactor
699 communities were analysed at respective days (black solid line) and each triplicate reactor

700 setup is indicated with grey lines. The anode biofilms were very different from the reactor
701 community (Supplementary Figure S5) and, therefore, not included in the plot. (B) At the end
702 point of the experiments (day 20) the microbial community composition was determined with
703 T-RFLP for the bacterial reactor community and the biofilms at the working electrodes.

704

705 **Figure 5: Division of labour in the eAD reactor microbiome:**

706 Overview on anaerobic digestions pathways and involvement of electrode biofilms in single-
707 chamber setup: Primary and secondary fermentation of the substrate by bacteria leads to the
708 formation of the key intermediates acetate, H_2 and C_1 -compounds (for better readability
709 referred to by CO_2 as major compound). They are further utilized by methanogenic archaea to
710 yield CH_4 and CO_2 . In addition to methane formation in the bulk liquid further reactions can
711 take place at the electrode surfaces. Anodic biofilms utilize key intermediates (e.g. acetate)
712 and further biogenic methane production at the cathode is possible. (Chemical compounds and
713 intermediates that were measured are highlighted in blue.)

714

715 **8 Tables and Figures**716 **Table 1:** Overview on eAD studies setups and results.

Reference	Operating conditions	Substrate	Microbial source	Operational parameters			Effect on methane production
				E_{cell} [V]	J_{geom} [A m ⁻²]	J_{vol} [mA L _R ⁻³]	
9	- mesophilic UASB reactor - single-chamber setup - anode: titanium mesh - cathode: stainless steel mesh - electrolysis conditions	synthetic wastewater	mesophilic anaerobic granular sludge from a wastewater plant	2.8–3.5	n.d.	60 – 110	increase of methane production (10-25%)
25	- mesophilic CSTR - single-chamber setup - anode: IrO ₂ -covered titanium mesh - cathode: stainless steel mesh	cow manure, switch grass	substrate, anaerobic sludge	n.d.	n.d.	0 – 33.3	increase of methane production (26%)
28	- mesophilic UASB reactor - single-chamber setup - anode: hollow FE cylinder - cathode: graphite axle	synthetic wastewater with increasing salinity	laboratory-scale UASB reactor	1.2	<i>max. 0.3</i>	<i>23.4</i>	n.d.
11	- mesophilic CSTR - single-chamber setup - carbon felt electrodes	waste activated sludge, molasses	anaerobic sludge from a municipal sludge digester	0.5, 1	3.36 – 6.78	25.2 – 50.85	no direct effect but stabilization
10	- mesophilic septic tank with sequential compartments for anode and cathode reaction - single-chamber setup - electrolysis conditions	simulated black wastewater	primary wastewater sludge, pig manure	2	0.9 – 5.0	0.8 – 7.4	increase of methane production (factor 5)
26	- thermophilic modified H-type MFC - dual-chamber setup - carbon electrodes	artificial garbage slurry (+ 0.2 mM of 2,6-anthraquinone disulfonate)	thermophilic anaerobic digester, with garbage slurry	-0.3, -0.6, -0.8*	-0.0625 – 0.3825	-1.0 – 6.12	increase of methane production (81%)

717 n.d. not determined, the value was not determined or not given in the reference

718 values in italics were calculated based on the data given in the publication

719 *working electrode potential vs. Ag/AgCl reference electrode

720 **Table 2:** Overview of all studied setups, all having 2.5 g corn silage as carbon source.
721

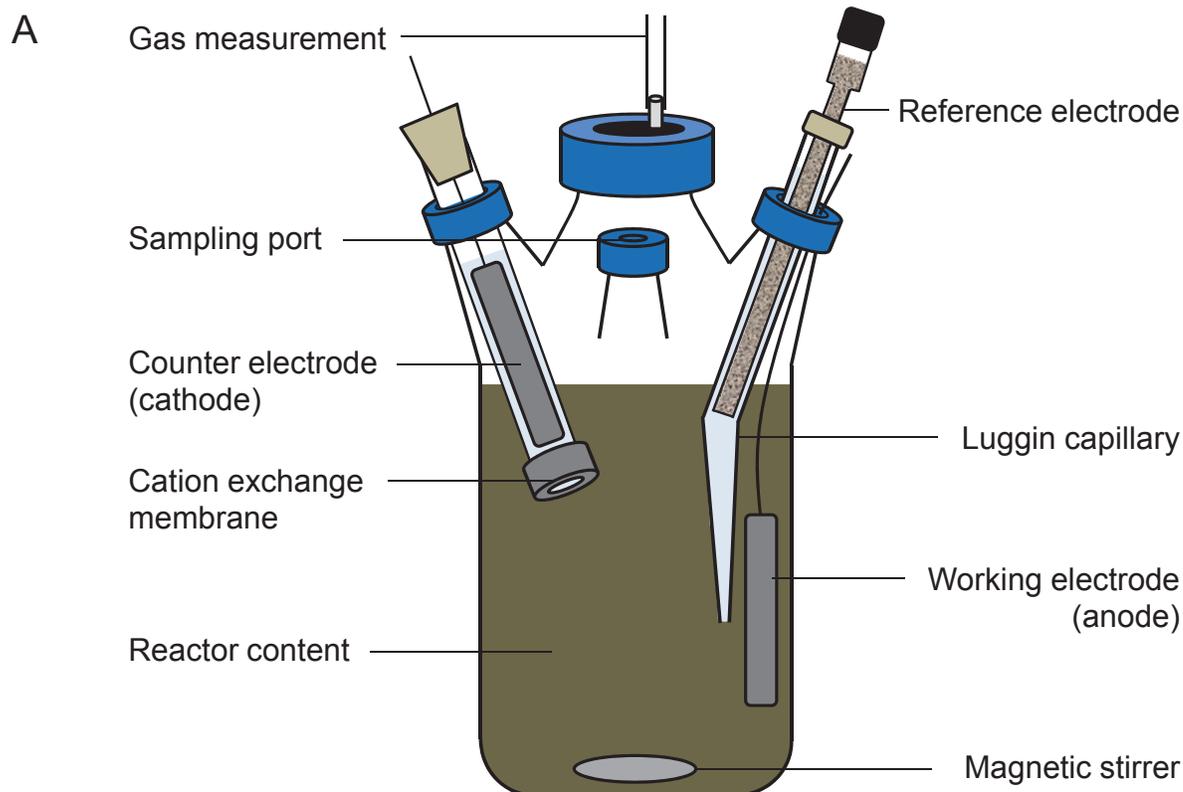
Denomination	Electrochemical conditions*	Single-chamber (SC) or dual-chamber (DC) setup	Number of replicates	
			seeding sludge 50%	seeding sludge 5%
Setting I (standard AD conditions)				
SC _{-0.2V}	- 0.2 V	SC	3	
SC _{+0.2V}	+ 0.2 V	SC	3	
DC _{-0.2V}	- 0.2 V	DC	3	
DC _{+0.2V}	+ 0.2 V	DC	3	
AD	OCP	SC	8	
Setting II (growth conditions)				
SC _{-0.2V}	- 0.2 V	SC		3
SC _{+0.2V}	+ 0.2 V	SC		3
DC _{-0.2V}	- 0.2 V	DC		3
DC _{+0.2V}	+ 0.2 V	DC		3
AD	OCP	SC		9
Setting III (electrolysis conditions)				
DC _{+0.2V}	+ 0.2 V	DC		3
SC _{-1.2mA}	- 2.1 mA (electrolysis)	SC		2
AD	OCP	SC		3

722

723 *potential vs. Ag/AgCl reference electrode

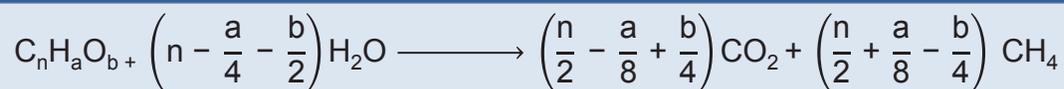
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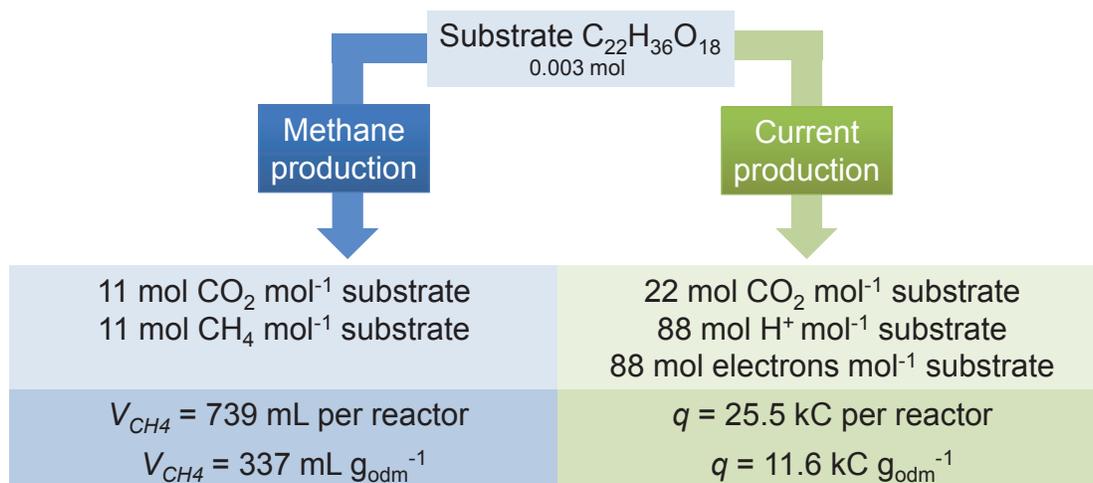
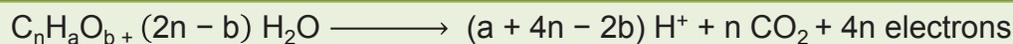


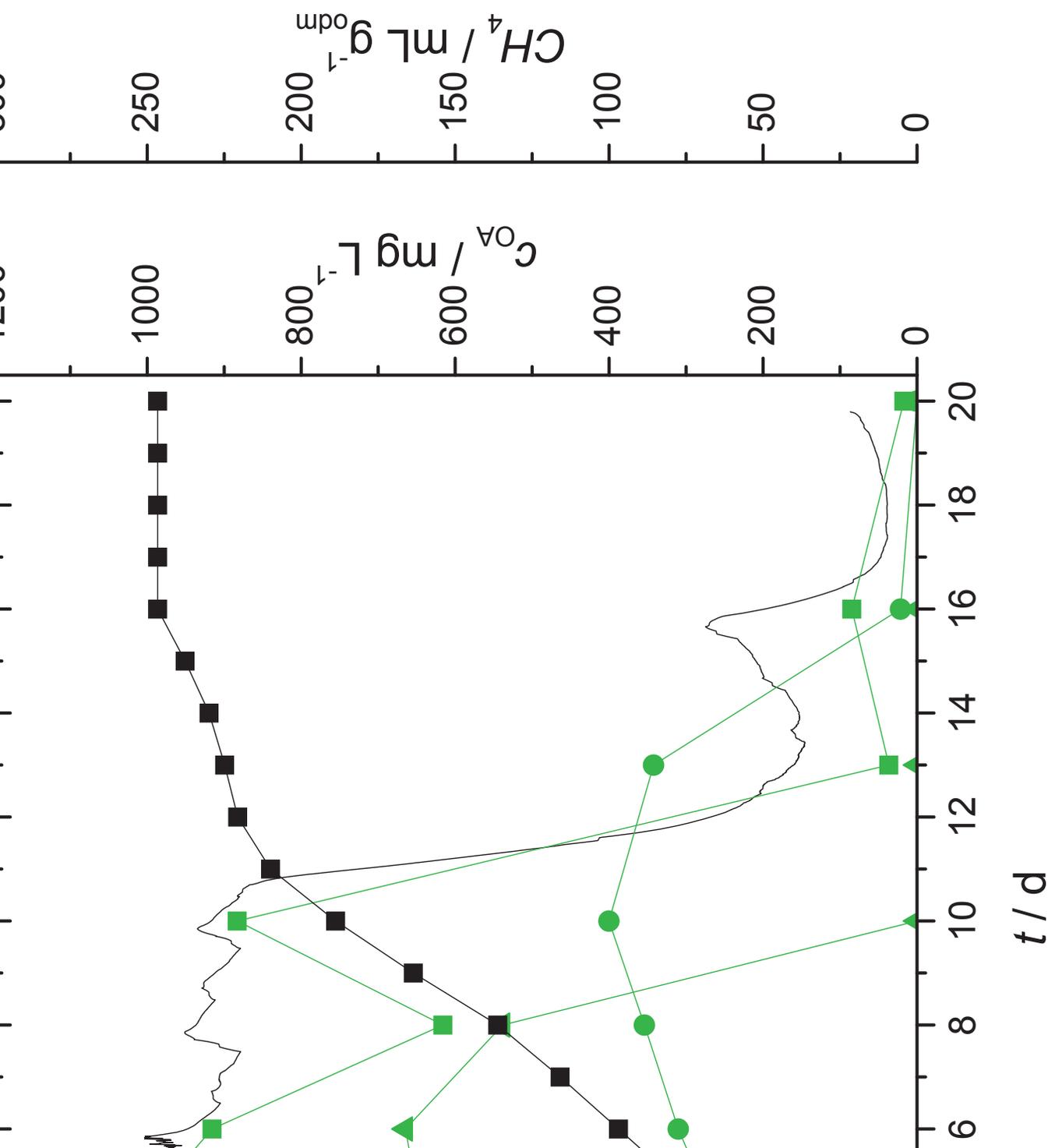
B

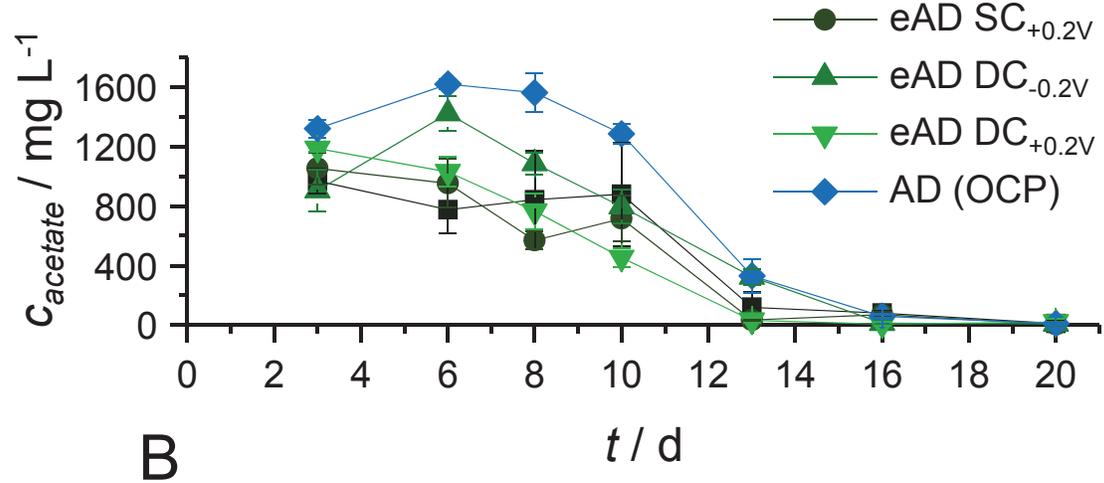
Methane production by anaerobic digestion:



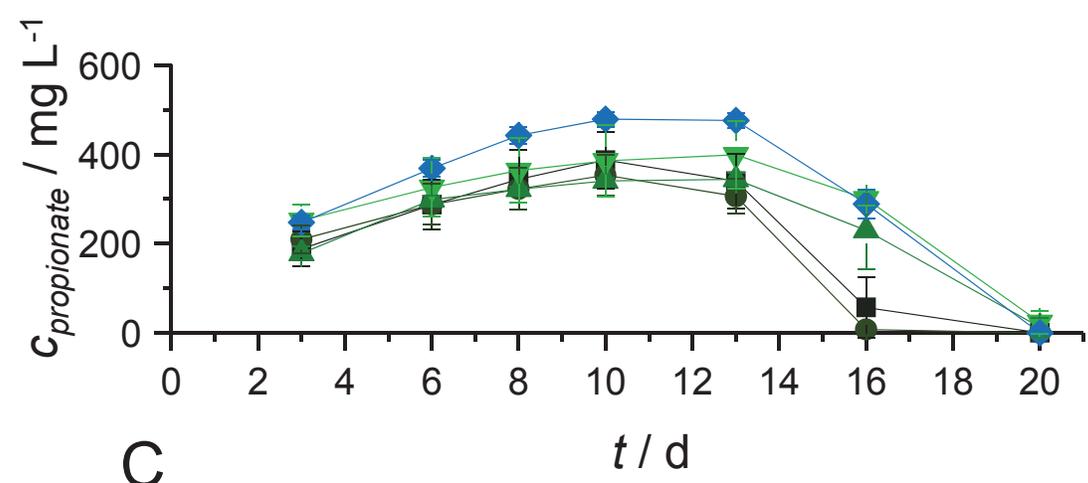
Electric current production:



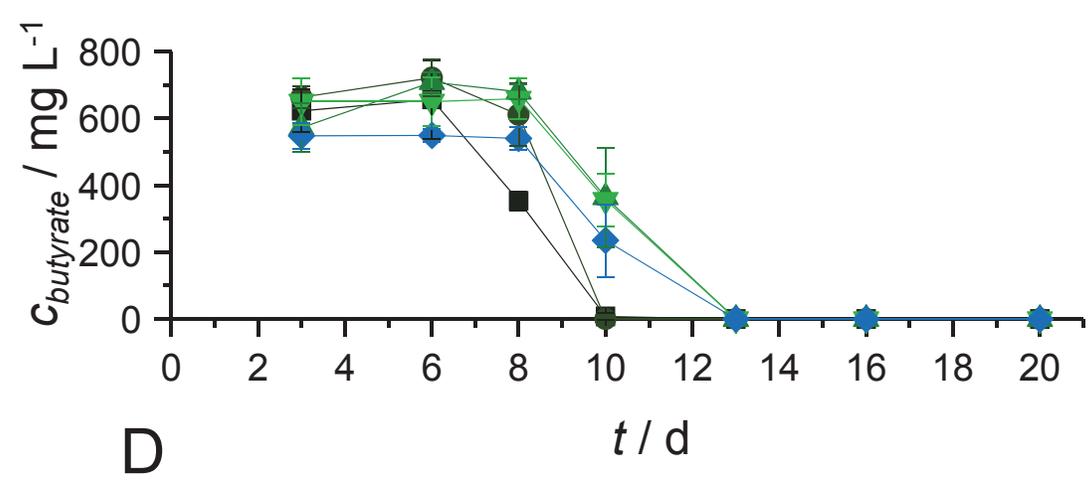




B



C



D

