Microbial fuel cells for polishing effluents of anaerobic digesters under inhibition, due to organic and nitrogen overloads

Short title: Microbial fuel cells for polishing effluents of inhibited anaerobic digesters

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ABSTRACT

BACKGROUND: Bioelectrochemical systems have been proposed as a possible polishing step for anaerobic digestion (AD). They can also be useful to overcome AD instability in case of AD inhibition while, at the same time recovering ammonia. Continuous assays with a microbial fuel cell (MFC) fed with digested pig slurry were performed to evaluate its operation during malfunction periods of the AD reactor and its feasibility as a strategy to recover ammonia, either by introducing VFA pulses in the MFC or by inducing AD inhibition. A microbial community assessment was performed to study MFC changes over its operation when fed with the digestate.

RESULTS: The MFC achieved COD removal efficiencies of 50% during AD inhibition, reaching a maximum of ammonium removal of 31% (11.19 g_N m^{-2} d^{-1}). A high throughput *16S rRNA* gene based sequencing assessment revealed that the anode biofilm was different from the digestate fed, showing a reduction in microbial population diversity in the anode after a 182-day-operation period with digested pig slurry. The main enriched populations in the anode belonged to *Bacteroidetes* (*Flavobacteriaceae), Chloroflexi (*fermentative bacteria *Anaerolineaceae), Methanosarcinaceae* and hydrogenotrophic methanogens belonging to *Methanobacteriaceae.*

CONCLUSION: MFCs have proven to be a reliable technology to complement the operation of AD, improving the quality of the effluent and recovering ammonia, particularly during AD inhibition.

Keywords

Microbial Fuel Cell (MFC), Anaerobic digestion, Ammonia recovery, System stability, Integrated system, High throughput sequencing.

INTRODUCTION

Microbial fuel cells (MFC) are bioelectrochemical devices with a wide range of applications, from power sources to biosensors.[1] MFCs have recently proven to be a promising technology when combined with anaerobic digestion (AD), mainly as a polishing strategy to increase the quality of the effluent, either by reducing its organic matter content or by removing nutrients.[2-5] Different kind of substrates have been used: landfill leachate,[6] wastewater from potato-processing industries,[7] sludge,[8] pig slurry,[9, 10] a mixture of swine manure and rice bran[2] or molasses wastewater.[5]

MFCs can be particularly useful, when the AD suffers from inhibition, as a buffer system to complement the AD process until it recovers. AD processes, especially when performed at thermophilic temperature ranges, are known to be sensitive to several substances that may be present in the waste stream, such as ammonia.[11] In the case of inhibition of the AD, an increase in VFAs in the effluent will take place[12] and a system such as a MFC will be necessary to maintain the quality of the effluent.[8]

Aside from the suitability of MFCs as a means to reduce VFAs,[13] they have also been used for ammonia recovery, since in a two chambered system a flux of cations travels through the cation exchange membrane, from the anode to the cathode compartment, in order to maintain the system's electroneutrality.[14] A subsequent stripping and absorption step will allow recovered ammonia to be reused as a fertiliser.[9,15-18] This application is particularly interesting since AD does not modify the total amount of N in the digestate, and thus needs to be combined with other processes for N removal or recovery, particularly when working with high strength wastewaters such as livestock manure. Recently, a hybrid system consisting of a continuous stirred tank reactor and a submersible microbial desalination cell has been developed for counteracting ammonia inhibition during AD when working with synthetic wastewater.[19] A later work has coupled MFC to AD to study not only COD removal, but also nitrogen balance, although focused on nutrient removal instead of recovery.[2]

The combination of thermophilic AD with a MFC under perturbation has been previously evaluated by the addition of a severe acetic acid load.[20] However, MFC performance against AD destabilisation has not been studied in depth, nor its influence in ammonia recovery. A previous

study performed under microbial electrolysis cell (MEC) mode, thus applying a low amount of energy to the system to boost the process, has shown that VFA accumulation in pig slurry digestates can be removed in a MEC reactor while recovering ammonium at the same time.[21, 22] That study also showed that higher MEC performances were attained when the AD was under an unstable or inhibited state. Therefore, when it comes to reactor sustainability, it is interesting to assess whether this behaviour is also observed in an energy producing MFC without the addition of external energy.

Furthermore, the combination of AD with a MFC system may modify the anode's biofilm biomass, both due to the constant feed of allochthonous biomass from the digestate and to the anodophilic enrichment on the anode. Therefore, microbial population evolution under these conditions is a field that needs an in-deep study in order to gain insight on the stability of microbial biofilms established on the anode in bioelectrochemical system (BES) reactors. A previous study has shown a reduction in anode biodiversity when integrating pig slurry AD with a MEC system,[22] but more data are needed to understand these changes with a MFC system when no additional energy is supplied to the BES.

Taking it all together, a MFC is developed here at lab-scale as a downstream system for AD of high strength wastewater, able not only to polish the digestate, but also to buffer the AD under inhibited state. At the same time, the MFC will recover the ammonia content of the digestate, being thus a system with three combined objectives, filling the gaps existing in the work described previously.

The main aim of this study is to assess the performance of a MFC operated in combination with a pig slurry thermophilic AD, as a system to overcome AD destabilisation and inhibition periods due to organic and nitrogen overloads. The effluent quality will be assessed in terms of COD, VFA and ammonium removal. Also, changes in the microbial composition of the MFC anode will be assessed.

EXPERIMENTAL

Experimental set-up

The MFC reactor consisted in a two chambered cell, made of methacrylate, previously operated in batch mode with digested pig slurry for 2 months, as it was described elsewhere.[9] Each compartment had a volume of 0.5 L, and was separated by a cation exchange membrane (CEM, dimensions: 14 x 12 cm; Ultrex CMI-7000, Membranes International Inc., Ringwood, NJ, USA). The anode (carbon felt) and the cathode (stainless steel mesh) were connected through an external 100 Ω resistance. An Ag/AgCl reference electrode (Bioanalytical Systems, Inc., USA, +197 mV vs. standard hydrogen electrode (SHE)) was inserted in the anode (working electrode) compartment. A potentiostat (VSP, Bio-Logic, Grenoble, France) was used for data monitoring in a three-electrode mode. All potential values in this paper are referred to SHE. A personal computer connected to the potentiostat recorded electrode potentials and current, every 5 min, using EC-Lab software (Bio-Logic, Grenoble, France). Digested pig slurry was used as feeding solution in the anode compartment (Table 1), previously filtered through a stainless steel 125 μm sieve. The feeding solution for the MFC cathode chamber contained (per litre of deionised water): KH_2PO_4, 3 g; Na_2HPO_4, 6 g (pH of the buffer of 9.1). The solutions of both the anode and the cathode compartments were continuous fed with a pump at a rate of 23 mL h^{-1} and mixed by recirculation with an external pump.

Reactor operation

The MFC was operated in continuous mode for 182 days with a 34-hour hydraulic retention time (HRT). The stability of the AD-MFC integrated operation was assessed in two different experiments, using digestates from two different sources. Table 1 shows the main characteristics of the feeding solutions.

In Phase 1, the first block of experiments, the MFC was fed for 40 days with the digestate from a pig slurry mesophilic AD plant with a HRT of 40 days (Vila-Sana, Lleida, Spain), diluted with tap water (1:2) to obtain the desired COD. The organic loading rate (OLR) of the MFC anode compartment was set at 18.22 g_{COD} L^{-1} d^{-1}, similar to that of a previous work in MEC mode. [22] As a

preliminary performance evaluation of the MFC at a punctual overload episode of the AD system, a series of pulses of pure and mixed VFAs were performed on the anode compartment (Table 2). Samples were taken from the anode and the cathode compartment before each pulse, and 1, 4, 7, and 24 hours after each pulse.

The second block of experiments, Phases 2a and 2b, started a week after the last pulse of Phase 1. To assess its performance with a real inhibited AD effluent, the MFC was fed for 142 days with the effluent of a 4 L lab-scale thermophilic AD, which has been previously described.[22] This AD was fed with pig slurry with a HRT of 10 days. In Phase 2a, the MFC was fed for 80 days with the effluent of the AD performing in a stable state, whilst in Phase 2b (62 days) the effluent resulted from the AD destabilised by an organic and nitrogen overload. The resulting OLR of the MFC anode compartment was of 10.59±1.78 and 24.10±5.20 g_{COD} L^{-1} d^{-1} in Phase 2a and 2b, respectively. The Nitrogen Loading Rate (NLR) was of 0.90±0.05 and 1.84±0.06 g_N L^{-1} d^{-1}, also respectively. All the assays were performed at room temperature (23±2 ºC).

Analytical methods and calculations

The pH of the bulk solution, COD, alkalinity, ammonium ($N\text{-}NH_4^+$), VFAs and methane composition of the samples were measured following methods which were previously described (APHA, 1999).

Methane production (determining the amount of dissolved methane in the solution), ammonium and COD removal efficiencies, current density (A m^{-2}) and coulombic efficiency (CE), were calculated according to methods which were previously reported.[22]

Anaerobic biodegradability assay

In order to assess the biodegradability of the remaining organic matter of the digestates used as feed solution for the MFC, anaerobic biodegradability tests (ABT) of the two different digestates used in Phase 2, were performed. ABT were performed in duplicate using serum bottles (120 mL) according to Soto et al.[23] and Angelidaki et al.[24] These serum bottles were filled with a 50 g solution comprised of the inoculum (5 g_{VSS} L^{-1}), substrate (5 g_{COD} L^{-1}), macronutrients, micronutrients, and bicarbonate (1 $g_{NaHCO3-}$ $g_{CODadded}^{-1}$). The digested sludge from a mesophilic lab-scale anaerobic digester was used as inoculum. A control in duplicate, without the digestate substrate, was included in the setup. The bottles were sealed with rubber stoppers and capped

with aluminium crimp caps. The headspace was purged with N_2 for 5 min in order to remove O_2. The bottles were incubated at 37±2 ºC for 62 days. Methane production was monitored periodically taking a gas sample (0.2 mL) from the head space with a syringe and analysing the gas composition by gas chromatography, equipped with a thermal conductivity detector (TCD).

Microbial community analysis

The bacterial communities attached to the anode material in the MFC at the beginning and at the end of the experiments, as well as those present in the digested pig slurry in Phase 1, were analysed using culture-independent molecular techniques such as quantitative real-time PCR (qPCR) and high throughput *16S rRNA* gene sequencing (MiSeq, Illumina). The microbial diversity and structure of the MFC influent (digestate), in Phase 2a and 2b, have been previously described as part of a recent study, there respectively described as Phase 1 and Phase 2.[22] This referred study showed that *Pseudomonadaceae* (20%), and *Clostridiaceae* (20%) were the eubacteria families respectively predominant in Phase 2a and 2b, while *Methanobacteriaceae* was the predominant archaea family (98%) in both digestates.

Quantitative PCR assay (qPCR)

Total DNA was extracted in triplicate from known weights of each sample with the PowerSoil® DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA), following the manufacturer's instructions. Gene copy numbers of eubacterial *16S rRNA* gene and *mcrA* gene (methanogenic archaeal methyl coenzyme-M reductase) were quantified by quantitative real-time PCR (qPCR). Each sample was analysed in triplicate by means of the three independent DNA extracts. The analysis was carried out following a protocol which was described elsewhere.[22] Standard curve parameters of the qPCRs performed showed a high efficiency, and were as follows for *16S rRNA* and *mcrA*, respectively: slope of -3.407 and -3.591; Y-intercept of 39.26 and 39.48; correlation coefficient of 0.999 and 0.998; efficiency of 97 and 90%.

High throughput sequencing and data analysis

The same total DNA obtained from the anode of the MFC and Phase 1 AD effluent used for qPCR analysis was also used for high throughput *16S rRNA* gene based sequencing by means of MiSeq technology (Illumina). The specific steps of MiSeq analysis for eubacteria and archaea were

carried out as it was described elsewhere.[22] The taxonomic assignment of the obtained Operational Taxonomic Units (OTUs) was performed using the Naïve Bayesian Classifier tool (16S rRNA training set 14) –with an 80% bootstrap cut-off value– from the Ribosomal Database Project (RDP).[25]

The data obtained from sequencing datasets were submitted to the Sequence Read Archive of the National Centre for Biotechnology Information (NCBI), under study accession number SRP070839, for eubacterial and archaeal populations.

To evaluate the diversity of the samples, the number of OTUs, the inverted Simpson index, Shannon index, Goods coverage and Chao1 richness estimator were calculated using the Mothur software v.1.34.4 (http://www.mothur.org).[26] All the estimators were normalised to the lowest number of reads among the different samples. Multivariate statistical correspondence analysis of MiSeq data (relative OTU distribution matrix) was performed by means of XLSTAT 2014 software (Addinsoft, Paris, France).

RESULTS AND DISCUSSION

MFC performance under AD instability: assays with VFA pulses in the feed.

To test the performance of the MFC when an AD instability event occurred, the MFC was fed with the digestate, plus different pulses of VFA to simulate the composition of the digestate under these periods (Table 2). During the first 7 days of Phase 1, before any VFA pulse was performed, the MFC produced current densities in a range of 100-300 mA m^{-2}. When the pulses of VFA started to be applied, the current density was maintained at around 150 mA m^{-2}. Although some peaks in current were registered, a clear correspondence between VFA introduction and the increase in current density could not be established (Figure 1a). After each VFA pulse, VFA concentrations showed a fast decrease, returning to values existing before the addition of VFA in less than 24 h. The pH of the bulk solution in the anode compartment remained in a range of 7.3-8.8, being thus appropriate for microbial activity (Figure 1b). In a previous work, using a MEC, current density increased in response to each VFA pulse.[21] This behaviour agrees with the low coulombic efficiencies achieved when working with MFCs (1-4%) compared to those achieved in MEC mode

(11-18%).[9] Notwithstanding energy recovery, these results show that MFCs can absorb a punctual increase in VFA without showing signs of destabilisation.

MFC performance under AD stable and inhibited states

During Phase 2a, the MFC was fed with the effluent of a lab-scale thermophilic AD at steady state operation (Table 1). Average current density was of 121 mA m^{-2}. At the beginning of this period, the COD removal efficiency in the MFC was as high as 48%, gradually decreasing to values between 10% and 20%, at the end of the period (Figure 2), as the COD of the influent gradually decreased from 18.70 to 12.50 g L^{-1}. This reduction of the COD of the influent shows an improvement in performance of the AD. On day 80 the influent was changed, shifting to the effluent obtained from an AD inhibited as a result of an increase in its organic and nitrogen loading rates (Phase 2b). The MFC reacted showing an increase in COD removal efficiency up to values of 50%, stabilising around 30% to 40%. At the end of Phase 2a, the COD of the MFC effluent was of 10.06 g kg^{-1}, representing a 1.57 g_{COD} L^{-1} d^{-1} removal. At the end of Phase 2b, a 3.2-fold increase in COD removal was achieved with respect to Phase 2a (5.04 g_{COD} L^{-1} d^{-1}). Despite the increase of influent COD and removal rates, the average current density was similar to the previous phase (112 mA m^{-2}). The COD removal efficiency achieved in Phase 2b is slightly lower than the obtained by Kim et al. (2015) working with a two-chambered MFC (external resistance of 1 kΩ) fed in batch with digested swine wastewater (61.1%).[10]

Since the COD removal efficiency of the MFC may be limited by the biodegradability of the substrate, anaerobic biodegradability tests (ABT) were performed on the digestates. Results showed that the maximum biodegradability of the digestates utilised in Phase 2a and Phase 2b was of 53% and 81%, respectively. The MFC maximum COD removal efficiency in Phase 2a was 81% of the achieved in the ABT, while it was 62% in Phase 2b. So in this case, the biodegradability of the substrate was not a limiting factor in the MFC, and longer HRTs would help to achieve higher COD removal efficiencies.

Average CEs were 1.07±0.59% and 0.30±0.06% in Phase 2a and Phase 2b, respectively, much lower than those obtained in batch experiments with digested pig slurry and the same reactor and external resistance (100 Ω).[9] A CE as high as 19% has been achieved in an AD-MFC combined

system (external resistance of 500 Ω) fed in fed-batch with a mixture of swine manure and rice bran (6.8 g_{COD} L^{-1}).[2] In that work, COD removal efficiency was 17.6%, much lower than the one obtained in this system. Despite achieving a higher COD removal in Phase 2b than in Phase 2a, an increase in current density was not observed. A previous study has found that an increase in the organic loading rate is not always followed by a significant increase in current generation, since the increase in the loading rate needs to be accompanied by a decrease of the external resistance in order to increase the continuous current generation.[27] The conversion of COD to methane only represented between 0.5% and 3.5% of the electron loss in this MFC in Phase 2a, and between 0.3% and 0.9% in Phase 2b, concurring with the dissolved methane detected in the samples. So, other complex processes are taking part in the anode compartment, and other electron acceptors may be present in the substrate, reducing the number of electrons reaching the electric circuit.

Ammonium removal efficiencies oscillated between 12% and 27% in Phase 2a, and between 14% and 31% in Phase 2b. $N\text{-}NH_4^+$ concentrations in the effluent were of 1.03 g L^{-1} and 2.23 g L^{-1} at the end of Phase 2a and Phase 2b, respectively. These values represent an $N\text{-}NH_4^+$ flux through the CEM of 4.76 g_N m^{-2} d^{-1} and 11.19 g_N m^{-2} d^{-1}, also respectively. Previous assays, performed in a batch mode MFC with the same external resistance, achieved a flux of 8.86 g_N m^{-2} d^{-1}, although working with a lower COD and ammonia concentrations; in that case, it was also found that ammonia transport in an open circuit was as high as the one obtained in a closed circuit. Hence, all ammonium transport was being produced by diffusion.[9] Ammonia transport by diffusion may still play an important role in this system, probably due to cation exchange with the cathodic buffer. In Phase 2b, ammonia diffusion may be favoured by the 2-fold higher ammonium concentration in the influent. Nitrogen removal rates, obtained in other studies so far, range between 2.94 and 162.18 g_N m^{-2} d^{-1}.[28] The removal rate in this study is higher than the 3.3 g_N m^{-2} d^{-1} value obtained with a MFC working with urine at a higher current density (500 mA m^{-2}) and a 3-fold higher concentration of N in the influent (4050 mg_N L^{-1}).[17] An increase in the electron recovery efficiency of the MFC system will allow for a higher current density and higher ammonia recovery rates.

Figure 3 shows VFA concentrations both for the influent and the effluent of the MFC. At the end of Phase 2a, removal efficiencies for acetate established themselves in a range between 50% to 80%

(with a concentration in the effluent of 155-387 mg L^{-1}). In turn, propionate removal achieved 100%. Thus, the effluent of the AD was polished, removing residual VFA concentrations of the digestate. During Phase 2b, an increase in the VFA of the influent was observed, particularly acetate and propionate, as a result of the destabilisation of the AD. The reduction of VFA concentrations in the MFC at the end of this period was in the order of 52%-64% for acetate and 55%-70% for propionate. Minor VFAs such as iso- and n-butyrate and iso- and n-valerate, were reduced in 68%-70%, 92%-98%, 71%-77% and 87%-100%, respectively. Average VFA concentration for the acetic equivalent remained under 2000 mg L^{-1} despite the influent concentration being in a range of 4200-6500 mg L^{-1}. The MFC is able to polish the AD effluent when the reactor is under inhibition, being the integration of both systems a valuable approach to maintain the quality of the effluent.

Microbial community analysis

The evolution of the anodic microbial community in the MFC, due to the integrated operation with the AD, was studied by means of qPCR and high throughput sequencing.

Quantitative evolution of total eubacteria (*16S rRNA* gene) and archaeal methanogenic populations (*mcrA* gene) attached to the anode material as biofilm is shown in Figure 4. After 182 days of MFC operation (at the end of phase 2b), the eubacterial population suffered a 33-fold decrease. In turn, archaeal population was reduced 3.6 times –being relatively enriched and accounting for 1.5% of the total bacterial community– but remaining in the same order of magnitude. This slight reduction in methanogenic populations may explain the reduction in methane production during Phase 2b. On the other hand, the decrease in eubacteria and archaea populations may be due to the increase in the NLR during the last phase, since some microbial populations may be sensible to high ammonia concentrations in the influent. Concentrations of free ammonia nitrogen (FAN) above 900 mg L^{-1} were reached during Phase 2b, and at these levels the first signs of inhibition may occur according to previous studies.[12,29]

Regarding high throughput *16S rRNA* gene sequencing assessment (MiSeq analysis), reads –ranging from 34,632 to 16,875 reads per sample–, and coverage obtained for bacteria and

archaeal community for each sample are shown in Table SI1. Figure SI1 shows rarefaction curves –with all the samples close to approaching a plateau when plotting OTUs vs. number of *16S* rRNA– concomitant to high coverage values (99%-100%) (Table SI1). Regarding eubacterial diversity, the inverted Simpson, Shannon, and Chao-1 indexes showed that the sample of the MFC at the beginning of the assays was the most diverse (36.63, 4.75 and 820.25, respectively), whilst the MFC anode biofilm at the end of the assays was the least diverse (8.01, 3.03 and 591.25, respectively) (Table SI1). These diversity and richness results are in concordance with a previous study that also showed a reduction of the BES biodiversity after being operated with digested pig slurry.[22]

The dominant eubacterial *phylum* identified in the anode biofilm sample of the MFC at the beginning of Phase 1 was *Bacteroidetes* (40%), followed by *Firmicutes* (20%) and *Proteobacteria* (14%) (Figure 5a). These *phyla* are also the predominant ones in previous studies.[18,22,30-33] At the end of the experiment (Phase 2b), *Bacteroidetes* increased its relative abundance in the anode up to 66%. This increase was concomitant to an increase of a minor *phylum* at the initial biofilm, *Chloroflexi* (17%), and the decrease of *Firmicutes* (8%) and *Proteobacteria* (below 0.5%). Regarding the eubacterial community, the influent (digestate) in Phase 1 and the anode biofilm were closely similar at the beginning of the experiments. The anode compartment of the MFC had been previously fed in batch mode with digested pig slurry for 2 months, as it was described elsewhere.[9] At family level (Figure 5b), the digestate in Phase 1 was dominated, as in the case of the MFC biofilms, by *Flavobacteriaceae* (24%). Indeed, *Flavobacteriaceae* (13%) was the predominant family at the initial biofilm, followed by three families with the same relative abundance, *Desulfuromonadaceae*, *Porphyromonadaceae* and *Acholeplasmataceae* (8%). The anode biofilm in the MFC at the end of the experiment (Phase 2b) showed a 2.6-fold increase of *Flavobacteriaceae* (34%, within the phylum *Bacteroidetes*). At the same time, the *Anaerolineaceae* family, mainly *Longilinea* sp., (harbouring fairly known fermentative bacteria belonging to the phylum *Chloroflexi*) increased from 1% to 17%. This change is probably due to a higher bioavailability of fermentative substrates on the digestate from the unstable anaerobic digester (Phase 2b). In a previous study, both families were identified in the anode of a MEC,[22] as well as

Anaerolineaceae in the anode of an MFC.[30] 32% and 36% of the OTUs of the initial and final biofilms of the MFC were unclassified at family level, mainly belonging to *Bacteroidetes* and *Firmicutes* phyla. They represented 47% and 34% of the unclassified OTUs in the initial MFC biofilm, respectively; and 81% and 15% in the final biofilm. These OTUs, which cannot be assigned to a known family, may be novel taxa or perhaps still poorly defined in the RDP database.

With respect to archaeal diversity, different results were obtained depending on the index considered (Table SI1). The inverted Simpson index showed the highest diversity for the initial sample of the MFC anode biofilm, being followed by the final sample and the AD effluent. Shannon index identified the final anode biofilm as the sample with the highest diversity, followed by the initial anode biofilm. However, by using Inverted Simpson indexes, which are more sensitive to samples with low diversities (i.e. Archaea), it was revealed that the diversity of archaeal community on the anode was maintained throughout Phase 2, being higher than those harboured in the inflow digestate. Chao-1 revealed complex eubacterial populations in all samples (anode and digestate) ranging from 591 to 820 OTUs, whereas archaeal communities were quite less complex, accounting for 62 to 94 OTUs.

Methanomassiliicoccaceae (49%) and *Methanotrichaceae* (45%) –formerly known as *Methanosaetaceae*– were the predominant archaea families in the anode of the MFC at the beginning of the experiments (Figure 6). After the VFA pulses experiment and inhibited AD feeding, a clear shift was observed showing a distinct enrichment of the *Methanosarcinaceae* family (70%), when in the initial anode it only represented 2% of the population. Also the *Methanobacteriaceae* family, which was under 0.5% in the initial MFC biofilm, increased up to 9% at the final sample. Both families are classified as hydrogenotrophic methanogens, *Methanosarcinaceae* being able to generate methane also by means of the aceticlastic way. On the other hand, Methanotrichaceae ("*Methanosaetaceae"*) is strictly aceticlastic. Previous studies have stated that *Methanosarcina* sp. *s*eems to be more tolerant towards ammonium stress than other methanogens, particularly *Methanosaeta* sp.[34] Thus, the increase in NLR of the MFC may have favoured an increase in relative abundance of the first one. Interestingly, Methanosarcinaceae was not a predominant group in the MFC influent, neither in Phase 1,

dominated by *Methanotrichaceae* (78%), or in Phase 2, dominated by the genus *Methanothermobacter* (98%) belonging to the *Methanobacteriaceae* family, as it was also described in a previous study.[22]

Correspondence analysis for eubacterial and archaeal populations (Figures 7a and 7b, respectively), including in this case, samples of the digested pig slurry in Phase 2a and 2b (AD-Ph2a and AD-Ph2b, respectively), cluster similarly in both cases. The MFC initial biofilm was clustered together with the digested pig slurry in Phase 1 (AD-Ph1). On the contrary, the final biofilm was located far from this cluster, and the second one formed by the digested pig slurry in Phases 2a and 2b. This shows that the evolution of the anode biofim is independent from the population present in the digestates feeding the MFC.

CONCLUSIONS

The MFC has proven to be a useful technology to improve the quality of the effluent following a possible malfunction of AD reactors, since it has been able to remove high levels of VFA from AD effluents while at the same time recovering ammonia. On punctual VFA pulses, simulating a malfunction of the AD process, the MFC showed a poor conversion of COD increase into current density. This may happen because other complex reactions might be taking place in the anode compartment, or because other electron acceptors might be present in the substrate even if VFAs were totally removed. The MFC operating under a stable period of the AD achieved 10%-20% of COD removal and 12%-27% of ammonium removal whilst, when feed with the effluent of the inhibited reactor, these removal efficiencies increased to 30%-40% and 14%-31%, respectively. A serial operated MFC after the anaerobic digestion of pig slurry has proven to induce a reduction in biodiversity of the microbial population, as well as a decrease in total population, particularly for eubacteria.

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Table 1. Characterisation of the digested pig slurries used as feeding in the MFC.

Parameter	Phase 1	Phase 2a	Phase 2b
pH (-)	8.21 ± 0.05	7.85 ± 0.03	7.74 ± 0.03
COD (g O_2 kg^{-1})	26.04 ± 3.73	15.13 ± 2.46	34.43 ± 7.43
N-NH_4^+ (g L^{-1})	2.05 ± 0.04	1.29 ± 0.07	2.41 ± 0.34
TS (%)	1.37 ± 0.10	1.05 ± 0.02	1.93 ± 0.51
VS (%)	0.83 ± 0.10	0.62 ± 0.03	1.18 ± 0.33

Table 2. Operational conditions of the MFC reactor during the series of pure and mixed VFA pulses (Phase 1).

Day	VFA addition (mg)			Added COD (mg)
	Acetate	Propionate	Butyrate	
1-7	0	0	0	-
8	250	0	0	267
9 ,14 and 15	500	0	0	534
19 and 20	0	500	0	757
21 and 28	500	500	0	1291
29 and 33	1000	200	85	1525

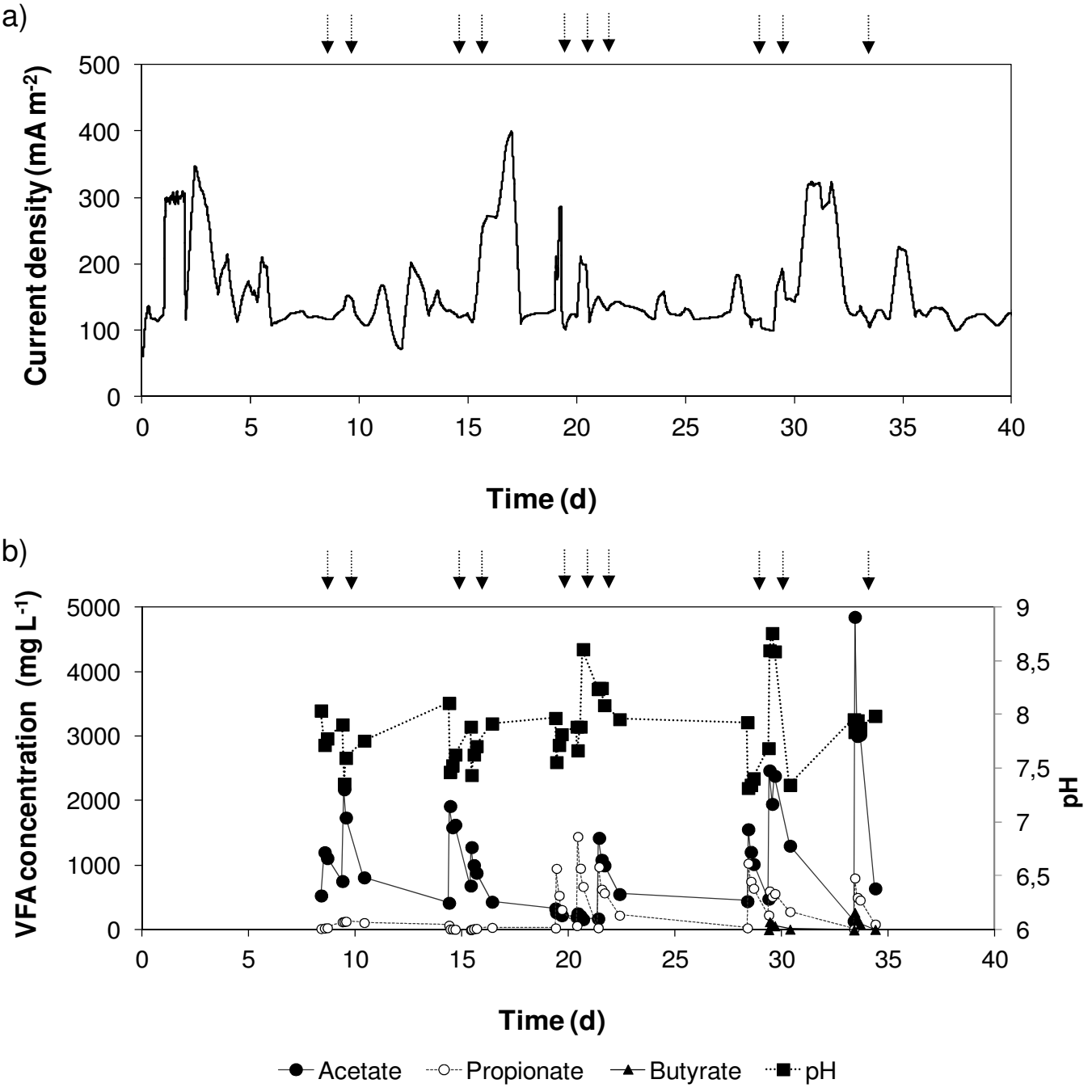


Figure 1 a) Current density and b) VFA concentration and pH in the anode compartment obtained in Phase 1 during the pure and mixed VFA pulses. Arrows show when each pulse was performed.

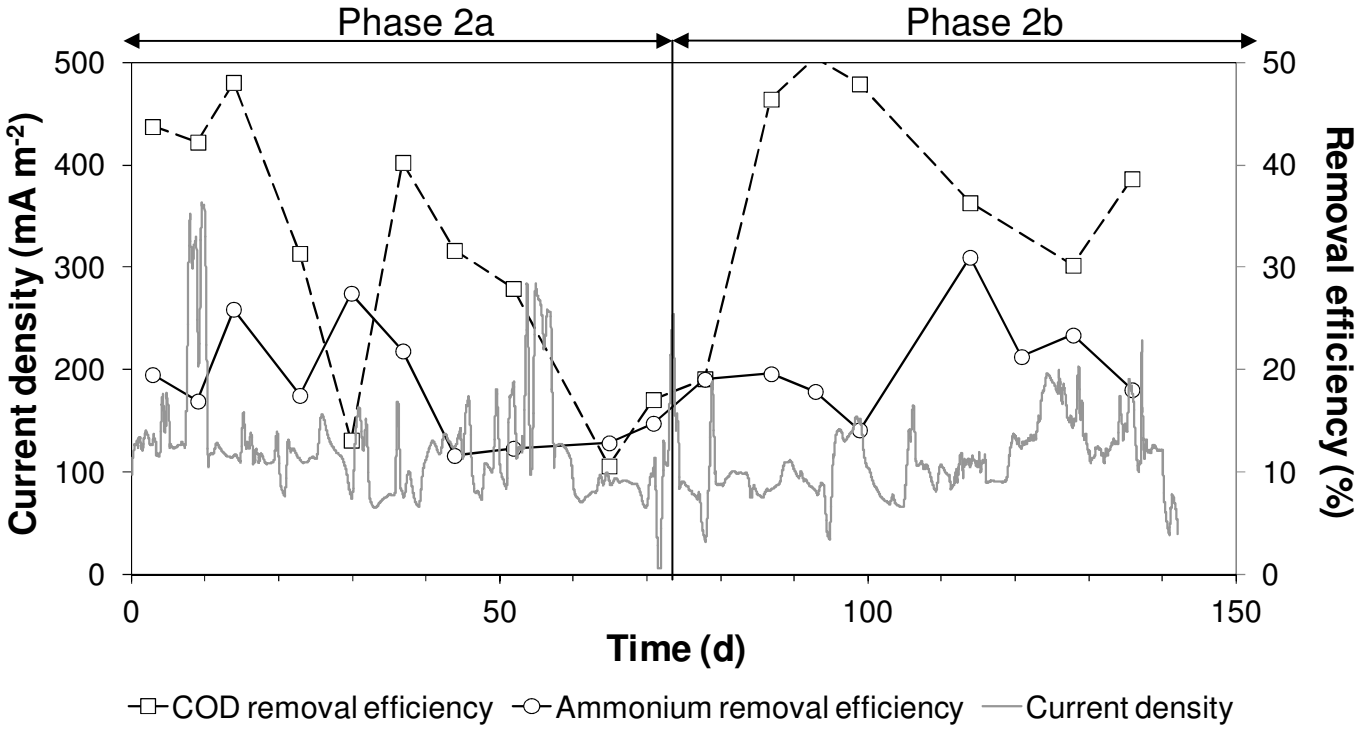
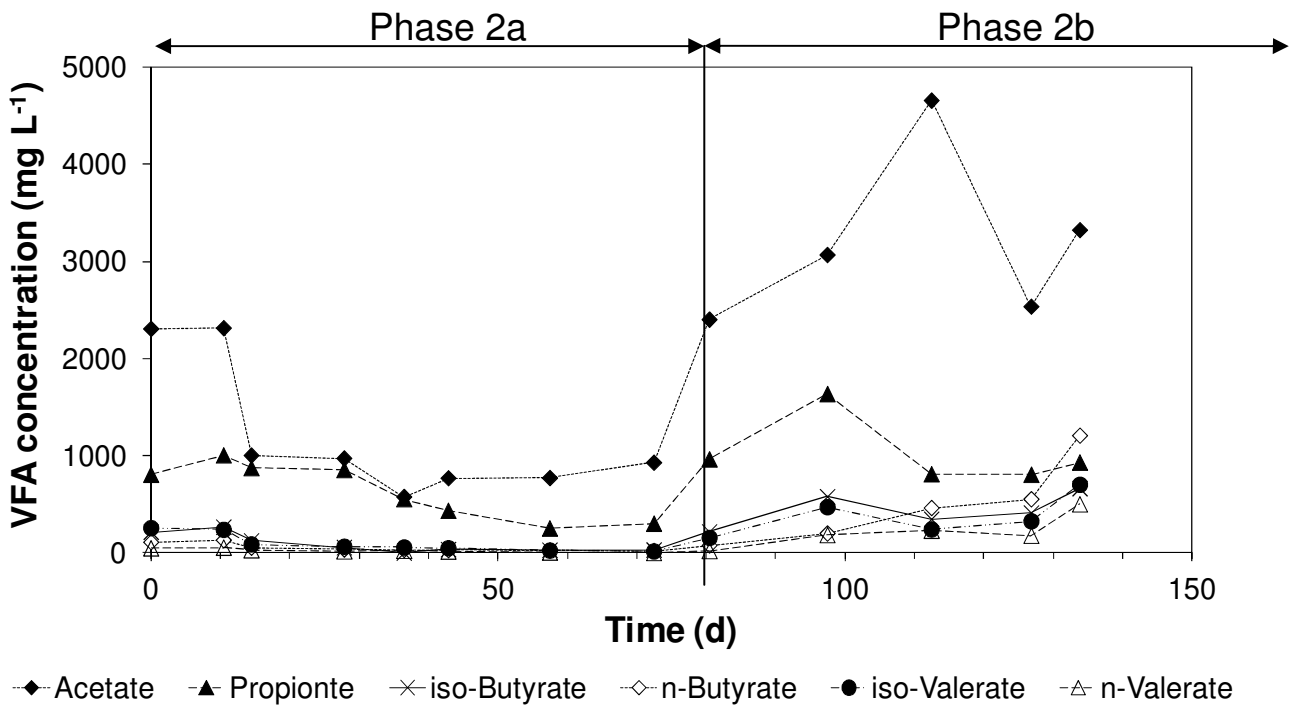


Figure 2 Current density and COD and ammonium removal efficiencies obtained in Phase 2.

a)



b)

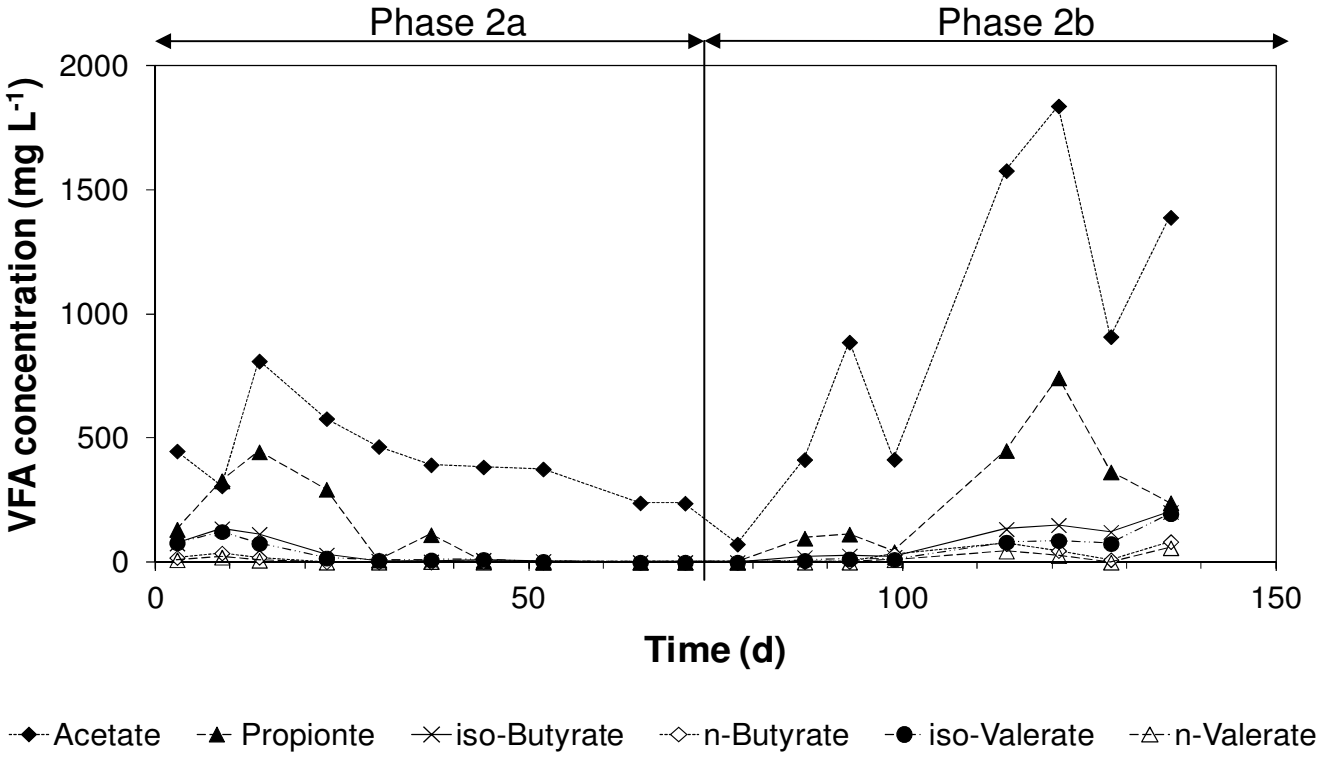


Figure 3 VFA concentration in a) the influent and b) the effluent in Phase 2.

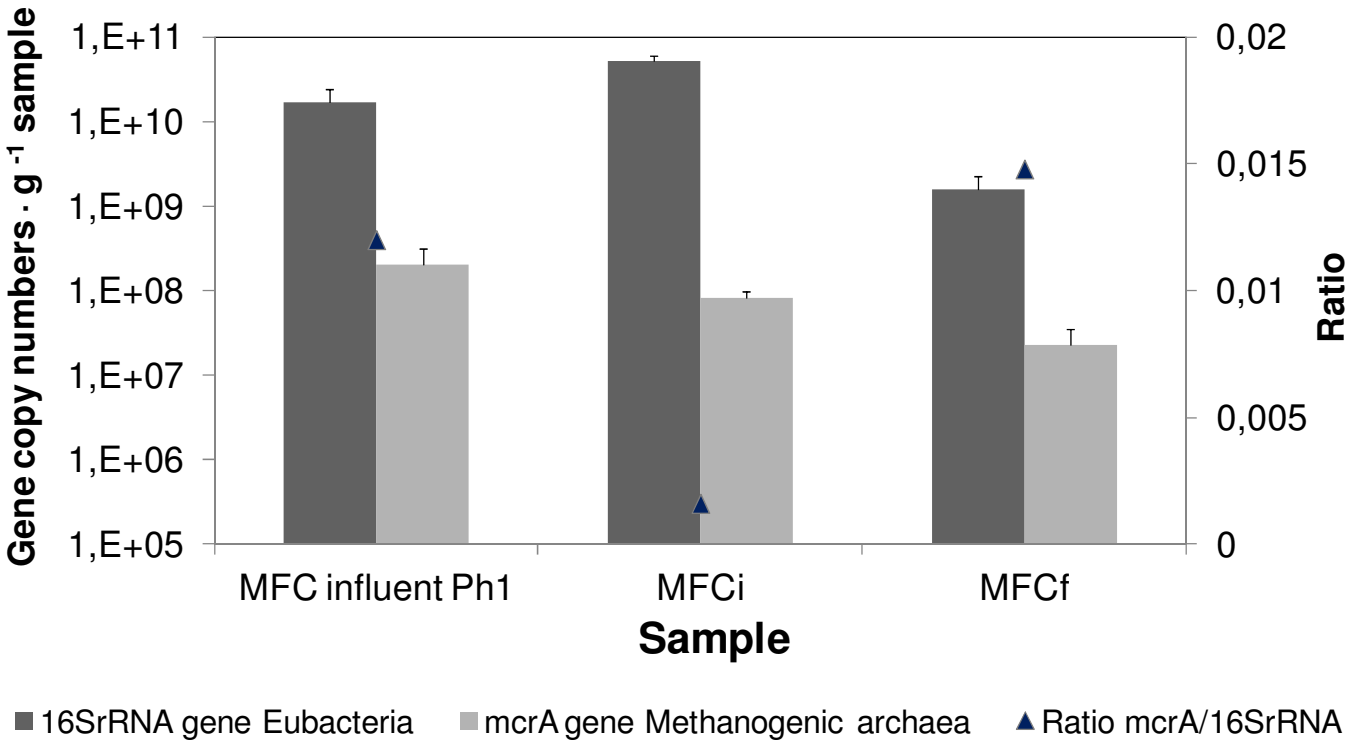
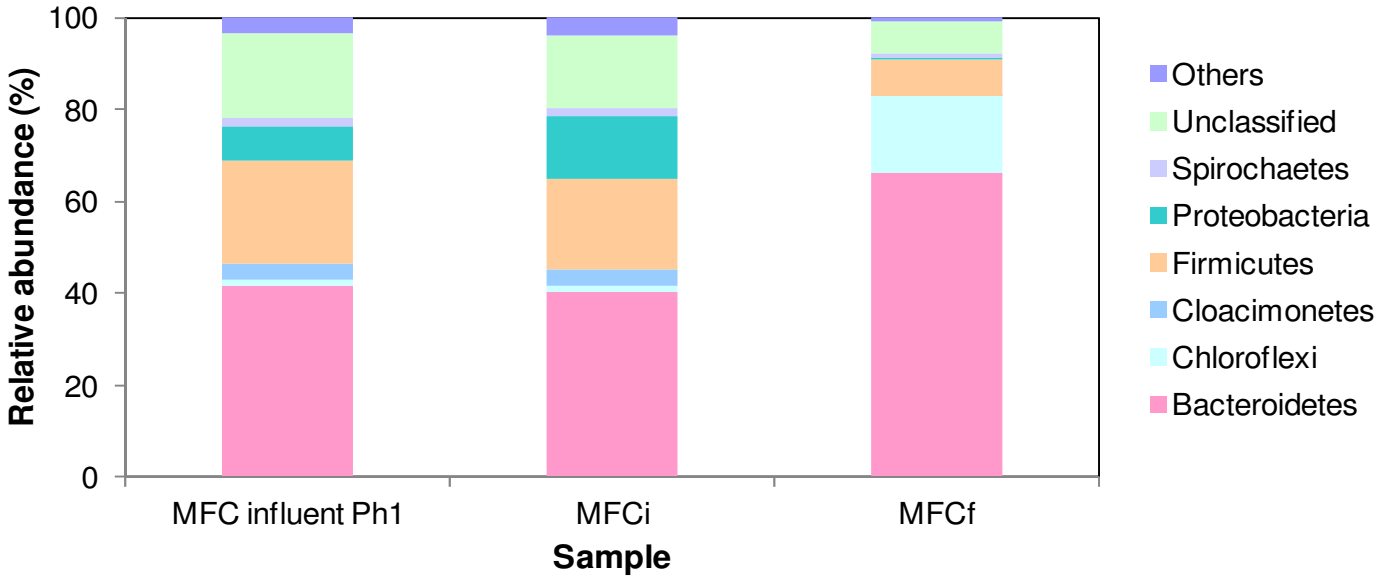


Figure 4 Gene copy numbers for *16S* rRNA and *mcrA* genes and ration between them, of the initial and final MFC anode biofilm (MFCi and MFCf, respectively), and the MFC influent in Phase 1.

a)



b)

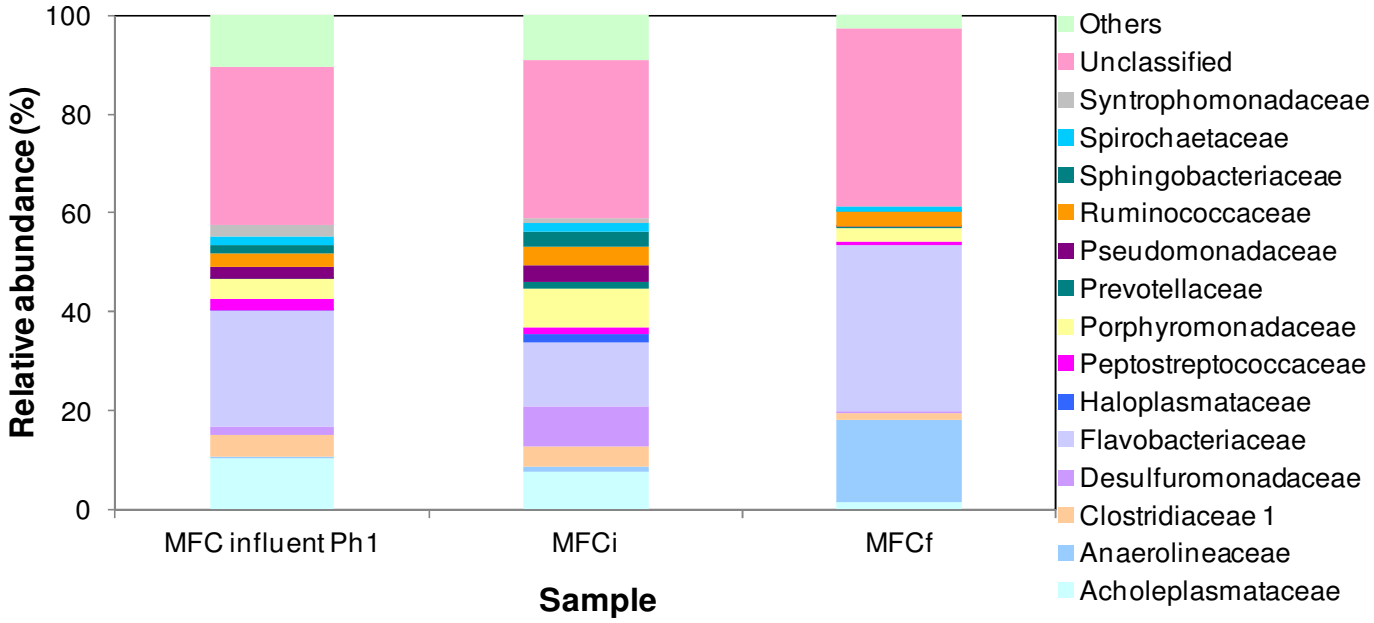


Figure 5 Taxonomic assignment of sequencing reads of Eubacterial community of the initial and final MFC anode biofilm (MFCi and MFCf, respectively), and the MFC influent of Phase 1, at a) phylum b) family levels. Relative abundance was defined as the number of reads (sequences) affiliated with any given taxon divided by the total number of reads per sample. Phylogenetic groups with a relative abundance lower than 1% were categorised as "others".

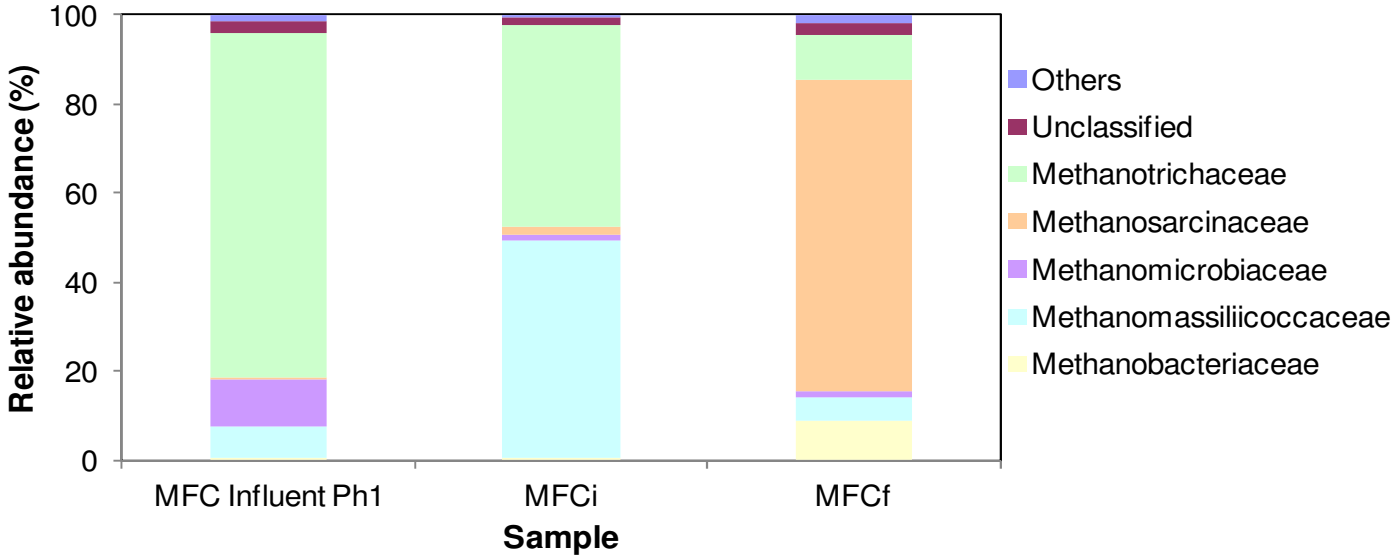
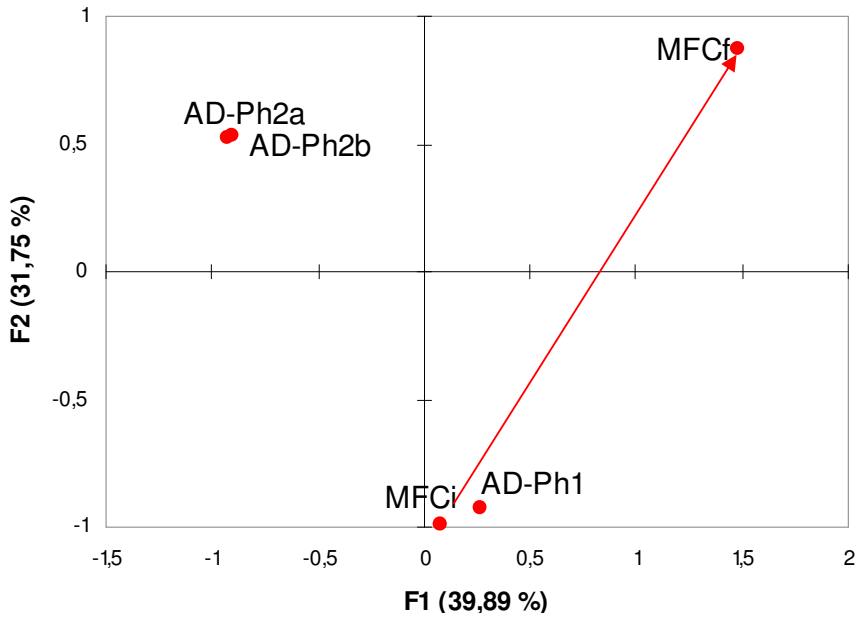


Figure 6 Taxonomic assignment of sequencing reads of Archaeal community of the initial and final MFC anode biofilm (MFCi and MFCf, respectively), and the MFC influent of Phase 1, at family level. Relative abundance was defined as the number of reads (sequences) affiliated with any given taxon divided by the total number of reads per sample. Phylogenetic groups with a relative abundance lower than 1% were categorised as "others".

a)



b)

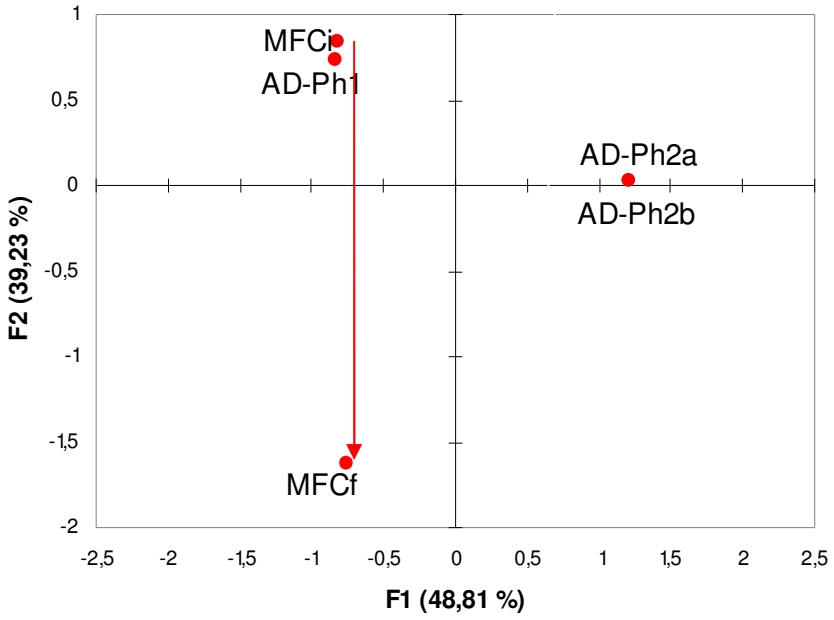


Figure 7 Correspondence Analysis for initial (MFC_i) and final (MFC_f) MFC anode samples and the 3 AD influents (AD-Ph1, AD-Ph2a and AD-Ph2b) regarding (a) Eubacterial and (b) Archaeal community.