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Short Communication

Evaluating inhibition conditions in high-solids anaerobic digestion of organic fraction of municipal solid waste

Andrea Schievano [a], Giuliana D'Imporzano [a], Luca Malagutti [b], Emilio Fragali [c], Gabriella Ruboni [c], Fabrizio Adani [a,*]

[a] Gruppo Ricicla – Dipartimento di Produzione Vegetale – Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy
[b] Dipartimento di Scienze Animali – Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy
[c] Linea Ambiente srl, Via XXV Aprile 18 25038 Rovato (BS), Italy

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ABSTRACT

High-solids anaerobic digestion (HSAD) processes, when applied to different types of organic fractions of municipal solid waste (OFMSW), may easily be subjected to inhibition due to organic overloading. In this study, a new approach for predicting these phenomena was proposed based on the estimation of the putrescibility (oxygen consumption in 20 h biodegradation, OD_{20}) of the organic mixtures undergoing the HSAD process. Different wastes exhibiting different putrescibility were subjected to lab-scale batch-HSAD. Measuring the organic loading (OL) as volatile solids (VS) was found unsuitable for predicting overload inhibition, because similar VS contents corresponded to both inhibited and successful trials. Instead, the OL calculated as OD_{20} was a very good indicator of the inhibiting conditions (inhibition started for $OD_{20} > 17$–18 g O_2 kg^{-1}). This new method of predicting inhibition in the HSAD process of diverse OFMSW may be useful for developing a correct approach to the technology in very different contexts.

1. Introduction

Regarding full-scale anaerobic digestion (AD) implementation, the wet processes (<10% dry matter – DM – in the feed) are actually the most diffused. However, many waste materials, typically lignocellulosic and green wastes, are not suitable for the wet processes, as they have a solid bulk structure. In contrast, the high-solids AD (HSAD) processes (also known as dry-AD or anaerobic composting) (>10% DM in the feed) are more versatile in terms of the kinds of materials (liquid plus solid) and may be implemented more broadly (Bolzonella et al., 2006). In some northern-EU countries, HSAD is more developed than southern-EU (e.g. Italy), where there is the chance of spreading this technology.

It is well known that during the AD process, the fermentation of hydrolyzed organics to volatile fatty acids (VFA) may result in VFA accumulation, along with a drop in pH if the acids are not metabolized by methanogens. As fermentative microbial consortia have much faster kinetics than methanogens, high organic loadings may lead to volatile fatty acid accumulation and inhibition of the methanogenic bacteria (Gorris et al., 1989). Failure to maintain the balance between these two groups of microorganisms, due to an increase in the concentration of organic compounds in the feed (organic overload) or in the feed flow rate (hydraulic overload), is the primary cause of reactor instability (Demirel and Yenigün, 2002). Thus, even if the HSAD processes are reported to tolerate higher organic loadings in terms of volatile solids (VS) per volume unit (Hartmann and Ahring, 2006), i.e., high organic loadings (OL), measured in kg VS m^{-3}digester, low operational stability still limits the diffusion of the HSAD process (Dupla et al., 2004). In fact, many problems may arise depending on the quality of the fed VS and their degradation kinetics (Buffiere et al., 2006).

The putrescibility (i.e. short-term biodegradability) of the fresh fed materials was reported to be an important information for preventing failures, estimating biogas production, and managing the digestion process (Buffiere et al., 2006). Therefore, wide differences in the waste composition and putrescibility may lead to different behaviors during the HSAD process. In particular, HSAD is often used to treat the organic fraction of municipal solid waste (OFMSW), which is composed of very diverse materials depending on the context, the method of separate-collection, and the season (Barrena et al., 2009). In many northern-EU countries, the OFMSW is typically composed of vegetable, fruit, and garden waste (VFG); on the other hand, in Italy, for example, lignocellulosic materials are collected separately from household kitchen waste, which also includes cooked food residues and, sometimes, food-industry by-products (Veeken and Hamelers, 1999). This may lead to a higher

* Corresponding author.
E-mail address: fabrizio.adani@unimi.it (F. Adani).

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putrescibility of the OFMSW compared to VFG, which is typically used in the HSAD facilities in north-Europe.

In HSAD plants, to establish and maintain a stable digestion process, fresh material (F) is usually mixed (at defined ratios) with digested material, which is re-circulated and functions as inoculum (I). When applying HSAD to different types of OFMSW, either the organic loading (OL, kg VS m^{-3}) or the F/I ratios should be adapted to that particular type of waste. An estimation of the putrescibility of the fresh materials would help in preventing overload inhibition.

The aims of this work were to apply the HSAD process to highly putrescent OFMSW and to define a parameter (based on the putrescibility of the fed mixture) for predicting overload inhibition. This would help in implementing HSAD in very different contexts where the production and collection of waste differ substantially.

2. Methods

2.1. OFMSW material collection and preparation

The waste materials studied were OFMSW from household door-to-door separate collections that differed in their provenience and composition. The first sample (Sample W_1) was a typical separate-collection OFMSW coming from a northern-EU country, i.e., mostly containing vegetable, fruit, and garden waste, mixed with lignocelluloses material at the ratio 70/30 w/w (ratios referred to wet weight basis). The waste was sampled in a Swiss HSAD facility. In contrast, the second type of waste (Sample W_2) was sampled at an Italian OFMSW composting facility near Milano, Italy. The waste was highly putrescent compared to the W_1, as the collection included consistent fractions of any kind of kitchen residue and cooked food. A source-separated municipal garden waste (MGW) was also considered, to be mixed with OFMSW for mitigating its putrescibility. The W_2 was mixed with the MGW at two different ratios: W_2/MGW at 70/30 w/w (the same ratio used in the Swiss AD plant) for the first mix (Sample M_1) and for the second one, in order to lower the putrescibility of the mix, W_2/MGW at 50/50 w/w (Sample M_2) (ratios referred to wet weight basis). Then, a sample of digested solid material was collected in the Swiss HSAD plant and used as inoculum (I) for the HSAD assays.

2.2. Chemical characterization

Representative samples of wastes were used to carry out all the analyses. Dry matter (DM) and volatile solids (VS) were determined according to standard procedures (APHA, 1998). Total Kjeldhal Nitrogen (TKN) was determined (using fresh material) according to the analytical method for wastewater sludges (IRSA-CNR, 1994). Total phosphorus (as P_2O_5) content was determined using the standard methods for the examination of water and wastewater (APHA, 1998). Fiber analyses were performed for neutral detergent fiber (NDF), neutral detergent acid detergent fiber (NDADF), and acid detergent lignin (ADL) following Van Soest's method (Van Soest et al., 1991). Cell solubles (CS), lignin plus unhydrolyzable lipid (ADL), cellulose (NDADF–ADL), and hemicellulose (NDF–NDADF) were calculated according to Van Soest et al. (1991). All analyses were performed in duplicate. The analysis of VFA in the bulk samples was performed on a 10-times-diluted solution of 2.5 g of wet sample filtered to 0.45 nm. The liquid solutions (1 ml) were then injected into a gas chromatograph (Varian, CP-3800) with a capillary column 25 m × 0.32 mm in diameter and a flame ionization detector (FID). Helium at a pressure of 20 kPa was used as carrier gas, and the temperatures of the injector and FID were 220 and 240 °C, respectively.

2.3. Bio-methane potential (BMP) and specific oxygen uptake rate (SOUR) assays

The BMPs of all samples were determined by using a standardized method reported in Schievano et al. (2008).

The SOUR test is an aerobic biological assay. It is a measure of the oxygen uptake rate in a water solution during the microbial respiration in degrading a suspended solid matrix. The microbial respiration works out in standardized moisture conditions, and in maximized conditions of both oxygenation and bacteria-substrate interaction, amplifying the differences among the different samples. The potential oxygen uptake was determined as the cumulative oxygen demand during the 20 h test (OD_{20}: g O_2 kg^{-1} FM $20\ h^{-1}$). This test provides a measure of the short-term biodegradability (putrescibility) of the organic matter, and was performed as reported by Schievano et al. (2009).

All the tests were performed in duplicate.

2.4. High-solids batch bio-methane potential assay (HSMP)

The samples W_1, W_2, M_1, and M_2 underwent high-solids batch bio-methane potential assay in order to discriminate the threshold of the inhibiting conditions from the appropriate conditions for performing HSAD. The assays were performed at three different feedstock/inoculum (F/I) ratios (on FM w/w basis): 1/1, 1/2, and 1/3. Based on the three different F/I ratios used, the total DM content in the trials ranged from 17.7% to 28.1%. In 500 ml serum bottles, 50, 33.5, and 25 g of fresh material, respectively, were added to 50, 66.5, and 75 g of inoculum. The total amount of material was always 100 g, and the batches had 900 dm^3 of headspace. Control blanks were prepared using 100 g of inoculum (I). The overall pH in the mixtures ($F + I$) was measured at the beginning of the test and always found in the range of 6.8–8. All bottles were sealed with Teflon hermetic caps, flushed with a N_2 atmosphere, and incubated in thermophilic conditions (55 ± 1 °C) until no further biogas production was detected (about 60 d). The batch digesters were periodically analyzed for both quantitative and qualitative determination of biogas production. Quantitative biogas production was estimated by withdrawing extra-pressure gas with a 60 ml syringe. The biogas production of blank control batches was subtracted from the biogas production of every sample. Qualitative characterization of the biogas was performed with a gas chromatograph (Carlo Erba Megaseries 5300 with a capillary column 25 m × 0.32 mm in diameter and a flame ionization detector [FID]) to determine the CH_4–CO_2 ratio in the biogas (v/v). The carrier gas was nitrogen at 20 kPa pressure, and the temperatures of the injector and FID were 130 and 150 °C, respectively. Comparison of obtained peak areas was carried out with a standard gas mixture at 30:70 (CH_4:CO_2) ratio. All tests were run in duplicates. Inoculum with stable methanogenic activity (CH_4 > 60% in biogas, v/v) was obtained using the output digestate of a full-scale HSAD plant operating in Switzerland.

3. Results

3.1. Chemical and biological parameters

The chemical characterization of the considered materials (Table 1) reflected and confirmed the differences in the types of wastes. Probably because of the strong presence of lignocellulosic materials (LCM), W_1 and MGW had higher DM contents compared to W_2 (Table 1). On the other hand, the VS content was lower in W_1 and MGW, and the nitrogen (TKN) and phosphorous (P–P_2O_5) contents were about half of those of W_2 (Table 1). Moreover, the VS compositions showed noticeable differences, as fiber analysis indi-

Table 1
Chemical characterization of the considered materials.

	DM[a] (g kg^{-1})	VS[b] (g kg^{-1} DM)	pH	TKN[c] (g kg^{-1} DM)	Total P (g kg^{-1} DM as P_2O_5)
W_1	407 ± 5	662 ± 2	5.18 ± 0.04	7.0 ± 0.3	2.4 ± 0.1
W_2	242 ± 9	916 ± 2	4.38 ± 0.07	20.5 ± 1.4	5.7 ± 0.4
MGW	400 ± 12	626 ± 3	6.13 ± 0.07	4.0 ± 0.1	1.5 ± 0.1
M_1	290 ± 23	796 ± 4	4.91 ± 0.03	13.7 ± 0.5	4.0 ± 0.8
M_2	321 ± 16	736 ± 2	5.26 ± 0.05	10.2 ± 1.1	3.1 ± 0.9
Inoculum (*I*)	155 ± 26	613 ± 30	8.11 ± 0.01	23.4 ± 1.9	8.4 ± 1.1

[a] DM = dry matter.
[b] VS = volatile solids.
[c] TKN = Total Kjeldal Nitrogen.

cated (Table 2). The ADL in W_1 was twice higher than in W_2, confirming the high presence of LCM fractions in W_1 (Table 2). On the contrary, the soluble-cell material (CS) in W_2 was higher than in W_1 (Table 2). MGW showed relatively low VS, nitrogen, and phosphorus contents (Table 1); high lignin content; and low soluble-cell content (Table 2). M_1 and M_2 showed midrange values between the characteristics of W_2 and MGW for all parameters (Tables 1 and 2). The inoculum (*I*) used in the HSMP tests showed the lowest VS and CS contents (Tables 1 and 2), but the highest mineral nutrient content and ADL concentrations (Tables 1 and 2).

The putrescibility, assessed by measuring the OD_{20}, was progressively lower for W_2, M_1, M_2, W_1, and MGW, respectively (Table 3). In particular, the OD_{20} of MGW was similar to that of W_1 and much lower than that of W_2, so that the mixes M_1 and M_2 resulted in mitigated putrescibility when compared to W_2. The OD_{20} of the inoculum (*I*), as expected, was the lowest among those of all the considered materials (Table 3). The bio-methane potential (BMP) was similar for W_1 and MGW (Table 3). As expected, the highest BMP was measured for W_2, while the lowest value was for the inoculum (*I*) used in the HSMP test (Table 3).

Table 2
Fiber contents in the considered materials.

	Cellulose[a] (g kg^{-1} DM) (ash free)	Hemicellulose[b] (g kg^{-1} DM) (ash free)	Lignin plus unhydrolyzable lipid[c] (g kg^{-1} DM) (ash free)	Soluble cell material[d] (g kg^{-1} DM) (ash free)
W_1	156 ± 22	39 ± 23	126 ± 4	679 ± 7
W_2	103 ± 2	39 ± 7	65 ± 2	793 ± 7
MGW	310 ± 36	50 ± 27	189 ± 27	452 ± 1
M_1	232 ± 2	46 ± 2	142 ± 6	581 ± 6
M_2	267 ± 8	48 ± 4	163 ± 2	522 ± 8
Inoculum (*I*)	221 ± 6	−26 ± 26	221 ± 9	585 ± 26

[a] Cellulose: acid detergent fiber (ADF) – acid detergent lignin (ADL).
[b] Hemicellulose: neutral detergent fiber (NDF) – acid detergent fiber (ADF).
[c] Lignin plus unhydrolizable lipid:acid detergent lignin (ADL).
[d] Soluble cell material: 1000 g kg^{-1} – neutral detergent fiber (NDF).

Table 3
Biochemical methane potential (BMP) and putrescibility (OD_{20}: oxygen demand in 20 h biodegradation) of the considered samples.

	BMP Ndm^3 CH_4 kg^{-1} DM[a,b]	OD_{20} g O_2 kg^{-1} FM[c]
W_1	196 ± 13	33 ± 1
W_2	448 ± 51	75 ± 6
MGW	236 ± 3	23 ± 1
M_1	384 ± 37	67 ± 6
M_2	342 ± 27	59 ± 4
Inoculum (*I*)	101 ± 5	4 ± 1

[a] DM = dry matter.
[b] The gas volumes were normalized at 25 °C and 1 atm.
[c] FM = fresh matter.

These results outlined the diversity of the waste materials considered in terms of chemical composition, quality, biodegradability, and putrescibility of the organic matter (OM).

3.2. Results of the HSMP assay

Based on the different degrees of putrescibility measured for the considered materials, the HSMP assay yielded different results for W_1 and W_2 (Table 4). W_1 resulted in a satisfactory bio-gasification (HSMP/BMP > 60%) at 1/2 and 1/3 *F*/*I* ratios, while the 1/1 *F*/*I* ratio determined inhibition in the anaerobic process, i.e., the biomethane yield was very low, and the methane content in biogas stood steadily around 5% (Table 4). W_2 showed inhibition at any of the tested *F*/*I* ratios. The biogas production was negligible, i.e., the blank trial (only inoculum) produced more biogas than the fed batches, and the CH_4 concentration in the biogas was detected always in the range of 2–14% (Table 4). The sample M_1 gave similar results, i.e., negligible production of biogas and very low methane concentration during the test: around 1% for the 1/1 *F*/*I* ratio, 4% for 1/2 *F*/*I*, and up to 22% for the 1/3 *F*/*I* ratio. M_2 also resulted in negligible generation of biogas and CH_4 concentrations for the 1/1 and 1/2 *F*/*I* ratios, while it achieved satisfactory production (HSMP/BMP > 60%) for the 1/3 *F*/*I* ratio, with a methane concentration of 62% (Table 4). This was the only HSMP trial containing W_2 that resulted in stable methane production.

The VFA concentrations in all the inhibited trials were found to be more than 10 times higher than those in the not-inhibited ones (Table 5). W_1 (at *F*/*I* ratios of 1/1 and 1/2) and M_2 (at a *F*/*I* ratio of 1/3) were the only three trials that showed total VFA contents below 1 g kg^{-1} (as acetic acid) and butyric acid concentrations below 0.3 g l^{-1}. At the same time, the inhibited trials showed butyric acid concentrations ranging from 1.80 to 8.90 g l^{-1} as well as detectable concentrations of the other VFAs. These data proved that the inhibition was strictly linked to organic overloading, which led to VFA accumulation.

4. Discussion

From the obtained results, it was found that the HSAD process can be strongly influenced by the type of waste material used. If one substrate can be digested without the risk of meeting inhibiting conditions, under identical process conditions (i.e. *F*/*I* mixing ratio) other kinds of feedstock may result in collapsing the methanogenesis. Thus, the *F*/*I* ratio was found to be an unsuitable parameter for identifying a threshold of inhibitory conditions in a HSAD process (Table 4).

The organic loading (OL) is usually measured in terms of the total VS fed into the digesters (Hartmann and Ahring, 2006). In this study, however, the OL calculated as total VS fed (*F* + *I*) did not detect a clear inhibition threshold (Table 4). For example, W_2 at 1/3 *F*/*I* ratio (OL of 127 g VS kg^{-1} FM) resulted in overload inhibition (Table 4), while a higher OL (153 g VS kg^{-1} FM) yielded a successful

Table 4
Results of the solid state batch methane potential (HSMP) assay.

Material	Feedstock to inoculum ratio (*F*/*I*)	OL[a] as VS of the bulk mixture (*F* + *I*) g_{VS} kg^{-1} FM[a,d]	OL[a] as OD_{20} of the bulk mixture (*F* + *I*) g O_2 kg^{-1} FM[a,d]	HSMP Ndm^3 CH_4 kg^{-1} DM[a,e]	CH_4 content in biogas[c] (% v/v)	Process yield (% of BMP)
W_1	1/1	182	19	1 ± 1	5 ± 1	<1
	1/2	153	14	134 ± 18	57 ± 2	68
	1/3	139	11	130 ± 7	56 ± 1	67
W_2	1/1	159	39	0[b]	2 ± 1	0[b]
	1/2	137	27	0[b]	10 ± 2	0[b]
	1/3	127	22	0[b]	14 ± 3	0[b]
M_1	1/1	163	36	0[b]	1 ± 1	0[b]
	1/2	140	25	1 ± 1	4 ± 1	<1
	1/3	129	20	2 ± 7	22 ± 3	<1
M_2	1/1	166	31	0[b]	1 ± 2	<1
	1/2	142	22	2 ± 3	8 ± 1	<1
	1/3	130	17	219 ± 30	62 ± 3	64

[a] FM = fresh matter, DM = dry matter, OL = organic loading.
[b] The methane production of the inoculum was higher than the production of the batch.
[c] Mean of methane concentration in the biogas during the HSMP assay.
[d] Values calculated as sum of the contributions of the fresh materials and the inoculum.
[e] The gas volumes were normalized at 25 °C and 1 atm.

Table 5
Volatile fatty acids (VFAs) concentrations in the digested materials, after the solid state batch methane potential (HSMP) assay.

Material	Feedstock to inoculum ratio (*F*/*I*)	Total VFA (g kg^{-1} as acetic acid)	Acetic acid (g kg^{-1})	n-Butyric acid (g kg^{-1})	Iso-butyric acid (g kg^{-1})	n-Valeric acid (g kg^{-1})	Iso-valeric acid (g kg^{-1})	Propionic acid (g kg^{-1})
W_1	1/1	16.6	11.3	6.2	0.2	0.3	0.3	0.7
	1/2	0.8	0.7	0.3	0[a]	0[a]	0[a]	0[a]
	1/3	0.9	0.8	0.2	0[a]	0[a]	0[a]	0[a]
W_2	1/1	6.6	4.9	2.5	0[a]	0[a]	0[a]	0[a]
	1/2	11.1	5.2	8.3	0[a]	0[a]	0[a]	0.2
	1/3	16.5	9.0	7.7	0.3	0[a]	0.5	2.2
M_1	1/1	12.2	7.0	6.2	0.2	0[a]	0.4	0.8
	1/2	9.2	4.6	6.3	0[a]	0[a]	0.2	0.2
	1/3	11.6	4.7	8.9	0[a]	0[a]	0.2	0.9
M_2	1/1	6.9	4.6	2.4	0[a]	0[a]	0.2	0.7
	1/2	2.4	1.1	1.8	0[a]	0[a]	0.1	0.1
	1/3	0.2	0.1	0.1	0[a]	0[a]	0[a]	0[a]

[a] Concentrations below 0.05 g kg^{-1}.

HSAD process (W_1 at 1/2 *F*/*I* ratio, Table 4). This demonstrates that the OL, based on the VS fed, would not be a useful parameter for predicting process failure, when the material treated is very putrescent.

To understand the factors that determine the suitability of HSAD process conditions for a particular substrate, a deeper analysis must be carried out. It is well known that the reasons for the organic overload inhibition of a methanogenic process are particularly related to the putrescibility of the OM, i.e., the content of soluble and/or easily hydrolysable fractions, such as sugars, alcohols, short chain fatty acids, amino acids, and soluble proteins (Vidal et al., 2000). In fact, the fermentative microflora have higher kinetics compared to methanogens, so that high concentrations of soluble substrate (i.e. readily available to fermentative microbes) may lead to excessively fast VFA production and, consequently, VFA accumulation (Gorris et al., 1989).

For these reasons, assessing the putrescibility (as OD_{20}) of the organic materials may be a strategy for predicting the overload inhibition in HSAD processes. In fact, other parameters, such as VS, give only quantitative information about the fed OM, while none provide qualitative data in terms of solubility and degradability (Schievano et al., 2008). The BMP, in contrast, also reports information about the quality of the OM, but it is a long-term (around 60 days) biodegradation process, and thus influenced by both the soluble/readily hydrolysable compounds (sugars, VFAs, alcohols, amino acids, etc.) and the insoluble/hardly hydrolysable molecules (cellulose, hemicellulose, long-chain lipids, etc.). In fact, the insoluble fractions of the OM, such as crystalline cellulose, hemicelluloses, long-chain lipids, complex proteins, and polyamides, usually require more than a few hours for completing hydrolysis and biodegradation, while soluble components can be directly fermented by the microflora. Crystalline cellulose, for example, was reported to be hydrolyzed by enzymatic treatments in at least 3 days (Hendriks and Zeeman, 2009). Thus, the OD_{20}, as it is a measure of the O_2 consumption in short-term biodegradation (20 h), is more strongly influenced by the soluble and easily hydrolysable compounds than by the insoluble/more recalcitrant fractions of the OM (D'imporzano and Adani, 2007).

To better describe the HSAD process conditions, the OLs were recalculated as the overall OD_{20} of the *F* + *I* mixtures for each trial in the HSMP test (Table 4). The obtained OD_{20} (*F* + *I*) values were found to be coherent with the occurrence of inhibition in the HSMP test. In fact, a clear threshold was defined for the OD_{20} (*F* + *I*) values (about 17–18 g O_2 kg^{-1} FM), above which inhibition started to occur (Table 4). The OL, calculated as OD_{20}, may be used as a suitable indicator for defining the ideal process conditions when approaching new and different types of wastes in HSAD processes.

5. Conclusions

The F/I ratio and the OL, calculated as VS fed, are normally the most used parameters for defining the operational conditions of full-scale HSAD processes. In this study, these parameters were found unsuitable for preventing process failures due to organic overloading, especially when the quality and putrescibility of the waste are very different. A new parameter, the OL measured as the total OD_{20} of the bulk mixture ($F + I$) undergoing the HSAD process, was found useful in predicting the possible occurrence of process failures for organic overloading. This strategy may help in spreading the application of the HSAD process in new and very diverse contexts. Further studies on a wider variety of waste materials should be carried out, for broadening the applicability of this new approach.

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