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Unravelling the active microbial community in a thermophilic anaerobic digester-microbial electrolysis cell coupled system under different conditions

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ABSTRACT

Thermophilic anaerobic digestion (AD) of pig slurry coupled to a microbial electrolysis cell (MEC) with a recirculation loop was studied at lab-scale as a strategy to increase AD stability when submitted to organic and nitrogen overloads. The system performance was studied, with the recirculation loop both connected and disconnected, in terms of AD methane production, chemical oxygen demand removal (COD) and volatile fatty acid (VFA) concentrations. Furthermore, the microbial population was quantitatively and qualitatively assessed through DNA and RNA-based qPCR and high throughput sequencing (MiSeq), respectively to identify the RNA-based active microbial populations from the total DNA-based microbial community composition both in the AD and MEC reactors under different operational conditions. Suppression of the recirculation loop reduced the AD COD removal efficiency (from 40% to 22%) and the methane production (from 0.32 to 0.03 m^3 m^{-3} d^{-1}). Restoring the recirculation loop led to a methane production of 0.55 m^3 m^{-3} d^{-1} concomitant with maximum MEC COD and ammonium removal efficiencies of 29% and 34%, respectively. Regarding microbial analysis, the composition of the AD and MEC anode populations differed from really active microorganisms. *Desulfuromonadaceae* was revealed as the most active family in the MEC (18%–19% of the RNA relative abundance), while hydrogenotrophic methanogens (*Methanobacteriaceae*) dominated the AD biomass.

1. Introduction

Anaerobic digestion (AD) is a technology that has been widely used since the beginning of the twentieth century to treat animal, municipal and industrial wastes, producing biogas, a form of renewable energy (Yenigün and Demirel, 2013) as a by-product. One of its weak points is the sensitivity of methanogens, one of the main groups involved in the process, to chemical and environmental stressors, especially under thermophilic conditions (Chen et al., 2008). Certain inhibitory substances or process conditions may lead to the anaerobic reactor upset and failure indicated by a decrease in methane gas production and the accumulation of volatile fatty acids (VFA). Different strategies for recovering inhibited reactors have been evaluated, such as reactor feeding patterns, dilution and addition of absorbents for fast recovery after the inhibition of an AD reactor due to the presence of long chain fatty acids (LCFA) (Palatsi et al., 2009); electrochemical nutrient recovery (Desloover et al., 2014; Sotres et al., 2015a) or the use of membrane contactors (Lauterböck et al., 2012) for ammonia toxicity control; pH reduction or the addition of zeolite, biomass or humic acid have also been strategies used to recover ammonia-inhibited thermophilic processes (Ho and Ho, 2012). Ammonia inhibition is one of the main issues to deal with when treating high strength wastes such as livestock manure, hence being the topic in a wide range of studies and reviews (Yenigün and Demirel, 2013).

Combining AD and bioelectrochemical systems (BES) such as microbial electrolysis cells (MEC) has been previously reported as a new processing strategy aiming to recover energy and nitrogen (Cerrillo et al., 2016b). On the one hand, this system can help to produce additional energy and to polish the AD effluent, especially when malfunction of the AD reactor occurs due to an organic overload, attaining a more stable and robust system. And on the other hand, ammonium can be removed and recovered, taking advantage of this process to reduce ammonia inhibition in the AD (Cerrillo et al., 2016b). In a previous study, the microbial community assessment revealed that changes in biomass composition

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were appreciated with a certain delay with respect to the AD observed performance, in terms of VFA accumulation or methane production (Cerrillo et al., 2016b). This fact points out that an RNA-based approach in AD-BES systems could help us to gain insight on the resilient active microbial key players during an inhibition process. A previous work also demonstrated that the ammonia inhibition of methanogenesis in AD was largely due to the repression of functional gene transcription, rather than to a decrease in total population of methanogenic archaea (Zhang et al., 2014). In fact, DNA only provides information about the existence of bacteria in the reactors, but it cannot provide information about their activity and gene expression, which is important to understand which groups are being enhanced by certain environmental or operational conditions. Transcription analysis enables exclusive detection of short-lived messenger RNA (mRNA) produced by active organisms without the potential bias of DNA detection from dormant or dead cells (Munk et al., 2012). In addition, total rRNA, is dependent of ribosome abundance in a bacterial cell, and can be significantly shifted (10–100 folds) from dormant cells in comparison with growing cells (Neidhardt et al., 1996). For this reason, direct extraction of total bacterial RNA (basically mRNA and rRNA) from samples is a key procedure for the subsequent application of qPCR or high throughput sequencing techniques.

The main aim of this study is (1) to assess the combination of the AD process with a microbial electrolysis cell and a recirculation loop as a system to recover AD reactors that have experienced a process failure, and (2) to study the microbial population in the AD-MEC system during the inhibited and recovered state of the AD process, with respect to the predominant presence of eubacteria and archaea in the biomass, and also with regards to metabolically active populations, by means of DNA and RNA-based methods.

2. Materials and methods

2.1. Experimental set-up

An anaerobic thermophilic 4 L lab-scale continuous stirred tank reactor (AD) was used to study its performance when treating pig slurry. The AD reactor was connected in series to the anode compartment of a two-chambered MEC for ammonia recovery and had a recirculation loop set up between both reactors, as previously described (Cerrillo et al., 2016b). An Ag/AgCl reference electrode (Bioanalytical Systems, Inc., USA) was inserted in the anode compartment of the MEC (+197 mV vs. SHE, all potential values in this paper are referred to SHE) and a potentiostat (VSP, Bio-Logic, Grenoble, France) was used to poise the anode (working electrode) potential at 0 mV in a three-electrode configuration. The potentiostat was connected to a personal computer recording electrode potentials and current every 5-min using EC-Lab software (Bio-Logic, Grenoble, France). The solutions of both the anode and the cathode compartment were fed in continuous mode with a peristaltic pump at 21 mL h^{-1} and mixed by recirculation with an external pump. The MEC was operated at room temperature during the entire assay (23 ± 2 °C). An overview of the complete AD-MEC integrated system can be seen in Cerrillo et al. (2016b).

2.2. Reactors operation

The raw pig slurry used as AD feed was collected from a farm in Vila-Sana (Lleida, Spain) (Table 1). The hydraulic retention time (HRT) was fixed at 10 days. The reactor was operated during 118 days in 2 different phases, with organic (OLR) and nitrogen (NLR) loading rates of 6.10 ± 1.88 kg_{COD} m^{-3} day^{-1} and 0.35 ± 0.04 kg_N m^{-3} day^{-1}, respectively (Table 2). The AD had been previously operated for 37 days with a recirculation loop set up between the AD and the MEC, to reduce ammonia inhibition phenomena (Cerrillo et al., 2016b). At the beginning of this study, corresponding to Phase 1, the recirculation loop was suppressed, and later enabled again in Phase 2 (50% of the AD feed flow rate), with the aim to evaluate the effectiveness of this processing strategy to recover AD after a failure event and to study the changes in biomass. For each experimental condition, specific methane yields (m^3_{CH4} d^{-1}), specific methane production rates (m^3_{CH4} m^{-3} d^{-1}) and COD removal efficiencies were used as control parameters. As well as biogas composition, alkalinity, NH_4^+-N and VFA concentrations in the effluent.

Table 1
Characterisation of the pig slurry used as feeding in the anaerobic digester (AD) (n = number of samples).

Parameter	Value
pH (−)	7.0 ± 0.1
COD (g_{O2} kg^{-1})	62.63 ± 2.96
TKN (g L^{-1})	3.65 ± 0.11
NH_4^+-N (g L^{-1})	2.66 ± 0.27
TS (g kg^{-1})	35.80 ± 0.72
VS (g kg^{-1})	23.50 ± 1.21
n	10

As for the MEC, the digested pig slurry obtained from the AD was used as feed for the anode compartment previously filtering it through a 125 μm stainless steel sieve. The catholyte solution for the cathode chamber contained (in deionised water) NaCl 0.1 g L^{-1}. Samples were periodically taken to analyse pH and NH_4^+-N concentration in the anode and cathode effluents, besides COD and VFA concentration in the anode effluent.

2.3. Analytical methods and calculations

Chemical oxygen demand (COD), Kjeldahl nitrogen (TKN), ammonium (NH_4^+-N), pH, total solids (TS), volatile solids (VS), volatile fatty acids (VFAs), biogas composition (N_2, CH_4, CO_2), partial, total and intermediate alkalinity (PA, TA and IA, respectively), free ammonia nitrogen (FAN), COD and ammonium removal efficiency and the current density (A m^{-2}) obtained in the MEC, were determined and calculated as described elsewhere (Cerrillo et al., 2016b).

PA alkalinity, which roughly corresponds to bicarbonate alkalinity, was obtained by titration, from the original pH sample, to pH 5.75. TA determination (titration to pH 4.3) allowed for IA calculation (titration from 5.75 to 4.3, roughly corresponding to VFA alkalinity) (Ripley et al., 1986). IA:TA ratio was used as a tool to monitor anaerobic digestion, considering that the process was stable when IA:TA was below 0.3.

The Coulombic efficiency (CE), or the fraction of electrons resulting from the consumption of COD effectively transformed into current density (Equation (1)), was calculated as

$$CE = \frac{M \int_0^t I\,dt}{F\,b\,q\Delta COD} \quad (1)$$

where M is the molecular weight of the final electron acceptor, I is the current (A), F is Faraday's constant, b is the number of electrons transferred per mole of O_2, q is the volumetric influent flow rate (L s^{-1}), and ΔCOD is the difference between the influent and effluent COD (g L^{-1}).