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An electrode-assisted anaerobic digestion process for the production of high-quality biogas

K. Yanuka-Golub, K. Baransi-Karkaby, A. Szczupak, L. Reshef, J. Rishpon, R. Shechter, U. Gophna and I. Sabbah

ABSTRACT

Biogas is a sustainable, renewable energy source generated from organic waste degradation during anaerobic digestion (AD). AD is applied for treating different types of wastewater, mostly containing high organic load. However, AD practice is still limited due to the low quality of the produced biogas. Upgrading biogas to natural gas quality (>90% CH_4) is essential for broad applications. Here, an innovative bio-electrochemically assisted AD process was developed, combining wastewater treatment and biogas upgrading. This process was based on a microbial electrolysis cell (MEC) that produced hydrogen from wastewater at a relatively high efficiency, followed by high-rate anaerobic systems for completing biodegradation of organic matter and an *in situ* bio-methanation process. Results showed that CH_4 production yield was substantially improved upon coupling of the MEC with the AD system. Interestingly, CH_4 production yield increase was most notable once circulation between AD and MEC was applied, while current density was not markedly affected by the circulation rates. The microbial community analysis confirmed that the MEC enhanced hydrogen production, leading to the enrichment of hydrogenotrophic methanogens. Thus, directing soluble hydrogen from the MEC to AD is plausible, and has great potential for biogas upgrading, avoiding the need for direct hydrogen harvesting.

Key words | anaerobic digestion, biogas upgrading, methanogenic hydrogenotrophs, microbial electrolysis cells, wastewater treatment

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ABBREVIATIONS

AD	Anaerobic digestion
COD	Chemical oxygen demand
MEC	Microbial electrolysis cell
OCV	Open circuit voltage
PCoA	Principal coordinates analysis
TS	Total solids
VFA	Volatile fatty acids
VS	Volatile solids

INTRODUCTION

Anaerobic digestion (AD) is a microbial process that occurs in oxygen-free environments by which organic waste is decomposed by complex metabolic pathways, eventually leading to the formation of biogas, a mixture of carbon dioxide and methane ($CO_2 + CH_4$), that is considered as a renewable energy source that can potentially replace fossil fuels (Chen *et al.* 2008). The production of biogas by AD has been implemented in the treatment of waste for over a century, and in recent years a substantial increase in the number of installations of industrial-scale reactors for wastewater treatment has been observed (Appels *et al.* 2011). In wastewater treatment, AD offers several advantages compared with other technologies, including lower sludge production and lower energy requirements along with a high potential for energy recovery. Additionally, the produced biogas of the existing anaerobic system will be more economically valuable when meeting the quality of natural gas to be utilized as either vehicle fuel or to be connected to the natural gas infrastructure. Nevertheless, AD is not yet widely applied world-wide

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because it often has low operational stability (Chen *et al.* 2008), including activated biomass washout, lengthy periods of acclimation, low quality of the produced biogas, in addition to sensitivity to xenobiotics and micropollutants that may inhibit process stability and performance (Ruel *et al.* 2002; Rajagopal *et al.* 2013).

Although biogas is considered a potentially important energy resource, it has limited application and utilization, since it typically consists of 60%–70% CH_4 and 30%–40% CO_2, and often contains additional undesirable gases, (i.e., traces of water vapor, H_2S and H_2, and other contaminants). Therefore, although biogas can be used for heating or to generate power, the large volume of CO_2 reduces the heating value of the biogas, increases compression and transportation costs and limits its economic feasibility (Ruel *et al.* 2002; Zhao *et al.* 2010; Muñoz Torre *et al.* 2015). Thus, low-quality biogas cannot be used as a vehicle fuel or integrated into the natural gas energy system before removing substantial amounts of the CO_2 component. In this regard, upgrading it to natural-gas quality (CH_4 content of 90% or higher) will enable broad applications, substantially increasing its value.

Most of the methods for biogas upgrading are based on chemical and physical techniques that remove CO_2 with minimal loss of CH_4 (Niesner *et al.* 2013), yet most of them are not cost-effective or sustainable in terms of capital investment and operational costs, and require expensive rare elements for chemical catalysis (Guebitz *et al.* 2015; Angelidaki *et al.* 2018). About 200 biogas upgrading plants operated world-wide make use of five main technologies for bio-methane processes including those able to produce bio-methane to the required purity (Niesner *et al.* 2013). In contrast, biogas upgrading can also be accomplished by biologically based methods, where hydrogenotrophic methanogens utilize H_2 (from an external source) as an electron donor to reduce CO_2 to CH_4 (Luo *et al.* 2012; Rachbauer *et al.* 2016; Kougias *et al.* 2017). This leads to higher CH_4 concentrations than those achieved by the methanogenic microbial community in a conventional AD reactor during wastewater treatment. This approach, which mitigates CO_2 that is present in the system, can be implemented under mild operational conditions, and thus is considered a sustainable process for both biogas upgrading and, on top of that, reducing anthropogenic greenhouse gas emissions from the wastewater treatment process. While many studies chose to inject pure hydrogen to experimentally investigate biological biogas upgrading (Bassani *et al.* 2015; Agneessens *et al.* 2017; Wahid *et al.* 2019), microbial electrolysis cells (MECs) can be used as an alternative low-cost approach for hydrogen production (Logan *et al.* 2008; Yu *et al.* 2018).

In this study, a novel method for treating wastewater while simultaneously producing biomethane (upgraded biogas) by an integrated three-chamber AD-MEC-AD system is presented. The method has the significant advantage of avoiding the necessity of pre-harvesting the H_2 that is essentially produced from the same source of treated wastewater. The objectives of this research were two-fold: (1) to design an innovative biological approach combining a process for CO_2 reduction to CH_4 with a hybrid high-rate anaerobic–microbial electrolysis cell (MEC) system for low-cost hydrogen production; and (2) as the upgrading process is biologically based on the activity of hydrogenotrophic methanogens, it was aimed to enhance the stability of methanogenesis through the utilization of the hybrid system, and to better understand the prokaryotic community dynamics that potentially govern the biogas upgrade capacity in the hybrid high-rate anaerobic–microbial electrolysis cell system. Unlike recent applications in which electrodes were implanted directly into AD reactors (*in situ* electrode-configuration) (e.g. Bo *et al.* 2014; Liu *et al.* 2017), here, an *ex situ* electrode-configuration was used (*in(ex) situ* electrode-configuration and *in situ* bio-methanation are distinguished).

MATERIALS AND METHODS

Anaerobic bioreactor inoculation and operation conditions

Two laboratory-scale anaerobic reactors (AD_1 and AD_2) were inoculated separately, using an anaerobic granular biomass that was collected from a well operated, up-flow anaerobic sludge blanket (UASB) bio-reactor used to treat the wastewater of a citrus-based soft drink factory (PRIGAT) at Kibbutz Givat Haim, Israel. The volume ratio between the two reactors was 75% for the first stage (AD_1) and 25% for the second tank (AD_2), as illustrated in Supplementary Figure S1 (available with the online version of this paper). The second reactor operated under conditions to enrich primarily hydrogenotrophic methanogen activity, as described below. The active volumes of the two bioreactors were 2,300 mL and 800 mL, respectively. Both reactors were equipped with cylindrical double jackets maintaining a constant temperature of 37 °C by circulating heated water. The system was fed with synthetic wastewater (kept at 4 °C), where the effluent of the first unit (AD_1) was fed into the subsequent reactor, and the produced biogas of the first methanogenic stage was mixed with hydrogen from

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an external source (gas bag) at a ratio of 4:1 (H_2:CO_2), and the entire mixture was fed into the second-stage tank (AD_2). In addition, a circulation of 1.5–2 times the biogas flow rate was applied to the second AD reactor in order to increase the solubility of hydrogen, and hence, to enhance its bioavailability and allow the enrichment of the hydrogenotrophic microorganisms.

Microbial electrolysis cell (MEC)

The MEC (active volume 2,100 mL) had a spirally wound design comprised of a carbon cloth electrode for the anode attached to another carbon cloth electrode for the cathode. Each electrode surface area was 900 cm^2. The electrodes (with a flow spacer and an ion permeable separator) were connected to a potentiostat (IviumStat, Ivium Technologies, The Netherlands) under an applied voltage of 1.0 V. After an acclimation period, the MEC was coupled to the combined-AD reactor. Polarization assays were performed once the current stabilized after every increase in organic loading rate by applying various external voltages (OCV, 1,500, 1,300, 1,100, 900, 700 and 500 mV), with each voltage being applied for 25 minutes, and the current was recorded by the potentiostat. The electrodes' potential measurements (vs Ag/AgCl) were manually recorded routinely, as well as for every applied voltage during polarization assays.

Operation conditions

The integrated AD_1-MEC-AD_2 system operated continuously, in series by implementing two consecutive methanogenesis steps: the effluent of the first AD unit was fed into the subsequent reactor (MEC); and the produced biogas of the first methanogenic stage was also fed to the second AD system, as illustrated in Supplementary Figure S1. The system was continuously fed with synthetic wastewater containing glucose as a carbon source. The used synthetic wastewater was prepared based on three stock solutions. All chemicals, of analytical grade, were obtained from Sigma-Aldrich and used as received without any further purification (concentrations of the chemicals given below are in g L^{-1} in distilled water):

A. NH_4Cl, 100; $MgCl_2{\cdot}6H_2O$, 10; NaCl, 10; $CaCl_2{\cdot}2H_2O$, 5; $(NH_4)_2HPO_4$, 40.

B. $K_2HPO_4{\cdot}3H_2O$, 152.6.

C. Trace-metal and saline solution: $FeCl_2{\cdot}4H_2O$, 2; H_3BO_3, 0.05; $ZnCl_2$, 0.05; $CuCl_2{\cdot}2H_2O$, 0.038; $MnCl_2{\cdot}4H_2O$, 0.05; $(NH_4)_6Mo_7O_{24}4H_2O$, 0.05; $AlCl_3$, 0.05; $CoCl_2{\cdot}6H_2O$, 0.05; $NiCl_2{\cdot}6H_2O$, 0.092; ethylenediamine tetra acetate, 0.5; concentrated HCl, 1 mL; $Na_2SeO_35H_2O$, 0.1.

To a volume of 900 mL of distilled water, 10 mL of stock solution (A), 2 mL of stock solution (B) and 1 mL of stock solution (C) were added, also 0.2 g yeast extract, 0.33 g peptone, 1.5 g glucose, 2.6 g $NaHCO_3$ and 0.25 g $Na_2S{\cdot}9H_2O$ were added.

Analytical methods and calculations

The produced biogas in the anaerobic system was collected in 3 L Tedlar Air® sampling bags. Collected biogas volume was measured daily and the CH_4 and CO_2 contents were analyzed with special CH_4 sensors (Guardian Plus, model 97,460, Edinburgh Sensors and Guardian NG Edinburgh Sensors respectively). H_2 was analyzed using an (F-12D ATI) ATI hydrogen sensor by using a gas transmitter. Chemical oxygen demand (COD), total solids (TS) and volatile solids (VS) were measured according to the 18th edition of *Standard Methods for the Examination of Water and Wastewater* (APHA 2005). The concentration of volatile fatty acids (VFA) was analyzed using a combination of the potentiometric titration methods for acidity (Method 2310B) and alkalinity (Method 2320B) with sludge sample preparation techniques according to the 18th edition of *Standard Methods* (APHA 2005). The total alkalinity was calculated using the amount of acid needed to titrate the sample from the starting pH to pH 4; the volatile acids were calculated using the amount of hydroxide needed to titrate the sample from pH 4 back to pH 7 (APHA *et al.* 2005).

To evaluate the contribution of combining the MEC with an enriched hydrogenotrophic reactor (AD_2) for upgrading the produced CH_4 from a fixed amount of organic substrate, methane production yield (mL CH_4/g COD removed) was calculated based on biogas and COD removal measurements taken for three different operation conditions of the systems. The three different operation conditions were: AD alone (without MEC), AD-MEC without circulation (AD-MEC, without circulation) and AD-MEC under applied circulation between MEC-AD_2 ×9 the flow rate (AD-MEC, with circulation). As described by Speece (1983) and Jiménez *et al.* (2018), 1 g of COD removed typically corresponds to the production of 350 mL CH_4 when the COD is used only by methanogens under standard conditions. However, this volume can be recalculated to 337.5 mL under NTP conditions (20 °C) while taking into account that 10% of the substrate was assimilated by microorganisms for cell growth.

CH_4 production yield (mL/g COD removed) was calculated based on biogas and COD measurements taken from

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AD_2, using the following equation:

$$CH_4\,production\,yield\left(\frac{ml\,CH_4}{g\,COD\,removed}\right) = \frac{\frac{ml\,CH_4}{Hr}}{\frac{g}{L}\,(COD_{in} - COD_{out})\cdot\frac{L}{Hr}}$$

DNA extraction

Water samples from each of the three-reactor systems were collected at different time points for bacterial and archaeal community analysis. Total DNA was extracted from suspended cells in the water samples using the PowerWater DNA isolation kit (DNeasy PowerWater, QIAGEN, Israel), according to the manufacturer's instructions. For cell suspension, 20 mL of each of the three-reactor systems were filtered through a 0.22 μm sterile filter paper. The filter paper was inserted into the Power Water® bead tube to extract microbial DNA according to the manufacturer's instructions (DNeasy PowerWater, QIAGEN, Israel). Additionally, metagenomic DNA was extracted from another AD reactor as a control for AD community composition without an integrated MEC. Quality and quantity of the genomic DNA were determined using NanoDrop (Thermo-Fisher Scientific, Waltham, MA) and agarose gel electrophoresis.

Analysis of the 16S rRNA gene sequences

For community analysis, fragments of the bacterial and archaeal 16S rRNA genes were separately amplified (for archaea using the Arc344F/Arc806R primer set and for bacteria using the universal Bac515F/Bac806R primer set) while incorporating linkers required for subsequent sequencing with the MiSeq 500 sequencer platform (Illumina). PCR amplification, quality control and sequencing were performed at Hy Laboratories Ltd (Rehovot, Israel). Data analysis is described in the Supplementary Methods (available online).

RESULTS AND DISCUSSION

Applying circulation between AD and MEC enhances hydrogen solubility and increases its availability for the hydrogenotrophic community

The specific objective of this study was to design and test an innovative biological approach converting CO_2 and H_2 to CH_4 using a process that incorporates a hybrid high-rate anaerobic–microbial electrolysis cell (AD-MEC) for low-cost hydrogen production. Since the MEC system has the potential to produce hydrogen in a cost-effective manner from wastewater, the reduction of CO_2 to CH_4 in the AD system has the potential to increase the yield of CH_4. The enriched-hydrogen liquid was directly fed into the methanogenic community to upgrade the biogas without any harvesting step. In order to increase hydrogen solubility, circulation between the AD and MEC was employed at a circulation ratio of 9 (compared with the flow rate of the feed). Figure 1 shows the observed CH_4 production yields of the AD reactor in the different operation phases. The theoretical CH_4 yield (337.5 mL/g COD, dashed line) corresponds to the maximum CH_4 that can potentially be produced from the anaerobic digestion process without upgrading. The observed CH_4 production yield refers to the total CH_4 produced from anaerobic digestion combined with the biogas upgrading. CH_4 production did not change significantly upon coupling of the MEC to the AD (AD-MEC, without circulation) relative to the AD without MEC (Figure 1). These results indicate that CH_4 production was a result of the primary anaerobic stage (AD_1), as expected, before upgrading. Importantly, coupling MEC and AD_2 without circulation did not significantly improve the production yield of methane.

On the other hand, a significant increase in the observed CH_4 production yield was noted following the application of the circulation of the effluent between AD_2 and MEC (Figure 1). These results clearly indicate that circulation enhances hydrogen solubility and, therefore, increases the

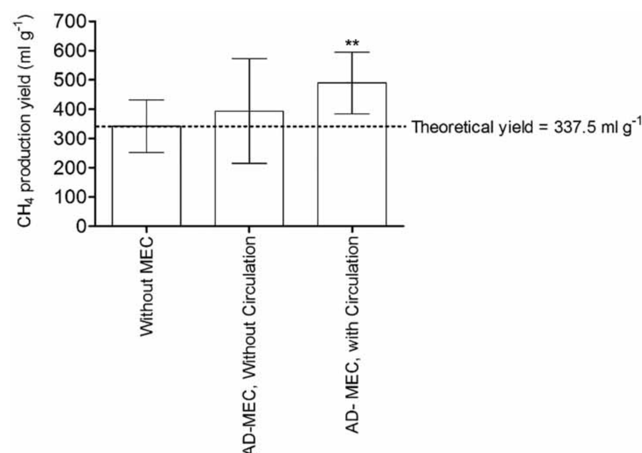

Figure 1 | CH_4 production yield (±SD) produced by the AD_2 reactor in the different operation phases (calculations are described in Materials and methods section). Dashed line denotes the theoretical CH_4 production yield obtained from the removal of 1 g of COD.

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bioavailability of hydrogen for the hydrogenotrophic methanogenic community. Other studies have reported that coupling AD and MEC reactors increased methane production. Bo *et al.* (2014) showed a 2.3-fold increase in methane yield when the coupled AD-MEC reactor was applied with 1 V. Liu *et al.* (2017) found that only a two-chamber configuration enabled methane enrichment in biogas compared with a single-chamber AD-MEC comprised of an *in situ* electrode-configuration. In essence, most of the studies combining the AD reactor with MEC have directly applied the electrodes within the AD reactor (Bo *et al.* 2014; Park *et al.* 2018; Yu *et al.* 2018). However, Xu *et al.* (2014) compared between an *in situ* and an *ex situ* electrode-configuration in which an external digester was fed with glucose. In contrast to results presented by Liu *et al.* (2017), Xu *et al.* (2014) found that the *in situ* biogas upgrading system was more effective than the *ex situ* one, however, no circulation was applied in both studies. Therefore, here is the first time that methane production was improved through increasing hydrogen solubility by applying the electrodes in a separate MEC reactor combined with high-rate circulation of the hydrogen-rich effluent flowing into the hydrogenotrophic-enriched AD reactor. The results show that circulation was instrumental in substantially increasing methane production yields relative to the AD reactor without MEC, as well as the combined AD-MEC without circulation.

Importantly, it was shown that while the CH_4 production yield of AD without MEC was identical to the theoretical value, the combined system showed 1.17 and 1.45 increases for AD-MEC without and with circulation, respectively. These results correlate with other studies that reported little improvement of methane production after combining an AD-MEC relative to theoretical maximum yields (Park *et al.* 2018). Overall, the results emphasize the potential of the combined AD-MEC system with circulation to substantially increase CH_4 production yields beyond the theoretical expected values.

The observed current density of the MEC reactor in the different operation phases is shown in Figure 2. The current density is produced as a result of organic matter oxidation in the anode surface by electrochemically active microorganisms that transfer electrons to the anode and secrete protons to the liquid media. The electrons travel through the external circuit and react on the cathode surface with the protons that diffuse toward it from the anode. Since an additional voltage is applied to the circuit, hydrogen formation becomes thermodynamically possible (Logan *et al.* 2008). Figure 2 shows that current density was not affected by the increased circulation. This result indicates that MEC performance was robust under the different conditions without any lack of organic matter, especially VFA, on the anode. The observed current density was lower than that reported ($1\ A\ m^{-2}$) for an *in situ* electrode-configuration fed with glucose (Xu *et al.* 2014), but similar to that obtained by the *ex situ* electrode-configuration in the same study ($0.4\ A\ m^{-2}$). Yet, current densities were substantially higher compared with other combined AD-MEC reactor systems fed with glucose (Gajaraj *et al.* 2017). The differences in current densities may have resulted from gas–liquid transfer limitations that occurred in the different systems and differences in overall MEC internal resistance.

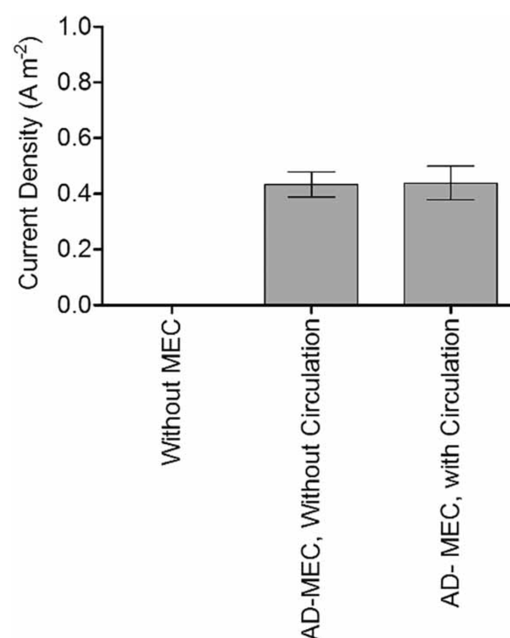

Figure 2 | Current density produced by the MEC reactor in the different operation phases.

MEC performance is highly dependent on the organic load influent

The MEC was connected to the AD reactors starting on day 117 and until the last day of the experiment (255 days). The average current density throughout the experiment was relatively constant, and predicatively it was highly dependent on the VFA concentration of the inlet (Figure 3). There were two time-points (day 143 and day 180) in which the current was considerably altered due to a sudden drop in VFA concentrations (hereafter starvation period, days 143 and 180). Subsequently, at these time-points the anode and cathode potentials became more positive (on day 143: −93 mV, −293 mV, respectively, and on day 180: −153 mV, −293 mV, respectively), as shown in Figure 4.

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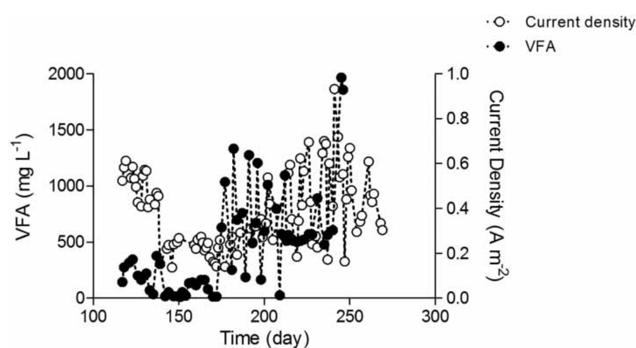

Figure 3 | VFA concentration in the inlet into the MEC and current density as a function of time.

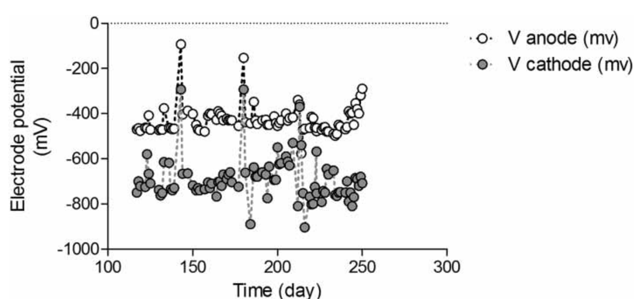

Figure 4 | Anode and cathode potentials (vs Ag/AgCl).

Once current density reached steady-state values following the starvation period (Figure 3), the circulation ratio was increased to nine times the feed flow rate between AD_2 and MEC. Comparing the polarization curve for MEC performance on day 187 (pre-circulation) and on day 215 (post-circulation), it was observed that MEC performance was considerably improved for the entire range of the applied voltage (−500–1,500 mV, Supplementary Figure S2, available with the online version of this paper). The maximal current and power density for pre- and post-circulation were 0.50 and 1.01 A m^{-2}, respectively. Maximal power density was 744.6 and 1,517.4 mW m^{-2}, respectively.

Microbial community analysis of the hybrid AD-MEC

The amplicon sequencing generated more than five million bacterial and more than three million archaeal raw reads with median sequence lengths of 251 and 377, respectively. Samples were taken routinely from the combined system (AD-MEC), as well as an additional AD, which was not connected to an MEC reactor (control AD reactor). The control reactor operated for over a year, from which seven samples were taken for community analysis during an 80-day time-period. Additionally, the control AD reactor was injected with pure hydrogen under different rates and concentrations. Importantly, all time-points except one (day 396) exhibited steady-state conditions and accordingly, the bacterial and archaeal compositions were relatively stable (Supplementary Figure S3, available online). For the combined AD-MEC system, microbial analysis results are presented for samples taken from the MEC and AD_2 (enriched hydrogenotrophic reactor) as circulation was applied between these reactors to enhance biogas upgrading. Thus, the microbial dynamics of those specific reactors were of the most interest.

A principal coordinates analysis (PCoA) was plotted for both the bacterial and archaeal samples (Figure 5). The plots differ in the degree of separation between the samples taken from the combined and non-combined systems. The bacterial plot (Figure 5(a)) shows a slightly stronger separation between the AD-MEC samples and control samples (ANOSIM $p = 0.0005$, $R = 44\%$) relative to the archaea (ANOSIM $p = 0.0002$, $R = 37\%$). Additionally, the bacterial plot shows a stronger separation between the

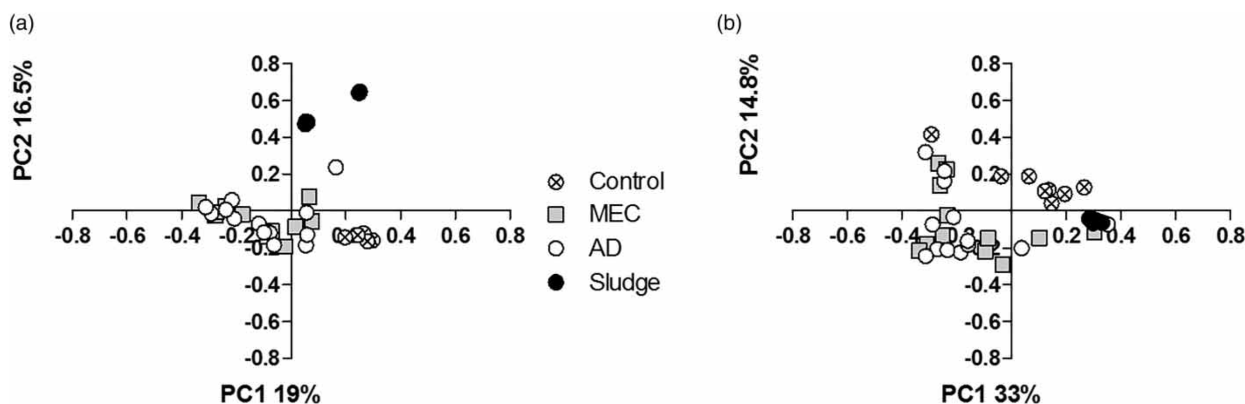

Figure 5 | Principal coordinates analysis (PCoA) based on the Bray Curtis distance metric between (a) bacterial microbial communities and (b) archaeal microbial communities of the samples from the different reactors, as indicated by the different colors. PC: principal coordinate.

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combined system-derived samples and the sludge samples, while the archaeal samples separated less well from the sludge sample used for inoculation (Supplementary Tables 1 and 2, available online). This indicates that there were bacterial community members appearing in higher abundances in the combined system (i.e. electroactive species known to be enriched primarily in microbial electrochemical systems).

As expected, known electroactive species (e.g. *Geobacter* and *Desulfuromonas* spp.) that are typically enriched primarily in microbial electrochemical systems (Yu *et al.* 2018) were more abundant in the combined system relative to control AD reactors and the sludge samples (Figure 6).

Figure 7 shows the composition of the different methanogenic orders in the reactors at the different time-points sampled. All reactors were composed primarily of three orders: *Methanobacteriales*, *Methanomicrobiales* and *Methanosarcinales*, in agreement with previous reports on AD communities (Yu *et al.* 2006). While these three groups were generally evenly distributed in the control reactors and sludge samples (Figure 7(a) and 7(b)), in the combined system, *Methanomicrobiales* predominated in both reactors (Figure 7(c) and 7(d)). Interestingly, while the control reactors were relatively stable with *Methanosarcinales* relative abundance throughout their operation, an increase in this group's abundance in the combined system was correlated with time-points at which there was a technical problem. Essentially, there was a switch between the *Methanomicrobiales* and *Methanosarcinales* groups on day 150 in both of the combined reactors (AD and MEC, Figure 7(c) and 7(d)). Yet, this switch did not persist, as the *Methanomicrobiales* relative abundance almost immediately increased and stabilized for the rest of the operation until the last time-point at which an additional technical failure occurred. Thus, it is assumed that in such systems, the appearance of *Methanosarcinales* can be defined as a marker for system failure in such enriched hydrogenotrophic reactors, since these methanogens can utilize a variety of non-hydrogen substrates. Under steady-state conditions when hydrogen is bioavailable in the system, *Methanomicrobiales* dominate the system and outcompete other methanogens (mainly acetoclastic). However, a rapid shift from *Methanomicrobiales* to *Methanosarcinales* could predict a technical or possible system failure probably reflecting a reduction in hydrogen availability in the bioreactor.

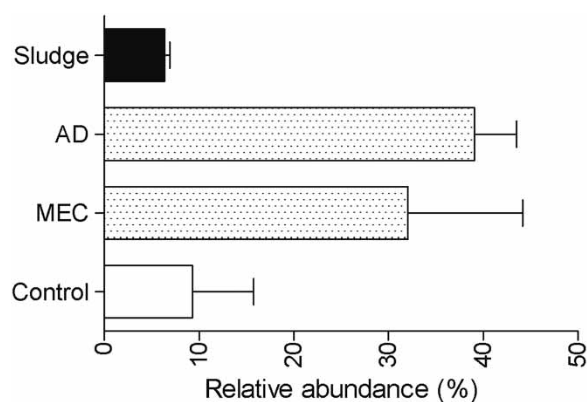

Figure 6 | Relative abundance of the electroactive spp. (sum of *Geobacter* and *Desulfuromonas* relative to the sum of the Deltaproteobacteria class) in the control reactor, the combined system reactors and sludge samples. Both *Geobacteraceae* and *Desulfuromonadaceae* are known as electroactive bacterial groups and they are closely related both phylogenetically and functionally.

Key changes of both bacterial and archaeal family-level groups were followed in AD_2 relative to the control AD reactor to further examine the dynamics of the above-mentioned switch (around day 150 of the experiment), as presented in Figure 8. Key groups were chosen based on significant fold-changes of relative abundance compared with their average abundance observed in the sludge samples used for initial inoculation of the reactor, as well as compared with the relative abundance in the control AD reactor that was not connected to the MEC. According to control AD (Figure 8(a)), while the order *Methanobacteriales* was relatively stable throughout the time, *Methanomicrobiales* and *Methanosarcinales* were highly dynamic and their abundance oscillated antagonistically to each other. Similar observations were made for the combined system (Figure 8(b)). Family-level investigation revealed that while *Methanosaetaceae* was the more dynamic group in the control AD, *Methanosarcinaceae* belonging to the *Methanosarcinales* order was highly stable through time (Figure 8(c)). On the other hand, *Methanosarcinaceae* abundance in the AD-MEC system substantially increased on day 150 and *Methanosaetaceae* was less affected by the technical problem that occurred around that day. Conversely, the abundance of the hydrogenotrophic methanogen family, *Methanospirillaceae*, substantially decreased at this time-point which presumably allowed the rise of the acetoclastic methanogen *Methanosarcinaceae* (Figure 8(d)). Interestingly, the abundance of the other highly abundant groups, *Methanobacteriaceae*, *Methanoregulaceae* (*Methanomicrobiales*) and *Methanosaetaceae* (*Methanosarcinales*) were dynamically consistent with each other in the combined reactor. The hydrogenotrophic *Methanospirillaceae* was previously shown to be the key CO_2 reducer when applying sufficient voltage for H_2 production in other combined AD-MEC systems (Bo *et al.* 2014). The other archaeal groups were also reported previously

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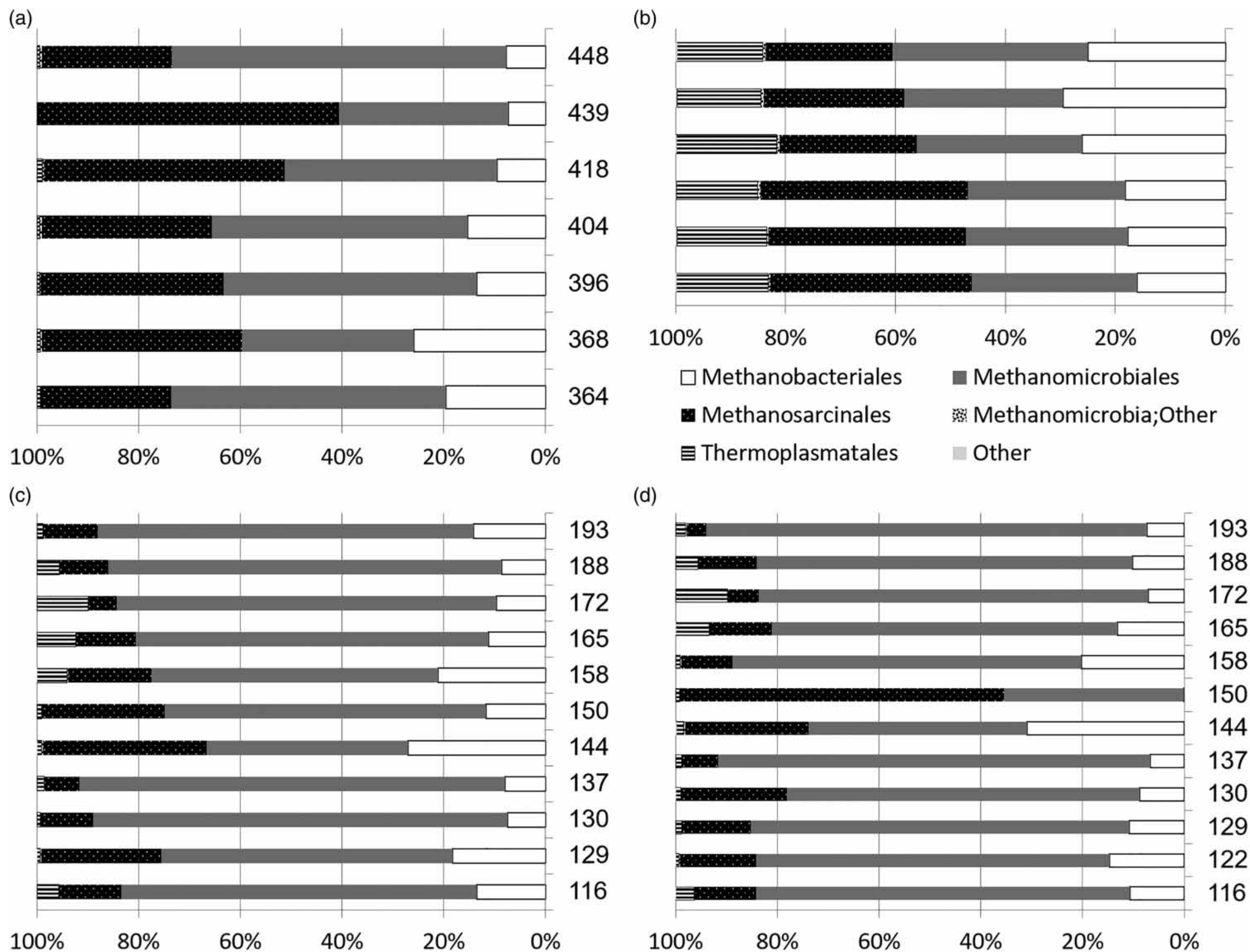

Figure 7 | Relative abundance of archaeal orders belonging to the Euryarchaeota phylum in the different reactors: (a) samples collected from the control AD reactor not connected to an MEC; (b) samples collected from six inoculum sludge samples; (c) samples collected from the MEC; (d) samples collected from the AD_2 (the combined system). Each color on the graph represents a different order of methanogenic archaea.

(Bassani *et al.* 2015). Thus, *Methanospirillaceae* is suggested to be the primary methanogen family responsible for the biogas upgrading in our system, while *Methanoculleus* was also recently identified as a dominant hydrogenotroph in such systems (Treu *et al.* 2018).

In accordance with bacterial communities reported in previous studies, *Bacteroidetes*, *Proteobacteria*, and *Firmicutes* were the most abundant groups in the system, and were likely involved in substrate degradation (Nelson *et al.* 2011; Yi *et al.* 2016). Methanogens, and especially hydrogenotrophs, generally metabolically interact with different bacterial community members (Stams *et al.* 2003; Treu *et al.* 2018). Thermodynamically, hydrogenotrophic methanogens are crucial for keeping a low H_2 concentration that maintains a favorable reaction rate of acetogenic reactions that channel acetic acid into H_2 and CO_2 (Stams *et al.* 2003), but are often outcompeted by other microbes. These methanogens are the key players in ensuring complete conversion of the organic waste to methane. Thus, the purpose of separating the AD reactor into AD_1 and AD_2 was to overcome this thermodynamic challenge, allowing a function-based subdivision between the following: (1) AD_1 – the primary steps of biomass degradation are hydrolysis, fermentation and acetogenesis. The latter includes bacterial members that are highly sensitive to high hydrogen concentration. (2) AD_2 – the biogas upgrading process that requires an external input of hydrogen and CO_2 that leads to the enrichment of hydrogenotrophic methanogens.

Interestingly, the bacterial community composition in the control reactor exhibited a dramatic shift on day 396. While all other time-points had a stable hydrogen

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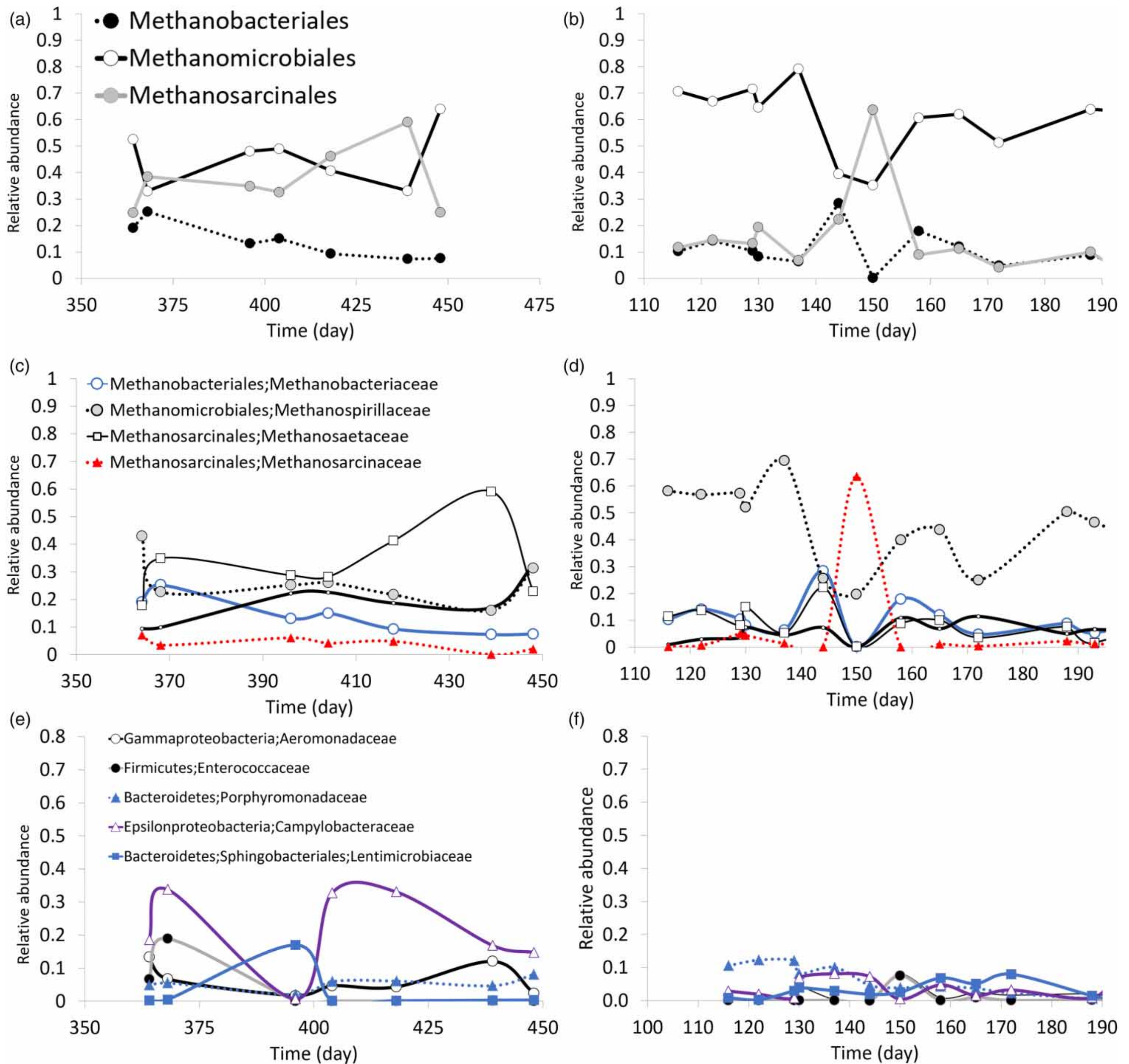

Figure 8 | (a) Relative abundance of most abundant archaeal orders belonging to the Euryarchaeota phylum in control AD; (b) relative abundance of most abundant archaeal orders in AD_2 (the combined system); (c) relative abundance of most abundant archaeal families in control AD; (d) relative abundance of most abundant archaeal families in AD_2; (e) relative abundance of most abundant bacterial families in control AD; (f) relative abundance of most abundant bacterial families in AD_2.

consumption rate, on day 396 a reduced consumption rate was observed (data not shown), leading to a temporary, short-term higher concentration of hydrogen in the system. Although the archaeal community composition was not affected by this change (Figure 7(a)), the bacterial community revealed an increase in *Bacteroidetes* and a reduction in *Proteobacteria* (Figure S3). In contrast to the combined reactor's critical time-point (around day 150) at which, due to the technical failure, the MEC directed a lower portion of hydrogen-enriched liquid into AD_2, the same bacterial community groups were affected in an opposite manner (Figure 8(e) and 8(f)). While the abundance of the same specific proteobacterial family (*Campylobacteraceae, Epsilonproteobacteria*) was affected similarly in both cases, the *Bacteroidetes* families were different (*Lentimicrobiaceae* belonging to the order *Sphingobacteriales* in the control

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reactor and *Porphyromonadaceae* belonging to the order *Bacteroidales* in AD_2 of the AD-MEC system). Bassani et al. (2015) reported that an unclassified member of *Sphingobacteriaceae* was found to decrease after the addition of H_2. Here, these closely related members of *Bacteroidetes* responded differently to hydrogen exposures. Remarkably, *Lentimicrobiaceae* was found to grow syntrophically with the hydrogen-consuming methanogen *Methanospirillum hungatei* (Sun et al. 2016), supporting its enhanced growth once hydrogen availability increased at this time-point. Essentially, the bacterial composition dynamics indicated specific groups that flourish under high and low hydrogen concentrations, as well as important bacterial–archaeal interactions that occurred under those conditions.

CONCLUSION

The results show that the combination of MEC with AD significantly improved the performance of an anaerobic treatment system, while substantially upgrading the potential for a higher-quality biogas in a cost-effective manner. Yet, the successful upgrading process was achieved only when circulation of the hydrogen-enriched liquid was applied between the MEC and the second AD reactor, leading to a higher hydrogen bioavailability for hydrogenotrophic methanogens. Accordingly, microbial community analysis confirmed that the process was enriched with electroactive species in the MEC-based enhanced hydrogen production module of the system. It was also observed that while in the control reactor there was a relatively even distribution of acetoclastic and hydrogenotrophic methanogens, in the combined system hydrogenotrophic methanogens were more abundant at most of the time-points. However, at critical time-points when failure occurred, the dominance of acetoclastic methanogens suddenly increased, as well as other bacterial members that thrive under hydrogen-limiting conditions, clearly indicating an association between such combined anaerobic system performance and an increased abundance of specific microbial groups. Consequently, the microbial results show proof of concept that upgrading the produced biogas by using soluble hydrogen directly from a microbial electrochemical device is plausible, and thus has great potential for biogas upgrading without the need for direct hydrogen harvesting. Nevertheless, this process, undoubtedly, requires further optimization from the engineering and microbial perspectives, to facilitate a more practical upscaled application for commercial wastewater treatment systems.

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