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Mitigation of Volatile Fatty Acid Build-Up by the Use of Soft Carbon Felt Electrodes: Evaluation of Anaerobic Digestion in Acidic Conditions

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Abstract: Anaerobic digestion and bioelectrochemical systems have great potential to recover energy from waste streams and help overcome common hurdles associated with this process, as integrated technologies. In this study, the benefit of integrating an electrogen-enriched bioanode in a batch anaerobic digester was explored under ambient temperature conditions associated with organic overloading and reactor acidity. An increase in CH₄ production was observed in the electrode-containing reactors (0.56 L CH₄ kg_{VS}⁻¹ h⁻¹) in comparison with the conventional anaerobic digester (0.14 L CH₄ kg_{VS}⁻¹ h⁻¹) during the initial stages of operation. In addition, the mere presence of electrodes operating in open circuit mode resulted in a delay in volatile fatty acid (VFA) build-up. This seems to be associated with the enhancement in VFA consumption due to biomass proliferation on the electrode surface, rather than on electrochemical activity.

Keywords: anaerobic digestion; bioelectrochemical systems; glucose; methane production; acidic conditions

1. Introduction

There have been numerous attempts to utilize geothermal, wind, and solar power as clean and renewable sources of energy for electricity production or cooling processes [1]. Different technologies are also available to turn organic wastes and lignocellulosic biomass into biofuel (i.e., ethanol and biogas) from fermentation processes. The great demand of energy and the associated impact of fossil fuel consumption on the environment have elicited extensive research on alternative sources of traditional petroleum-derived products [2]. The use of wastes allows the recycling of nutrients, in addition to the production of energy, and offers a solution for the disposal of organic materials that are otherwise difficult to reuse (e.g., weeds, animal blood, manure) [3,4].

Anaerobic digestion (AD) has become the main biological process by which to treat organic wastes due to its flexibility and robustness [5]. This process allows the combined treatment of different waste streams taking advantage of their carbon and nitrogen content, thus making co-digestion a suitable option for increasing biogas yields [6]. Biogas is a renewable energy source that mainly consists of methane and carbon dioxide, and it can be upgraded to about 97% biomethane, which is used as popular renewable forms of energy such as car fuel [7].

AD is an efficient alternative to aerobic processes when high-strength organic wastewaters need to be treated, thanks to the lower energy demand associated with the lack of aeration [8].

As a result, the number of anaerobic digesters installed worldwide is continuously increasing [9]. However, AD also has vulnerabilities. The process needs to be maintained under either mesophilic or thermophilic conditions for an acceptable degradation rate to be attained. Operating at lower temperatures often results in an increase in the reactor volume and a decrease in the organic loading rate. In addition, when the composition of the organic stream is based on simple organic molecules, the high sensitivity and slow growth rate of methanogenic microbiota in acidic conditions may result in the accumulation of volatile fatty acids (VFAs) [10,11]. The production of acetate from H_2 by homoacetogens [12] also contributes to VFA accumulation, modifying the microbial populations in the digester [13] and even collapsing the anaerobic process. Hence, improving VFA consumption rates is key to maintaining stable operations in anaerobic systems [14].

Bioelectrochemical systems (BESs) have recently emerged as feasible alternatives to conventional wastewater treatment [15]. Microbial electrolysis cells (MECs), in particular, are one of the most promising bioelectrochemical devices [16]. In MECs, exoelectrogenic microorganisms such as *Geobacter* or *Shewanella* degrade organic matter. The microorganisms are immersed in an anaerobic environment and, using a small electrical input, convert organic materials into valuable products (e.g., H_2 or CH_4). MECs can work with a wide range of substrates, with VFA-rich streams representing the ideal, since most organic acids can be readily converted to electricity by electrogens. This has allowed the use of MECs as a post-treatment technology for fermentative processes, where the concentrations of VFAs are usually high due to thermodynamic limitations [17,18].

Fermentative processes and BES have been integrated in the same reactor, representing a significant improvement. Implementing these two processes in a single reactor avoids the need for two separate stages, reduces operational costs and energy losses, and maximizes substrate utilization [19]. In addition, a combined AD and BES (AD-BES) reactor could represent a useful way to prevent inhibition of the AD process in pre-installed digesters, optimizing the efficiency and treatment capacity of pre-existing infrastructure. In fact, this combined approach has been evaluated for the treatment of different substrates, such as fecal wastewater [20], waste activated sludge [21,22], molasses [23], domestic wastewater [24], and toxic contaminants [25]. However, these studies were performed at non-inhibitory organic loads and offered no information on how the combined AD-BES reactor behaves when VFAs accumulate. This is important because the integration of fermentative and bioelectrocatalytic processes may contribute to the stabilization of the anaerobic digester through the consumption of the excess VFAs by electrogens.

In this work, the integration of an electrogen-enriched electrode in an anaerobic digester was evaluated under ambient temperature conditions at which the digestion process was prone to acidic inhibition. The combined configuration was assessed in terms of VFA evolution profiles, electric charge, and CH_4 production. Different adaptation strategies were considered in order to favor the acclimation of the electrogenic anodophilic biomass to conditions in the digester.

2. Materials and Methods

2.1. Reactor Design

The study was performed under acidic conditions associated with organic overloading. The performance of the combined AD-BES configuration was studied under conditions of acid inhibition due to VFA accumulation. In order to define these conditions, batch digestion tests using glucose as a substrate and digested sludge as an inoculum were performed in duplicate. The digested sludge was obtained from the wastewater treatment plant in León, Spain. This sludge was further stabilized in the laboratory by storage at room temperature. This allows for a reduction of the remaining degradable sludge material and thus avoids interferences when evaluating the biogas production during the experimental set-up. The physicochemical characteristics of this sludge are shown in Table 1.

Glucose was added to the experiments in quantities appropriate for inducing acidification. Therefore, the substrate–inoculum (S:I) ratio of volatile solids (VS) for the acid conditions experiment was 2.0; a lower S:I ratio of 0.25 was also tested to quantify differences associated with the production of organic acids. Batch digestion tests were evaluated until the complete removal of VFA from the 0.25 ratio test. The pH of reactors working under acidic conditions was controlled by the addition of an alkali solution (mixture of NaOH, KH_2PO_4 , and KOH). The batch digestion tests were carried out in 250 mL Erlenmeyer flasks that were continuously stirred. A thermostatic bath was used to maintain the temperature at 25 °C. This temperature value, which is below the traditional mesophilic range, was selected based on previous work [26] performed by the authors on the development of low-cost BES. The results obtained at these temperatures will aid in the future development of low-tech reactors capable of working in tropical regions and improve the design of dry fermentation digesters that present acceptable biogas yields at 25 °C [27].

The AD and BES were integrated in 3 L methacrylate reactors with a working volume of 2 L. A glucose solution was mixed with the digested sludge to maintain the previously defined S:I ratio for the acid conditions; it attained an initial glucose concentration in these reactors of 17 g L⁻¹. The reactors worked in batch mode and were continuously stirred. The temperature was maintained at 25 °C using a thermostatic jacket. Three reactors equipped with gas and liquid inflow and outflow connections were used for this experiment. In two reactors, the electrolytic modules (anode and cathode) were submerged in the reactor liquor. The third reactor denoted “AD” was a conventional anaerobic reactor used as the control. This anaerobic digester also worked under the same conditions of the AD-BES reactors, that is, at a temperature of 25 °C, a S:I ratio of 2.0, and using digested sludge as inoculum. The other anaerobic reactors were equipped with two pairs of bioelectrodes. One operated in open-circuit mode (the OC reactor) and the other in closed-circuit mode (the CC reactor), at a voltage of 0.9 V. The electrodes were inoculated using the mixed culture derived from the effluent of another MEC treating wastewater that had been operating for more than a year, supplemented with 1 g L⁻¹ of sodium acetate [28].

Table 1. Characterization of the anaerobic inoculum used in batch digestion experiments for evaluating digestion performance under overloading conditions.

Parameter	Value
pH	7.36
Organic matter (%)	1.12 ± 0.05
Total nitrogen (%)	0.21 ± 0.01
C/N ratio	3.15
Total solids (g L ⁻¹)	16.71 ± 0.80
Volatile solids (g L ⁻¹)	11.93 ± 0.59
NH ₄ ⁺ (g L ⁻¹)	1.13 ± 0.05
Alcalinity (g-CaCO ₃ L ⁻¹)	2.32 ± 0.10
VFA (g L ⁻¹)	0.5 ± 0.001
Acetate (g L ⁻¹)	0.030 ± 0.001
PO ₄ ³⁻ (ppm)	440 ± 13
Ca ²⁺ (ppm)	576 ± 17
Mg ²⁺ (ppm)	108 ± 3
K ⁺ (ppm)	232 ± 7
Na ⁺ (ppm)	48.4 ± 1.0
Mn (ppm)	3.4 ± 0.1
Fe (ppm)	263 ± 7
Cu (ppm)	3.4 ± 0.1
Zn (ppm)	17.1 ± 0.5

2.2. Electrode Preparation and Operation

Electrodes for the OC and CC reactors were prepared with two layers of soft carbon felt (Sigratherm GFD 2, SGL Carbon Group), each 1 cm thick, as the anode and a titanium electrode as the cathode. One piece of polyester cloth, 0.6 mm thick, was sandwiched between the anode and the cathode to prevent any electrical contact between the two electrodes. The inter-electrode separation distance was set to 1 mm. The dimensions of both the anode and the cathode were 100 mm × 100 mm. Two pairs of electrodes were inserted in each reactor, resulting in an anode surface/reactor volume ratio of 10 m² m⁻³. Although this ratio could be improved, it was selected to adapt the experiment to the shape and volume of pre-installed anaerobic digesters.

Electrogenic biomass was previously inoculated in the electrodes using the effluent from a MEC reactor in the laboratory as described in Section 2.1. To properly evaluate the combined AD-BES reactor, three different electrogen adaptation protocols were attempted for a 7 day period, with the applied voltage kept at 0.9 V in all cases. In a first approach, the electrodes were acclimated in a salt solution, the composition of which has been defined elsewhere [26]. The carbon source was sodium acetate (500 mg Ac L⁻¹), which is considered one of the preferred substrates for electrogenic populations. All solutions were filter sterilized and stored at 4 °C to prevent microbial growth. Distilled water was used to prepare this solution, and chemicals and reagents used were of analytical grade. The second approach tested electrodes previously acclimated using digested sludge, also with sodium acetate as carbon source. This was denoted the Acetate (Ac) test. The third acclimation test also consisted of the electrodes being adapted to digested sludge; however, in this case, increasing concentrations of glucose were used as the carbon source. The organic loading rate applied during this period was increased from 0.4 to 1.0 g L⁻¹ d⁻¹. This run was denoted the Glucose (Gl) test.

The Ac and Gl tests were performed in duplicate. The combined AD-BES reactors were denoted OC-Ac and CC-Ac (electrode-containing reactors at open-circuit and closed-circuit conditions, respectively, adapted with acetate) and OC-Gl and CC-Gl (open-circuit and closed-circuit conditions, respectively, adapted with glucose) (Figure 1). The term “electrode-integrated digesters” is used in this text to refer to the set of experiments OC-Ac, CC-Ac, OC-Gl, and CC-Gl as a whole. As explained below, the initial test in which the electrode was adapted using the salt solution presented poor results, and therefore it is not represented in Figure 1.

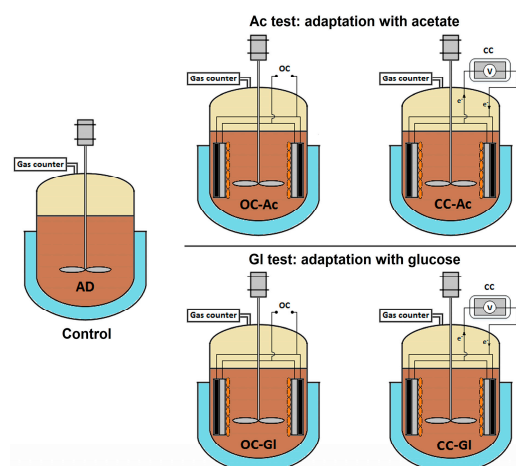


Figure 1. Schematic view of the reactors used in this study and nomenclature definition for the different experiments tested: AD (conventional anaerobic digester), OC-Ac, and OC-Gl (combined AD-BES reactors working in open-circuit mode, pre-adapted with acetate and glucose, respectively) and CC-Ac and CC-Gl (combined AD-BES reactors working in closed-circuit mode at $V_{app} = 0.9$ V, pre-adapted with acetate and glucose, respectively).

A 16 Ω resistor was added to the circuit for online current measurements at 10 min intervals using a data acquisition system (PCI-6221, National Instruments, Austin, TX, USA). The same interval was used to record gas flow rates, which were measured with a milli-gas counter (MGC-1, Ritter Co., Bochum, Germany). An adjustable DC power supply (BK Precision 9120, Yorba Linda, CA, USA) maintained the applied voltage at the pre-set level of 0.9 V. The performance of the combined AD-BES reactor was assessed in terms of (i) specific CH₄ production (L-CH₄ kg_{VS}⁻¹); (ii) current production (A); and (iii) VFA removal rates (%).

2.3. Analytical Measurements and Calculations

Gas composition and VFAs were analyzed as described by Martínez et al. [29]. Conductivity and pH were measured in accordance with the American Public Health Association (APHA) methods [26] and were regularly quantified during the AD/electrocatalytic process. Total solids and VS were also measured in accordance with the APHA methods [30]. Total organic carbon, total carbon, and total nitrogen content were measured using a total organic carbon analyzer (multi N/C 3100, Analytik Jena, Jena, Germany). Total Ca, K, and Na were analyzed using a PerkinElmer Optima 2000 DV (Waltham, MA, USA) inductively coupled plasma atomic emission spectrometer. For the analysis, 0.3 g of the sample was digested in 10 mL of 65% nitric acid in a microwave accelerated reaction system (MARS-5, CEM Corporation, Matthews, NC, USA). The samples were submitted at 100 °C for 5 min initially and subsequently at 190 °C for 30 min, with a ramp time of 10 min.

3. Results and Discussion

3.1. Batch Digestion Tests

A high concentration of glucose was applied to cause VFA accumulation, creating acidic conditions that would inhibit methane production. Table 2 shows the results obtained from batch digestion tests carried out at S:I ratios of 2.0 and 0.25.

Table 2. Results obtained from batch digestion test under non-inhibitory and inhibitory conditions. Specific CH₄ production and total volatile fatty acid (TVFA) production for glucose biodegradability tests at the two ratios tested of volatile solids for substrate and inoculum (VS-substrate/V_S-inoculum = 0.25 and 2). Production profiles of acetate, butyrate, and propionate are presented for the VS substrate/inoculum ratio of 2 (n.d. = no detected; - = no data available).

Time (h)	Specific CH ₄ Production (L-CH ₄ kg _{VS} ⁻¹)		VFA Production (mg L ⁻¹)				
	Ratio 0.25	Ratio 2.0	Ratio 0.25		Ratio 2.0		
			TVFA	Acetate	Butyrate	Propionate	TVFA
0	0 ± 0	0 ± 0	37	36	6	0	42
15	105 ± 15	11 ± 3	225	491	1043	18	1560
23	153 ± 24	23 ± 2	170	820	2139	24	2988
40	234 ± 22	30 ± 10	240	1190	2372	173	3814
64	240 ± 15	34 ± 2	4	1496	2415	364	4513
88	245 ± 17	37 ± 14	3	1902	2942	356	5576
112	253 ± 15	39 ± 0	-	-	-	-	-
136	268 ± 15	43 ± 16	84	2300	3280	415	6458
160	283 ± 15	47 ± 16	6	2298	3325	413	6383
184	293 ± 25	48 ± 18	n.d.	-	-	-	-
208	299 ± 15	50 ± 15	n.d.	-	-	-	-

Specific CH₄ production for the S:I ratio of 0.25 was close to the theoretical CH₄ production from glucose, which is 415 L CH₄ kg_{VS}⁻¹ based on calculations using Buswell’s formula [31]. In contrast, the acid test was clearly affected by the inhibitory conditions, with an extremely low value of methane

produced and total VFA (TVFA) concentration values reaching slightly above the inhibitory threshold (6 g L^{-1} TVFA) reported by Siegert and Banks [32]. Moreover, TVFA values for the S:I ratio of 2.0 were 20 times higher than those of the 0.25 ratio. Therefore, the acid test ratio was selected to induce inhibitory conditions due to overloading in subsequent experiments.

3.2. Combined AD-BES Reactors

The first set of experiments used electrodes adapted with the salt solution. When fed with the acidic substrate, all reactors underwent rapid acidification, and negligible amounts of CH_4 were produced. Moreover, the AD reactor recovered partially after 60 h, whereas the electrode-integrated digesters did not resume CH_4 production. This led to the hypothesis that the poor performance observed in the combined AD-BES reactors could be attributed to improper or insufficient adaptation of the anodic microorganisms to the operating conditions within the anaerobic digesters; the anodes used in this first test were grown with a synthetic effluent, using acetate as substrate and no digested sludge. Therefore, different adaptation alternatives were used; the bioanodes were re-inoculated and grown with anaerobic sludge; one was amended with acetate in one case, and glucose in the other. The following sections present results of the sludge-adapted electrodes, since the first protocol ended in failure.

3.2.1. Methane Production and Current Profiles

The different reactors displayed varied behaviors right from the beginning of the experiment. The specific CH_4 production curves in Figure 2a show higher values for the electrode-containing reactors, except for the one testing the CC-Gl combination, at least during the first 100 h of the experiments. Although CH_4 production was far below the theoretical CH_4 production from glucose, there was an initial improvement in the methanogenic response from the combined AD-BES reactors. However, methane production continued to grow steadily in the AD reactor beyond the first 100 h, while it decayed in the electrode-based reactors. This showed that the presence of electrodes may help reduce the recovery period for the AD process. In other words, electrodes may play a role in accelerating, rather than inducing, the recovery of the anaerobic processes.

The open-circuit reactors (OC-Ac and OC-Gl) had higher methane production rates during the initial hours of the experiment. The AD reactor had an initial methane production rate of $0.14 \text{ L CH}_4 \text{ kg}_{\text{VS}}^{-1} \text{ h}^{-1}$, while this value was, on average, $0.56 \text{ L CH}_4 \text{ kg}_{\text{VS}}^{-1} \text{ h}^{-1}$ for the OC reactors during the first 40 h. The electrodes adapted to acetate as a carbon source had the highest values of specific methane production within their homologous systems. This is probably because they contained greater microbial populations capable of metabolizing acetate. In digestion processes, acetate and H_2/CO_2 are converted to CH_4 by acetotrophic and hydrogenotrophic methanogens, respectively [33]. In addition, acetate may also be oxidized to CO_2 and H_2 by syntrophic acetate oxidizing bacteria coupled with the reduction of CO_2 to CH_4 by H_2 -utilizing methanogens [34,35]. The presence of the electrode may aid the consumption of VFAs, either by providing a rich consortium of acetate consuming microorganisms or by aiding VFA consumption due to enhancements in electron transfer.

Direct interspecies electron transfer has been suggested as a probable mechanism for electron exchange in systems in which cathodic methane/hydrogen production is expected [36]. Although acetoclastic/hydrogenotrophic methanogenesis cannot be ruled out, the introduction of the electrodes enhanced the methanogenic processes as a consequence of better biomass proliferation, as observed from the responses obtained from OC experiments versus CC tests. In these experiments, applying an electric charge did not cause higher methane production rates, which was in accordance with results reported by De Vrieze et al. [23]. The measurement of the electric current of the closed-circuit system allows for the calculation of the methane produced by electromethanogenesis using the stoichiometry of this reaction. Figure 2b represents the theoretical maximum amount of methane calculated from the electric charge reaching the cathode in the applied voltage reactors. For the CC-Ac reactor, the amount of CH_4 produced by this route was small (dark grey color) compared with the total amount of methane

measured. On the other hand, for the CC-Gl test, this value was higher but still somewhat lower than 20%. This behavior indicates that the overall methane keeps a close relation with the biomass present in the reactor instead of with the electrogenic process.

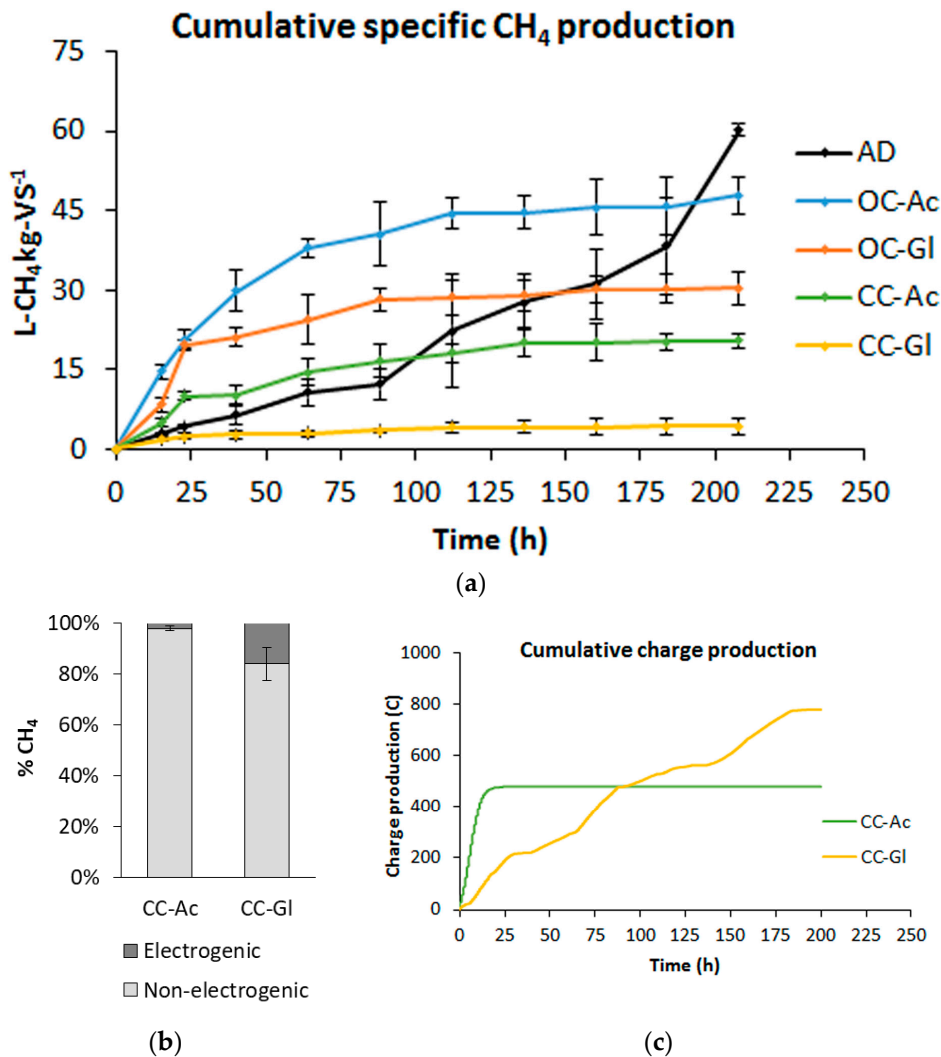


Figure 2. Results from combined AD-BES reactors working under acidogenic conditions: (a) specific CH₄ production for reactors; (b) theoretical CH₄ production by electromethanogenic processes; and (c) current consumption profiles of the closed-circuit (CC) reactors.

Despite the production of this low charge, it was observed that the electrodes adapted to acetate (CC-Ac) produced charges at a faster rate during the initial hours of the test, although they rapidly become stagnant (Figure 2c), probably because the anodophilic microorganisms were not properly adapted. In contrast, the glucose-adapted reactors produced electrical charges at lower rates steadily throughout the entire experiment, possibly indicating that adaptation to glucose prevents osmotic shock and subsequent failure of electrogenic processes, therefore producing more stable anodes.

3.2.2. Analysis of Volatile Fatty Acids

Figure 3a presents the VFA concentration profiles for all the reactors. It clearly shows that the presence of a bioanode attenuated VFA build-up in the electrogen-based reactors, compared to the AD reactor, at least during the first 100 h of testing and regardless of the operational mode of the former.

This result agrees with those discussed in the previous section, where the methane production rate improved in three of the four electrogen-based digesters during the initial stages.

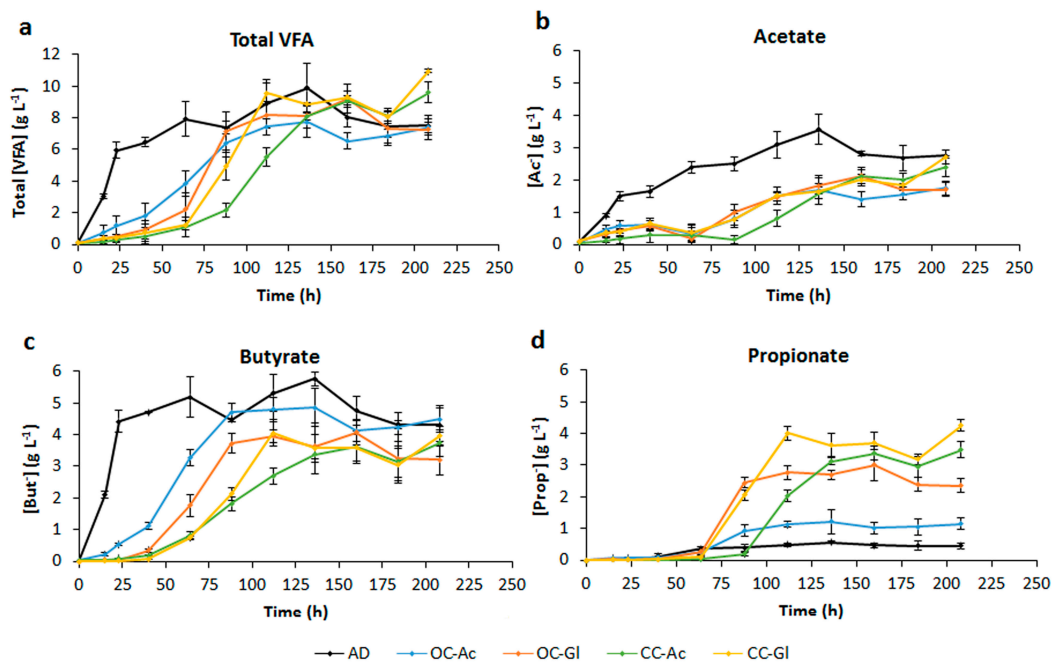
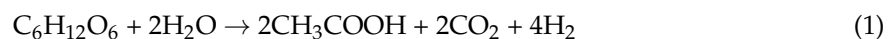


Figure 3. VFA evolution of the combined AD-BES reactors and anaerobic digester working under acidogenic conditions. Total concentrations of (a) VFAs; (b) acetate; (c) butyrate; and (d) propionate for all conditions tested. AD: Anaerobic digester; OC-Ac and OC-Gl: open-circuit reactors adapted with acetate and glucose, respectively; CC-Ac and CC-Gl: closed-circuit reactors adapted with acetate and glucose, respectively.

Two possible situations could explain this attenuation in the VFA build-up: (a) no VFAs were produced in electrode-integrated digesters, or (b) VFAs were produced but then rapidly degraded by anodophilic microorganisms before measurement was possible. If the first hypothesis is true, the amount of CO₂ produced should in theory be stoichiometrically correspondent with VFA (i.e., if VFA levels are low, CO₂ production should be low as well), according to Equations (1) and (2) below. Conversely, if the second hypothesis is true, there should be no direct correspondence between CO₂ production and VFA concentrations. The stoichiometry of the degradation reactions of glucose to acetate and butyrate are as follows:



Therefore, to identify the most likely explanation, a mass balance test was performed for both the AD and combined AD-BES reactors (Figure 4). Since the latter presented similar behaviors (i.e., attenuated VFA accumulation), the OC-Ac test was selected as representative, for the sake of simplicity. During the initial stages of the experiment, CO₂ production in the combined AD-BES reactor was much higher than in the control, and therefore not stoichiometrically coincident with VFA production. The ratio reported in Figure 4 was calculated using the measured CO₂ values, as well as the theoretical CO₂ expected using measured VFA values and the stoichiometry of Equations (1) and (2). The combined AD-BES reactor produced much more CO₂ during the initial stage than theoretically expected, suggesting that VFAs were being produced but probably degraded immediately by anodophilic microorganisms, supporting the second hypothesis. In fact, the correspondence between

real and theoretical production is better in the AD reactor, which also shows higher absolute levels of VFAs.

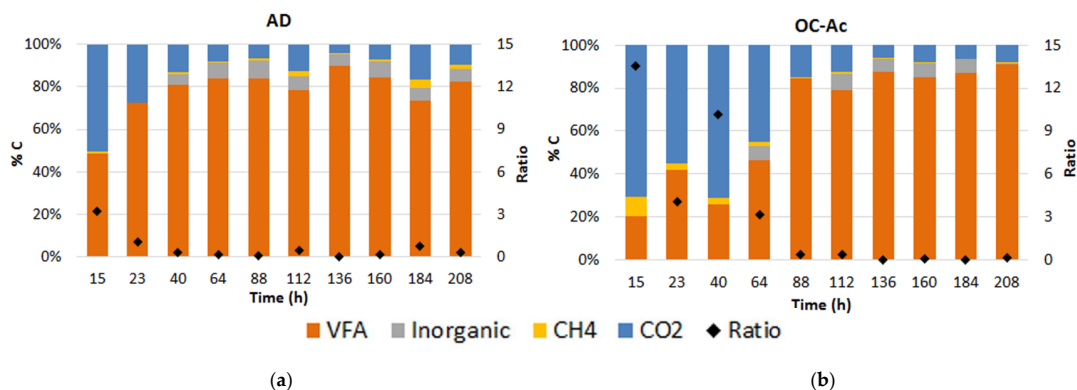


Figure 4. Comparison of performance of the (a) anaerobic digester and (b) the combined AD-BES system (OC-Ac): left-hand y-axis: proportion of degraded carbon compared to different compounds: VFAs, inorganic carbon, CH₄, and CO₂. Right-hand y-axis: ratio between the measured CO₂ values and theoretical CO₂ production on a stoichiometric basis, after VFA production.

Figure 3b,c shows that the presence of anodes in the digester kept levels of acetate and butyrate low. Acetate (Figure 3b) follows a similar pattern for all the combined AD-BES reactors regardless of the operational mode or adaptation process, whereas it is present in much higher concentrations in the AD reactor. This suggests that the mere presence of the electrodes has an important effect on acetate degradation rates, irrespective of voltage applied. On the other hand, the evolution of butyrate concentrations (Figure 3c) is different between the OC and CC reactors; it is lower for longer in the CC tests. Therefore, it seems that the application of voltage improves the consumption rate of this acid.

In contrast to butyrate and acetate, propionate concentrations increased rapidly in the combined AD-BES and for most of the tests; their levels were well above those found in the control reactor (Figure 3d). Ma et al. [37] indicated that propionate values should be kept below 1.5 g L⁻¹ to attain stable CH₄ production in digestion reactors. However, Wang et al. [38] reported that high concentrations of propionic acid do not necessarily indicate an imbalance in the AD process, and are not inhibitory even if added directly or formed as an intermediary. This agrees with the stable performance of reactors when propionic acid concentrations are high, such as during phenol digestion, as reported by Pullammanappallil et al. [39]. In their experiment, the metabolism of phenol led to an elevated propionic acid content (2750 mg L⁻¹). Similarly, stable operation has also been reported in reactors carrying out co-digestion of glycerin and swine manure, in which concentrations of propionic acid went above 4000 mg L⁻¹ [40].

In the present experiments, propionate accumulation more or less coincided, over time, with the stagnation of methane production. This behavior suggests that the poor performance observed in combined AD-BES reactors is probably connected to propionate build-up, but also with pH levels. The pH of these reactors was kept between 6 and 6.5 after an initial drop. These pH levels are still appropriate for methanogenic processes; however, a higher proportion of undissociated VFAs is present, with TVFA levels in the combined AD-BES reactors ranging between 211 and 366 mg L⁻¹ in undissociated form. This value was also high for the AD reactor (340 mg L⁻¹), but in this reactor an increasing trend was maintained in the methane production rate.

Inhibition of propionate degradation has been reported to be caused by the undissociated forms of VFAs [41], suggesting that propionate degradative processes stopped after the decrease in pH levels and subsequent increase in undissociated VFA forms. This is probably the main cause of the plateau in the methane curves obtained from the combined AD-BES reactors, but it does not explain the behavior of the AD reactor, in which low levels of propionic acid were maintained and increasing volumes

of methane were produced. The high levels of acetate and butyrate present in this system indicate the prevalence of *Clostridium* species. In fact, a common way to enrich fermentative H_2 -producing systems is to induce an overload in CH_4 -producing microflora [42,43]. In the present experiment, H_2 was not detected in biogas measurements, but this may be explained by the activity of H_2 -utilizing methanogens. The predominant microflora seemed more resistant to the undissociated forms of VFAs, but not in the combined AD-BES reactors.

The accumulation of propionic acid was the consequence, not the cause, of the process imbalance observed in the combined AD-BES reactors. The OC-Ac reactor was the integrated configuration with the lowest levels of propionate and produced the most methane (Figure 2a), but still reached an inhibitory state after 100 h, further supporting the idea of a strong relationship between process imbalances and propionate accumulation. Figure 3d also shows that the acetate adapted bio-electrodes seemed to delay and even reduce the accumulation of propionate better than glucose adapted electrodes, with the better performance occurring under the OC configuration. On the other hand, glucose pre-growth produced better electrogenic characteristics, indicating that adapting the electrogens to operational conditions prior to electrode introduction produces a better response. Overall, the integration of electrogen-enriched electrodes in an anaerobic reactor under conditions of inhibitory acidification increased CH_4 production and delayed VFA accumulation during the initial stages of operation.

Further tests of reactors under continuous modes should be conducted in future experiments, to evaluate the effect of hydraulic retention time on the response to methane production or VFA accumulation. Moreover, the ratio between the surfaces of the electrode and the reactor volume might be able to be optimized further. It is reasonable to assume that an increase in electrode surface area would improve performance, producing more biogas and consuming VFAs faster, as a result of increased availability of anodophilic microorganisms.

4. Conclusions

The feasibility of integrating anaerobic digestion and bioelectrochemical technologies was assessed using different electrode adaptation protocols. Electrogen-enriched electrodes were introduced in a batch anaerobic digester operating under conditions of organic overloading and low temperature. The presence of electrodes improved the initial stages of the operation by delaying VFA build-up and enhancing the CH_4 production rate. The amelioration of VFAs seems to be based on the presence of the electrode as a solid support for biomass proliferation and on the electrochemical activity of the electrodes, with the closed circuit system presenting the lower VFA build-up during the initial stages of the fermentation. However, the effect of voltage application is comparatively less relevant than the impact of biomass proliferation. Bioaugmentation achieved during the adaptation protocols of the electrodes may also explain the better results obtained for the AD-BES reactors regardless of the application of voltage. However, propionate concentrations started to increase dramatically after the combined AD-BES reactors operated for 100 h, completely stopping methanogenic processes. In order to prevent propionate accumulation, designing pre-adaptation protocols for bacteria is advisable.

The significance of these results is associated with the future development of the low-cost anaerobic digester operating in tropical regions where temperature control by the use of external heating systems results in additional costs and technical constraints. The decrease in the temperature of the operation may set limitations on the treatment capacity of the system. Therefore AD-BES integration is thought of as way to operate high-loading anaerobic digesters in tropical regions.

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Author Contributions: Adrián Escapa, Olegario Martínez, and Xiomar Gómez conceived and designed the experiments; Rubén Moreno and Elia J. Martínez performed the experiments; Rubén Moreno, Rebeca Díez-Antolínez, and Xiomar Gómez analyzed the data; Adrián Escapa and Elia J. Martínez contributed with reagents/materials/analysis tools; Xiomar Gómez, Adrian Escapa, and Rubén Moreno wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

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