



Overcoming organic and nitrogen overload in thermophilic anaerobic digestion of pig slurry by coupling a microbial electrolysis cell



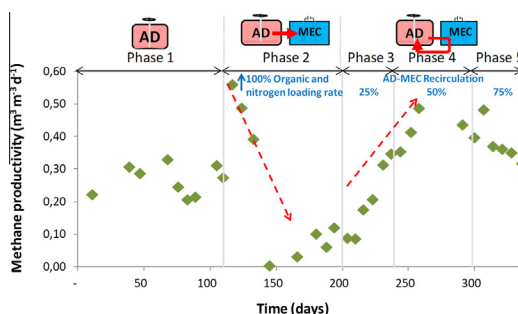
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HIGHLIGHTS

- An AD inhibited by organic and nitrogen overload was recovered by coupling a MEC.
- A 55% increase in methane production was achieved with the AD-MEC-loop system.
- The AD-MEC polished the effluent removing 46% of COD and recovering 40% of N.
- The AD microbial population increased in biodiversity with the MEC loop connection.
- A more stable and robust system which supports organic and N overload was achieved.

GRAPHICAL ABSTRACT



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ABSTRACT

The combination of the anaerobic digestion (AD) process with a microbial electrolysis cell (MEC) coupled to an ammonia stripping unit as a post-treatment was assessed both in series operation, to improve the quality of the effluent, and in loop configuration recirculating the effluent, to increase the AD robustness. The MEC allowed maintaining the chemical oxygen demand removal of the whole system of $46 \pm 5\%$ despite the AD destabilization after doubling the organic and nitrogen loads, while recovering $40 \pm 3\%$ of ammonia. The AD-MEC system, in loop configuration, helped to recover the AD (55% increase in methane productivity) and attained a more stable and robust operation. The microbial population assessment revealed an enhancement of AD methanogenic archaea numbers and a shift in eubacterial population. The AD-MEC combined system is a promising strategy for stabilizing AD against organic and nitrogen overloads, while improving the quality of the effluent and recovering nutrients for their reutilization.

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1. Introduction

Anaerobic digestion (AD) of livestock manure and other wastes results in organic matter stabilization and biogas production, a

biofuel containing mainly methane and carbon dioxide that can be used in power generation systems to obtain heat and electricity. This energy recovering technology is nowadays widely used to treat various kinds of wastes (Angenent et al., 2004). AD process is complex, since it involves many different groups of microorganisms, especially methanogens, that are particularly sensitive to organic overloads and diverse substances that may be present in the waste stream such as ammonia (Angelidaki and Ahring, 1993; Yenigün and Demirel, 2013). AD can mainly take place at

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two different ranges of temperatures, either mesophilic (25–40 °C) or thermophilic (45–60 °C). The later one is more favourable to obtain a high digestion rate, since high loading rates or short retention times can be applied, due to higher growth rates of bacteria at higher temperatures. Moreover, improved solids settling and destruction of microbial pathogens is attained (Angelidaki and Ahring, 1994). On the other hand, thermophilic AD has a lower process stability than mesophilic AD, it being more sensitive to high ammonia concentrations since free ammonia (NH₃), the active component causing ammonia inhibition, increases with an increase in pH and temperature (Angelidaki and Ahring, 1994). Reactor upset will be indicated by a reduction in biogas production and/or biogas methane content, and the accumulation of volatile fatty acids (VFA) that may lead to reactor failure (Chen et al., 2008). At a microbiology level, and due to the complex interdependence of microbial activities for the adequate functionality of anaerobic bioreactors, the genetic expression of *mcrA*, which encodes the α subunit of methyl coenzyme M reductase –the enzyme that catalyses the final step in methanogenesis–, has been proposed as a parameter to monitor the process performance (Alvarado et al., 2014; Morris et al., 2013).

Besides monitoring the AD process by means of CH₄ production, it is interesting to explore new technologies that can help AD to maintain effluent quality within the desired limits despite AD failure. So far, different strategies for stabilizing AD reactors under high organic loading rates and for controlling ammonia toxicity have been evaluated, ranging from the more classical approaches, such as co-digestion with carbon-rich substrates to equilibrate the carbon to nitrogen ratio (Chiu et al., 2013), introduction of adaptation periods (Borja et al., 1996), reduction of ammonia content of the substrates by air stripping (Bonmati and Flotats, 2003; Laurenzi et al., 2013), or dilution of the substrates (Hejnfelt and Angelidaki, 2009); to more innovative ones, such as the use of an electrochemical system aimed at NH₄⁺ extraction coupled to an upflow anaerobic sludge blanket (UASB) in the recirculation loop to help control ammonia toxicity with high nitrogen loading conditions (Desloover et al., 2014).

An alternative to these techniques is the use of bioelectrochemical systems (BES) in combination with an AD process. BESs are bioreactors that use microorganisms attached to one or both electrode(s) in order to catalyse oxidation and/or reduction reactions. These systems are also useful for recovering nutrients, such as ammonium (Sotres et al., 2015a). BESs have proven to be useful as post-treatment for anaerobic digesters in order to reduce organic matter content and recover ammonium (Cerrillo et al., 2016). Different AD-BES configurations have been previously studied, mainly aimed to improve biogas production in the AD (Tartakovsky et al., 2011; Zhang and Angelidaki, 2015). But more research in terms of combined system behaviour against factors that may destabilise the AD process is needed, as well as a more global approach of the AD-BES system integrating stabilization of the process, microbial community stability, improvement of the quality of the effluent, and nutrient recovery.

Since the effluent of a BES is expected to have a lower content of organic matter and ammonium, a combined AD-BES system with a recirculation loop between both components may offer some advantages in order to increase the stability of the system, mainly improving its resistance against organic and nitrogen overloads. The combination of BES with AD, as a system to reduce ammonia inhibition, has been previously demonstrated using a submersible microbial desalination cell fed with synthetic wastewater, although in that case the BES was not exploited to reduce organic matter content (Zhang and Angelidaki, 2015). On the other hand, although combined AD-BES systems have been tested against strong perturbations (Weld and Singh, 2011), the effect of stress on microbial synergies (eubacterial and archaeal communities) is

scarcely known, especially on methanogenic archaea and their evolution when operating in a coupled system under inhibited and recovered stages.

The main aim of this study is to assess the combination of the AD process with a microbial electrolysis cell (MEC) both in series operation, as a system to improve the effluent quality, and in loop configuration to recirculate the effluent, as a technique to increase the stability and robustness of the AD process, while recovering ammonia with a stripping and absorption unit. Furthermore, microbial community dynamics have been assessed in both reactors to understand the reactor set-up effects, as well as microbial resilience at different operational conditions, even under an inhibited AD operation.

2. Materials and methods

2.1. Experimental set-up

A lab-scale continuous stirred tank reactor (CSTR) was used to study its performance when treating pig slurry at a thermophilic temperature range. The anaerobic digester (AD) consisted of a cylindrical glass reactor (25 cm diameter) with a 4 L working volume. The digester was fitted with a heat jacket with hot water circulating to keep the temperature at 55 °C. Thermophilic conditions were chosen as AD is more sensitive to the presence of inhibitors such as ammonia at this range of temperature. A temperature probe was fitted into the reactor lid for temperature monitoring. Continuous mixing was also supplied using an overhead stirrer. A gas counter was used to measure biogas production (μ Flow, Bioprocess Control AB, Sweden). The digester was initially inoculated with 2550 mL (64% of the AD volume) of the effluent of another lab scale thermophilic AD fed with sewage sludge from a wastewater treatment plant.

A two chamber cell BES reactor described elsewhere (Cerrillo et al., 2016) which had been previously operated in MEC mode with digested pig slurry was used for the experiments. The anode was a carbon felt (dimensions: 14 × 12 cm; thickness: 3.18 mm; Alfa Aesar GmbH and Co KG, Karlsruhe, Germany); and a 304 stainless steel mesh was used as cathode (dimensions: 14 × 12 cm; mesh width: 150 μ m; wire thickness: 112 μ m; Feval Filtros, Spain). Both compartments (0.5 L each one) were separated by a cation exchange membrane (CEM, dimensions: 14 × 12 cm; Ultrex CMI-7000, Membranes International Inc., Ringwood, NJ, USA). A potentiostat (VSP, Bio-Logic, Grenoble, France) was used to poise the anode (working electrode) potential at 0 mV in a three electrode mode, with an Ag/AgCl reference electrode (Bioanalytical Systems, Inc., USA) inserted in the anode compartment (+197 mV vs. standard hydrogen electrode, SHE). All potential values in this paper are referred to SHE. The potentiostat was connected to a personal computer which recorded electrode potentials and current, every 5 min, using EC-Lab software (Bio-Logic, Grenoble, France).

A stripping and absorption system was used to recover the ammonium transferred from the anode to the cathode compartment. It consisted of two glass columns (70 cm height; 7 cm $\emptyset_{\text{external}}$; 5.5 cm $\emptyset_{\text{internal}}$) filled with glass rings (5–7 mm length). The cathode effluent was initially conducted to the top of the stripping column, and later circulated through the filling towards the bottom while air was pumped in the opposite direction. The air leaving the top of the column was directed to the absorption column, which was filled with an acidic solution (H₂SO₄, 10% v/v). Fig. 1 shows the scheme of the complete AD-MEC-Stripping/Absorption combined system.

2.2. Reactors operation

The AD was fed in a continuous mode with raw pig slurry from a farm in Vila-Sana (Lleida, Spain) with a hydraulic retention time

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