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Methanosarcina plays a main role during methanogenesis of high-solids food waste and cardboard

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ABSTRACT

Anaerobic digestion of food waste is a complex process often hindered by high concentrations of volatile fatty acids and ammonia. Methanogenic archaea are more sensitive to these inhibitors than bacteria and thus the structure of their community is critical to avoid reactor acidification. In this study, the performances of three different inocula were compared using batch digestion tests of food waste and cardboard mixtures. Particular attention was paid to the archaeal communities in the inocula and after digestion. While the tests started with inocula rich in *Methanosarcina* led to efficient methane production, VFAs accumulated in the reactors where inocula initially were poor in this archaea and no methane was produced. In addition, higher substrate loads were tolerated when greater proportions of *Methanosarcina* were initially present in the inoculum. Independently of the inoculum origin, *Methanosarcina* were the dominant methanogens in the digestates from the experiments that efficiently produced methane. These results suggest that the initial archaeal composition of the inoculum is crucial during reactor start-up to achieve stable anaerobic digestion at high concentrations of ammonia and organic acids.

1. Introduction

Novel technologies for treatment and valorization of the organic fraction of municipal solid waste (OFMSW) must be developed to deal with an increasing production and new international regulations. Anaerobic digestion (AD) is a well-known process used for efficient treatment of organic waste with high total solids (TS) contents (≥20 %), converting them into biogas and digestate, both added-value end-products. However, AD of highly biodegradable substrates such as food waste (FW), which is a major component of OFMSW, is often associated with accumulation of volatile fatty acids (VFAs), which are detrimental to the AD process. In addition, FW is rich in organic nitrogen, which is reduced to ammonia during AD, leading to high concentrations of total ammonia nitrogen (sum of NH_3 and NH_4^+; TAN) in the digesters (L. Zhang et al., 2012). Accumulation of both VFA and/or TAN might lower the methane yields and can even lead to failure of the AD process (Banks et al., 2008). The reactors are particularly vulnerable to these inhibitions during the start-up period (Fernández et al., 2001). This occurs because the microbial communities are not adapted to the stressful conditions imposed by the substrates and the operational parameters (*i.e.* high organic loading rates). Therefore, to achieve efficient methane yields and productivities with FW as substrate, it is crucial to have well-adapted microbial communities in the digesters, which are resistant to high VFA and free ammonia nitrogen (NH_3; FAN) concentrations.

Methanogenic archaea are generally more sensitive to inhibitors than bacteria and thus methanogenesis is usually the first process affected by common inhibitors, such as FAN or VFAs (De Vrieze et al., 2012). Nonetheless, not all methanogenic archaea have the same resistance to these inhibitors and thus the composition of the archaeal microbial community varies according to the operating conditions (Abbassi-Guendouz et al., 2013). Due to their high substrate affinity, acetotrophs such as *Methanosaeta* are generally predominant under unstressed conditions and thus acetotrophic methanogenesis is the predominant pathway for methane production. On the other hand, under stressful AD conditions, these methanogens are preferentially inhibited and mixotrophic microorganisms (*i.e.* able to consume acetate and hydrogen to produce methane), such as *Methanosarcina* which are more resistant to inhibitors (*i.e.* FAN or VFAs), become predominant (De Vrieze et al., 2012; Venkiteshwaran et al., 2016). In fact, while *Methanosaeta* cannot grow at TAN concentrations greater than 3 $g{\cdot}L^{-1}$, *Methanosarcina* have been found at much higher TAN concentrations (De Vrieze et al., 2012; Poirier et al., 2016).

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As an illustration, Capson-Tojo et al. (2017a) found *Methanosarcina* to be the dominant methanogens at TAN concentrations up to 3.7 $g{\cdot}L^{-1}$ (795 mg $FAN{\cdot}L^{-1}$) using FW as substrate in AD batch tests.

Over the past years, the importance of the microbial communities for efficient AD processes has gained attention and many studies have been carried out to further understand the structures of the communities of both bacteria and archaea in AD reactors. In a recent study carried out by Zhang et al. (2016) with sewage sludge and FW as substrates (with final NH_4^+ concentrations up to 2.01 $g{\cdot}L^{-1}$), it was observed that *Methanosaeta* were the main archaea at the beginning of the batch experiment (71 % of the operational taxonomical units; OTUs). Afterwards, *Methanosarcina* grew during acid production (with transient VFA concentrations up to 24 $g{\cdot}L^{-1}$) and overpassed in abundance *Methanosaeta* because of their greater resistance to VFA and TAN inhibition. Finally, other hydrogenotrophic methanogens (*i.e. Methanoculleus*) grew once acetate was totally consumed. Using a high solid-state AD box-type container fed with FW at high TS contents (from 34.4 to 44.5 %) and TAN concentrations (2.5 $g{\cdot}L^{-1}$), Walter et al. (2016) observed that *Methanosarcina* were the dominant species accompanied by different hydrogenotrophs (*i.e. Methanobacterium*, *Methanoculleus* and *Methanocorpusculum*). Consistently, Zamanzadeh et al. (2016) found *Methanosaeta* as the main archaea in mesophilic continuous AD of FW at low concentrations of FAN ($\leq$200 $mg{\cdot}L^{-1}$). This further supports that the concentration of TAN-FAN is a key factor that can result in shifts of the archaeal populations. In a recent batch study, Poirier et al. (2016) identified the key microbial phylotypes resisting to extreme ammonia concentrations (up to 50 g $TAN{\cdot}L^{-1}$). They achieved high methane yields at TAN concentrations as high as 25 g $TAN{\cdot}L^{-1}$, with *Methanosarcina* and *Methanoculleus* as main methanogens and with relative abundances of *Methanosaeta* lower than 5 % in all AD reactors.

The objective of this study was to evaluate, for the first time, the AD performance of three microbial inocula from different origins and with different initial archaeal compositions using FW and cardboard (CB) as substrates. These wastes are the main components of OFMSW (Kim and Oh, 2011; Y. Zhang et al., 2012) and are generally collected at the same facilities, and thus their co-digestion is facilitated. Also, they constitute a good waste model substrate, since the initial proportions of carbon and nitrogen could be easily adjusted. Batch tests were performed at different substrate loads, TS contents ($\geq$20 %) and co-digestion proportions. Special attention was paid to the archaeal communities and to the FAN and VFA levels.

2. Materials and methods

2.1. Substrate and microbial inoculum

A synthetic FW was prepared according to the VALORGAS report (VALORGAS, 2010). It was composed of fruits and vegetables (80.7 %), meat (8.2 %), pasta (4.8 %), bread (6.2 %), dairy products (1.9 %) and biscuits (1.9 %). Its precise composition has been detailed elsewhere (Capson-Tojo et al., 2017a). Being FW and CB the most common components of OFMSW, CB (branded "Cartonnages Michel" and shredded to less than 1 mm) was added as co-substrate to simulate this waste (Hogg et al., 2002), increasing at the same time the C/N ratio of the substrate and thus diluting the TAN concentrations in the reactors and favoring the AD process (Capson-Tojo et al., 2017a). Three different inocula from industrial plants were used: mixture of a centrifuged granular sludge issued from a mesophilic industrial UASB reactor treating sugar factory effluents with a dried digestate. This digestate was used to increase the TS content of the inoculum and was sampled in a thermophilic industrial plant treating OFMSW (Inoc-UASB1); a mixture of sludge and dried digestate issued from the same sources than Inoc-UASB1 but sampled at a different moment (Inoc-UASB2); a sludge issued from an AD industrial plant treating a mixture of different organic waste streams at 35 °C mixed with dried compost (99 % TS; 81 % VS) to increase the TS content of the inoculum (Inoc-OW). The amounts of dried digestate and compost added were 0.5 g per g of inoculum (w/w) (Inoc-UASB1 and Inoc-UASB2) and 0.17–0.34 g per g of inoculum (w/w) (Inoc-OW) respectively, depending on the desired TS content and the initial water content of the sludge.

2.2. Dry batch anaerobic co-digestion tests

Different co-digestion ratios (4–1 g TS FW·g TS CB^{-1}), initial TS contents (20–35 %) and substrate to inoculum (S/X) ratios (0.25–1.00 g VS·g VS^{-1}) were tested. These values were selected according to previous results and to data gathered from the literature (Capson-Tojo et al., 2016, 2017a). Table 1 summarizes the 10 different experimental conditions that were considered in this study. Each tested condition was run in triplicate. This experimental set-up allowed to produce results which primarily depended on the inoculum source, while evaluating at the same time different initial conditions (*i.e.* S/X and co-digestion ratios and initial TS contents). Therefore, the obtained results were not dependent on the particular operational conditions applied, but only on the type of inoculum used. With this set-up the performance of each reactor was also totally independent between them.

After adding the required volumes of sludge into the flasks, the corresponding amounts of substrates (according to Table 1) were supplemented. Finally, the TS contents were adjusted adding water and the flasks were flushed with nitrogen and sealed.

As aforementioned, to allow working at the high TS contents desired, the inocula used were mixed with dried digestates (Inoc-UASB1 and Inoc-UASB2) and compost (Inoc-OW). Different blank reactors were carried out to account for the biogas production that could have been produced by the degradation of these materials (Capson-Tojo et al., 2017a, 2017b, 2018). In addition,

Table 1
Operational conditions of the batch experiments and obtained methane yields. "UASB1", "UASB2" and "OW" stand for the inoculum.

Inoculum	Substrate	Substrate C/N ratio	Co-dig. ratio (g TS FW·g TS CB^{-1})	S/X (g VS·g VS^{-1})	Initial TS (%)	Methane yield (mL CH_4·g VS^{-1})
UASB1	FW + CB	23.1	1.75	0.25	30.0	11 ± 3*
UASB1	FW + CB	27.8	1.00	0.25	30.0	8 ± 2*
UASB1	FW + CB	27.8	1.00	0.25	35.0	17 ± 2*
UASB1	FW	16.3	–	0.25	20.0	1 ± 1*
UASB2	FW + CB	22.7	1.86	0.25	27.5	409 ± 11
UASB2	FW + CB	27.8	1.00	0.25	27.5	393 ± 9
UASB2	FW + CB	27.8	1.00	0.25	35.0	401 ± 16
UASB2	FW + CB	19.3	4.00	1.00	27.5	0 ± 0*
OW	FW	16.3	–	0.25	20.0	464 ± 14
OW	FW	16.3	–	1.00	20.0	375 ± 17

* These values were considered as indicators of an inefficient AD process.

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