High-Solids Anaerobic Digestion requires a tradeoff between Total Solids, Inoculum-to-Substrate Ratio and Ammonia Inhibition

ABSTRACT

Increasing Total Solids on anaerobic digestion can reduce the methane yield, by the interaction of highly-complex bio-physical-chemical mechanisms. Therefore, understanding those mechanisms and their main drivers becomes crucial to optimize high-solids anaerobic digestion at industrial scale. In this study, seven batch experiments were conducted to investigate the effects of increasing the Total Solids content on high-solids anaerobic digestion of the organic fraction of municipal solid waste. With an Inoculum-to-Substrate Ratio = 1.5 g VS/g VS and maximum Total Solids ≤ 19.6 %, mono-digestion of the organic fraction of municipal solid waste showed a methane yield of 174-236 NmL CH_4/g VS. With an Inoculum-to-Substrate Ratio ≤ 1.0 g VS/g VS and maximum total solids ≥ 24.0 %, similar mono-digestion experiments resulted in acidification. Co-digestion of the organic fraction of municipal solid waste and beech sawdust permitted to reduce the Inoculum-to-Substrate Ratio to 0.16 g VS/g VS while increasing Total Solids up to 30.2 %, though achieving a lower methane yield (i.e. 117-156 NmL CH_4/g VS). At each Inoculum-to-Substrate Ratio, a higher Total Solids content corresponded a to higher ammonia and volatile fatty acid accumulation. Thus, a 40 % lower methane yield of the organic fraction of municipal solid waste was observed at a NH_3 concentration ≥ 2.3 g N-NH_3/kg Reactor Content and Total Solids = 15.0 %. Meanwhile, the addition of sawdust to the organic fraction of municipal solid waste lowered the nitrogen content, being the risk of acidification exacerbated only at Total Solids ≥ 20.0 %. Therefore, the biodegradability of the substrate, as well as the operational Total Solids and the Inoculum-to-Substrate Ratio, are closely-interrelated parameters determining the success of methanogenesis, but also the risk of ammonia inhibition on high-solids anaerobic digestion.

Keywords: High-solids Anaerobic Digestion; Organic Fraction of Municipal Solid Waste; Batch Experiments; Co-digestion; Thermophilic; Methane Yield; Volatile Fatty Acids.

1 INTRODUCTION

Anaerobic digestion (AD) is a biochemical treatment technology in which an organic waste (OW) is decomposed to a mixture of gases – mainly CH_4 and CO_2 – known as biogas, and a partially stabilized organic material known as digestate. Biogas has a high calorific content, while the nutrient-concentrated digestate has the potential to be used as soil amendment (De Baere and Mattheeuws 2013). AD takes place through a sequential set of fermentative steps carried out symbiotically by different microbial consortia (Gerardi 2003). The main AD steps are hydrolysis, acidogenesis, acetogenesis and methanogenesis, while the AD biochemistry strongly depends on a balance between volatile fatty acid (VFA) production by acidogens/acetogens and VFA consumption by methanogens. When an imbalance occurs, VFA and/or H_2 accumulate, potentially leading to AD failure by acidification (i.e. pH ≤ 6.0) (Motte et al. 2014; Staley et al. 2011). Other inhibitory substances may also accumulate during AD, such as free ammonia (NH_3) and cations (e.g. Na^+, K^+) (Chen et al. 2008; Riggio et al. 2017).

Depending on the total solid (TS) content, AD can be operated under 'wet' (i.e. TS < 10 %), 'semi-solid' (i.e. 10 ≤ TS < 20 %) and 'dry' (i.e. TS ≥ 20 %) conditions (Abbassi-Guendouz et al. 2012; Pastor-Poquet et al. 2018). High-solids AD (HS-AD) includes the two last cases, and has some advantages such as the use of a smaller digester volume, and a reduced need for water addition and dewatering operations, enhancing the process economy (André et al. 2018; Kothari et al. 2014). However, HS-AD also shows some drawbacks such as a high risk of reactor acidification by substrate overload, and a reduced mass transfer associated to the low content of free water in the system (Benbelkacem et al. 2015; Bollon et al. 2013; García-Bernet et al. 2011).

Moreover, as the TS content is rather high in HS-AD, a lower amount of water is available to dilute potential inhibitors (i.e. NH_3) than in 'wet' AD.

HS-AD of the organic fraction of municipal solid waste (OFMSW), including food waste (FW) and green/lignocellulosic waste (GW), is widely used. Indeed, the high TS content (i.e. 20-50 %) and the high biodegradation potential of OFMSW are particularly favorable to lower the operational costs of HS-AD (De Baere and Mattheeuws 2013). In this line, batch systems for OFMSW treatment at industrial scale can be operated up to 40 % TS, provided that leachate is continuously recirculated as a source of microorganisms and partial mixing (André et al. 2018; Riggio et al. 2017).

The operational TS of HS-AD mainly depends on the TS and volatile solid (VS) of the OW, but also its biodegradability under anaerobic conditions, since AD of OFMSW might yield a 30-80 % reduction of the substrate TS (Pastor-Poquet et al. 2018). Thus, the presence of lignocellulosic substrates (i.e. GW or paper/cardboard) in OFMSW usually permits to increase the operational TS content in HS-AD, due to the higher TS content but also lower biodegradability of these substrates, in comparison to OFMSW (Pastor-Poquet et al. 2018). Nonetheless, the addition of lignocellulosic materials might reduce the biodegradability rate of the overall mixture due to the slower hydrolysis (Brown and Li 2013; Pastor-Poquet et al. 2018, In Press). On the other hand, the addition of lignocellulosic substrates might reduce simultaneously the chances of NH_3 inhibition in HS-AD due to the lower protein content.

Laboratory-scale batch experiments are normally used to obtain valuable information about the main operating parameters and/or the AD dynamics for a given OW at industrial scale. One of the main parameters is the inoculum-to-substrate ratio (ISR) to be used avoiding acidification. For example, when assessing the maximum methane yield of highly biodegradable substrates (i.e. FW) during a biomethane potential (BMP) test, a relatively high ISR (i.e. 2-4 g VS/g VS) is recommended (Holliger et al. 2016). However, as a sole parameter, the ISR is inadequate to avoid HS-AD acidification (Schievano et al. 2010). Indeed, a given mixture substrate-inoculum sets simultaneously the ISR (i.e. g VS/g VS) and the maximum TS, according to the VS and TS mass balances, respectively. Therefore, adapted combinations of ISR (i.e. 0.25-4 g VS/g VS) and FW:GW ratio (i.e. 0-100 %) are required to circumvent acidification, while maximizing the TS content in HS-AD experiments (Capson-Tojo et al. 2017; Schievano et al. 2010).

The effects of increasing the initial TS content on HS-AD batch tests are not yet fully understood, since a higher initial TS has been reported to reduce the methane yield of substrates such as cardboard (Abbassi-Guendouz et al. 2012) and OFMSW (Forster-Carneiro et al. 2008b; Liotta et al. 2014), but not of lignocellulosic substrates (Brown et al. 2012). Importantly, whether the TS increase inside the digester results in a lower methane yield, the overall HS-AD efficiency decreases, potentially compromising the OFMSW treatment economy (Fernández et al. 2010; Mata-Álvarez 2003).

This study evaluates the effects of increasing the initial TS content on the methane yield, TS removal and chemical oxygen demand (COD) conversion in HS-AD

laboratory-scale batch bioassays at 55ºC, using mono-digestion of OFMSW and co-digestion of OFMSW and beech sawdust. Sawdust simulates the addition of biodegradable GW (e.g. branches and leaves) to OFMSW, permitting to stabilize HS-AD at high TS (i.e. ≥ 20 %). To maximize TS while avoiding acidification, different ISR and/or co-digestion ratios were used. Furthermore, this study highlights the important interrelationship between the initial conditions (i.e. TS and ISR) and the main AD inhibitors (i.e. NH_3) in HS-AD of OFMSW, by evaluating the pH, TS, VFA and ammonia dynamics during sacrifice experiments. More in particular, the interaction between TS and the NH_3 content determines the overall methane yield, and set the basis for an optimal HS-AD configuration when treating OFMSW at industrial scale.
This study was conducted at the Department of Civil and Mechanical Engineering of the University of Cassino and Southern Lazio (Italy) from June 2016 to September 2017.

2 MATERIALS AND METHODS

2.1 Organic Substrates and Inoculum

OFMSW consisted of a mixture of household waste, restaurant waste, spent coffee collected and GW (i.e. organic soil, small branches and leaves) collected in Cassino (Italy). The wastes were gathered independently during one month while stored in buckets at 4ºC, and eventually mixed into a 100 L barrel. In total, 60 kg of waste were collected with an approximated weight proportion of 45, 35, 15 and 5 % (w/w) for household waste, restaurant waste, spent coffee and GW, respectively. The mixed waste was minced twice to a pastry material with a particle size smaller than 5-10 mm by means of an industrial mincer (REBER 9500NC), fully homogenized and stored in 5 L buckets at -20ºC, aiming to minimize the composition fluctuations during the experimental period.

To increase the TS content in the batch experiments, 1-2 kg of OFMSW were dried for 7-10 days at 55ºC until constant weight right before each experiment. The resulting agglomerate was further minced with mortar and pestle, homogenized to a flour-like material with a particle size ≤ 2 mm, and stored in air-tight containers until use. Goldspan® beech sawdust with a 1.0-2.8 mm particle size was used as co-substrate.

Three 'wet' and six high-solids inocula were used in this study, since different experiments were started at different periods. All inocula were sampled from a 30 L methanogenic reactor fed with OFMSW under thermophilic (55ºC) conditions. Prior to being used in the experiments, all inocula were degassed for 7-10 days at 55ºC and subsequently filtered through a 1 mm mesh to remove coarse materials. These inoculums were considered 'wet' since TS was ≤ 5 %. To increase simultaneously the TS and ISR of batch experiments, the 'wet' inoculums were centrifuged at 6000 rpm for 10 min with a bench-scale centrifuge (REMI XS R-10M, India), right before each experiment – high solids inoculum. The supernatant was separated and the remaining viscous material was manually homogenized. Finally, micronutrients were added to each inoculum as recommended by Angelidaki and Sanders (2004).

2.2 Batch Experiments

2.2.1 Experimental Setup

Seven batch experiments were performed to evaluate the effects of increasing the initial TS from 10.0 to 33.6 % in HS-AD. Dried OFMSW and/or sawdust were used as organic substrates under different mono- and co-digestion conditions [Table 1]. Because of availability, experiments were performed in 160 or 280 mL serum bottles (Wheaton, USA), all incubated at 55ºC. The different TS were obtained by an adequate combination of substrate, inoculum and distilled water addition. To minimize the occurrence of experimental biases, each bottle contained exactly the same amount of substrate and inoculum, while the amount of distilled water depended on the desired TS. Thus, different medium volumes were obtained within the same set of batch experiments [Table 1].

The bottles were sealed with butyl rubber stoppers and aluminum crimps, and flushed with inert gas (helium or nitrogen), before adding 0.2 mL of 10 g/L Na_2S to guarantee an adequate redox potential (Angelidaki and Sanders 2004). All batch assays lasted until the gas production was negligible (i.e. < 1 mL/d) during three consecutive measurements. The bottles were manually agitated when the gas production was measured. For each experiment, blank assays were conducted in triplicate to evaluate the biomethane production of the sole inoculum. Blank assays contained the same amount of inoculum, while further distilled water was used to compensate for the absence of substrate [Table 1].

2.2.2 HS-AD Biodegradability Indicators

Five out of seven batch experiments were aimed to evaluate the effects of increasing the initial TS on the HS-AD methane yield, TS removal and COD conversion, using initial

TS contents from 'wet' (i.e. TS = 10 %) to 'dry' conditions (i.e. TS ≥ 20 %) [Test 1-5, Table 1]. Mono-digestion experiments were run with a homogeneous mixture of dried OFMSW and high-solids inoculum at an ISR of 0.50, 1.00 and 1.50 g VS/g VS, for Test 1, 2 and 3, respectively. The ISR increase resulted in lower initial TS [Table 1]. In the fourth experiment (Test 4), HS-AD of sawdust was investigated by using a mixture of beech sawdust and 'wet' inoculum at an ISR = 0.04 g VS/g VS. In the fifth experiment (Test 5), co-digestion of dried OFMSW and sawdust was performed with high-solids inoculum. The OFMSW:sawdust ratio was 1:4 g TS:g TS and the overall ISR was 0.16 g VS/g VS. All TS conditions were evaluated in triplicate.

2.2.3 Sacrifice Tests

To evaluate the main dynamics (i.e. TS, VFA, ammonia nitrogen and COD conversion) during HS-AD, two batch experiments were performed as sacrifice tests [Tests 6 and 7, Table 1]. 15 replicates were used in each test. After measuring the gas volume and composition, a single bottle was emptied and the content was analyzed (i.e. for VS, VFA and ammonia) every 3 to 5 days during the first two weeks, and every 7 to 10 days until the end of the experiment. In Test 6, dried OFMSW was used as the sole substrate in presence of high-solids inoculum. The initial TS and ISR were 15.0 % and 1.00 g VS/g VS, respectively. Test 7 was performed to study the co-digestion of OFMSW and beech sawdust with an initial TS = 19.4 % and an ISR = 0.60 g VS/g VS. The ratio OFMSW:sawdust was 1.0:1.1 g TS:g TS.

2.3 Biomethane potential of OFMSW and beech sawdust

The individual BMP of the raw OFMSW and beech sawdust at 55ºC was estimated according to Angelidaki and Sanders (2004) and Holliger et al. (2016). The BMP assay with OFMSW was performed in 280 mL bottles using 6 replicates and an ISR = 2.00 g VS/g VS, whereas the BMP of sawdust was assessed in 160 mL bottles using 3 replicates and an ISR = 1.00 g VS/g VS [Table 1]. In the BMP test for OFMSW, the distilled water addition served to minimize the chances of ammonia inhibition. In contrast, ammonia build-up was not expected in the BMP test of sawdust, due to the low nitrogen content of this substrate, as shown in next section. The lower biodegradability of sawdust permitted to use also a lower ISR.

2.4 Physical-Chemical Analyses

The pH and alkalinity were measured right after 1) diluting the (semi-)solid sample with distilled water, 2) homogenization, 3) centrifugation at 6000 rpm for 15 min and 4) supernatant titration to a pH of 5.75 and 4.3 for the carbonate (ALK_P) and total (ALK_T) alkalinity, respectively (Lahav et al. 2002). The intermediate alkalinity (ALK_I) was the difference between ALK_T and ALK_P. The TS and VS, total Kjeldahl (TKN) and ammonia nitrogen (TAN), and specific weight (ρ_s) analyses were carried out according to the standard methods (APHA 1999; EPA 2015).

The density (ρ) – containing the air-filled porosity (ε) – was approximated using a 1-2 L calibrated cylinder and a ± 0.01 g precision scale. The NH_3 was approximated as in Capson-Tojo et al. (2017). The COD of (semi-)solid samples was determined as described by Noguerol-Arias et al. (2012). The soluble COD (CODs) was determined with the same method by immediately analyzing the supernatant filtered through a 0.45

μm polypropylene membrane. The VFA (acetic, propionic, butyric and valeric acids) analysis of 0.45 μm pre-filtered samples was conducted with a LC-20AD HPLC (Shimadzu, Japan) equipped with a Rezex ROA-Organic Acids 8+ column (Phenomenex, USA) coupled to a 210 nm UV detector. The column was maintained at 70ºC with a 0.0065 M H_2SO_4 mobile phase flowing at 0.6 mL/min. Lactate and ethanol were measured by the same method but using a RID detector. However, these last compounds were not detected in any of the batch conditions assessed in this study.

The biogas production was evaluated with a two-vessel water displacement system. The first vessel contained 4 N NaOH to capture the produced CO_2, while the second vessel was filled with distilled water to be 'displaced'. Once measured the biogas production, the reactor headspace was sampled with a 250 μL pressure-lock syringe for the analysis of the biogas composition in terms of CH_4, CO_2, H_2, O_2 and N_2 with a 3400 GC-TCD (Varian, USA) equipped with a Restek Packed Column. The carrier gas was argon.

2.5 Calculations

Whether not stated otherwise, the above physical-chemical analyses were reported per kilogram (kg) of the overall inoculum-and-substrate mixture, including water (i.e. overall reactor content in wet basis).

The methane yields obtained in the seven batch experiments, as well as the BMP values for OFMSW and for beech sawdust, were expressed as the normalized methane production (P = 1 bar, T = 0ºC), excluding the endogenous methane production of the inoculum, divided by the added substrate VS (VS_{subs}). The Dixon's test was applied as recommended by Holliger et al. (2016) to discard any outlier in the batch experiments or BMP tests. The overall methane or hydrogen production at the end of each experiment was expressed as a normalized volume of gas (P = 1 bar, T = 0 ºC) measured by water displacement, divided by the VS added (VS_{added}) – including the substrate and inoculum. The hydrogen production by the VS removed ($VS_{removed}$) was also calculated in some acidified reactors.

The TS removal was the difference between the initial and final TS contents, divided by the initial TS. Noteworthy, the TS removal is roughly equivalent to the VS removal. The global COD conversion included the overall methane and/or hydrogen production and the VFA content at the end of each experiment, divided by VS_{added}. In sacrifice tests [Tests 6 and 7, Table 1], the progressive COD conversion was evaluated as the produced methane, hydrogen and VFA at a specific time interval, divided by VS_{added}. In this study, the COD conversion permitted to compare the VFA accumulation and the biogas production among methanogenic and acidified experiments, but also to evaluate the NH_3 inhibition between different initial TS contents in methanogenic reactors. The reactor content volume (V_{Global}) for each initial mixture was obtained as $\sum(M/\rho)$, being M the mass of each compound in the batch experiments (i.e. inoculum, substrate and water). The liquid-solid volume (V_{Real}) for the inoculum-substrate mixture was obtained as $\sum(M/\rho_s)$. ε was obtained as 1 - V_{Real}/V_{Global}. In this study, all the initial batch configurations were designed to be porosity free (i.e. ε = 0; $V_{Global} = V_{Real}$), since gas reduces the metabolite mass transfer in comparison to liquid media (Bollon et al. 2013).

In the HS-AD experiments used to assess the main biodegradability indicators (Section 2.2.2), the repeatability (i.e. average ± standard deviation) was assessed using all triplicates at each initial TS content. On the other hand, in the sacrifice tests (Section 2.2.3), the biogas production and composition included the average ± standard deviation of all the (remaining) replicates at a given experimental time, including that being subsequently emptied. The rest of physical-chemical analyses (e.g. TS, TAN, VFA) were performed in triplicate for the punctually-emptied replicate. In all these batch experiments, the water loss (in terms of vapor) regarding the initial amount of water in each substrate-inoculum mixture was considered negligible (i.e. < 3%, data not shown).

3 RESULTS AND DISCUSSION

3.1 Bio-Physical-Chemical Characterization of Substrates and Inoculum

Table 2 shows the average composition of the raw OFMSW, dried OFMSW and sawdust. The TS of the raw OFMSW was 26 %, in agreement with reported values for source-sorted OFMSW (Christensen 2011; Schievano et al. 2010). The TS of the dried OFMSW was 92 %. A relatively lower TAN, CODs/COD and COD/TKN ratios were observed for the dried compared to the raw OFMSW, while the VS/TS was maintained approximately constant and ε increased [Table 2]. Therefore, some volatilization of organic material (e.g. VFA, TAN) occurred when drying OFMSW at 55ºC. However, drying was an adequate conditioning for assessing the effect of TS increase in HS-AD of raw OFMSW, since the macroscopic composition was maintained relatively constant [Table 2]. A similar conditioning was used by Forster-Carneiro et al. (2008a) to increase the TS in HS-AD batch reactors. The TS of beech sawdust was 94 % [Table 2], similar to that obtained by Brown and Li (2013) for GW.

The BMP of the raw OFMSW and sawdust at 55ºC was 497 ± 58 NmL CH_4/g VS_{subs} [Figure 1a] and 161 ± 12 NmL CH_4/g VS_{subs} [Figure 1b], respectively, indicating the lower biodegradability of sawdust than of OFMSW under anaerobic conditions. Moreover, reaching the maximum methane yield took a considerably longer for sawdust than OFMSW (i.e. 130 and 56 days, respectively), suggesting also a reduced hydrolysis rate for lignocellulosic substrates (Pastor-Poquet et al. 2018, In Press; Vavilin et al. 2008). The higher standard deviation in the BMP for raw OFMSW was attributed to the waste heterogeneity. The BMP values were equivalent to those observed for source-sorted OFMSW and GW (Brown and Li 2013; Schievano et al. 2010).

The average composition of the 'wet' and high-solids inocula is reported in Table 2. Only minor deviations in macroscopic characteristics (i.e. TS and TKN) were observed between 'wet' and high-solids inocula sampled at different times. Centrifugation increased the TS content, and ALK_I/ALK_P, COD/TKN and VS/TS ratios compared to the 'wet' inoculum [Table 2]. A similar inoculum conditioning was used by Brown and Li (2013) to increase the TS in 'dry' co-digestion. Other inoculum pretreatments to increase TS in HS-AD include inoculum filtration (Liotta et al. 2014) or drying at 105ºC (Capson-Tojo et al. 2017), though heating the inoculum at 105ºC might result in methanogenesis inhibition (Ghimire et al. 2015).

3.2 Batch Experiments

3.2.1 Acidified Experiments

Mono-digestion of OFMSW with an ISR of 0.5 and 1.0 g VS/g VS (Test 1 and Test 2) allowed to increase the TS up to 33.6 and 24.0 %, respectively [Table 1]. However, all the TS conditions resulted in acidification (i.e. pH ≤ 6.0), likely due to the low ISR used (Angelidaki and Sanders 2004). Methanogenesis inhibition led to H_2 production and VFA accumulation. The highest H_2 production with an ISR = 0.5 g VS/g VS (Test 1) was achieved at the lowest TS (i.e. 10.2 %) and progressively decreased with increasing TS [Figure 2b], likely due to the reduced mass transfer in high-solids conditions. The H_2 production (i.e. 2-20 NmL H_2/g VS_{added} = 7-60 NmL H_2/g $VS_{removed}$) was comparable to that reported by Valdez-Vazquez and Poggi-Varaldo (2009) for OFMSW (i.e. 10-50 NmL H_2/g $VS_{removed}$). With an ISR = 1.0 g VS/g VS (Test 2), the H_2 production was ≤ 1 NmL H_2/g VS_{added}. A reduced H_2 production can be attributed to a higher ISR.

In both experiments, an inverse relationship between the TS removal and the initial TS was observed [Figure 2c]. Meanwhile, the global COD conversion described an average 0.35 g COD/g VS_{added} at an initial TS of around 10 % and a similar downward trend with increasing TS in both experiments [Figure 2d]. The COD conversion in acidified reactors corresponded from 87 to 96 % of the VFA accumulation. This confirms that H_2 production and/or VFA accumulation potentially reduced the hydrolysis rate (Cazier et al. 2015; Vavilin et al. 2008), playing a major role on the organic degradation at higher TS, due to the low water available (García-Bernet et al. 2011).

3.2.2 Methane-Producing Experiments

Despite mono-digestion of OFMSW at an ISR = 0.5 g VS/g VS (Test 1) acidified at all TS contents, methanogenesis occurred in 2 out of 3 replicates performed at 28.3 % TS, leading to an average methane yield of 64 ± 6 NmL CH_4/g VS_{subs} [Figure 2a] – 87 % lower than the BMP of raw OFMSW – and a 23 % TS removal [Figure 2c]. The methanogenic onset observed in the two bottles at 28.3 % TS might relate to a favorable mass transfer in the high-solids mixture, as discussed in Section 3.2.4, since all the bottles contained exactly the same amount of substrate and inoculum.

Methanogenesis succeeded in all TS contents with mono-digestion of OFMSW using an ISR = 1.5 g VS/g VS (Test 3), though only a maximum 19.6 % TS was reached under these conditions [Figure 2a]. A methane yield of 236 ± 5, 199 ± 32, 174 ± 47 and 222 ± 62 NmL CH_4/g VS_{subs} was observed at initial TS of 10.8, 13.4, 16.4 and 19.6 %, respectively [Figure 1c and 2a], i.e. 52-65 % lower than the BMP of OFMSW. These methane yields corresponded to a volumetric productivity of 8.8 ± 0.2, 9.3 ± 1.5, 10.2 ± 2.8 and 15.8 ± 4.4 NmL CH_4/L Reactor Content (data not shown) at initial TS of 10.8, 13.4, 16.4 and 19.6 %, respectively, being the higher volumetric productivity at increasing TS one of the main advantages of HS-AD (Brown et al. 2012). Interestingly, the standard deviation of the methane yield increased alongside the TS [Figure 2a], likely due to mass transfer effects and/or a higher heterogeneity of the initial mixture, as discussed in Section 3.2.4. In contrast, the TS removal decreased at increasing initial TS contents [Figure 2c]. The global COD conversion was approximately 0.38 ± 0.05 g COD/g VS_{added} at all TS, but showing a higher standard deviation at an initial TS = 19.6 % [Figure 2d & Table 3]. It should be noted that the TS removal (i.e. VS removal) and the COD conversion yield similar information about the overall organic degradation in methanogenic experiments. Nonetheless, the COD conversion was considered as a more informative assessment of the VFA accumulation in these experiments, as indicated in Section 2.5. Particularly, it can be observed how the COD standard deviation is obscured when assessing the TS removal [Figure 2c & Figure 2d].

Mono-digestion of sawdust (Test 4) showed a methane yield of 64 ± 3, 92 ± 3, 94 ± 4, 81 ± 32 NmL CH_4/g VS_{subs} at initial TS of 9.8, 14.6, 19.3 and 24.1 %, respectively [Figures 1d and 2a]. The methane yield at 9.8 % TS was approximately 30 % lower than that obtained at higher TS. After 100 days, the methane yield was 55-70 % lower than the BMP of sawdust, probably due to the lower ISR (i.e. 0.04 g VS/g VS) slowing down the biochemistry (Holliger et al. 2016), and/or the higher TS used. An 8-fold-higher standard deviation was observed at 24.1 % TS, likely due to inaccessible substrate regions at high TS – mass transfer limitations. The TS removal at initial TS = 24.1 % was around 50 % lower than that obtained at lower TS [Figure 2c]. The global COD conversion showed a downward trend from 14.6 to 24.1 % TS [Figure 2d].

With co-digestion of dried OFMSW and sawdust (Test 5), methane was produced only at 10.0 and 15.0 % TS, while co-digestion reactors at higher TS contents resulted in acidification [Figure 2], potentially due to the higher organic content at higher TS. The methane yield reached 138 ± 1 and 156 ± 19 NmL/g VS_{subs} at 10.0 and 15.0 % TS, respectively [Figure 1e]. Interestingly, 1 out of 3 replicates performed at 30.2 % TS also showed methanogenesis likely due to mass transfer effects in HS-AD, reaching a methane production of 117 NmL/g VS_{subs}. The H_2 yield – during the first week – decreased with increasing TS [Figure 2b]. The TS removal was also reduced at an increasing TS content [Figure 2c].

3.2.3 Main Effects when Increasing the Initial TS in HS-AD

The TS increase in HS-AD led to an increased biomethane volumetric productivity with mono-digestion of OFMSW (Test 3), but also resulted in acidification by substrate overload at higher initial TS with co-digestion of OFMSW and sawdust (Test 5). Moreover, higher standard deviations in the methane yields at higher TS, as well as the occurrence of methanogenesis only in some of the replicates at 28.3 and 30.2 %, were observed. These last results were likely due to mass transfer effects in HS-AD experiments, which influenced the occurrence of acidification and/or inhibition.

The low water content of a high-solids mixture hinders the accessibility of microorganisms to large portions of the substrate (Bollon et al. 2013), possibly explaining the increasing standard deviation in the methane yield at TS ≥ 10 % [Figure 2a]. Particularly, 'dry' AD (i.e. TS ≥ 20 %) is associated to the presence of spatially-differentiated acidogenic/methanogenic centers (Staley et al. 2011; Xu et al. 2014). In such systems, the convective transport is minimum, while the metabolite diffusion increases in importance, since the free-to-bound water ratio is low (Bollon et al. 2013; García-Bernet et al. 2011). Besides limiting the organic degradation, this phenomenon also reduces the chances of acidification of all the methanogenic centers in case of overload, likely explaining the methanogenesis onset observed in some replicates at 28.3 % TS (Test 1) and 30.2 % TS (Test 5). Homogenization devices, such as reactor stirrer or leachate recirculation, might help to prevent the influence of mass transfer limitations in HS-AD (André et al. 2018; Kothari et al. 2014).

3.2.4 Maximizing the TS in HS-AD of OFMSW by Sawdust Addition

In this study, the physical-chemical characteristics of the substrate and inoculum (e.g. VS/TS and biodegradability) and the operational TS and ISR were found closely interrelated parameters determining the methane production or acidification in HS-AD. The ISR and the maximum TS were simultaneously adjusted in mono-digestion experiments according to the TS and VS balances of the substrate-inoculum mixture, since only one degree of freedom is available in a binary mixture (i.e. TS or ISR). Particularly, whether TS are higher in the substrate than in the inoculum, higher initial TS contents of a given substrate-inoculum mixture are obtained by lowering the ISR [Tests 1-3, Table 1]. Nonetheless, the ISR must be sufficiently high to avoid acidification, as a function of the substrate biodegradability (Angelidaki and Sanders 2004; Schievano et al. 2010). For example, the high biodegradability of OFMSW required a higher ISR (i.e. 1.5 g VS/g VS), yielding a lower maximum TS (i.e. 19.6 %) [Figure 2]. In contrast, the lower methane potential and biodegradability rate of sawdust – as an example of lignocellulosic substrate – allowed the use of an extremely low ISR (i.e. 0.04 g VS/g VS) and a higher TS (i.e. 24.1 %).

In the case of co-digestion, two degrees of freedom are available in a ternary mixture (i.e. TS, ISR or OFMSW:GW ratio). Thus, a great number of combinations exists depending on the particular substrate and/or inoculum characteristics (e.g. VS/TS), explaining the different TS, ISR and FW:GW ratios used in literature for co-digestion. In this line, Brown and Li (2013) showed that, for a fixed ISR in 'dry' AD, the acidification risk increases by increasing the FW:GW ratio, due to the higher biodegradability of the inoculum-substrate mixture. Moreover, a higher FW:GW exacerbates the risk of TAN buildup and NH_3 inhibition in HS-AD.

Summarizing, adding sawdust to OFMSW reduces the biodegradability and TAN content of the substrate-inoculum mixture in comparison to mono-digestion of OFMSW, favoring the simultaneous TS and ISR increase in HS-AD. Thus, a OFMSW:sawdust ratio of 1:4 g TS:g TS was chosen in this study mainly to increase the maximum TS of co-digestion up to 30 %, but reducing the chances of NH_3 inhibition and acidification. Nonetheless, the addition of GW to OFMSW in industrial applications depends on the availability of co-substrates, the reactor design and/or the overall process economy (Christensen 2011; Kothari et al. 2014).

3.2.5 HS-AD Dynamics and NH_3 Inhibition

During the sacrifice test for mono-digestion of OFMSW (Test 6) [Figure 3], the daily methane production peaked around day 28, while the cumulative methane yield stabilized by day 65 reaching a value of 296 ± 13 NmL CH_4/g VS_{subs}, i.e. 40 % lower than the BMP of OFMSW. Because of the organic degradation, TS showed a 34.7 % TS removal. Acetic acid peaked to 8.40 g/kg (day 8) and was extensively consumed within 30 days from the reactor startup. Propionic, butyric and valeric acids increased significantly along the experiment. TAN started at 2.4 g N/kg and reached 3.8 g N/kg. At the same period, pH started at 7.3, decreased to a minimum of 6.3 and increased above 8. The TAN and pH increase resulted in a NH_3 concentration up to 2.5 g N/kg. The global COD conversion was 0.63 g COD/g VS_{added}.

These results suggest that the high ammonia levels were responsible for the reduced methane yield, TS removal and COD conversion in HS-AD, since all biodegradability indicators significantly slowed down in the mono-digestion sacrifice (Test 6) as NH_3 reached 2.3 g N/kg from day 45 [Figure 3]. Depending on the methanogens acclimation, NH_3 concentrations of 0.2-1.4 g N/L have been reported inhibitory (Chen et al. 2008; Fricke et al. 2007; Prochazka et al. 2012). In this study, the NH_3 increase correlated well with the propionic/valeric accumulation in Test 6 [Figure 3], being the VFA buildup a likely consequence of methanogenic inhibition (Demirel and Scherer 2008).

The above results indicate that the ammonia buildup most probably hampered the methane production also in the mono-digestion experiment using an ISR = 1.5 g VS/g VS (Test 3) [Figure 2]. Thus, the nitrogen content (i.e. TKN, TAN and NH_3) was observed to increase in Test 3 alongside the higher initial TS, because of the lower amount of water initially used for dilution, potentially exacerbating the NH_3 inhibition and VFA accumulation at higher TS [Table 3]. With all the above, the NH_3 accumulation can determine the overall anaerobic degradation (i.e. methane yield, TS removal and COD conversion) during HS-AD, particularly at higher initial TS contents. These results complement the main bio-physical-chemical effects arising in HS-AD due to the TS increase (i.e. reduced organic degradation by mass transfer effects), as mentioned in Section 3.2.3. In other words, the TS increase can limit the organic degradation in HS-AD of OFMSW due both to mass transfer effects and NH_3 inhibition. With the aim to reduce the risk of NH_3 inhibition while increasing the TS content, a co-digestion sacrifice was performed.

3.2.6 Other Factors Influencing Acidification in HS-AD

In co-digestion sacrifice (Test 7) [Figure 4], methanogenesis was inhibited from day 3, linked to a pH drop from 7.4 to 6.0. Thus, only a 10.3 % TS removal was observed, while TAN increased from 1.5 to 3.0 g N/kg, and acetic, propionic, butyric and valeric acids substantially increased. The overall H_2 production was 0.18 NmL H_2/g VS_{added} and the global COD conversion was 0.18 g COD/g VS_{added}.

The pH drop observed right after starting the HS-AD batch experiments (initial 0-3 days) was crucial to discern about the potential acidification in Tests 6 and 7. The initial pH drop is normally observed in AD when acidogenic outcompetes methanogenic growth (Gerardi 2003), and becomes particularly important in HS-AD of OFMSW due to the high organic content used. Both mono- (Test 6) and co-digestion (Test 7) sacrifice tests showed an initial pH ≥ 7.3 (day 0) that rapidly dropped due to the VFA accumulation. In mono-digestion (Test 6), the pH = 6.4 from day 3 to 11 likely determined the low cumulative methane production (i.e. 6.3 NmL CH_4/g VS_{subs}) observed during these days, whereas the pH = 6.0 in the co-digestion sacrifice (Test 7) potentially inhibited methanogenesis (Demirel and Scherer 2008; Staley et al. 2011).

The ALK_P and likely also the microbial activity of the inoculum used as a seed in a HS-AD reactor played a major role to determine the acidification or methanogenesis onset, since ALK_P is the main pH buffer in AD (Prochazka et al. 2012). These factors mainly depend on the source reactor performance, the degassing period and the inoculum pretreatment. Thus, the ALK_P of the inoculum in this study determined the initial ALK_P of the inoculum-substrate mixture [Table 2], by the ALK_P mass balance.

At high TS, external buffer addition might help to circumvent HS-AD acidification. For example, Liotta et al. (2014) added $NaHCO_3$ to stabilize the acidogenic stages in HS-AD. However, whether inorganic buffering is used, particular attention is needed to minimize the TS dilution, while maintain an optimal cationic (i.e. Na^+) concentration for microorganisms (Chen et al. 2008). Moreover, both the $NaHCO_3$ concentration and the $NaHCO_3$-to-organics ratio (i.e. g $NaHCO_3$/g TS) need to be the same along different initial TS, to allow comparison among these. Thus, $NaHCO_3$ addition was not used in this study to reduce the ‘external’ influencers in HS-AD.

In either case, acidification in this study did not associate to a low ALK_P, nor to a high ALK_I/ALK_P ratio – data not shown. For example, mono-digestion Test 1 acidified at an initial ALK_P of 1.7-5.6 g $CaCO_3$/kg and ALK_I/ALK_P = 0.88, whereas acidification was avoided in mono-digestion Test 6 with ALK_P of 2.6 and ALK_I/ALK_P = 2.12. Similarly, methanogenesis failed to start in Test 2, operated at the same ISR than Test 6 (i.e. 1.0 g VS/g VS), though the initial ALK_P and ALK_I/ALK_P ratio were 1.5-3.8 g $CaCO_3$/kg and 1.51, respectively, in the acidified experiment (Test 2).

In conclusion, other factors related to the initial inoculum-substrate mixture, and not assessed here, influenced also the HS-AD acidification. Some of these might include the different (micro-)nutrient or inhibitory content, but also the mass transfer, reactor homogenization, reactor headspace volume, particle size and/or inoculum activity (André et al. 2018; Bollon et al. 2013; Chen et al. 2008; Holliger et al. 2016; Motte et al. 2014). Therefore, all these factors should be considered alongside the TS, ISR, ALK_P and nitrogen content to evaluate HS-AD of OFMSW. All the above results corroborate that HS-AD is an extremely complex bio-physical-chemical process, with an elevated number of interrelated mechanisms and operational variables, where a thorough experimental assessment is required, in order to fully understand the overall bio-physical-chemistry and eventually optimize HS-AD of OFMSW at industrial scale.

4 CONCLUSIONS

This study shows that both the initial TS and ISR determine the success of methanogenesis in HS-AD of OFMSW. During mono-digestion of OFMSW, increasing the maximum TS required a lower ISR, enhancing the risk of acidification. Meanwhile, $NH_3 \geq 2.3$ g N/kg at 15.0 % TS resulted in VFA accumulation (i.e. 0.13-0.14 g COD/g VS_{added}) and 40 % lower methane yield. Adding sawdust to OFMSW permitted to increase simultaneously the TS and ISR, by reducing considerably the biodegradability and nitrogen content of the mixture, in comparison to mono-digestion of OFMSW. This also led to acidification occurring only at higher TS (i.e. ≥ 20 %). Therefore, the initial inoculum-substrate mixture in HS-AD must result from a tradeoff between the maximum TS and the optimum ISR, but also the buffering capacity and the nitrogen content, to circumvent acidification and NH_3 inhibition.

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TABLE CAPTIONS

Table 1 Summary of high-solids batch experiments and biomethane potential tests (BMP)

Table 2 Bio-physical-chemical characterization of substrates and inoculum

Table 3 Effect of total solids on the performances of high-solids anaerobic digestion of the organic fraction of municipal solid waste using an inoculum-to-substrate ratio of 1.5 g VS/g VS (Test 3)

FIGURE CAPTIONS

Fig. 1 Cumulative methane production: a) Biomethane potential (BMP) test for the organic fraction of municipal solid waste (OFMSW); b) BMP test for sawdust; c) mono-digestion of 55ºC-dried OFMSW at an ISR of 1.50 g VS/g VS (Test 3); d) mono-digestion of beech sawdust at an ISR of 0.04 g VS/g VS (Test 4); and e) co-digestion of 55ºC-dried OFMSW and beech sawdust at an ISR of 0.16 g VS/g VS (Test 5)

Fig. 2 Main anaerobic biodegradability indicators: a) methane yield; b) hydrogen yield; c) total solid removal; and d) total chemical oxygen demand (COD) conversion

Fig. 3 Sacrifice test with mono-digestion of organic fraction of municipal solid waste (Test 6). a) Daily and cumulative methane production, and pH; b) volatile fatty acids; c) total (TS) and volatile (VS) solids, and total (TAN)and free (FAN) ammonia nitrogen; and d) chemical oxygen demand (COD) conversion

Fig. 4 Sacrifice test with co-digestion of organic fraction of municipal solid waste and beech sawdust (Test 7). a) Daily and cumulative methane production, and pH; b) volatile fatty acids; c) total (TS) and volatile (VS) solids, and total (TAN) and free (FAN) ammonia nitrogen; and d) chemical oxygen demand (COD) conversion