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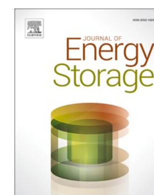

Research Papers

Microbial electromethanogenesis for energy storage: Influence of acidic pH on process performance

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ABSTRACT

Microbial electromethanogenesis (EM) has positioned itself as a promising technology for electrical energy storage using CO_2 as a feedstock. However, the selectivity of the final product remains a challenge, being highly dependent of the operating conditions (temperature, pH, conductivity, etc.). This study tries to understand the role that pH plays on the start-up, performance and the structure of microbial communities of an EM system. To that end, two EM reactors were started at pH 7.0 and 5.5 respectively and were subsequently subjected to pH variations between 7.5 and 3.5. The reactor inoculated at pH 5.5 started to produce CH_4 earlier than that inoculated at pH 7.0, and the acetogenic activity was gradually displaced by methanogenesis during the start-up period, regardless of the pH. In addition, as the pH of the catholyte became more acidic, the performance improved in terms of methane production, current density and columbic efficiency. Acidic environments – pH around 4.5 – promoted higher methane production due to the selection of *Methanobacterium*, an acid-tolerant hydrogenotrophic archaea. When pH was set at 3.5, the overall performance declined sharply, probably because it induced unfavourable physiological conditions.

1. Introduction

The decarbonisation of the energy and chemical sectors, which has become a global priority, has fuelled the research and development of microbial electrosynthesis (MES), a technology that has emerged in the recent years as an alternative to more conventional organic synthesis processes [1]. MES is based on the ability of certain microorganisms (usually referred to as electrotrophs [2]) to catalyse the electrochemical reduction of CO_2 to a wide range of organic compounds [3–6]. When the end product is methane, MES is usually termed as microbial electromethanogenesis (EM). EM represents a novel power-to-gas alternative for electrical energy storage [7], and in recent years it has attracted a considerable interest among scientists and engineers because of its advantages in comparison to conventional abiotic electrocatalytic methanogenesis [8]. To illustrate, EM can be carried out at ambient conditions (temperature and pressure) using microorganisms as catalysts, which potentially makes EM a more environmentally friendly and cost-effective way of energy storage and methane production. However, important technological constraints are hampering the development of MES and EM to a practical scale [9]. One of them is the low selectivity of MES, that mainly depends on reactor configuration [10,11], and on various operating parameters such as cathode potential, pH, temperature, buffering capacity and the composition and concentration of catholyte [12,13]. Among them, pH plays a key role, as it has a direct impact not only on the process itself but also on the selection of the microorganisms that will populate the biofilm. When mixed cultures are used as inoculum, methanogens and homoacetogens can compete for H_2 [1], a common intermediate usually found in biocathodes during the conversion of CO_2 to both methane and carboxylates [14]. It is known that most mesophilic methanogens have an optimal growth pH in the range between 6.5 and 8.0 [15] and that their methanogenic activity is inhibited when the pH drops below 4.5 [16,17]. In contrast, homoacetogens prefer a mild acidic to neutral pH and are inhibited at high alkaline pH [18]. Acetogenesis and chain elongation in particular have an optimal range between 7 and 5 [19,20], and it has been observed that reducing the pH from 7 to 5.8 increases substrate (CO_2) availability and enhances production rate [21].

In addition, since hydrogen is a typical intermediate in EM, an acidic pH would favour (from a thermodynamic point of view) the hydrogen evolution reaction and, as a result, methane productivity. However, as

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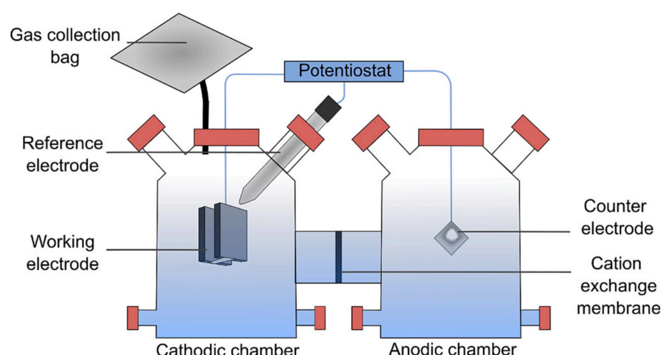

Fig. 1. Reactor diagram: the anodic chamber contains a platinum mesh anode (counter electrode) and is open to air. The cathodic chamber contains a pair of carbon felt electrodes (biocathode). The diagram also includes a bag to store gases, reference electrode and connections for sampling and bubbling N_2 between cycles.

discussed above, pH also affects the physiology of the microbial biomass that catalyse the cathodic reactions and so, although a low pH would benefit the cathodic reactions, it would be detrimental for methanogens. Moreover, an acidic pH could also create a favourable environment for acetogens, a group of microorganism that compete with methanogens for hydrogen. In summary, pH would be playing an ambivalent role in EM that might be having a definite impact on both, performance (e.g.: current density, productivity, energy efficiency, etc.) and product selectivity. To our best knowledge, this topic has not been addressed before in the literature. However, we believe it is worth investigating the role of pH in bioelectromethanogenesis before undertaking any serious scale-up attempt, as this parameter might affect not only product selectivity and performance, but also other critical factors such as CO_2 solubility. Therefore, in this paper we try to study and understand the influence that pH has on product selectivity and on product formation during the inoculation and start-up of an EM system. To that end, we inoculate two biocathodes at different pH values: 5.5 (more favourable for the development of homoacetogenic microorganisms) and 7.5 (more suitable for methanogens proliferation). In addition, since acidic pH would favour (in theory) the cathodic reaction, we also aim at investigating the effect of pH values in the range between 6.5 and 3.5 on EM performance (in terms of current density, product formation and current efficiency) and on the resilience of cathodophilic communities (specially of electrotrophs and methanogens).

2. Materials and methods

2.1. Reactors

Two standard H-type reactors with an internal volume of 500 mL per chamber were used. The biocathodes were made of two 2 × 6 × 0.5 cm carbon felt plates (SGL Group, Germany) attached by a titanium wire and suspended inside the cathodic chamber (surface area: 0.0064 m^2). Prior to inoculation they were pre-treated by subsequent immersion in nitric acid (1 M), acetone (1 M) and ethanol (1 M) during 24 h each to avoid hydrophobicity and impurities [22]. A pre-treated cation exchange membrane (CMI7000, Membranes International, USA) was used to separate the anodic and cathodic compartments. The counter electrodes were made of 2 × 2 cm platinum mesh (Goodfellow, UK) suspended inside the anodic chamber with titanium wire.

All cells worked on a three-electrode configuration using an Ag/AgCl commercial reference electrode (Sigma-Aldrich, USA) (0.20 vs. SHE; the stability of the reference electrode was checked prior to every batch cycle) at an applied potential of −1 V vs Ag/AgCl. All the electrochemical tests were performed with a Biologic VSP potentiostat assisted by EC-Lab® software (v11.30).

Table 1
Experimental conditions.

Reactor name	Set pH (cathodic chamber)	Actual pH	
		pH	Standard deviation
P7	7.5	7.2	0.27
P5	6.5	6.49	0.10
	5.5	5.64	0.05
	4.5	4.48	0.08
	3.5	3.46	0.05
Abiotic	3.5	3.45	0.07

Appropriate connections and sealings were designed for sampling ports and substrate supply as illustrated in Fig. 1. Gas was collected from 1 L bag (Ritter, Germany). Reactors were kept in a constant temperature chamber (30 ± 1 °C).

The catholyte was continuously stirred using a magnetic stirrer at 200 rpm in order to prevent mass transfer limitations [23].

2.2. Electrolytes

The anolyte consisted of 0.1 M potassium phosphate buffer in deionised water. The catholyte consisted of 20 mM potassium phosphate buffer, macronutrients (280 mg·L^{-1} NH_4Cl, 5.7 mg·L^{-1} $CaCl_2$, 10 mg·L^{-1} $MgSO_4 \cdot 7H_2O$, and 90 mg·L^{-1} $MgCl_2 \cdot 6H_2O$), 1 mL·L^{-1} of a micronutrients solution, and 1 mL·L^{-1} of a vitamin solution as described in [24].

At the beginning of each batch cycle 300 mL of CO_2 were fed to the cathodic chamber by means of the gas bag. The pH of the bulk catholyte was set (see Section 2.3) by adding HCl 1 M.

2.3. Operation

The influence of pH was determined at 2 different stages: during start-up and on already developed biocathodes.

2.3.1. Start-up operation

Anaerobic sludge obtained from an anaerobic digester in a local wastewater treatment plant was used as primary inoculum (1:5 proportion) as it has been described before [25]. Although single species can provide better controlled environments and higher specific production, mixed cultures offer higher flexibility and resilience [14]. Reactors were operated in batch-mode in cycles of 7 days duration. The electrolytes and the gas bag were fully replenished at the beginning of each cycle. The cathodic chamber of one of the reactors – that will be referred to as P7 – was inoculated and operated during the whole start-up period at pH 7, while the other reactor (referred to as P5) was inoculated and operated during the same period at pH 5.5. The start-up duration was 14 cycles.

2.3.2. Operation of mature electrodes

As it will be shown in Section 3, the reactor P5 showed a much better performance than P7. Consequently, it was hypothesized that since a relatively low pH benefits electro-methanogenesis, it might be possible to obtain an improved performance by further reducing pH. Therefore, after the start-up, and once the reactors achieved a stable performance, the pH in reactor P5 (that was already acclimated to acidic conditions) was modified according to the program presented in Table 1.

All the tests were conducted in triplicates, and the sequence of experiments randomised to avoid habituation effects.

To assess the possible occurrence of abiotic hydrogen production, an additional reactor (called "abiotic") was assembled with an identical configuration to that described in Section 2.1. It was operated in abiotic conditions using the same electrolytes described in Section 2.2, although the pH of the catholyte was set at 3.5 to create the most favourable condition for abiotic hydrogen production (catholyte pH 3.5).

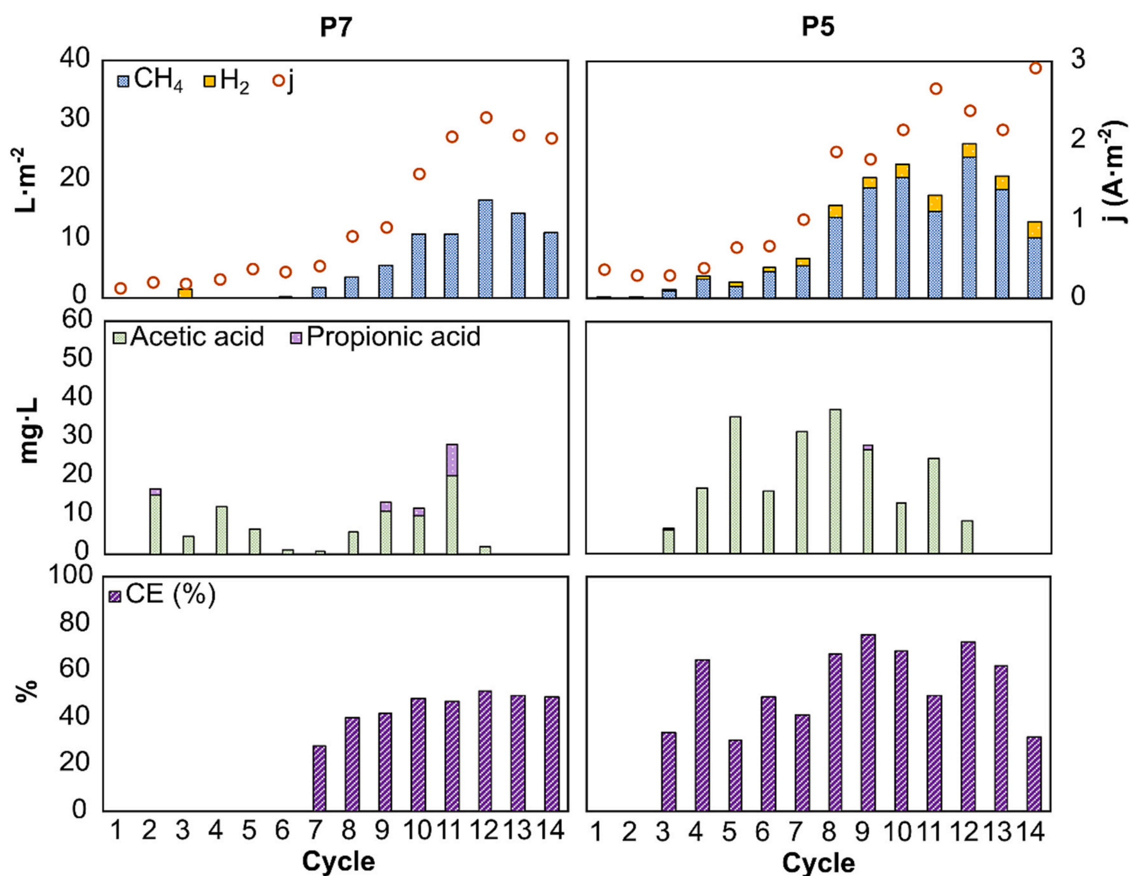

Fig. 2. Performance of reactors P5 and P7 during the start-up. Top: methane and hydrogen production normalised to projected electrode surface area (bars), and current density (dots). Middle: acetic and propionic acid production. Bottom: current efficiency (CE).

2.4. Analytical techniques

Liquid samples were analysed for total organic carbon (TOC), total inorganic carbon (IC), total nitrogen (TN; Multi N/C 3100, Analytikjena) and volatile fatty acids (VFAs) from C_2 to C_6 (Bruker 450-GC with a flame ionisation detector (FID)). Dissolved oxygen (Hach, HQ40d two-channel digital multimeter), redox (pH Meter, pH 91; Wissenschaftlich Technische Werkstätten, WTW), pH (pH Meter BASIC 20+, Crison) and ammonium (781 pH/Ion Meter, Metrohm) were measured following standard methodologies [22].

At the end of each batch cycle, the gas bag was disconnected from the reactor and the amount of gas in the bag (V_g) was measured with the aid of a gastight syringe (50 mL, Hamilton SampleLock syringe). Gas composition (H_2, CO_2, O_2, N_2 and CH_4) were determined by a gas chromatography (Varian CP3800 GC) equipped with a thermal conductivity detector (TCD) [22]. The volume of hydrogen and methane produced in each cycle was calculated from V_g and the gas mole fraction in the gas bag, and was corrected to the standard temperature and pressure (STP) conditions.

The electrochemical performance of the biocathodes was characterised by means of cyclic voltammograms (CV) tests using a Biologic VSP potentiostat. CV tests were performed in turnover and non-turnover conditions (i.e., in the presence and absence of CO_2 respectively) between −1.0 and 0.1 V vs. Ag/AgCl and at a scan rate of 1 mV·s^{-1} at a temperature of 30 °C.

2.5. Molecular biology techniques

Cathode samples were taken from both reactors. Samples of reactor P7 were taken at the end of the experiment while samples of reactor P5 were taken at the end of the conditions 5.5 and 3.5 (Table 1).

These samples were used to characterise the microorganisms that had developed in the methane-producing biocathode. The inoculum was also analysed.

Microbial communities were analysed and followed along the experimental time by high throughput sequencing of massive 16S rRNA gene libraries. Total Bacteria and Archaea were analysed. Genomic DNA was extracted with a DNeasy PowerSoil Kit (Qiagen) according to manufacturer's instructions. All PCR reactions were carried out in a Mastercycler (Eppendorf, Hamburg, Germany), and PCR samples were checked for size of the product on a 1 % agarose gel and quantified by NanoDrop 1000 (Thermo Scientific). The entire DNA extract was used for high-throughput sequencing of 16S rRNA gene-based massive libraries with 16S rRNA gene-based primers V4 515F-806R for Bacteria and V4 515F to 806R for Archaea. The Novogene Company (Cambridge, UK) carried Illumina sequencing out using a HiSeq 2500 PE250 platform.

The obtained DNA reads were compiled into FASTq files for further bioinformatics processing carried out using QIIME software version 1.7.0 [26]. Sequence analyses were performed by Uparse software (v7.0.1001) using all the effective tags. Sequences with ≥97 % similarity were assigned to the same OTUs. Representative sequence for each OTU was screened for further annotation. For each representative sequence, Mothur software was performed against the SSUrRNA database of SILVA Database [27] for species annotation at each taxonomic rank (Threshold: 0.8–1).

The quantitative analysis of all samples was carried out by means of quantitative-PCR (qPCR) using PowerUp SYBR Green Master Mix (Applied Biosystems) in a StepOnePlus Real-Time PCR System (Applied Biosystems) as described previously [25]. The qPCR amplification was performed for the 16S-rRNA gene in order to quantify the entire eubacterial community and for the mcrA gene to quantify the total

methanogen community. The primer sets 314F qPCR (5′-CCTACGG-GAGGCAGCAG-3′) and 518R qPCR (5′-ATTACCGCGGCTGCTGG-3′) at an annealing temperature of 60 °C for 30 s was used for Bacteria and Arc 349F (5′-GYGCASCAGKCGMGAAW-3′) and Arc 806R (5′-GGAC-TACVSGGGTATCTAAT-3′) for Archaea quantification.

3. Results and discussion

3.1. Impact of pH on the start-up process

After inoculation, both biocathodes were cultured for a period of 14 batch cycles (Fig. 2). Results seem to indicate that the acidic conditions in P5 favoured not only a faster start-up, (P5 required only 3 cycles to produce any measurable amount of methane, while it took 7 cycles for P7) but also a better performance: P5 produced, on average, 38 % more methane per cycle and 35 % more current than P7. In addition, the acidic conditions promoted a more efficient use of electrons, as the averaged current efficiency along the 14 cycles was 63 % in P5 against 49 % calculated for P7.

The acidic environment in P5 also seemed to stimulate the production of VFAs: cumulative acetic acid production along the 14 cycles was 216.8 $mg \cdot L^{-1}$ in P5, well above the 88.3 $mg \cdot L^{-1}$ measured in P7. In any case, from cycle 11 onwards, the production of VFAs in both reactors decreased as methane yield reached stabilisation, and it totally stopped on the last two cycles (13 and 14). A similar behaviour has been reported in previous studies: in [28] for instance, the increase in methane content in the biogas during the start-up coincided with the decline in acetic acid levels, indicating a possible displacement by competitive advantage of methanogenic organisms over acetogenic organisms. Methane and VFAs production in MES/EM systems are hydrogen mediated [29,30], so most probably both groups of microorganism (acetogens and methanogens) would be competing for this intermediate in our reactors. Moreover, acetogenic bacteria have a growth rate almost three times higher than methanogenic archaea [31], which would explain the rapid onset of VFAs production during the start-up. However, and despite these competitive advantage of acetogens, methanogens can gain more energy from the consumption of H_2 and CO_2 [28] and therefore, we can expect in the long run a gradual increase in methane production accompanied by a drop in VFAs production, which is consistent with the results presented in Fig. 2. In addition, and as it will be discussed in Section 3.3 (microbiology analyses), hydrogenotrophic methanogens experienced a remarkable growth compared to acetogenic microorganisms, a result that would support this hypothesis.

3.2. Impact of acidic pH on EM performance

Current density began to be repeatable in both reactors on cycles 10 to 14, so it was assumed that the cathodic biofilms were mature enough to start the next experimental stage. The aim of this new experimental phase was to investigate the impact of acidic pH on methane production. To that end, the pH of the catholyte in reactor P5 was gradually modified (from 6.5 to 3.5), while the pH in P7 was maintained at around 7 (Table 1). This resulted in significant variations in the performance of P5: on the one hand, current density tended to increase as the pH dropped (Fig. 3 and Fig. 4 top), probably because of the greater availability of H_3O^+ at low pH, that reduces concentration overpotentials and favours current production. However, when the pH was further reduced to 3.5, current density declined sharply, which might be revealing unfavourable physiological conditions for electrotrophic bacteria when the pH falls below a certain limit. According to our results, this threshold would be somewhere in the range between 3.5 and 4.5, although it must be noted that since current did not collapse at pH 3.5, the biofilm would probably endure a more acidic pH (in this study we decided not to go beyond 3.5 to avoid any permanent harm of the biofilm). In addition, to confirm that the measured current is due exclusively to the biological activity of electrotrophs, the electrodes were polarized at the same potential, although in abiotic conditions (i.e.: non-inoculated electrodes). Results indicate that even in the most favourable conditions for the abiotic cathode (pH 3.5) the measured current was negligible (Figs. 4 and 5).

Interestingly, methane production followed a similar trend as current, doubling at pH 4.5 (in relation to that measured at pH 7.5), and sharply declining at pH 3.5 (Fig. 4 top). This confirms a close connection between current and methane, with hydrogen acting as an intermediary

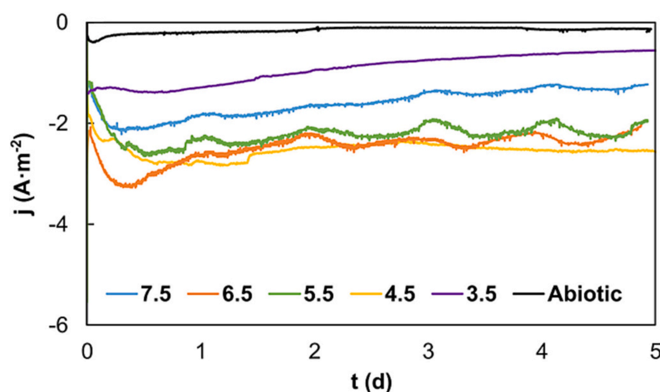

Fig. 3. Typical current density profiles obtained for each experimental condition.

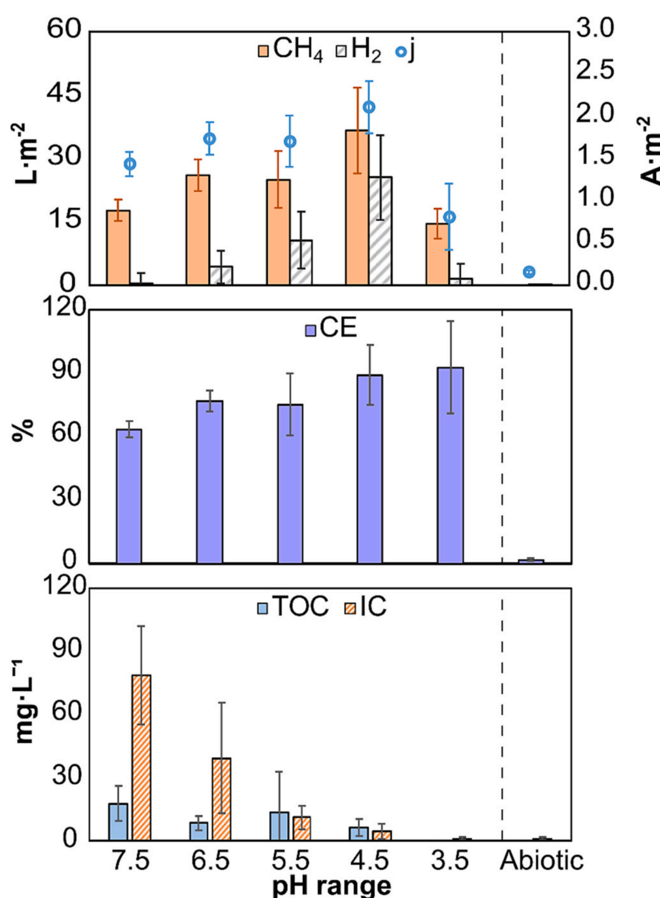

Fig. 4. Experimental results averaged across 3 replicates for each experimental condition. Top: methane and hydrogen production per electrode surface area (bars) and current density (dots). Middle: current efficiency. Bottom: total organic carbon (TOC) and inorganic carbon (IC) measured in the catholyte.

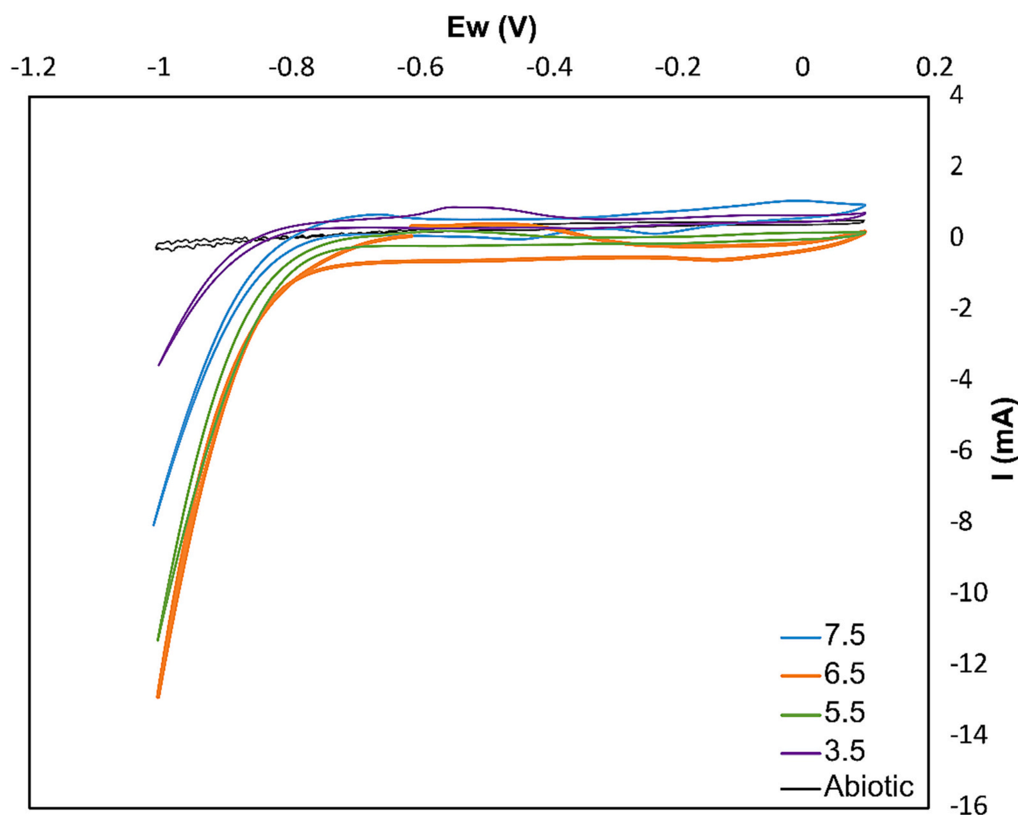

Fig. 5. Cyclic voltammograms for each experimental condition and for the abiotic cathode.

between them (evidenced by the presence of this gas in the reactor headspace). It is also important to note that the amount of hydrogen increased steadily as the pH dropped (up until pH 4.5) (Fig. 4 top), which seems to indicate that acidic pH induces a metabolic imbalance between electrotrophs and methanogens, probably caused by the inability of the latter to absorb the surplus of hydrogen produced by the former, or even by the relatively low growth rate of archaea (methanogens) compared to bacteria (electrotrophs) [31].

Contrary to our expectations, the current efficiency also increased as the catholyte became more acidic, reaching its maximum at pH 3.5 (Fig. 4 middle). This implies that even though hydrogen and methane yields were severely limited at pH 3.5, the electrons were being used more efficiently in the production of these gases, which in turn means that less electrons are being diverted to other secondary products at low pH. The only possible electron sinks alternative to hydrogen and methane in our reactors are dissolved organic compounds and biomass proliferation. Organic compounds – measured as the concentration of total organic carbon (TOC) at the end of the batch cycles (Fig. 4 bottom) – were detected only sporadically in the catholytes and at relatively low concentrations (TOC reached its maximum concentration of 18 $mg \cdot L^{-1}$ at pH 7.5). In addition, the composition of the TOC was very inconsistent throughout cycles, consisting mainly of acetic acid (maximum concentration of 7 $mg \cdot L^{-1}$) and small amounts of propionic and butyric acids (results not shown). Results also indicate that TOC tends to decrease as the pH drops (Fig. 4 bottom), and therefore the only plausible explanation for the improvement in current efficiency can be found in a reduced growth rate of bacteria and archaea (it is known that at such a low pH the growth rates of both domains gets greatly diminished [32,33]). This hypothesis is supported by the results of the microbial analysis (see Section 3.3) that showed a notable decline in the population of both bacteria and archaea. In short, it seems that as the pH drops, less electrons are diverted to biomass proliferation, and therefore (in proportion) more electrons are available for methane production.

In addition, all these results would corroborate the syntrophic relationship between the hydrogenic electrotrophs and the hydrogenotrophic methanogens regardless of the bulk pH. CV analyses also seem to confirm the role of hydrogen as a metabolic intermediary between these two groups of microorganisms: the voltammograms shown in Fig. 5 exhibited the characteristic reduction waves associated to the H_2 evolution reaction [34,35]. In addition, the voltammograms at pH 6.5 and 5.5 showed the largest current peaks, being both notably larger than the peak obtained at pH 7.5, which is coherent with the better performance observed during the batch tests (Fig. 4).

The low signal detected at pH 3.5 would also be consistent with the partial inhibition of electrotrophs as discussed above. As expected, the abiotic electrode failed to produce any observable signal.

3.3. Impact of acidic pH on EM microbiology

To further understand the implications of acidic pH on EM performance, biofilm samples for microbiology analyses were taken at the end of the experiments at pH 7.5, 5.5 and 3.5. Relative abundances analyses revealed large differences between the microbial composition of the inoculum and that of the cathodic biofilms (Fig. 6). The most striking feature was perhaps the considerable proliferation of *Methanobacterium*, a hydrogenotrophic methanogenic archaeon [14,36,37] with an optimal growth pH of 7, and whose proliferation can be inhibited at pH $\leq$ 5.5 [38–40]. The reason why this genus thrived – and even maintained productivity – in our biofilms cultured at pH 5.5 and 3.5 is uncertain. A possible explanation can perhaps be found in the relatively high local pH caused by mass transfer limitations on the surface of the biocathodes [41], that might be preventing the biofilm from been exposed to the harmful low bulk pH. This, in combination with the availability of H_2 on the biofilm, may have favoured its abundance against other methanogenic archaea such as *Methanobrevibacter* or *Methanosaeta* [42,43].

Regarding bacteria, it is noteworthy the relatively high abundances of *Desulfovibrio* and *Petrimonas* at pH 5.5 and 3.5. These are two electroactive microorganisms that have been described in biocathodes as

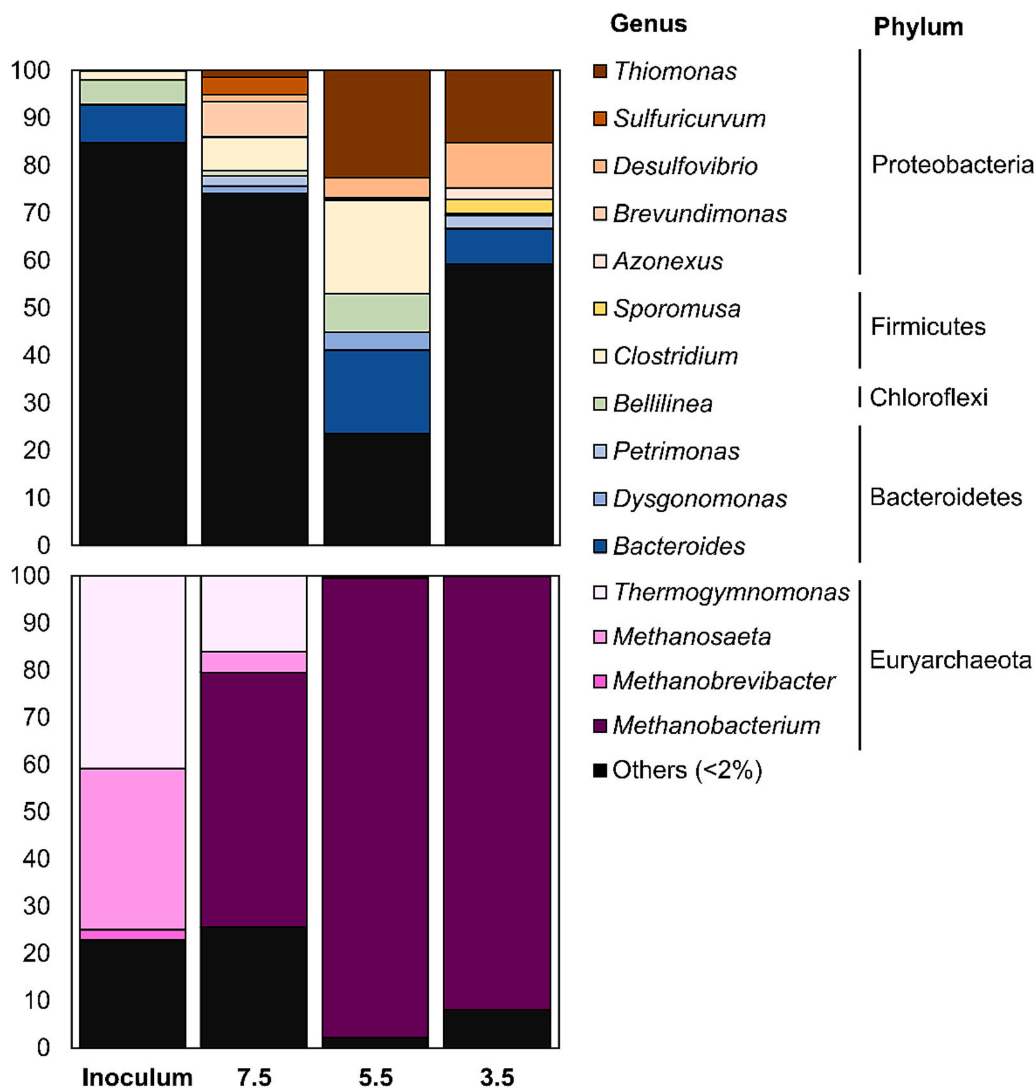

Fig. 6. Relative abundance for bacteria (top) and Archaea (bottom).

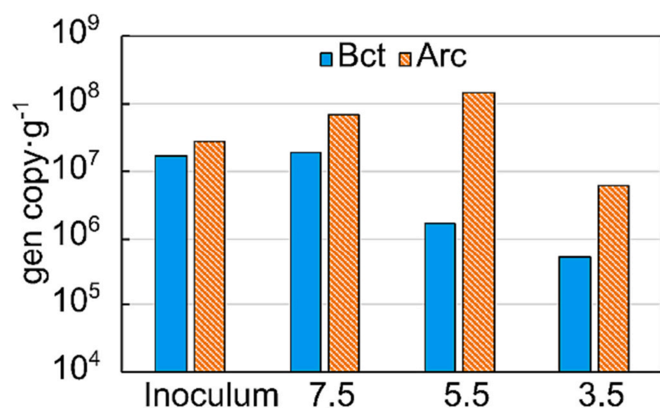

Fig. 7. qPCR in terms of total bacteria (Bct) and archaea (Arc) for the inoculum and at the different pH tested.

being capable of producing H_2 via direct extracellular electron transfer [34,44,45], and whose presence, together with *Methanobacterium*, supports the theory of a syntrophic relationship between electrotrophs and methanogens. *Desulfovibrio* species have been found to be capable of growing in biocathodes at a pH as low as 3.0 [46], although most of its members have an optimal growth range between 5.5 and 7.0 [47]. For *Petrimonas*, the optimal pH is found in the range between 7.1 and 7.8 [48]. As discussed above, the relatively high pH on the surface of the cathode would provide a suitable environment for the proliferation of these two genera, even when the bulk pH drops down to 3.5 (Fig. 6). Moreover, *Petrimonas* and *Desulfovibrio*, whose metabolism involves direct extracellular electron transfer, must grow in close contact with the cathode on the deep layers of the biofilm, where they are protected by the more superficial layers that are more exposed to the bulk pH.

Nevertheless, and despite the increase in their relative abundance, the total bacterial population experienced a decline of almost two orders of magnitude, as revealed by quantitative analyses (Fig. 7), which implies a decline in absolute numbers for both, *Petrimonas* and *Desulfovibrio*. This is not entirely coherent with the CV results (Fig. 5) and with the relatively high current densities measured in the batch tests at pH 5.5. It seems that, to compensate for the lower number of electrotrophic bacteria, the electron transfer process became more efficient as pH becomes more acidic, probably because as pH drops the thermodynamics of hydrogen production are more favourable.

Therefore, pH can be considered as having an ambivalent role on the performance of methanogenic biocathodes: on the one hand it affects negatively the viability of electroactive and methanogenic bacteria

while, on the other hand, it improves both the electron transfer processes and the conversion of electrical current into methane as revealed by current efficiency results in Fig. 4 middle.

Clostridium, a genus capable of reducing CO_2 to acetate in the presence of hydrogen [1,22], is another bacteria whose relative abundance experienced a significant increase at pH 7.5, and specially at pH 5.5, compared to the inoculum. *Sporomusa*, another well-known homoacetogenic bacteria usually found in biocathodes [49], also exhibited a relevant presence at pH 3.5. These two genera might be responsible for the acetate detected in the catholyte during the start-up (Fig. 2). The virtual absence of VFAs during the experimental phase (see Section 3.2), might be explained by their fast consumption by the acetoclastic methanogen *Methanosaeta* at pH 7.5 [50], or even by the outcompetition of homoacetogens by *Methanobacterium*, that experienced, as we have seen, a notable increase in relative abundance as pH becomes more acidic.

Finally, the biocathodes also furnished a suitable environment for the proliferation of *Thiomonas*, an extremophile genus occasionally found in biocathodes [51] that is related to sulphide removal and whose role in our biocathodes is not clear.

4. Conclusions

Two EM reactors were inoculated at a pH 5.5 and 7.5 respectively. pH did not seem to have any impact on product selectivity: in both situations, and during the first 11 cycles of batch operation, the main end products were acetate and methane. Nevertheless, the more acidic conditions contributed to a faster start-up and a faster product formation. In addition, acetate was almost totally displaced by methane after 11 batch cycles. In subsequent tests, as the pH of the catholyte was gradually reduced, current density and methane production increased and peaked at a pH of 4.5. Further reducing pH to 3.5 resulted in a notable deterioration of performance, although the cathodic efficiency improved slightly, which reveals that despite the decline in production rates, the current is used more efficiently in the production of methane.

The acidic conditions also favoured the proliferation of the hydrogenotrophic methanogenic archaea *Methanobacterium*, growing, probably, in syntrophy with hydrogen producing bacteria such as *Desulfovibrio* or *Petrimonas* confirming thus the role of H_2 as the main intermediate between electron uptake and CO_2 reduction.

CRediT authorship contribution statement

Guillermo Pelaz: Conceptualization, Investigation, Methodology, Writing-original draft.

Daniela Carrillo-Peña: Investigation, Methodology.

Antonio Morán: Supervision, Funding acquisition, Project administration.

Adrián Escapa: Conceptualization, Supervision, Formal analysis, Writing - reviewing and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

[1] P. Dessì, L. Rovira-Alsina, C. Sánchez, G.K. Dinesh, W. Tong, P. Chatterjee, M. Tedesco, P. Farràs, H.M.V. Hamelers, S. Puig, Microbial electrosynthesis: towards sustainable biorefineries for production of green chemicals from CO_2 emissions, Biotechnol. Adv. 46 (2021), 107675, https://doi.org/10.1016/j.biotechadv.2020.107675.

[2] X. Li, L. Ding, H. Yuan, Xiaoming Li, Y. Zhu, Identification of potential electrotrophic microbial community in paddy soils by enrichment of microbial electrolysis cell biocathodes, J. Environ. Sci. (China) 87 (2020) 411–420, https://doi.org/10.1016/j.jes.2019.07.016.

[3] S. Cheng, D. Xing, D.F. Call, B.E. Logan, Direct biological conversion of electrical current into methane by electromethanogenesis, Environ. Sci. Technol. 43 (2009) 3953–3958, https://doi.org/10.1021/es803531g.

[4] A.W. Jeremiasse, H.V.M. Hamelers, C.J.N. Buisman, Microbial electrolysis cell with a microbial biocathode, Bioelectrochemistry 78 (2010) 39–43, https://doi.org/10.1016/j.bioelechem.2009.05.005.

[5] K. Rabaey, S.T. Read, P. Clauwaert, S. Freguia, P.L. Bond, L.L. Blackall, J. Keller, Cathodic oxygen reduction catalyzed by bacteria in microbial fuel cells, ISME J. 2 (2008) 519–527, https://doi.org/10.1038/ismej.2008.1.

[6] C. Zhu, H. Wang, Q. Yan, R. He, G. Zhang, Enhanced denitrification at biocathode facilitated with biohydrogen production in a three-chambered bioelectrochemical system (BES) reactor, Chem. Eng. J. 312 (2017) 360–366, https://doi.org/10.1016/j.cej.2016.11.152.

[7] M. Shahparasti, S. Bouchakour, A. Luna, D. Molognoni, P. Bosch-Jimenez, E. Borràs, Simplified modelling of nonlinear electromethanogenesis stack for power-to-gas applications, J. Energy Storage 31 (2020), 101633, https://doi.org/10.1016/J.EST.2020.101633.

[8] N. Ali, M. Bilal, M.S. Nazir, A. Khan, F. Ali, H.M.N. Iqbal, Thermochemical and electrochemical aspects of carbon dioxide methanation: a sustainable approach to generate fuel via waste to energy theme, Sci. Total Environ. 712 (2020), 136482, https://doi.org/10.1016/J.SCITOTENV.2019.136482.

[9] C.M. Cordas, J.J.G. Moura, A. Escapa, R. Mateos, Carbon dioxide utilization—bioelectrochemical approaches, in: J.J.G. Moura, I. Moura, Louisa Maia, B. (Eds.), Enzymes for Solving Humankind's Problems, Springer, 2020, pp. 83–108, https://doi.org/10.1007/978-3-030-58315-6_3.

[10] B. Bian, S. Bajracharya, J. Xu, D. Pant, P.E. Saikaly, Microbial electrosynthesis from CO_2: challenges, opportunities and perspectives in the context of circular bioeconomy, Bioresour. Technol. (2020), https://doi.org/10.1016/j.biortech.2020.122863.

[11] Q. Fu, Y. He, Z. Li, J. Li, L. Zhang, X. Zhu, Q. Liao, Direct CO_2 delivery with hollow stainless steel/graphene foam electrode for enhanced methane production in microbial electrosynthesis, Energ. Conver. Manage. 268 (2022), 116018, https://doi.org/10.1016/J.ENCONMAN.2022.116018.

[12] H.Y. Yang, B.L. Bao, J. Liu, Y. Qin, Y.R. Wang, K.Z. Su, J.C. Han, Y. Mu, Temperature dependence of bioelectrochemical CO_2 conversion and methane production with a mixed-culture biocathode, Bioelectrochemistry 119 (2018) 180–188, https://doi.org/10.1016/j.bioelechem.2017.10.002.

[13] G. Pelaz, D. Carrillo-Peña, A. Morán, A. Escapa, Electromethanogenesis at medium-low temperatures: impact on performance and sources of variability, Fuel 310 (2022), 122336, https://doi.org/10.1016/j.fuel.2021.122336.

[14] A. ter Heijne, F. Geppert, T.H.J.A. Sleutels, P. Batlle-Vilanova, D. Liu, S. Puig, Mixed culture biocathodes for production of hydrogen, methane, and carboxylates, in: Advances in Biochemical Engineering/Biotechnology, Springer Science and Business Media Deutschland GmbH, 2019, pp. 203–229, https://doi.org/10.1007/10_2017_15.

[15] H. Zhou, D. Xing, M. Xu, Y. Su, J. Ma, I. Angelidaki, Y. Zhang, Optimization of a newly developed electromethanogenesis for the highest record of methane production, J. Hazard. Mater. 124363 (2020), https://doi.org/10.1016/j.jhazmat.2020.124363.

[16] A.A. Kovalev, D.A. Kovalev, E.A. Zhuravleva, I.V. Katraeva, V. Panchenko, U. Fiore, Y.V. Litti, Two-stage anaerobic digestion with direct electric stimulation of methanogenesis: the effect of a physical barrier to retain biomass on the surface of a carbon cloth-based biocathode, Renew. Energy 181 (2022) 966–977, https://doi.org/10.1016/J.RENENE.2021.09.097.

[17] Y. Li, Y. Zhang, Y. Liu, Zhiqiang Zhao, Zisheng Zhao, S. Liu, H. Zhao, X. Quan, Enhancement of anaerobic methanogenesis at a short hydraulic retention time via bioelectrochemical enrichment of hydrogenotrophic methanogens, Bioresour. Technol. (2016), https://doi.org/10.1016/j.biortech.2016.06.112.

[18] G. Mohanakrishna, J.S. Seelam, K. Vanbroekhoven, D. Pant, An enriched electroactive homoacetogenic biocathode for the microbial electrosynthesis of acetate through carbon dioxide reduction, Faraday Discuss. 183 (2015) 445–462, https://doi.org/10.1039/C5FD00041F.

[19] P. Izadi, J.M. Fontmorin, S.S. Lim, I.M. Head, E.H. Yu, Enhanced bio-production from CO_2 by microbial electrosynthesis (MES) with continuous operational mode, Faraday Discuss. 230 (2021) 344–359, https://doi.org/10.1039/D0FD00132E.

[20] I. Vassilev, F. Kracke, S. Freguia, J. Keller, J.O. Krömer, P. Ledezma, B. Virdis, Microbial electrosynthesis system with dual biocathode arrangement for simultaneous acetogenesis, solventogenesis and carbon chain elongation, Chem. Commun. 55 (2019) 4351–4354, https://doi.org/10.1039/C9CC00208A.

[21] P. Batlle-Vilanova, S. Puig, R. Gonzalez-Olmos, M.D. Balaguer, J. Colprim, Continuous acetate production through microbial electrosynthesis from CO_2 with

microbial mixed culture, J. Chem. Technol. Biotechnol. (2015), https://doi.org/10.1002/jctb.4657 n/a-n/a.

[22] R. Mateos, A. Sotres, R.M. Alonso, A. Escapa, A. Morán, Impact of the start-up process on the microbial communities in biocathodes for electrosynthesis, Bioelectrochemistry 121 (2018) 27–37, https://doi.org/10.1016/j.bioelechem.2018.01.002.

[23] M. Villano, F. Aulenta, C. Ciucci, T. Ferri, A. Giuliano, M. Majone, Bioelectrochemical reduction of CO_2 to CH_4 via direct and indirect extracellular electron transfer by a hydrogenophilic methanogenic culture, Bioresour. Technol. 101 (2010) 3085–3090, https://doi.org/10.1016/j.biortech.2009.12.077.

[24] M.C.A.A. Van Eerten-Jansen, A.B. Veldhoen, C.M. Plugge, A.J.M. Stams, C.J. N. Buisman, A. Ter Heijne, Microbial community analysis of a methane-producing biocathode in a bioelectrochemical system, Archaea 2013 (2013).

[25] R.M. Alonso, A. Escapa, A. Sotres, A. Morán, Integrating microbial electrochemical technologies with anaerobic digestion to accelerate propionate degradation, Fuel 267 (2020), 117158, https://doi.org/10.1016/j.fuel.2020.117158.

[26] J.G. Caporaso, J. Kuczynski, J. Stombaugh, K. Bittinger, F.D. Bushman, E. K. Costello, N. Fierer, A.G. Pẽa, J.K. Goodrich, J.I. Gordon, G.A. Huttley, S. T. Kelley, D. Knights, J.E. Koenig, R.E. Ley, C.A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J.R. Sevinsky, P.J. Turnbaugh, W.A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, R. Knight, QIIME allows analysis of high-throughput community sequencing data, Nat. Methods (2010), https://doi.org/10.1038/nmeth.f.303.

[27] Q. Wang, G.M. Garrity, J.M. Tiedje, J.R. Cole, Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy, Appl. Environ. Microbiol. 73 (2007) 5261–5267, https://doi.org/10.1128/AEM.00062-07.

[28] D.S. Molenaar, P. Saha, R.A. Mol, H.T. Sleutels, A. ter Heijne, J.C. Buisman, Competition between methanogens and acetogens in biocathodes: a comparison between potentiostatic and galvanostatic control, Int. J. Mol. Sci. (2017), https://doi.org/10.3390/ijms18010204.

[29] E. Blanchet, F. Duquenne, Y. Rafrafi, L. Etcheverry, B. Erable, A. Bergel, Importance of the hydrogen route in up-scaling electrosynthesis for microbial CO_2 reduction, Energ. Environ. Sci. 8 (2015) 3731–3744, https://doi.org/10.1039/C5EE03088A.

[30] G. Mohanakrishna, K. Vanbroekhoven, D. Pant, Imperative role of applied potential and inorganic carbon source on acetate production through microbial electrosynthesis, J. CO_2 Util. (2016), https://doi.org/10.1016/j.jcou.2016.03.003.

[31] M.R. Mattei, B. D'Acunto, G. Esposito, L. Frunzo, F. Pirozzi, Mathematical modeling of competition and coexistence of sulfate-reducing bacteria, acetogens, and methanogens in multispecies biofilms, Desalin. Water Treat. 55 (2014) 740–748, https://doi.org/10.1080/19443994.2014.937764.

[32] Q. Jin, M.F. Kirk, pH as a primary control in environmental microbiology: 1. Thermodynamic perspective, Front. Environ. Sci. 6 (2018) 21, https://doi.org/10.3389/FENVS.2018.00021/BIBTEX.

[33] G.M. Maestrojuan, D.R. Boone, Characterization of *Methanosarcina barkeri* MS(T) and 227, *Methanosarcina mazei* S-6(T), and *Methanosarcina vacuolata* Z-761(T), Int. J. Syst. Bacteriol. 41 (1991) 267–274, https://doi.org/10.1099/00207713-41-2-267/CITE/REFWORKS.

[34] F. Aulenta, L. Catapano, L. Snip, M. Villano, M. Majone, Linking bacterial metabolism to graphite cathodes: electrochemical insights into the H_2-producing capability of *Desulfovibrio* sp, ChemSusChem 5 (2012) 1080–1085.

[35] R.A. Rozendal, A.W. Jeremiasse, H.V.M. Hamelers, C.J.N. Buisman, Hydrogen production with a microbial biocathode, Environ. Sci. Technol. 42 (2008) 629–634, https://doi.org/10.1021/es071720+.

[36] J. Li, Z. Li, S. Xiao, Q. Fu, H. Kobayashi, L. Zhang, Q. Liao, X. Zhu, Startup cathode potentials determine electron transfer behaviours of biocathodes catalysing CO_2 reduction to CH_4 in microbial electrosynthesis, J. CO_2 Util. 35 (2020) 169–175, https://doi.org/10.1016/j.jcou.2019.09.013.

[37] H. Zhou, D. Xing, M. Xu, Y. Su, Y. Zhang, Biogas upgrading and energy storage via electromethanogenesis using intact anaerobic granular sludge as biocathode, Appl. Energy 269 (2020), 115101, https://doi.org/10.1016/J.APENERGY.2020.115101.

[38] N. Cuzin, A.S. Ouattara, M. Labat, J.L. Garcia, *Methanobacterium congolense* sp. nov., from a methanogenic fermentation of cassava peel, Int. J. Syst. Evol. Microbiol. 51 (2001) 489–493, https://doi.org/10.1099/00207713-51-2-489/CITE/REFWORKS.

[39] C. Joulian, B.K.C. Patel, B. Ollivier, J.L. Garcia, P.A. Roger, *Methanobacterium oryzae* sp. nov., a novel methanogenic rod isolated from a Philippines ricefield, Int. J. Syst. Evol. Microbiol. 50 (2000) 525–528, https://doi.org/10.1099/00207713-50-2-525/CITE/REFWORKS.

[40] S. Kotelnikova, A.J.L. Macario, K. Pedersen, *Methanobacterium subterraneum* sp. nov., a new alkaliphilic, eurythermic and halotolerant methanogen isolated from deep granitic groundwater, Int. J. Syst. Bacteriol. 48 (1998) 357–367, https://doi.org/10.1099/00207713-48-2-357/CITE/REFWORKS.

[41] Z. Wang, H. Deng, L. Chen, Y. Xiao, F. Zhao, In situ measurements of dissolved oxygen, pH and redox potential of biocathode microenvironments using microelectrodes, Bioresour. Technol. 132 (2013) 387–390, https://doi.org/10.1016/j.biortech.2012.11.026.

[42] N. Aryal, T. Kvist, F. Ammam, D. Pant, L.D.M. Ottosen, An overview of microbial biogas enrichment, Bioresour. Technol. (2018), https://doi.org/10.1016/j.biortech.2018.06.013.

[43] M. Diender, A.J.M. Stams, D.Z. Sousa, Pathways and bioenergetics of anaerobic carbon monoxide fermentation, Front. Microbiol. 6 (2015) 1–18, https://doi.org/10.3389/fmicb.2015.01275.

[44] H.T.T. Dinh, H. Kambara, Y. Harada, S. Matsushita, Y. Aoi, T. Kindaichi, N. Ozaki, A. Ohashi, Bioelectrical methane production with an ammonium oxidative reaction under the no organic substance condition, Microbes Environ. 36 (2021) ME21007, https://doi.org/10.1264/JSME2.ME21007.

[45] H. Kobayashi, N. Saito, Q. Fu, H. Kawaguchi, J. Vilcaez, T. Wakayama, H. Maeda, K. Sato, Bio-electrochemical property and phylogenetic diversity of microbial communities associated with bioelectrodes of an electromethanogenic reactor, J. Biosci. Bioeng. 116 (2013) 114–117, https://doi.org/10.1016/j.jbiosc.2013.01.001.

[46] H. Luo, W. Teng, G. Liu, R. Zhang, Y. Lu, Sulfate reduction and microbial community of autotrophic biocathode in response to acidity, Process Biochem. 54 (2017) 120–127, https://doi.org/10.1016/j.procbio.2016.12.025.

[47] D.V. Antsiferov, T.S. Fyodorova, A.A. Kovalyova, A. Lukina, Y.A. Frank, M. R. Avakyan, D. Banks, O.H. Tuovinen, O.V. Karnachuk, Selection for novel, acid-tolerant *Desulfovibrio* spp. from a closed Transbaikal mine site in a temporal pH-gradient bioreactor. Antonie van Leeuwenhoek, Int. J. Gen. Mol. Microbiol. 110 (2017) 1669–1679, https://doi.org/10.1007/S10482-017-0917-4/FIGURES/5.

[48] S. Hahnke, T. Langer, D.E. Koeck, M. Klocke, Description of *Proteiniphilum saccharofermentans* sp. nov., *Petrimonas mucosa* sp. nov. and *Fermentimonas caenicola* gen. Nov., sp. nov., isolated from mesophilic laboratory-scale biogas reactors, and emended description of the genus *Proteiniphilum*, Int. J. Syst. Evol. Microbiol. 66 (2016) 1466–1475, https://doi.org/10.1099/IJSEM.0.000902/CITE/REFWORKS.

[49] R. Mateos, A. Sotres, R.M. Alonso, A. Morán, A. Escapa, Enhanced CO_2 conversion to acetate through microbial electrosynthesis (MES) by continuous headspace gas recirculation, Energies 12 (2019) 3297, https://doi.org/10.3390/en12173297.

[50] A.E. Rotaru, P.M. Shrestha, F. Liu, M. Shrestha, D. Shrestha, M. Embree, K. Zengler, C. Wardman, K.P. Nevin, D.R. Lovley, A new model for electron flow during anaerobic digestion: direct interspecies electron transfer to *Methanosaeta* for the reduction of carbon dioxide to methane, Energ. Environ. Sci. 7 (2013) 408–415, https://doi.org/10.1039/C3EE42189A.

[51] E. Blázquez, J.A. Baeza, D. Gabriel, A. Guisasola, Treatment of real flue gas desulfurization wastewater in an autotrophic biocathode in view of elemental sulfur recovery: microbial communities involved, Sci. Total Environ. 657 (2019) 945–952, https://doi.org/10.1016/J.SCITOTENV.2018.12.037.