

Highlights

- AD and an electromethanogenic biocathode were operated as an integrated system.
- Methane production of $79 \text{ L m}^{-3} \text{ d}^{-1}$ was achieved in the biocathode.
- Ammonium removal in the MEC anode compartment achieved $14.46 \text{ g N-NH}_4^+ \text{ m}^{-2} \text{ d}^{-1}$.
- The MEC stabilised the AD when the organic and nitrogen loading rates were doubled.

1 Anaerobic digestion and electromethanogenic microbial electrolysis 2 cell integrated system: increased stability and recovery of ammonia and 3 methane

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8 ABSTRACT

9 The integration of anaerobic digestion (AD) and a microbial electrolysis cell (MEC) with an
10 electromethanogenic biocathode is proposed to increase the stability and robustness of the AD
11 process against organic and nitrogen overloads; to keep the effluent quality; to recover ammonium;
12 and to upgrade the biogas. A thermophilic lab-scale AD, fed with pig slurry, was connected in
13 series with the bioanode compartment of a two-chambered MEC. In turn, the biocathode of the
14 MEC was poised at -800 mV vs Standard Hydrogen Electrode and fed with CO₂ to increase the
15 methane production of the system. After doubling its organic and nitrogen loading rate, the AD
16 operation became stable thanks to the connection of a recirculation loop with the MEC effluent.
17 Ammonium removal in the anode compartment of the MEC achieved 14.46 g N-NH₄⁺ m⁻² d⁻¹, while
18 obtaining on average 79 L CH₄ m⁻³ d⁻¹ through the conversion of CO₂ in the cathode compartment.
19 The microbial analysis showed that methylotrophic *Methanossiliococcaceae* family
20 (*Methanomassiliicoccus* genus) was the most abundant among the metabolically active archaea in
21 the AD during the inhibited state; while, on the cathode, *Methanobacteriaceae* family
22 (*Methanobrevibacter* and *Methanobacterium* genus) shared dominance with
23 *Methanomassiliococcaceae* and *Methanotrichaceae* families (*Methanomassiliicoccus* and
24 *Methanotherix* genus, respectively).

25

26 Keywords

27 Electromethanogenesis, biocathode, anaerobic digestion, ammonia, biogas upgrading, RNA

28

29 **1. Introduction**

30 The increasing global demand for fossil fuels, their tendency to be scarcer, and the need to control
31 the greenhouse gas emissions when using them are requiring new strategies for energy
32 production. An alternative to conventional refineries for clean and renewable energy production is
33 the biorefinery. Biorefineries can recover nutrients and other products of interest from energetic
34 crops, organic wastes and other waste fluxes [1]. This concept goes beyond the philosophy of
35 petrochemical refineries, including sustainable management practices and closed cycle processes
36 whenever possible. Wastes, whether domestic, industrial, agricultural or from livestock are a great
37 opportunity to recover water, energy, chemical products and nutrients, and have a big potential for
38 application in biorefineries [2]. Not only meeting environmental protection objectives, but also
39 recovering energy and resources from wastes should be addressed in a circular economy [3].

40 The combination of anaerobic digestion (AD) and bioelectrochemical systems (BES) is an
41 integrated strategy that can be implemented with different objectives and configurations [4, 5] and
42 can attain the goals of the biorefinery concept [6, 7]. On the one hand, nutrients can be recovered
43 from ammonium-rich wastewater such as pig slurry or digested pig slurry thanks to cation or anion
44 transport through exchange membranes that takes places in BES [8]. The main example of this
45 application is the recovery of ammonium, which can be reused as fertiliser [9-13]. Otherwise, the
46 AD effluent would need to be processed or managed properly due to its high nutrient content.
47 Ammonia recovery has been demonstrated in various BES including microbial fuel cells (MFCs)
48 [10, 11] and microbial electrolysis cells (MECs) [14]. In MECs, a higher current density would
49 greatly enhance ammonium recovery, and thus MECs exhibit a better performance for ammonium
50 recovery than MFCs [15].

51 On the second hand, BES can operate with low organic loading rates and may be used to polish
52 the effluent of the AD [16-18] or even to absorb higher organic concentrations in the digestates due
53 to AD destabilisation or inhibition [18, 19]. Previous work has shown that integrated or multi-step
54 systems can increase the energy production from complex substrates [4]. The combination of AD
55 and BES for effluent polishing has been studied mainly using energy recovering MFC, to treat the
56 effluent of a two-stage biogas process [20], digested landfill leachate [21], digested swine

57 wastewater [22], a digested mixture of swine manure and rice bran [23] or digested wastewater
58 from processing potato industries [17]. Instead, the use of MEC mode proposed in this work for AD
59 effluent polishing has been scarcely reported [19].

60 On the third place, the combination of the previous advantages can be applied to increase the
61 stability of the AD process through the use of a submersible microbial desalination cell [24] or the
62 establishment of a recirculation loop with the BES [25]. The latter strategy has proven to be
63 effective for the control of AD inhibition due to organic and nitrogen overloads, while recovering
64 ammonia and maintaining the effluent quality and the methane production of the AD. Other studies
65 have reported an increased stability of the AD process when inserting electrodes in the reactor [26-
66 28]. However, this latter configuration does not allow for ammonia recovery, as proposed in the
67 present work.

68 Finally, BES have been applied to increase the methane content of the biogas produced in the AD
69 by the use of MECs with electromethanogenic biocathodes [29, 30]. Since biogas consists mainly
70 of methane (CH_4 , 40-75%) and carbon dioxide (CO_2 , 15-60%) it needs upgrading prior to its use as
71 vehicle fuel or for injection in the natural gas grid intended to adjust the calorific. Conventional
72 techniques for biogas upgrading focus on CO_2 removal without changing CH_4 mass [31], while
73 electromethanogenesis performed in MEC allows for the conversion of CO_2 into CH_4 [32-34]. An
74 alternative configuration to electromethanogenic MECs, by inserting electrodes in the AD reactor
75 and applying a potential, allows for the in situ biogas upgrading, reporting an increase in methane
76 yield [26, 35-37]. An increase of 59.7% in methane yield was achieved in a AD-MEC coupled
77 system compared to the AD alone [38]. MECs for CO_2 conversion to methane have been operated
78 mainly with synthetic medium [39, 40] and there is a lack of studies with real high strength
79 wastewater fed for the anode compartment. The present study contributes to the body of
80 knowledge by using a real high strength wastewater, digested pig slurry, to feed the anode
81 compartment.

82 The multiple ways of AD and BES combination suggest that a more comprehensive strategy can
83 help to settle most of the limitations of the AD process, which up to now has not been assessed.
84 Several studies have addressed the use of BES with a combined objective, such as Zepilli et al.

85 [40], who operated a methane-producing MEC with a synthetic solution of soluble organic
86 compounds, simulating the composition of a municipal wastewater, with the purpose of COD and
87 ammonium removal, and methane production. Instead, the scientific novelty of this study was to
88 investigate an integrated AD-MEC system fed with a high strength wastewater, such as pig slurry,
89 and designed with a multiple purpose: i) to increase the stability and robustness of the AD process
90 against organic and nitrogen overloads, ii) to keep the effluent quality, iii) to recover nutrients, and
91 iv) to upgrade the biogas. None of the reported bioelectrochemical systems has simultaneously
92 addressed these four purposes. Therefore, the application of MEC technology could be also
93 implemented to overcome simultaneously the main limitations of AD. Furthermore, further study on
94 the active microbial populations enriched in the bioanode and biocathode of the BES is needed to
95 gain insight on potential resilience strategies as well as to complement previous studies [30, 40].
96 Until now, studies were centred on the description of the most predominant existent
97 microorganisms on methanogenic biocathodes [29, 32, 34, 41, 42], while this study presents a
98 novel focus on the metabolically active biomass. This novel approach bridge the gap to distinguish
99 those active microorganisms from total microbial community in the biofilm communities in MECs.
100 The main aim of this study was to assess the performance of a lab-scale AD-MEC integrated
101 system as a strategy to stabilise a pig slurry thermophilic AD under an organic and nitrogen
102 overload, recover ammonia and increase the methane content of the biogas produced by the AD,
103 in terms of chemical oxygen demand and ammonia removal, methane yield and energy efficiency
104 of the process. The evolution of the active microbial community of the AD and the MEC
105 bioelectrodes (both the anode and the cathode) was evaluated in terms of composition and activity
106 by means of high throughput sequencing (16S rRNA *versus* 16S rDNA based Illumina-Miseq) and
107 quantifying total and metabolically active populations (*16SrRNA* and *mcrA* gene and transcripts) by
108 qPCR.

109 **2. Materials and methods**

110 **2.1 Experimental set-up**

111 A 4 L lab-scale thermophilic anaerobic continuous stirred tank reactor (AD) was used in the
112 assays. It consisted of a cylindrical glass reactor (25 cm diameter) with a 4 L working volume. The

113 digester was fitted with a heat jacket with hot water circulating to keep the temperature at 55 °C.
114 The AD reactor was connected in series with the anode compartment of a two-chambered MEC
115 (0.5 L in each compartment) and had a recirculation loop between both reactors. The anode of the
116 MEC was carbon felt (dimensions: 14 x 12 cm; thickness: 3.18 mm; Alfa Aesar GmbH & Co KG,
117 Karlsruhe, Germany) that had been inoculated with anode-inoculum from a mother MFC and had
118 been operated with digested pig slurry as feeding solution for 9 months. The cathode chamber was
119 filled with granular graphite with diameter ranging from 1 to 5 mm (Typ 00514, enViro-cell
120 Umwelttechnik GmbH, Oberursel, Germany), leaving a net volume of 265 mL. Prior to being used,
121 in order to remove metals and organic residues, the granular graphite was treated as described in
122 Sotres et al. (2016) [43]. An A304 stainless steel mesh was used as electron collector in each
123 compartment (dimensions: 14 x 12 cm; mesh width: 6 x 6 mm; wire thickness: 1 mm; Feval Filtros,
124 S.L., Barcelona, Spain). The anode and cathode compartments were separated by a cation
125 exchange membrane (CEM) (dimensions: 14 x 12 cm; Ultrex CMI-7000, Membranes International
126 Inc., Ringwood, NJ, USA). The cathode compartment was inoculated with 30 mL of a resuspension
127 of the anaerobic granular sludge of an UASB (volatile suspended solids content of 33 g L⁻¹) that
128 had been operated with methanol in order to enrich the biomass in methanogenic archaea, as
129 described elsewhere [44]. The resuspension was done by vortex mixing during 10 minutes in a 50
130 mL tube containing 30 g of granular sludge and 25 mL of Ringer 1/4 sterilised solution. A three
131 electrodes configuration was used, where the anode was the counter electrode, the cathode was
132 the working electrode and an Ag/AgCl reference electrode (Bioanalytical Systems, Inc., USA, +197
133 mV vs. standard hydrogen electrode (SHE)) was inserted in the cathode compartment. All potential
134 values in this paper are referred to SHE. A potentiostat (VSP, Bio-Logic, Grenoble, France) was
135 used for data monitoring, which was connected to a personal computer for electrode potentials and
136 current recording every 5 min using EC-Lab software (Bio-Logic, Grenoble, France). The anode
137 was fed with filtered digested pig slurry (125 µm) from the AD and the cathode compartment was
138 fed with a synthetic solution which contained (per litre of deionised water): 5 g L⁻¹ of NaHCO₃,
139 NH₄Cl, 0.87 g; CaCl₂, 14.7 mg; KH₂PO₄, 3 g; Na₂HPO₄, 6 g; MgSO₄, 0.246 g; and 1 mL L⁻¹ of a
140 trace elements solution as described elsewhere [45].

141 **2.2 Reactor operation**

142 The AD was fed with pig slurry (Table 1) and operated for 222 days in three different phases, with
 143 a hydraulic retention time (HRT) of 10 days (Table 2). In Phase 1, the organic loading rate (OLR)
 144 and nitrogen loading rate (NLR) were established at $3.92 \text{ kg}_{\text{COD}} \text{ m}^{-3} \text{ day}^{-1}$ and $0.22 \text{ kg}_{\text{N}} \text{ m}^{-3} \text{ day}^{-1}$,
 145 respectively. In Phase 2, the OLR and NLR were doubled to force the inhibition of the reactor [25].
 146 And finally, in Phase 3, keeping the OLR and NLR equal to Phase 2, a recirculation loop with the
 147 MEC was connected (50% of the AD feed flow rate) to recover the AD. Samples of the AD effluent
 148 were taken once a week in order to assess the operation of the reactor.

149 The MEC was operated in continuous in series with the AD poisoning the cathode potential at -800
 150 mV vs SHE. The solutions of both the anode and the cathode compartment were fed in continuous
 151 at 20 mL h^{-1} and mixed by an external pump. The HRT was of 32.4 h and 14.1 h for the anode and
 152 cathode compartment, respectively (with respect to the net volume of each compartment), with an
 153 OLR and NLR of the anode compartment for each phase specified in Table 2. The MEC was
 154 operated at room temperature during the entire assay ($23 \pm 2 \text{ }^\circ\text{C}$).

155 **Table 1.** Characterisation of the raw pig slurry used as feeding solution in the anaerobic digester (AD) in
 156 Phase 1 and Phases 2 and 3 (n=number of samples; mean \pm standard deviation).
 157

Parameter	Raw pig slurry	
	Phase 1 (n=6)	Phase 2 and 3 (n=12)
pH (-)	7.1 \pm 0.1	6.8 \pm 0.2
COD ($\text{g}_{\text{O}_2} \text{ kg}^{-1}$)	43.96 \pm 2.04	80.55 \pm 6.40
NTK (g L^{-1})	2.41 \pm 0.00	4.29 \pm 0.17
N-NH ₄ ⁺ (g L^{-1})	1.59 \pm 0.08	2.97 \pm 0.25
TS (g kg^{-1})	24.40 \pm 0.52	47.95 \pm 2.53
VS (g kg^{-1})	16.37 \pm 0.43	32.79 \pm 1.88

158

159 **Table 2.** Operational conditions for the AD reactor and the MEC (mean \pm standard deviation).

Phase	Period (d)	AD			MEC	
		OLR ($\text{kg}_{\text{COD}} \text{ m}^{-3} \text{ d}^{-1}$)	NLR ($\text{kg}_{\text{N}} \text{ m}^{-3} \text{ d}^{-1}$)	Recirculation (% feed flow rate)	OLR ($\text{kg}_{\text{COD}} \text{ m}^{-3} \text{ d}^{-1}$)	NLR ($\text{kg}_{\text{N}} \text{ m}^{-3} \text{ d}^{-1}$)
1	1 - 78	3.92 \pm 0.61	0.22 \pm 0.03	0	7.87 \pm 0.76	0.74 \pm 0.04
2	78 - 126	7.39 \pm 1.36	0.40 \pm 0.06	0	43.12 \pm 3.58	2.42 \pm 0.13
3	126 - 222			50		

160 2.3. Analytical methods and calculations

161 Samples were analysed for pH, chemical oxygen demand (COD) and ammonium (N-NH₄⁺)
162 according to Standard Methods 5220 [46]. Methane content of the biogas produced by the AD was
163 analysed using a gas chromatograph (CP-3800, Varian, USA). Methane was measured (through
164 the determination of dissolved methane) in the cathode samples according to Henry's Law and the
165 following method [47]. Around 2 mL catholyte samples were collected with a 5 mL syringe and
166 injected with a needle in a 4 mL vacutainer. The vacutainers were shaken vigorously for 30 s and
167 then allowed to stand for 1 h. Headspace gas was analysed for CH₄ using a gas chromatograph
168 (CP-3800, Varian, USA). Dissolved CH₄ was computed using the equation:

$$169 \quad X_L = \frac{C_{CH_4} \cdot MV_{CH_4} \cdot MW_{CH_4} \cdot (V_T - V_L + \alpha V_L) \cdot 1000}{V_L} \quad (1)$$

170
171
172
173 where X_L is the concentration of CH₄ (mg L⁻¹) in the solution, C_{CH_4} is the concentration of CH₄ (%)
174 in the headspace 1 h after shaking, MV_{CH_4} is the molar volume of CH₄ at 25 °C (0.041 mol L⁻¹),
175 MW_{CH_4} is the molecular weight of CH₄ (16 g mol⁻¹), V_T is the volume (mL) of the vacutainer, V_L is
176 the volume (mL) of the solution, and α is the water:air partition coefficient at 25 °C (0.03). Methane
177 production was normalised to the net volume of the cathode compartment (0.265 L).

178 Partial alkalinity (PA, titration from the original pH sample to pH 5.75, an alkalinity which
179 corresponds roughly to bicarbonate alkalinity) and total alkalinity (TA, titration to pH 4.3) were
180 determined to obtain intermediate alkalinity (IA, titration from 5.75 to 4.3, approximately the VFA
181 alkalinity) [48]. The IA:TA ratio was used as a tool to monitor anaerobic digestion, considering that
182 the process was stable when the IA:TA was below 0.3 [49]. COD and ammonium removal
183 efficiency in the MEC was calculated as the ratio of the difference between anode influent and
184 effluent concentrations and the influent concentration [25].

185 2.4. Electrochemical measurements and calculations

186 The current density (A m⁻²) of the MEC was calculated as the quotient between the intensity
187 recorded by the potentiostat (A) and the area of the anode (m²). The Coulombic efficiency (CE), or

188 the fraction of electrons obtained from the consumption of COD that are available for methane
 189 production at the cathode was calculated as:

$$190 \quad CE = \frac{M \int_0^t I dt}{F b q \Delta COD} \quad (2)$$

191 The energy efficiency relative to electrical input recovered as methane (EE_e , Equation 3), the
 192 energy efficiency relative to the energy content of the substrate (EE_s , Equation 4) and the energy
 193 efficiency with respect to the energy input and the energy in the substrate (EE_{e+s} , Equation 5) were:

$$194 \quad EE_e = \frac{n_{CH_4} \Delta G_{CH_4}}{\int_0^t I E_{ap} dt} \quad (3)$$

$$195 \quad EE_s = \frac{n_{CH_4} \Delta G_{CH_4}}{n_S \Delta G_S} \quad (4)$$

$$196 \quad EE_{e+s} = \frac{n_{CH_4} \Delta G_{CH_4}}{\int_0^t I E_{ap} dt + n_S \Delta G_S} \quad (5)$$

199 where M is the molecular weight of the final electron acceptor, I is the current (A), F is Faraday's
 200 constant (96485 C mol^{-1}), b is the number of electrons transferred per mole of O_2 , q is the
 201 volumetric influent flow rate ($L d^{-1}$), ΔCOD is the difference in the influent and effluent COD, n_{CH_4}
 202 are the moles of produced methane, ΔG_{CH_4} is the molar Gibbs free energy of CH_4 oxidation by
 203 oxygen to carbon dioxide ($-817.97 \text{ kJ mol}^{-1}$), E_{ap} is the applied voltage calculated as the difference
 204 between the cathode and anode potentials (V), n_S are the moles of acetate consumed and ΔG_S
 205 the molar Gibbs free energy of acetate oxidation to carbon dioxide ($-844.61 \text{ kJ mol}^{-1}$).

206 Finally, the cathodic methane recovery efficiency (R_{cat}), defined as the fraction of electrons
 207 reaching the cathode that are recovered as methane, was calculated as:

$$208 \quad R_{cat} = \frac{8 F n_{CH_4}}{\int_0^t I dt} \quad (6)$$

209 Cyclic voltammeteries (CV) in turnover conditions, i.e. in the presence of substrate, were performed
 210 using a potentiostat (VSP, Bio-Logic, Grenoble, France) after the cathode inoculation (day 0) and
 211 at the end of Phase 2 and 3 (day 78 and 222 of the assays, respectively), in order to study the

212 electroactive microbial biofilm developed on the cathode. The same three-electrode configuration
213 used for the MECs operation was maintained for the set up of the CV. The start (E_i) and vertex (E_f)
214 potentials were -800 and +400 mV vs SHE, respectively, and the scan rate was set at 1 mV s⁻¹.

215 **2.5. Microbial community analysis**

216 The bacterial communities in the biofilm harboured in the bioanode and the biocathode of the MEC
217 at the end of Phase 2 and 3 and in the AD biomass at the end of each Phase (1, 2 and 3) were
218 analysed by culture-independent molecular techniques such as quantitative PCR (qPCR) and high
219 throughput sequencing (MiSeq, Illumina) of partial 16S rDNA and 16S rRNA massive libraries. To
220 stabilise the nucleic acid, especially RNA, samples were stored at -80 °C. The inoculum of the
221 cathode and the biofilm settled up on the anode had been characterised in previous work [44, 50].
222 Briefly, regarding eubacteria, the cathode inoculum was dominated by *Bacteroidetes*, *Firmicutes*
223 and *Synergistetes* (61, 14 and 8%, respectively); *Methanosarcinaceae* (52%) was the predominant
224 archaea family (methylophs genus, *Methanomethylovorans* and *Methanolobus*) [44]. In the case
225 of MEC anode biofilm, *Firmicutes*, *Bacteroidetes* and *Proteobacteria* were the dominant eubacterial
226 *phyla* (35, 30 and 11%, respectively), and the predominant archaea family was *Methanotrichaceae*,
227 (87%) [44, 50].

228 **2.5.1 Nucleic acid extraction and cDNA synthesis**

229 Simultaneous total genomic DNA and RNA (including rRNA) were extracted in triplicate from
230 known weights of each sample, which consisted in effluent from the AD, a piece of carbon felt from
231 the anode (1 cm²) and granular graphite collected from the cathode compartments for the
232 biocathode biofilm. The PowerMicrobiome™ RNA Isolation Kit (MoBio Laboratories Inc., Carlsbad,
233 CA, USA) was used according to manufacturer's instructions, and cDNA was obtained from total
234 RNA following the protocol described elsewhere [50]. Henceforth, the term cDNA or 16S rRNA is
235 used to refer to the extracted RNA or 16S transcripts as amplicons from cDNA as a measure of
236 gene expression and microbial activity, whereas DNA or 16S rDNA terms are used referring to the
237 extracted genomic DNA and 16SrDNA amplicons from DNA.

238

239

240 **2.5.2 Quantitative PCR assay (qPCR)**

241 Total gene copy numbers and gene transcripts of eubacterial *16S rRNA* gene and *mcrA* gene
242 (methanogenic archaeal methyl coenzyme-M reductase) were quantified by means of qPCR. Each
243 sample was analysed in triplicate by means of the three independent DNA and RNA (cDNA)
244 extracts. The analysis was carried out following the protocol described elsewhere [25]. The
245 standard curve parameters of the qPCRs were as follows (for *16S rRNA* and *mcrA*, respectively): a
246 slope of -3.244 and -3.532; a correlation coefficient of 0.998 and 0.999; and an efficiency of 103
247 and 92%.

248 **2.5.3 High throughput sequencing and data analysis**

249 Simultaneous DNA and RNA (cDNA) extracts obtained from the AD and the bioanode and
250 biocathode biofilm, used for qPCR analysis, were also assessed by *16SrRNA* gene based- MiSeq,
251 following the specific steps described elsewhere [25]. The Bayesian Classifier tool of the
252 Ribosomal Database Project (RDP) was used to taxonomically classify the obtained Operational
253 Taxonomic Units (OTUs) [51]. The data obtained from sequencing datasets for eubacterial and
254 archaeal populations was submitted to the Sequence Read Archive of the National Center for
255 Biotechnology Information (NCBI) under the study accession number SRP072956.

256 A statistical multivariate analysis by means of correspondence analysis (CA) on the OTUs
257 abundance matrix of Eubacterial *and* Archaeal was performed. The obtained samples and OTUs
258 scores were depicted in a 2D biplot, which represented the phylogenetic assignment of the
259 predominant OTUs (relative abundance above 1 %). The diversity of the samples was evaluated
260 with the number of OTUs, the inverted Simpson index, Shannon index and Goods coverage using
261 the Mothur software v.1.34.4 (<http://www.mothur.org>) [52], normalising all the estimators to the
262 lowest number of reads among the different samples.

263

264 **3. Results and discussion**

265 **3.1 Performance of the AD (Phase 1 and Phase 2)**

266 The AD showed a stable operation during Phase 1 (Figure 1), with an average COD removal
267 efficiency of $54\pm 8\%$ and a methane production of $0.45\pm 0.06 \text{ m}^3 \text{ m}^{-3} \text{ d}^{-1}$ (Table 3). The IA:TA ratio

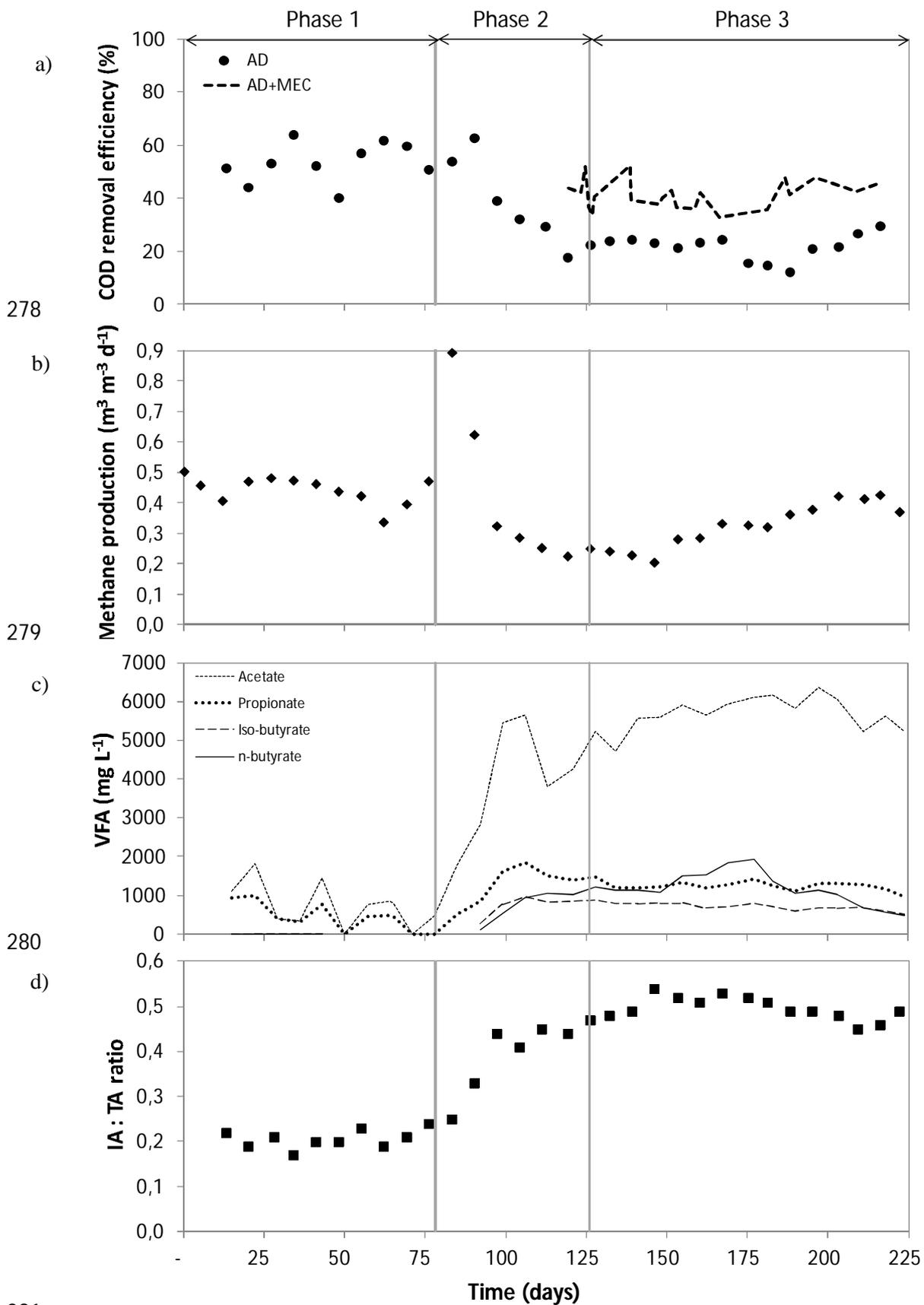
268 was kept below 0.3, corroborating the stability of the reactor. When the OLR and NLR of the AD
 269 were doubled in Phase 2, the reactor showed a fast inhibition, reducing the value of COD removal
 270 to 18% and the methane production to $0.23 \text{ m}^3 \text{ m}^{-3} \text{ d}^{-1}$, 50% of the obtained in the previous phase
 271 (Figure 1a and b). VFA accumulated, reaching values of 5670 mg L^{-1} for acetate, 1850 mg L^{-1} for
 272 propionate and over 1000 mg L^{-1} for butyrate (Figure 1c), and the IA:TA ratio increased to 0.44
 273 (Figure 1d) confirming the instability of the AD reactor.

274 **Table 3.** Summary of the main parameters for the AD and the MEC reactors in the different phases. Results
 275 for the AD correspond to the stable period of each phase (mean \pm standard deviation).

Parameter	Phase 1	Phase 2	Phase 3
AD			
CH ₄ production ($\text{m}^3 \text{ m}^{-3} \text{ d}^{-1}$)	0.45 \pm 0.06	0.27 \pm 0.04	0.38 \pm 0.04
COD removal efficiency (%)	54 \pm 8	28 \pm 8	22 \pm 5
IA:TA	0.22 \pm 0.04	0.40 \pm 0.08	0.50 \pm 0.03
pH	7.8 \pm 0.1	7.8 \pm 0.1	7.6 \pm 0.1
MEC			
COD removal efficiency (%)	24 \pm 8	14 \pm 5	21 \pm 6
N-NH ₄ ⁺ removal efficiency (%)	30 \pm 6	18 \pm 5	20 \pm 5
CE (%)	3.5 \pm 1.8	2.1 \pm 1.4	1.5 \pm 0.5
CH ₄ production ($\text{L m}^{-3} \text{ d}^{-1}$)	79 \pm 34	63 \pm 15	78 \pm 29
R _{cat} (%)	45 \pm 37	61 \pm 18	59 \pm 13
EE _e (%)	50 \pm 41	62 \pm 17	85 \pm 23
EE _s (%)	4.2 \pm 2.3	3.0 \pm 1.5	1.4 \pm 0.7
EE _{e+s} (%)	3.6 \pm 1.4	2.9 \pm 1.4	1.4 \pm 0.7
Energy input ($\text{kWh m}^{-3} \text{ CH}_4$)	39 \pm 33	15.8 \pm 4.5	11.5 \pm 3.4
AD-MEC			
COD removal efficiency (%)	-	42 \pm 6	41 \pm 6

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281

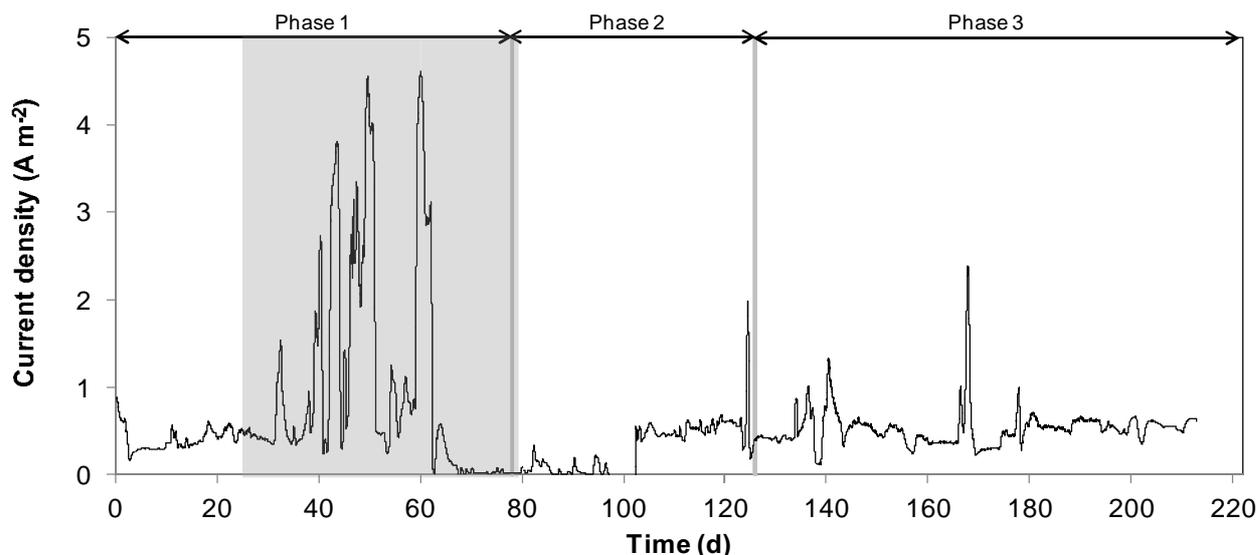
282 **Figure 1** Performance of the AD regarding (a) COD removal efficiency; (b) methane
 283 production; (c) VFA concentration; and (d) IA:TA ratio.

284 **3.2 Performance of the MEC (Phase 1 and Phase 2)**

285 The MEC fed with the effluent of the AD during Phase 1 showed a stable performance regarding
286 current density production during the first 30 days of operation (Figure 2), with an average current
287 density of 0.4 A m^{-2} . Afterwards, the current density production became instable, showing peaks of
288 up to 4.5 A m^{-2} (average of 1.5 A m^{-2}) and was drastically reduced from day 64 onward due to the
289 degradation of the stainless steel mesh used as electron collector in the anode compartment (data
290 corresponding to the instable period was not considered for calculations). The mesh was replaced
291 at the beginning of Phase 2. This change produced some period of instability at the beginning of
292 Phase 2, but afterwards the MEC showed a current density production similar to the obtained in
293 Phase 1. The COD removal efficiency of the MEC in Phase 1 was of $24 \pm 8\%$, with a maximum
294 removal of $3.2 \text{ kg}_{\text{COD}} \text{ m}^{-3} \text{ d}^{-1}$, and the CE was of $3.5 \pm 1.8\%$ (Table 3). In Phase 2 the COD removal
295 efficiency and the CE decreased to $14 \pm 5\%$ and $2.1 \pm 1.4\%$, respectively. Since CE is related with
296 the substrate concentration, the increase in COD of the influent resulted in a decrease in CE
297 [53]. The ammonium removal efficiency in Phase 1 was of $30 \pm 6\%$, corresponding to 6.64 g N-NH_4^+
298 $\text{m}^{-2} \text{ d}^{-1}$. The removal efficiency decreased to $18 \pm 5\%$ during Phase 2, although the absolute flux
299 was higher ($12.87 \text{ g N-NH}_4^+ \text{ m}^{-2} \text{ d}^{-1}$) as a result of the increased NLR. Previous work performed
300 with the same MEC but with an abiotic cathode fed with NaCl (0.1 g L^{-1}) and higher organic and
301 nitrogen loading rates ($28.50 \pm 1.80 \text{ kg}_{\text{COD}} \text{ m}^{-3} \text{ day}^{-1}$ and $1.73 \pm 0.09 \text{ kg}_\text{N} \text{ m}^{-3} \text{ day}^{-1}$, respectively)
302 achieved a nitrogen removal rate of $12.97 \pm 2.04 \text{ g N-NH}_4^+ \text{ m}^{-2} \text{ d}^{-1}$, similar to the one obtained in
303 Phase 2 and almost doubling the rate obtained in Phase 1 in this study [25]. And values as high as
304 $86 \text{ g N-NH}_4^+ \text{ m}^{-2} \text{ d}^{-1}$ were reported with a submersible microbial desalination cell fed with synthetic
305 solution [24].

306

307



308

309 **Figure 2** Current density profile obtained during the operation of the MEC. Data
 310 corresponding to the instable period (shaded) was not considered for calculations.

311 3.3 Performance of the AD-MEC combined system with recirculation loop (Phase 3)

312 When the recirculation loop with the MEC was connected in Phase 3, the performance of the AD
 313 started to recover, with COD removal values achieving 30% (below 20% in the end of Phase 2)
 314 and a maximum methane production of $0.43 \text{ m}^3 \text{ m}^{-3} \text{ d}^{-1}$, representing a 100% increase with respect
 315 to the production at the end of Phase 2, and nearly recovering the values achieved in Phase 1.
 316 However, the VFA concentration and the IA:TA ratio were similar to the previous phase.
 317 Interestingly, the overall performance of the AD-MEC integrated system maintained the COD
 318 removal in a range of 33-52%, in spite of the poorer performance of the AD, concomitant to an
 319 ammonium removal of $20 \pm 5\%$ ($14.46 \text{ g N-NH}_4^+ \text{ m}^{-2} \text{ d}^{-1}$). The MEC showed a current density
 320 production similar to the obtained at the beginning of Phase 1. The CE of the MEC decreased with
 321 respect to Phase 1, as described in Phase 2. The low CEs obtained are to be expected when
 322 working with complex substrates where other electron acceptors may be present [54-56]. Much
 323 higher CEs have been previously reported, such as 72-80% [40].

324 3.4 Biocathode operation performance

325 Methane production in the cathode compartment was around $0.079 \text{ m}^3 \text{ m}^{-3} \text{ d}^{-1}$. A volume of biogas
 326 of $0.2 \text{ m}^3 \text{ m}^{-3} \text{ d}^{-1}$ could be treated in this MEC with biocathode, assuming a typical biogas

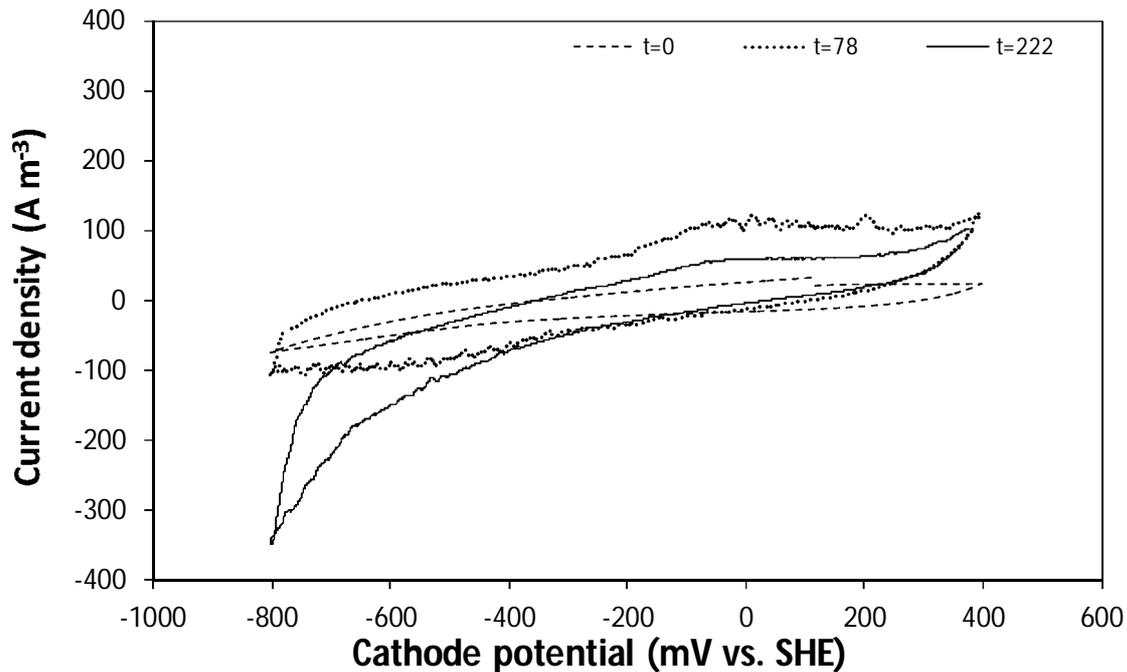
327 composition of 60% CH₄ and 40% CO₂ (490.78 kJ mol⁻¹) to obtain methane with a purity prochain
328 to 100% (817.97 kJ mol⁻¹) Higher methane productions have been achieved in previous works,
329 such as 0.16 m³ m⁻³ d⁻¹ [39], or 0.28 m³ m⁻³ d⁻¹ [57].

330 The highest R_{cat} was achieved in Phase 2 (61±18%), although the high variability of the obtained
331 results makes the observed differences between phases not to be significant (Table 3). A recent
332 review on bioelectrochemical power-to-gas, collected R_{cat} of previous studies between 19 to 156%,
333 were values >100% were likely the result of microbially influenced corrosion of the cathode by
334 methanogens [58]. The obtained results for R_{cat} in the present study are below the 96% previously
335 reported by Cheng et al. (2009) [32], the 84-86% obtained by Zeppilli et al. (2014) [40], or the 69%
336 reported by Batlle-Vilanova et al. (2015) [30]. But they are much higher than the 23.1% achieved
337 by Van Eerten-Jansen et al. (2001) [59] or the 24.2 ± 4.7% reported by Zhen et al. (2015) [42].

338 The E_{ee} was between 50 and 85%, values similar to the obtained in batch mode with a two-
339 chambered MEC using graphite granules as electrodes (57%) [33]. The low CE achieved in this
340 MEC made also the obtained EEs (between 1.4 and 3.6%) to be low in comparison with the
341 previous work (between 23 and 54%).

342 Figure 3 shows the cyclic voltammograms obtained after the cathode inoculation and at the end of
343 Phases 2 (78 days) and 3 (222 days). The curve obtained at the start of the operation showed a
344 low response to the different applied potentials, as a result of the recent inoculation. The
345 performance of the biocathode increased at the voltage of -800 mV at the end of the assay (Phase
346 3), coincidentally with the period of the best E_{ee}.

347



348

349 **Figure 3** Cyclic voltammograms of the MEC after the cathode inoculation (t=0 d) and at the
 350 end of Phase 2 and 3 ((t=78 d and t=222 d, respectively).

351 **3.5 Considerations about biogas upgrading and ammonia recovery application**

352 In dual-chamber MECs for methane production, water is usually used for electron donor in the
 353 anode chamber, since it is a widely available and cheap source [60]. However, the electric energy
 354 input is considerable higher compared to a BES using wastewater oxidation in the bioanode [58].
 355 In this study, the average energy input for biomethane production from CO_2 in the biocathode in
 356 Phase 3 was $11.5 \pm 3.4 \text{ kWh m}^{-3}$ (Table 3). In a previous work, Van Eerten-Jansen et al. [59]
 357 achieved an energy input of 73.5 kWh m^{-3} in an electromethanogenic biocathode operated in
 358 continuous with water as electron donor. The energy input was reduced to 15.4 kWh m^{-3} following
 359 the oxidation of hexacyanoferrate (II).

360 The values of electricity consumption obtained in this studies are similar to the ones corresponding
 361 for conventional biogas upgrading technologies, such as pressure swing adsorption (0.25 kWh m^{-3})
 362 ³), water scrubbing ($<0.25 \text{ kWh m}^{-3}$), organic physical scrubbing ($0.24\text{-}0.33 \text{ kWh m}^{-3}$) or chemical
 363 scrubbing ($<0.15 \text{ kWh m}^{-3}$) [61]. It is noteworthy that these values refer to the volume of biogas

364 treated, not to the volume of methane produced, as the previous ones. Furthermore, conventional
365 technologies are based on CO₂ removal, instead of CO₂ conversion to CH₄ proposed in this study.
366 Finally, it is noteworthy that the energy consumption of the MEC is not only invested in methane
367 production, but also used for ammonia recovery. The energy consumption for this latter aim
368 represented on average 3 kWh kg⁻¹_{N recovered}, while the values reported for conventional techniques
369 for ammonia removal range are 13 kWh kg⁻¹_{N removed} in a nitrification denitrification process, 5 kWh
370 kg⁻¹_{N removed} in a Sharon-Anammox process, or 9 kWh kg⁻¹_{N recovered} in NH₃ stripping [62].
371 There are still many challenges to overcome in order for AD-MEC coupled system with
372 electromethanogenic biocathode to be applied in large scale systems for biogas upgrading and
373 ammonia recovery. Mainly, the use of phosphate buffer in the cathode compartment to avoid pH
374 increase is not a sustainable approach for scaled systems [7] and an alternative should be
375 implemented. Furthermore, the use of the buffer hinders the recovery of ammonia by stripping of
376 the catholite and adsorption that has been used in previous work [25].

377 **3.6. Microbial community assessment**

378 The microbial community structure and activity of the samples taken from the AD and the biofilm
379 harboured on the electrodes of the MEC was characterised by means of qPCR technique and
380 sequenced by MiSeq.

381 **3.6.1 Quantitative analysis by qPCR**

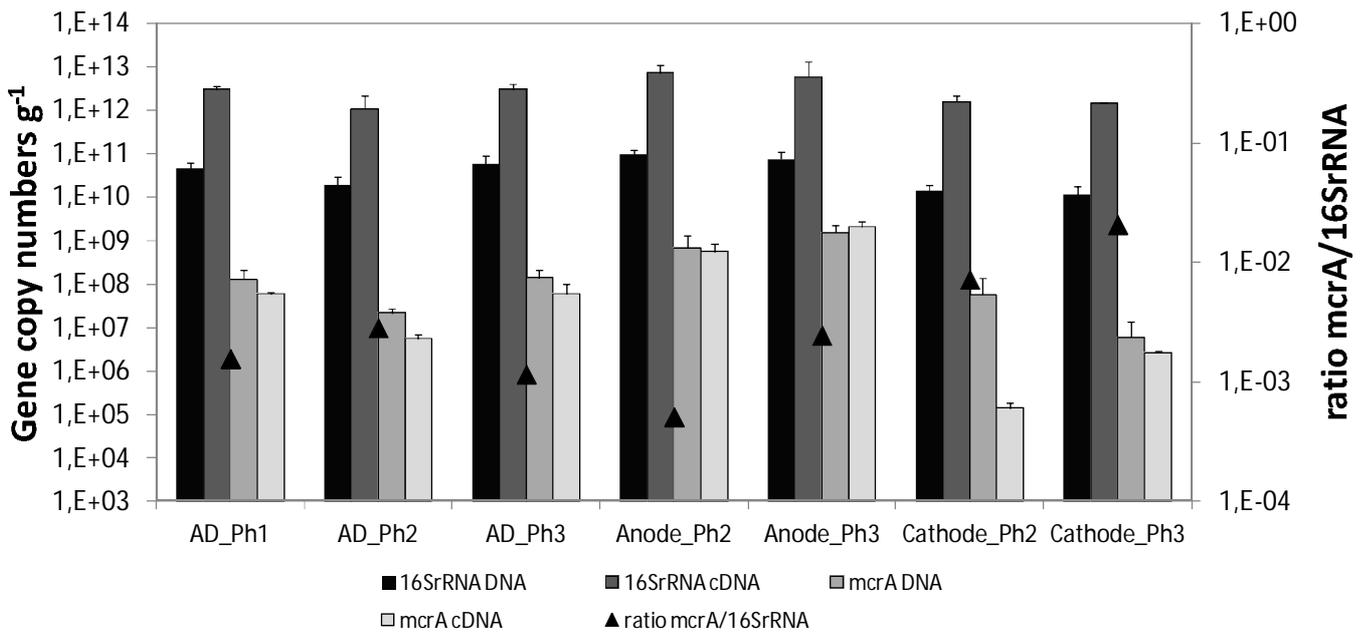
382 Figure 4 shows qPCR results for *16S* rRNA and *mcrA* gene copy numbers of the seven samples
383 analysed, either for DNA (present microorganisms) and cDNA (active microorganisms). In the AD
384 samples eubacteria remained in the same order of magnitude for *16S* rRNA gene copy numbers g⁻¹
385 during the stable, inhibited and recovered states; while showed a decrease of one order of
386 magnitude for *mcrA* (from 1.26·10⁸ to 2.18·10⁷ gene copy numbers g⁻¹) at the end of Phase 2 due
387 to the inhibition caused by doubling the OLR and NLR. The connexion of the recirculation loop
388 reduced the inhibition and helped to recover the methanogenic population, returning to levels
389 similar to those existing prior to the inhibition (1.38·10⁸ gene copy numbers g⁻¹). The same
390 behaviour was observed at cDNA-based qPCR, showing a decrease of one order of magnitude for
391 *mcrA* transcripts copy numbers in the AD sample of the end of Phase 2 and corroborating that the

392 methanogenic population was suffering inhibition. The magnitude of the reduction in *mcrA* gene
393 copy numbers when the AD was submitted to inhibition by an organic and nitrogen overload was
394 similar to the described in previous work [50, 63]. In Phase 1 free ammonia nitrogen (FAN)
395 concentration was in the range of 400-670 mg L⁻¹. Concentrations of FAN above 900 mg L⁻¹ were
396 reached during Phase 2, when the OLR and NLR were doubled, with a maximum of 1186 mg FAN
397 L⁻¹ at the end of the Phase. At these levels the first signs of inhibition may occur according to
398 previous studies [64, 65]. Once the recirculation loop was established in Phase 3, FAN levels
399 remained in a range of 365-740 mg L⁻¹.

400 Regarding the MEC, gene copy numbers for 16S rRNA for the anode and cathode biofilm were of
401 the same order of magnitude both for Phase 2 and 3 samples, either in DNA or in cDNA forms. On
402 the contrary, *mcrA* gene copy numbers of the anode sample increased at the end of the
403 recirculation phase, probably due to the parallel increase of methanogenic population in the AD. In
404 the case of the cathode biofilm, *mcrA* gene copy numbers g⁻¹ for DNA-based qPCR at the end of
405 the assay were within 10⁶ gene copy numbers g⁻¹, coincidentally with the values obtained in a
406 previous work with methanogenic biofilm harboured by granular graphite [30]. While a total
407 methanogenic population decrease was observed from the initial cathode to the final sample (DNA
408 level), *mcrA* expression belonging to methanogenic archaea increased one order of magnitude
409 (from 1.40·10⁵ to 2.63·10⁶ transcript copy numbers g⁻¹).

410 From these results, it is clear that the AD methanogenic population decreased due to the organic
411 and nitrogen overload and then could be recovered thanks to the establishment of the recirculation
412 loop with the MEC. Furthermore, the methanogenic cathode of the MEC was progressively
413 enriched in metabolically active methanogenic archaea.

414



415

416 **Figure 4** Gene copy numbers for 16S rRNA and *mcrA* genes and ratio between them of DNA
 417 and cDNA, of the AD effluent at the end of Phases 1, 2 and 3, and the biofilm harboured on the
 418 anode and cathode of the MEC at the end of Phase 2 and 3.

419

420 3.6.2 High Throughput Sequencing (16S-based MiSeq) results for eubacteria and archaea

421 Table 4 shows the number of reads obtained for the AD samples and the anode and cathode
 422 biofilms of the MEC for eubacteria (6822 OTUs) and archaea (900 OTUs). Figure 5a shows the
 423 relative abundance of eubacterial *phyla* for the seven samples, regarding DNA (present
 424 microorganisms) and cDNA (metabolically active microorganisms) forms. The three AD samples
 425 showed a similar eubacterial composition at DNA level, dominated by *Firmicutes*, *Bacteroidetes*
 426 and *Proteobacteria*, with relative abundances in the ranges of 64-68%, 12-18% and 5-10%,
 427 respectively. Previous studies, performed also in a thermophilic AD running on pig slurry, found
 428 that the *Firmicutes phylum* was the predominant one [25, 66]. The sample from the end of Phase 2
 429 showed a decrease in *Firmicutes* and *Proteobacteria phyla* with respect to Phase 1 and the sample
 430 at the end of Phase 3, while *Bacteroidetes* increased slightly. At cDNA level, although *Firmicutes*
 431 was still the predominant *phylum* (46-75% of relative abundance), *Proteobacteria* active
 432 microorganisms clearly surpassed *Bacteroidetes* (19-40% and 1-5%, respectively) and were
 433 increasingly more abundant over time, while *Firmicutes* showed the opposite tendency.

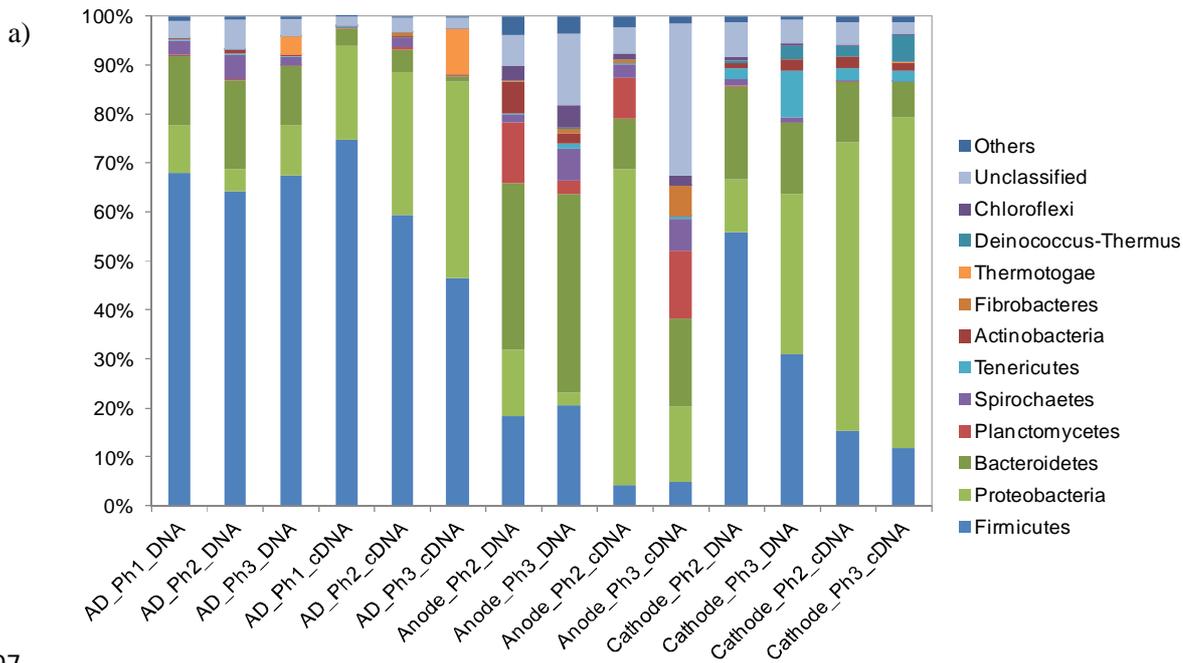
434 *Bacteroidetes* was the predominant *phylum* in the anode of the MEC both in Phase 2 and 3
435 samples (34 and 41%, respectively), according to DNA-based sequencing, while *Proteobacteria*
436 revealed as the most active one in Phase 2 sample (65%) and *Bacteroidetes* and *Proteobacteria*
437 shared dominance in the final sample (18 and 16%, respectively). These three phyla have been
438 identified in previous studies in BES [67, 68]. In the case of the cathode biofilm, the domination of
439 *Firmicutes* at DNA level in Phase 2 and 3 samples (56 and 31%, respectively), shared by
440 *Proteobacteria* in Phase 3 sample (33%), shifted to a clear dominance of *Proteobacteria* in both
441 samples at cDNA level (59 and 68%).

442 At family level *Clostridiaceae 1* and *Peptostreptococcaceae* were the most present and active
443 groups in the three AD samples (Figure 5b). *Porphyromonadaceae*, which was the third more
444 abundant family (6-9%) at DNA level, showed a low activity according to cDNA sequencing (below
445 1%). *Planococcaceae* and *Pseudomonadaceae* revealed as active families at the end of Phase 2
446 (21 and 12%, respectively), as *Campylobacteraceae* at the end of Phase 2 (13%), although they
447 were below 3% in DNA form abundance. Regarding the samples of the MEC anode,
448 *Planctomycetaceae* and *Porphyromonadaceae* stood out in the Phase 2 sample when looking at
449 DNA sequencing results (12 and 16%, respectively), but were replaced by *Desulfuromonadaceae*
450 and *Pseudomonadaceae* families according to cDNA (21 and 18%, respectively). In Phase 3
451 anode sample *Porphyromonadaceae* and *Planctomycetaceae* were the most abundant families at
452 DNA (12%) and cDNA (14%) levels, respectively. Finally, the cathode biofilm was dominated by
453 *Clostridiaceae* in Phase 2 and 3 samples at DNA level (34 and 12%, respectively), but by
454 metabolically active *Phodocyclaceae* and *Desulfovibrionaceae* according to cDNA results (14 and
455 37%, respectively). Thus, a clear differentiation between total eubacteria and active eubacterial
456 microorganisms, especially in the biomass harboured by the MEC electrodes, has been shown.

457 Figure 5c shows the relative abundance of archaea families of the seven samples. Although
458 *Methanobacteriaceae* showed a high abundance in the three AD samples (74-86%), the most
459 active archaeal populations were *Methanomicrobiaceae* in Phase 1 and Phase 3 AD samples (58
460 and 56%, respectively) and *Methanossiliococcaceae* in Phase 2 sample (54%). Members of
461 *Methanomassiliococcaceae* family have recently been described as obligate hydrogen-consuming

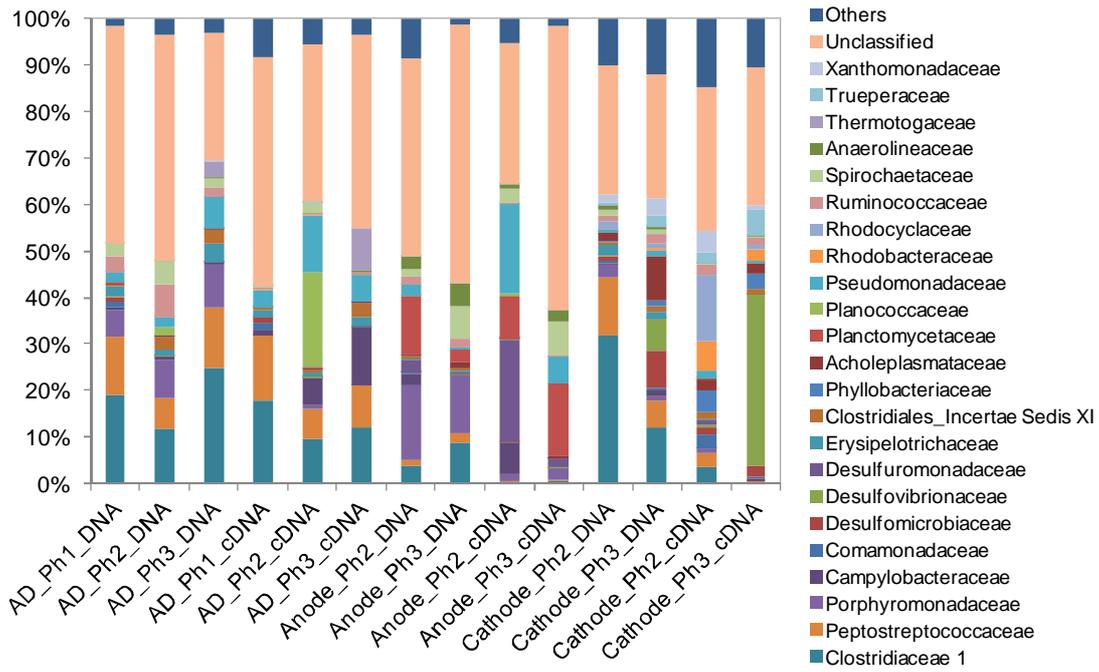
462 methanogens, reducing methanol and methylamines instead of carbon dioxide [69, 70]. A recent
463 study has reported an increase of this family at high OLR, and concluded that an additional
464 methanogenic pathway might contribute to methane production at high OLR [71]. It has also been
465 reported that this family emerged in the recovery process of a thermophilic AD after an organic
466 overload [72], since methanol could be produced fermentatively from lactate by a kind of
467 *Clostridium* species. Methanogenic population in the AD showed higher differences between
468 existing vs active composition than eubacteria. On the contrary, *Methanotrichaceae*, the
469 predominant family on the MEC anode Phase 2 and Phase 3 samples in presence (75 and 48%,
470 respectively) was also active (74 and 31%, respectively), independently of being on a low relative
471 abundance in the AD. On the other hand, *Methanossiliicoccaceae* and *Methanomicrobiaceae*
472 increased in presence and activity over time, probably due to the high activity of these families in
473 the AD. When looking at the cathode biofilm, *Methanotrichaceae* (genus *Methanotrix*, formerly
474 known as *Methanosaeta*) was the dominant family at the Phase 2 sample either in presence and
475 activity (53 and 68%, respectively), while in the Phase 3 sample it dominated at DNA but not at
476 cDNA level (36% and 0.3%, respectively). *Methanotrix* (*Methanosaeta*) genus was also detected in
477 a methanogenic cathode by Xu et al. (2014) [29] and Cai et al. (2016) [73]. It has been recently
478 described that *Methanotrix* (*Methanosaeta*) is capable of accepting electrons via direct interspecies
479 electron transfer (DIET) for the reduction of carbon dioxide to methane [74], so a deeper study is
480 necessary to understand the role of these species in the cathode biofilm of the methanogenic
481 MEC. *Methanomassiliicoccaceae* (genus *Methanomassiliicoccus*) and *Methanobacteriaceae*
482 (genus *Methanobacterium* and *Methanobrevibacter*) were also families with high relative
483 abundances at DNA level (15-31%), being the most active ones in the Phase 3 sample (57 and
484 33%, respectively) as well. Previous works have showed a clear dominance of
485 *Methanobacteriaceae* family in methanogenic biocathodes [29, 32, 34, 41, 42], differing from the
486 results obtained in this study. The high relative abundance of the methylotrophic
487 *Methanomassiliicoccaceae* family among the active archaea suggests that methanol must be
488 present in the cathode compartment, although it was not monitored in the present study. Methanol
489 produced in the anode compartment due to the fermentation of organic compounds could migrate

490 to the cathode compartment through the CEM. Methanol could be also produced at very low O₂
 491 partial pressure, from methane by ammonia oxidising bacteria (AOB) which can partially oxidise
 492 CH₄ to methanol when using ammonia as an energy source [75]. The genera *Nitrosomonas*, a well
 493 known AOB, was present in the biocathode samples, although at low relative abundance (<0.01%).
 494 Besides, some methane oxidising archaea and bacteria can also carry out anaerobic oxidation of
 495 methane [76], although so far they are poorly known and it cannot be determined if they are
 496 present in the biocathode.

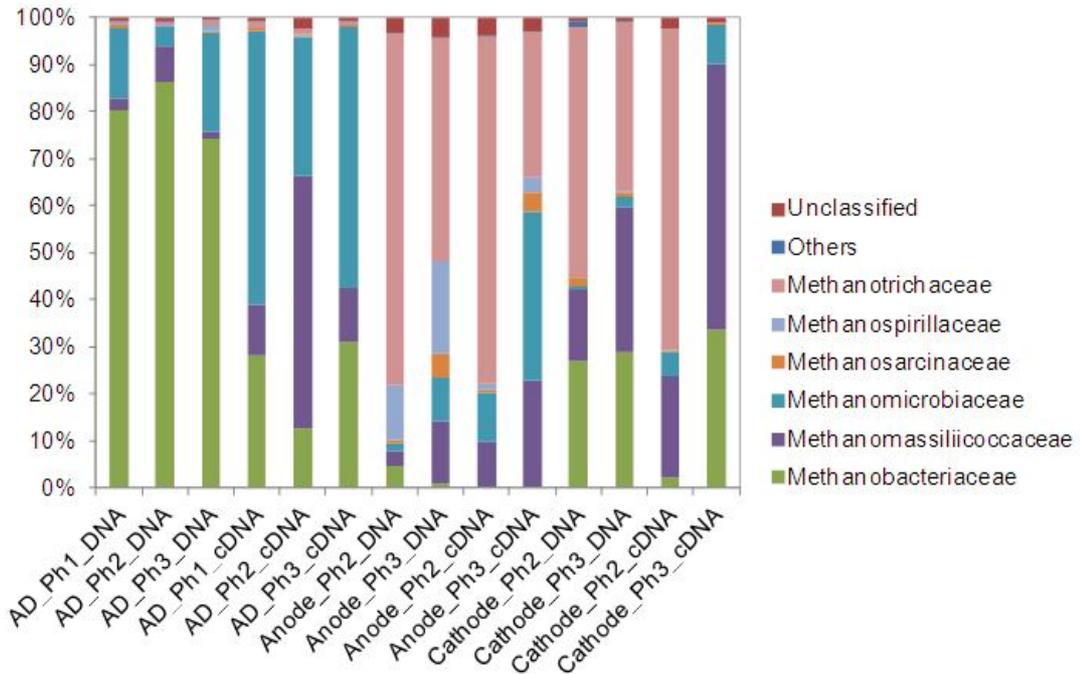


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b)



498 c)



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500 **Figure 5** Taxonomic assignment of sequencing reads from Eubacterial community of the AD
 501 effluent at the end of Phases 1, 2 and 3, and the biofilm harboured on the anode and cathode of
 502 the MEC at the end of Phase 2 and 3 for DNA and cDNA, at a) phylum b) family levels; and c) for
 503 Archaeal community at family level. Relative abundance was defined as the number of reads
 504 (sequences) affiliated with any given taxon divided by the total number of reads per sample.
 505 Phylogenetic groups with relative abundance lower than 1% were categorized as “others”.

506

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508 **3.6.3. Biodiversity analysis**

509 Table 4 shows the results for the biodiversity analysis performed on the AD samples and the
510 anode and cathode biofilms of the MEC samples. The Inverted Simpson and Shannon indexes of
511 the AD samples for eubacteria at DNA level decreased during Phase 2 and recovered in Phase 3,
512 showing the opposite behaviour for archaea. The biodiversity of the bioanode and the biocathode
513 increased regarding archaeal population, either in total (DNA) or in metabolically active (cDNA). On
514 the contrary, eubacteria decreased its biodiversity, result that agrees with the trend observed in
515 other studies [77].

516

517 **Table 4.** Diversity indexes for Eubacteria and Archaea community of the AD effluent at the end of Phases 1,
518 2 and 3, and the biofilm harboured on the anode and cathode of the MEC at the end of Phase 2 and 3 for
519 DNA and cDNA samples (mean±standard deviation). Normalised to the lowest number of reads (56424 and
520 59671 for eubacteria and archaea, respectively).

	Reads	Inverted Simpson	Shannon
Eubacteria			
ADPh1-DNA	116316	14.67±0.07	3.94±0.01
ADPh2-DNA	142940	13.00±0.07	3.85±0.01
ADPh3-DNA	117089	13.61±0.08	4.02±0.01
Anodei-DNA	95391	38.16±0.22	4.90±0.01
Anodef-DNA	96879	34.12±0.20	4.87±0.01
Cathodei-DNA	65906	16.12±0.06	4.29±0.00
Cathodef-DNA	61741	35.48±0.09	4.57±0.00
ADPh1-cDNA	163191	7.55±0.03	3.07±0.01
ADPh2-cDNA	152610	7.26±0.04	3.39±0.01
ADPh3-cDNA	214052	6.54±0.03	2.78±0.01
Anodei-cDNA	147740	8.58±0.05	3.71±0.01
Anodef-cDNA	171251	9.24±0.05	3.54±0.01
Cathodei-cDNA	56424	3.65±0.00	4.53±0.00
Cathodef-cDNA	93469	5.22±0.02	3.01±0.01
Archaea			
ADPh1-DNA	97511	2.87±0.01	1.67±0.01
ADPh2-DNA	59777	3.41±0.00	2.10±0.00
ADPh3-DNA	71736	3.33±0.01	1.84±0.00
Anodei-DNA	183422	1.58±0.00	1.18±0.01
Anodef-DNA	140800	2.83±0.01	1.90±0.01
Cathodei-DNA	220361	1.77±0.01	1.44±0.01
Cathodef-DNA	92290	4.92±0.02	2.40±0.01
ADPh1-cDNA	59671	3.95±0.00	2.26±0.00
ADPh2-cDNA	82628	5.97±0.02	2.61±0.00
ADPh3-cDNA	91203	6.75±0.02	2.54±0.00
Anodei-cDNA	125143	2.02±0.01	1.60±0.01
Anodef-cDNA	99696	6.35±0.02	2.55±0.01
Cathodei-cDNA	99324	2.37±0.01	1.76±0.01
Cathodef-cDNA	64740	4.61±0.01	2.23±0.00

522 **3.6.4 Correspondence analysis**

523 Correspondence analysis results for eubacteria and archaea community are shown in Figure 6a
524 and 6b, respectively. Regarding eubacteria, AD samples were clustered together, especially in the
525 case of DNA, while for cDNA form the samples were more disperse and detached from the first
526 ones. This cluster included the Phase 2 DNA sample of the cathode biofilm, which separated lately
527 at the end of the assay. When looking at the Phase 2 cathode sample for active microorganisms
528 (cDNA), it was nearer to the anode samples, although the Phase 3 sample differentiated
529 completely from all the samples analysed. Finally, the anode samples clustered clearly in two
530 groups, one for DNA and a second one for cDNA, showing that the metabolically active population
531 (cDNA sequencing) was different from total population (DNA sequencing).

532 Archaeal results for the AD biomass showed that the three DNA samples were prochain, so little
533 differences in composition were detected between inhibited and stable states. On the contrary,
534 cDNA samples, related to the active microorganisms, were distant from DNA samples and Phase 1
535 and 3 samples were clustered together while Phase 2 sample moved away from them. This means
536 that active microorganisms in the initial sample shifted to a different active community during
537 Phase 2 due to the inhibition and, once recovered with the recirculation loop, went back to the
538 previous composition. Regarding the anode and cathode communities, Phase 2 DNA and cDNA
539 samples resembled and an evolution was observed for the Phase 3 samples, moving away from
540 the initial composition, especially for cDNA. Interestingly, the biocathode cDNA Phase 3 sample
541 approached to the cDNA AD sample of Phase 2, suggesting that the active population of the
542 cathode resembled the AD population under inhibition.

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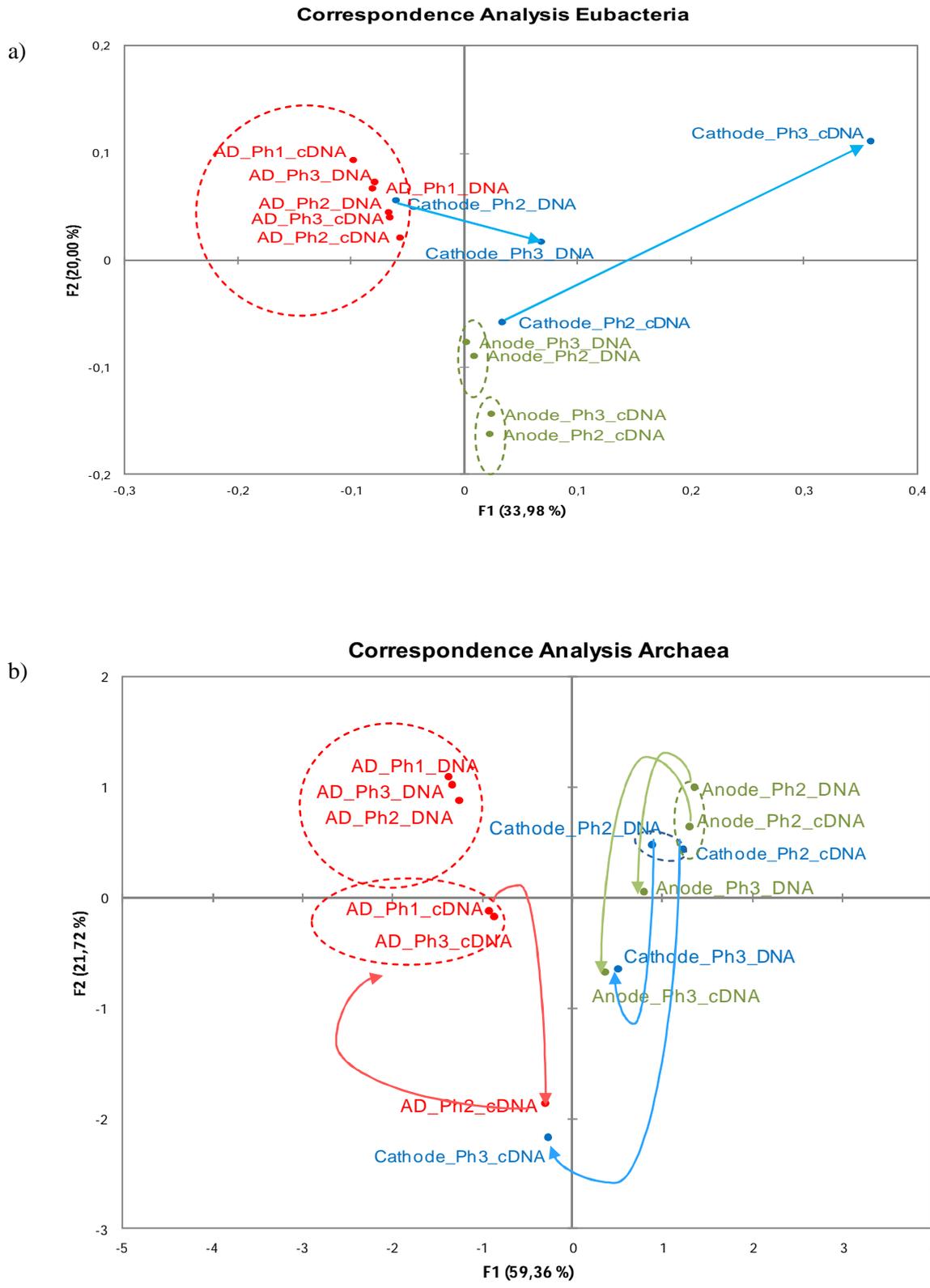


Figure 6 Correspondence Analysis of the AD effluent at the end of Phases 1, 2 and 3, and the biofilm harboured on the anode and cathode of the MEC at the end of Phase 2 and 3 for DNA and cDNA samples regarding (a) Eubacteria and (b) Archaea community.

579

580 **4. Conclusions**

581 The integration of anaerobic digestion (AD) and a microbial electrolysis cell (MEC) with a
582 methanogenic biocathode has proven to be a promising strategy to treat high strength wastewaters
583 with a multiple purpose. The methane production of the AD could be recovered after the inhibition
584 of the reactor due to the doubling of the organic and nitrogen loading rate thanks to the connexion
585 of a recirculation loop with the MEC. Ammonium removal in the anode compartment of the MEC
586 achieved $14.46 \text{ g N-NH}_4^+ \text{ m}^{-2} \text{ d}^{-1}$, while obtaining on average $79 \text{ L CH}_4 \text{ m}^{-3} \text{ d}^{-1}$ through the
587 conversion of CO_2 in the cathode compartment. The microbial analysis showed that methylotrophic
588 *Methanossiliicoccaceae* family (*Methanomassiliicoccus* genus) was the most abundant among
589 active archaea in the AD during the inhibited state. On the other hand, in the cathode
590 *Methanobacteriaceae* family (*Methanobrevibacter* and *Methanobacterium* genera), usually found to
591 be the most abundant in methanogenic biocathodes, shared dominance with
592 *Methanomassiliicoccaceae* (*Methanomassiliicoccus* genus) and *Methanotrichaceae* (*Methanotrix*
593 genus) families.

594

595

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