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Chemical characterization of municipal wastewater sludges produced by two-phase anaerobic digestion for biogas production

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ABSTRACT

In the present study, the chemical features of municipal wastewater sludges treated in two-phase separate digesters (one for acetogenesis and the other one for methanogenesis), were characterized by using chemical analysis, stable carbon isotope ratios ($\delta^{13}C$), HS-SPME–GC–MS, TG-DTA analysis and DRIFT spectroscopy.

The results obtained showed that sludges from acetogenesis and methanogenesis differed from each other, as well as from influent raw sludges. Both processes exhibited a diverse chemical pattern in term of VFA and VOC. Additional variations were observed for $\delta^{13}C$ values that changed from acetogenesis to methanogenesis, as a consequence of fermentation processes that led to a greater fractionation of ^{12}C with respect to the ^{13}C isotope. Similarly, the thermal profiles of acetogenesis and methanogenesis sludges greatly differed in terms of heat combustion produced. These changes were also supported by higher lipid content (probably fatty acids) in acetogenesis than in methanogenesis, as also shown by DRIFT spectroscopy.

1. Introduction

Anaerobic digestion of organic wastewater sludge has shown to be one of the most significant processes to reduce pollution (atmospheric and terrestrial) and produce fuels (e.g. methane) [1]. Most of the models reported in the literature refer to a single-stage, anaerobic digestion process, where hydrolysis, acidogenesis, acetogenesis and methanogenesis all take place in the same reactor. In these conditions, the understanding of the chemical and biological processes acting together is extremely difficult and it has actually been approached by working on single bacterial strains or under artificial conditions [2]. To recall a pertinent example, in order to maintain an appropriate environment for microorganisms in a whole-process single reactor, volatile fatty acid (VFA) production and utilization must be balanced, since a high VFA production might lead to a reactor failure [3]. Two-stage anaerobic processes have been proposed for dividing VFA and methane forming stages, so as to optimize each stage [4–7]; this, in fact, would allow a better analysis of the chemical process involved. Every decomposition stage of sludges is characterized by quali-quantitative variations of organic carbon. These changes can be directly identified by using a multiple analytical approach similar to that applied to chemical modifications occurring in the compost [8] or soil organic matter transformation [9].

The use of thermogravimetric analysis (TGA) has recently been extended to the study of sewage sludge [10]. This technique combined with differential thermal analysis (DTA) or differential scanning calorimetry (DSC) has enabled to follow the progress of organic matter stabilization during composting processes [9–14]. The common advantage of these techniques is the simplicity of sample preparation. By combining these techniques with diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy, a simple and rapid method to characterize the functional groups of composts [8,9] and soil organic matter [9,15], it is possible to obtain additional information about the structure of organic molecules involved in the transformation process.

Likewise, stable carbon isotope analysis represents a powerful monitoring tool to provide *in situ* detection of the organic matter transformation in methane [16], biodegradation of chlorinated hydrocarbons [17] and bacterial oxidation of methane [18]. Therefore, the $\delta^{13}C$ technique might improve our understanding of the transformation, utilization and stabilization of organic C during anaerobic digestion of wastes. Since a number of factors influence ^{13}C fractionation, its interpretation requires controlled laboratory experiments.

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Nomenclature

A	acetogenic digester
$\delta^{13}C$	carbon stable isotopic ratio
DRIFT	diffuse reflectance infrared Fourier transform
DTA	differential thermal analysis
HS-SPME–GC–MS	head-space solid phase microextraction combined with gas chromatography–mass spectrometry
HRT	hydraulic retention time (days)
M	methanogenic digester
$N–NH_4^+$	ammoniacal N ($g\,L^{-1}$)
SCOD	soluble chemical oxygen demand ($g\,L^{-1}$)
TA	total alkalinity ($mg\,L^{-1}$)
TG	thermogravimetric analysis
TSS	total suspended solids ($g\,L^{-1}$)
VFA	volatile fatty acids ($mg\,L^{-1}$)
VOC	volatile organic compounds
VSS	volatile suspended solid ($g\,L^{-1}$)

Several studies have highlighted the importance of VFA in methanogenesis [3]. Head-space solid phase microextraction combined with gas chromatography (SPME-GC) has been used for the determination of a variety of volatile organic compounds (VOC) in manures [19] and VFA in anaerobic processes [20]. The method is rapid and gives additional information about the anaerobic process.

In the present study, the chemical features of sludges from two separate phase digesters, one for the hydrolysis and acidification and the other one for methanogenesis, fed with municipal wastewater raw sludges, were investigated by using chemical analysis, $\delta^{13}C$ isotopic ratio, HS-SPME–GC–MS, TG-DTA analysis, and DRIFT spectroscopy, in order to detect chemical modifications that occur during acetogenesis and methanogenesis.

2. Materials and methods

2.1. Sludge

The raw sludge (influent) was obtained from a sequence of stabilization processes, carried out under aerobic–anaerobic conditions at an urban wastewater treatment plant in Bologna (Italy). The acetogenic and metanogenic inocula used in this experiment were collected from a two-phase anaerobic pilot plant fed by the same raw sludge and working in steady state conditions.

2.2. Experimental design

The entire process took place in two series of digesters fed by the same raw sludge and working in steady state conditions (Fig. 1); the first digester was for hydrolysis, acidification and acetogenesis (A), whereas the second one was for methanogenesis (M). To uniform distribution throughout the digesters, the sludges and the gas were injected through a distribution system.

The acetogenic sludge inoculum (about 1.8-L corresponding to 1.8 kg with a pH value of 4.5) was placed in each of ten 2-L total volume digesters (A1–A10) for acetogenesis. Since the first stage was operated at a hydraulic retention time (HRT) of 6 days, 300 mL d^{-1} corresponding to about 300 g d^{-1} total weight of influent were added in each acetogenic digester. The acetogenic cultures were incubated in a thermostatic chamber at a constant temperature of 25 °C ± 1.0. All acetogenic digesters (A) were continually flushed with 1.5 L d^{-1} of CO_2, resulting in a light mixing of the anaerobic culture without damage and a good CO_2 dissolution rate in the liquid phase.

The methanogenic sludge inoculum (about 1.8-L corresponding to 1.8 kg with a pH value of 7.0) was placed in each of ten 2-L total volume digesters (M1–M10) for methanogenesis. Since the second stage was also operated at a HRT of 6 days, 300 mL d^{-1} corresponding to about 300 g d^{-1} total weight of acetogenic sludge was passed from the acetogenic (A) to the corresponding methanogenic (M) digesters. The methanogenic cultures were incubated in a temperature-controlled water bath at 42 °C ± 2.0.

Agitation in methanogenic digesters (M) derives from the mixing caused by acetogenic sludges inflow and biogas produced.

During the experimental period, measurements of biogas production and composition (CH_4 and CO_2) were taken every 24 h; biogas collection and measurement was performed by water displacement method from both acetogenic and methanogenic digesters. The biogas composition was determined by using Geotech GA 2000 gas analysers (Keison Products, Chelmsford, UK). The gas output was more or less constant throughout the whole experiment and similar among replicates.

At the 6th day, the experiment was stopped and the sludges from each digester (both acetogenic and methanogenic) were freeze-dried to stop the biological activity and were subsequently analyzed.

2.3. Chemical analyses

Total C and N were measured on lyophilized sludge with an elemental analyser (CHNS-O mod. EA 1110, Carlo Erba, Italy). The percentage of C in the sample was calculated using acetanilide as a certified standard containing 71.09% of carbon.

The C isotopic ratio was measured by continuous flow-isotope ratio mass spectrometry (CF-IRMS mod. Delta Plus, Thermo Electron, Bremen, Germany).

The isotopic composition of the samples is expressed as units of $\delta^{13}C$ using Pee Dee Belemite (PDB) standard for C:

$$\delta^{13}C\,‰ = \frac{R_{sample} - R_{stdPDB}}{R_{stdPDB}}$$

where $R = {}^{13}C/{}^{12}C$.

All analyses were performed in triplicate. The reproducibility of the $\delta^{13}C$ values of the samples was better than 0.1‰ in 90% of the cases. The variation coefficient was <0.1%.

The determination of soluble chemical oxygen demand (SCOD), total suspended solids (TSS), volatile suspended solids (VSS), VFA, total alkalinity (TA), alkalinity ratio, $N–NH_4^+$ and pH was carried out according to APHA [21]. The pH, VFA and TA concentrations were measured off-line after taking samples from digesters. Total lipids were extracted using Folch's method [22].

2.4. Determination of volatile compounds by HS-SPME–GC–MS

This determination was performed according to a modified version of the method suggested by Vichi et al. [23]. About 10 mg of lyophilized sludge was weighed into a 2-mL vial and capped. A 50/30 μm divynilbenzene/carboxen/polydimethylsiloxane (DVB/Carboxen/PDMS) Stable Flex SPME fiber (Supelco, Bellefonte, PA, USA) was inserted through the septum into the vial, which was kept at 40 °C for 30 min. Vial penetration depth was set at 20 mm and, after 30 min of extraction, the SPME fiber was inserted into the injection port of the Shimadzu GC–MS–QP2010 Plus (Shimadzu, Tokyo, Japan). The injection penetration depth was set at 51 mm. The SPME fiber was desorbed at 260 °C for 10 min in the split mode. The chromatographic separation of volatile compounds was performed on a ZB-5ms fused-silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm) coated with 5% phenylpolysiloxane-95% poly (dimethylsiloxane) (Phenomenex,

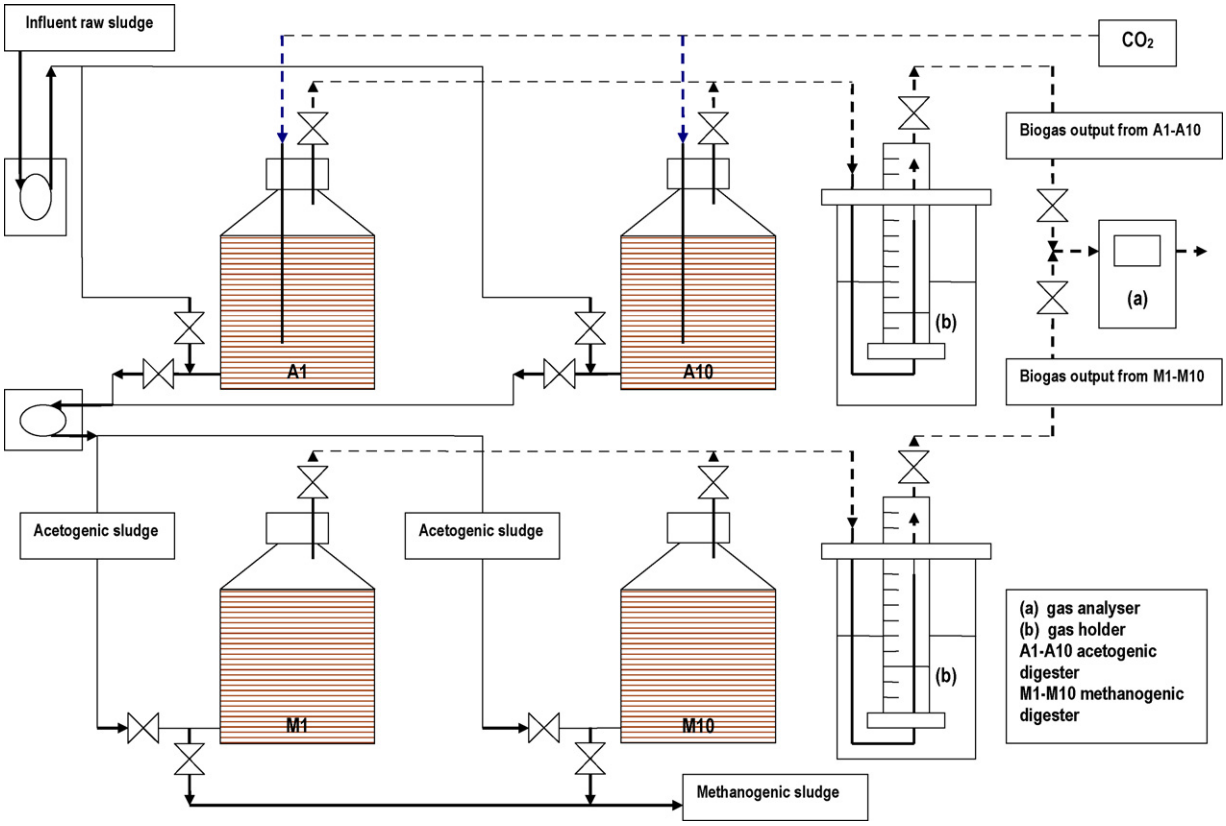

Fig. 1. Schematic diagram of anaerobic digesters: (A) acetogenenesis; (M) methanogenesis.

Torrance, CA, USA). The oven was programmed from 45 °C (kept for 10 min) to 200 °C at 3 °C/min and kept at this temperature for 8 min. The injector and transfer line temperatures were set at 260 and 210 °C, respectively. Helium was used as the carrier gas at a constant flow rate of 1 mL/min and a linear velocity of 36.2 cm/s; the split ratio was 1:30. The filament emission current was 70 eV. A mass range from 40 to 700 m/z was scanned at a rate of 1428 atomic mass units per s.

Identification of volatile compounds was performed by comparing their retention time and mass spectra with those of the corresponding standards, as well as data reported in the literature [23] and the NIST/EPA/NIH Mass Spectral Database (NIST 05, National Institute of Standards and Technology, Gaithersburg, MD, USA).

2.5. Thermal analyses

Thermogravimetric (TG) and differential thermal (DTA) analyses were carried out using a TG-DTA92 instrument (SETARAM, France); details on these procedures are described by Montecchio et al. [24]. The DTA integration area was used to estimate the heat of exothermic reactions.

2.6. Diffuse reflectance infrared spectroscopy

The spectra were recorded with a Bruker TENSOR series FT-IR Spectrophotometer (Ettlingen, Germany) equipped with an apparatus for diffuse reflectance (Spectra-Tech. Inc., Stamford, CT, USA). Spectra were collected as Kubelka–Munk units using KBr (Aldrich Chemical Co, WI, USA) as background reference. The spectra were collected from 4000 to 400 cm^{-1} and averaged over 100 scans (resolution $\pm 4\ cm^{-1}$). Analyses of spectral data were performed with Grams/386 spectral software (Galactic Industries, Salem, NH, USA).

3. Results and discussion

3.1. Chemical analyses

Table 1 shows the chemical composition of influent, acetogenic and methanogenic sludges. The pH values in the acetogenic

Table 1
Chemical parameters determined in the influent, acetogenetic and methanogenetic sludges.

	Influent average ± s.e. (n = 9)	Acetogenesis average ± s.e. (n = 9)	Methanogenesis average ± s.e. (n = 9)
T (°C)	rt	25 ± 1.0	42 ± 2.0
pH	6.0 ± 0.1	4.5 ± 0.2	7.1 ± 0.1
TSS ($g\,L^{-1}$)	35.4 ± 1.2	31 ± 0.9	26.2 ± 0.9
VSS ($g\,L^{-1}$)	24.3 ± 1.1	22 ± 0.8	16 ± 0.9
SCOD ($g\,L^{-1}$)	4.4 ± 1.0	20 ± 4.3	2.4 ± 0.9
$N–NH_4^+$ ($g\,L^{-1}$)	0.26 ± 0.03	0.34 ± 0.02	0.70 ± 0.02
VFA ($g\,L^{-1}$)	0.47 ± 0.01	7.57 ± 0.04	0.30 ± 0.01
TA ($g\,L^{-1}$)	1.50 ± 0.18	2.55 ± 0.10	2.92 ± 0.15
VFA/TA	0.31 ± 0.12	2.96 ± 0.13	0.10 ± 0.09

s.e.: standard error; rt: room temperature.

Table 2
Average daily production of CO_2, CH_4 and VFA in the influent, acetogenetic and methanogenetic sludges.

Influent average ± s.e. (n = 9)		Acetogenesis[a] average ± s.e. (n = 9)		Methanogenesis average ± s.e. (n = 9)		
CO_2 (L d^{-1})	VFA (g L^{-1})	CO_2 (L d^{-1})	VFA (g L^{-1})	CO_2 (L d^{-1})	CH_4 (L d^{-1})	VFA (g L^{-1})
nd	0.08 ± 0.02	0.9 ± 0.07	1.43 ± 0.06	0.6 ± 0.05	1.0 ± 0.06	0.06 ± 0.02

s.e.: standard error; nd = not detected.
[a] Acetogenic digesters were continuously flushed with 1.5 L d^{-1} of CO_2.

Table 3
Carbon and nitrogen contents and isotopic ratio ($\delta^{13}C$) of the influent, acetogenetic and methanogenetic lyophilized sludges after 6 days.

	Influent average ± s.e. (n = 3) d.m.	Acetogenesis average ± s.e. (n = 3) d.m.	Methanogenesis average ± s.e. (n = 3) d.m.
C (%)	35.59 ± 0.74	32.66 ± 0.36	28.22 ± 1.08
$\delta^{13}C$ (‰)	−25.88 ± 0.13	−24.19 ± 0.18	−23.75 ± 0.21
N (%)	3.52 ± 0.03	4.14 ± 0.23	3.55 ± 0.07
Total fat (%)	5.56 ± 0.91	12.35 ± 1.15	8.20 ± 0.98

s.e.: standard error. d.m.: dry matter.

digesters (at 25 ± 1 °C) and the methanogenic digesters (at 42 ± 2 °C) were similar to those found at the beginning of the study, without any pH adjustments.

Decrease of volatile suspended solids gave a good idea of the organic matter degradation in both digesters (A and M). The soluble COD values were very low in the influent and methanogenesis (Table 1). This suggested that a part of organic matter in the methanogenesis process was transformed in methane. In contrast, the increased amount of soluble COD in acetogenesis was unequivocally related to organic matter transformation into other compounds, such as low molecular weight organic substances or fatty acids (Table 1) that can give a high soluble COD value.

The difference observed between the VFA/TA ratio in acetogenesis with respect to the methanogenesis one, indicates an efficient phase separation process during the study. Furthermore, a VFA/TA ratio smaller than 0.3 is considered a good stability index in methanogenesis [25,26]. The highest ammonium amount (Table 1) was detected in the methanogenesis digesters (M); however, at this concentration, the methane production did not seem to be influenced. In general, these results are in agreement with those reported by other authors [27–29]. They did not find any inhibition effect of ammonium-nitrogen (NH_4^+–N) at concentrations between 200 and 1500 mg L^{-1} on methanogenesis.

Table 2 reports the daily biogas production measured for acetogenesis and methanogenesis. In the acetogenic digesters (A), only traces of CH_4 were detected, while a significant difference between CO_2 input (1.5 L d^{-1}) and output (0.9 L d^{-1}) was measured. Under this condition, the carbon dioxide dissolved in the sludge did not cause a variation of total alkalinity. However, the lower amount of CO_2 output and the considerable amount of VFA found in acetogenesis suggested that a part of CO_2 is transformed into fatty acids according to Wood–Ljungdahl pathway [30]. These results confirm that the digestion developed regularly.

The biogas output in the methanogenic digesters (M) shown in Table 2, fed with the sludge from the acetogenic series (A), was composed by about 60% of methane and 40% of carbon dioxide, while VFA were almost completely eliminated. The pathway of transformation of VFA to methane [31,32] gives an expected stechiometric ratio 1:1. No biogas production was estimated in influent sludge.

3.2. Chemical and isotopic composition in sludges

Table 3 shows the elemental (C and N) composition of freeze-dried sludges. The content in C and N decreased in methanogenesis with respect to acetogenesis, as expected.

The $\delta^{13}C$ value became less negative from influent to methanogenic sludge (Table 3). The fermentation processes led to a greater fractionation of ^{12}C over the ^{13}C isotope because of the lower energy required to break a ^{12}C–^{12}C bond as compared to a ^{13}C–^{12}C one. In fact, as shown by Krzycki et al. [33], the methane produced from sewage digesters or biodegradation of organic matter is strongly ^{13}C depleted ($\delta^{13}C$ from −47 to −89‰). As a consequence, the residue is enriched in heaver isotopes. This can justify the delta value in the methanogenesis sludge in this experiment. Moreover, the content of saturated lipids can furthermore affect the slight shift of the $\delta^{13}C$ value. The saturated lipids have less negative $\delta^{13}C$ values than the corresponding mono-and poly-unsaturated lipids [34]. Based on fatty acids characterization (unpublished results), it might be possible that the shift of the $\delta^{13}C$ depend on different unsaturated/saturated fatty acid ratio; in fact, it was higher in acetogenesis (0.32) than in methanogenesis (0.26).

A significant difference in the total lipid content (acetogenesis > methanogenesis) might explain the different roles of acetogenesis (fatty acids production from lipid hydrolysis) and methanogenesis (fatty acid degradation for methane production) [35] in the anaerobic treatments of sludges. Instead, no reasonable explanation can be provided for the $\delta^{13}C$ value in the influent, because no anaerobic fermentation is supposed to take place.

3.3. HS-SPME–GC–MS for VFA and VOC analysis

A different chromatographic pattern of VFA in acetogenesis and methanogenesis freeze-dried sludges, was observed (Fig. 2). In acetogenesis, the amount of short-chain VFA (from C-2 to C-6) was dominant (Fig. 2). This implies that homoacetogenic fermentation and acetate/butyrate fermentation were prevalent in this process. The presence of propionate, instead, indicated that hydrogen and formate are not low enough to activate the acetogenic bacteria involved in the decomposition of propionate [36]. In methanogenesis, acetate and *n*-butyrate are completely degraded, as supported by Gallert and Winter [37], while the propionate degradation seems to be a time-requiring process [37].

Other volatile organic compounds are present in the chromatograms. For instance, the hexanoic acid methyl ester (peak 10) appeared in influent and acetogenesis, but not in methanogenesis. The origin of this compound has been attributed to the anaerobic fermentation of lipids (2–12 C fatty acids) [38].

A group of alkanes (C_n-12 and C_n-13) were detected only in methanogenesis. These compounds usually derive from oils or

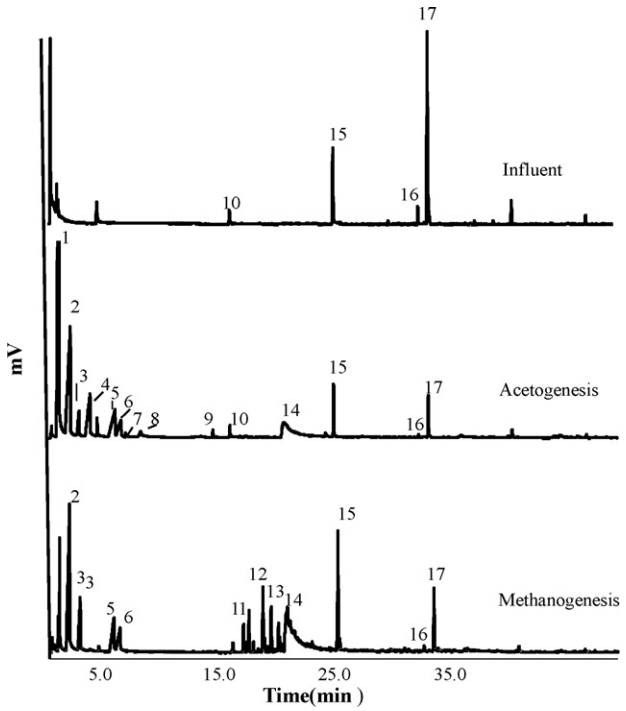

Fig. 2. HS-SPME–GC–MS of VFA and VOC from influent, acetogenic and methanogenic freeze-dried sludges: (1) acetic ac. ($C_2H_4O_2$); (2) propionic ac. ($C_3H_6O_2$); (3) isobutyric ac. ($C_4H_8O_2$); (4) butyric ac. ($C_4H_8O_2$); (5) isovaleric ac. ($C_5H_{10}O_2$); (6) butyric ac., 2-methyl ($C_5H_{10}O_2$); (7) 1-butanol, 2,3-dimethyl ($C_6H_{14}O$); (8) caproic ac. ($C_6H_{12}O_2$); (9) hexane, 2,2,4-trimethyl (C_9H_{20}); (10) hexanoic ac., 1,1-dimethyl ester ($C_{10}H_{20}O_2$); (11) 2,2-dimethyl decane ($C_{12}H_{26}$); (12) 2,2,6-trimethyl decane ($C_{13}H_{28}$); (13) 3,6-dimethyl undecane ($C_{13}H_{28}$); (14) *p*-cresole (C_7H_8O); (15) methylsiloxane; (16) 2,5-dimethyl decane; (17) hexadecane ($C_{16}H_{34}$).

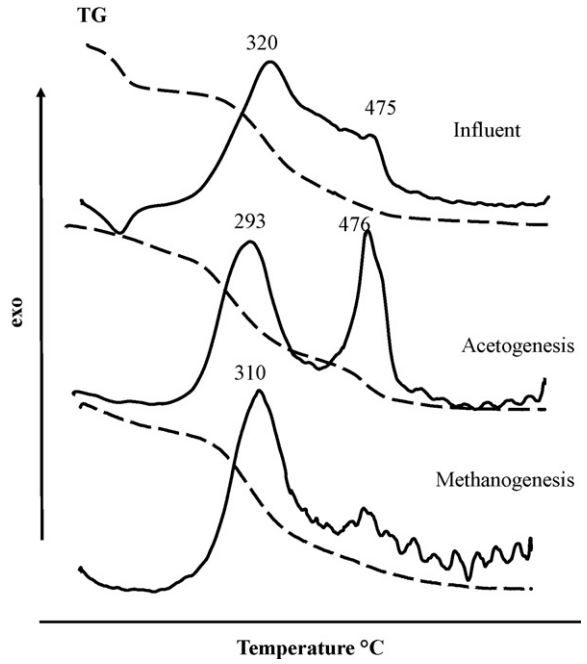

Fig. 3. TG-DTA curves of influent, acetogenic and methanogenic freeze-dried sludges.

fuels, but their presence in methanogenesis suggests a biological origin. According to the literature [39,40], the alkane chain residues are originated by β-oxidation of long-chain branched fatty acids. It is assumed to be the first mechanism of degradation of these molecules to acetate and butyrate and then they are converted into methane and CO_2.

The presence of *p*-cresol (peak 14) in acetogenesis and methanogenesis can be attributed to aromatic decomposition of amino acids, such as tyrosine [41], rather than to a disinfectant or preservative constituent. It has been extensively studied how the anaerobic degradation of *p*-cresol, via oxidation of the methyl substituent to *p*-hydroxybenzaldehyde and *p*-hydroxybenzoate and then via *n*-caproate [42], leads to the production of methane and CO_2. In influent, the low concentration of VFA (Table 2) might justify the absence of their GC peak, while the presence of some alkanes (C_n-12 and C_n-13) did not seem to be related to the fermentation processes. A volatile silicone compound (peak 15) was found in these samples; its origin is still debated, although it has already been detected in biogas from sewage digesters [43].

3.4. Thermal analysis (TG-DTA)

Fig. 3 shows the thermal study (TG-DTA) of the influent, acetogenesis and methanogenesis freeze-dried sludges. Very few thermo-analytical data for sludge have been reported in literature [12,13]. Data usually show that the thermal decomposition of thermolable component (proteins and carboxyl groups) of the organic matter produces strong exothermic reactions (~300 °C), while exothermic reactions at higher temperatures (~450 °C) are originated by decomposition of refractory C, such as aromatic rings, long-chain N-alkyl structures and saturated aliphatic chains [9,44].

In the present study, the DTA curves of the acetogenic and methanogenic digester sludges showed different, well-defined thermal events. In fact, the DTA curve of the acetogenic sludge exhibited two strong exothermic reactions at 293 °C (weight loss of 34.46%) and 476 °C (weight loss of 16.61%), attributed to the organic matter decomposition (Fig. 3). The bimodal thermal profile in acetogenesis is related to the presence of a complex mixture of organic components involved in the heating phase; the first peak at 293 °C was mainly produced by the decomposition of carboxyl groups (probably fatty acids), whereas the exothermic reaction at higher temperatures (~500 °C) was originated by decomposition of refractory C.

Due to the high amount of lipids, it might be possible that the double bonds of unsaturated fatty acids were broken during heating and that molecules, such as triglycerides, were modified into saturated structures characterized by a higher thermal stability.

A unique strong thermal reaction at 310 °C (weight loss of 49.12%) characterizes the methanogenesis DTA curve, which is evidently different from acetogenesis.

It is of particular relevance the variation of combustion heat released during thermal decomposition of sludges. The total energy per unit mass released during combustion is directly proportional to the total area of the DTA peaks. On this basis, the combustion heat of the acetogenesis sludge (−5802 μV s/mg) was greater than that of methanogenesis (−3553 μV s/mg), but it was not so different from that of the influent (−6453 μV s/mg). This variation in both fermentation processes seems to be related to the higher amount of lipids found in acetogenesis (Table 3). Experimental data reported in the literature on combustion heat values of vegetable oils [45] and fatty acids [46] seem to support these results. Moreover, a significant positive correlation was found between combustion heat and chain-length of fatty acids [46].

The DTA curve of influent sludge displayed a broad and unique exothermic reaction at 320 °C (weight loss of 50.38%) and a shoulder at 475 °C that may be related to organic matter with complex structures.

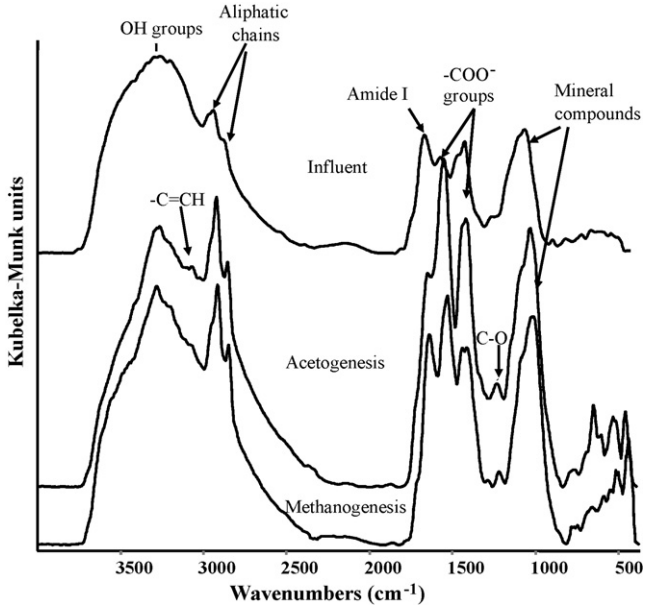

Fig. 4. DRIFT spectra of influent, acetogenic and methanogenic freeze-dried sludges.

3.5. DRIFT spectroscopy

The structural changes observed using TG-DTA analysis are supported by DRIFT spectra (Fig. 4). The spectra are dominated by a broad band at around 3300 cm^{-1}, due to OH stretching vibrations in acids and alcohols. Hydrocarbon chains exhibited strong bands between 3000 and 2800 cm^{-1}. These bands are attributed to the asymmetric C–H stretching of methyl (2958 cm^{-1}, shoulder) and methylene (2924 cm^{-1}) groups, and symmetric C–H stretching of methylene (2850 cm^{-1}) groups. The band at 3060 cm^{-1} indicated the presence of double bonds (=CH_2) within the carbon backbone [9,47]. Moreover, in the fingerprint region, the bands near 1450 and 730 cm^{-1} were due to the CH_2 scissoring deformation, and CH_2 rocking vibrations, respectively. The intense peak at around 1650 cm^{-1} was assigned to the stretching vibrations of –C=O group in ketones and amide I, while the peak at around 1550 cm^{-1} was mainly due to asymmetric stretching of carboxylate groups [9,47]. The fingerprint region also included the bands at 1420 and 1240 cm^{-1} assigned to symmetrical stretching of carboxylate groups and C–O stretching in carboxylic acids, respectively. Moreover, the bands between 1170 and 1000 cm^{-1} were mainly attributed to O–H stretching in mineral components.

A comparison of the DRIFT spectrum of the acetogenesis and methanogenesis evinced similar general features. However, some quantitative and qualitative differences can be observed. The relative intensity of the band corresponding to carbonyl/carboxyl groups was lower in methanogenesis and this band was shifted from 1561 to 1540 cm^{-1}, indicating a different content in acidic groups as also supported by VFA concentration and DTA analysis. The carbonyl frequency in fatty acids usually increases and decreases alternatively for odd and even carbon number [41]. The lack of a shift of the CH band frequency suggested that the aliphatic component in both sludges was similar and mainly characterized by methylenic chains [47].

4. Conclusions

The utilization of two separate phase digesters, one for acetogenesis and the other one for methanogenesis, allowed to gather important data to understand various metabolic aspects of each phase of the process.

The acetogenesis role is, in general, fundamental for lipid hydrolysis and the consequent VFA production. Acetogenesis confirmed to be a very complex process, characterized by an efficient transformation of the organic material into molecules with high energetic potential. Moreover, the data obtained proved that VFA production in acetogenesis can be increased, if it is kept separated from methanogenesis.

The decrease of the lipid content and unsaturated/saturated fatty acids ratio in methanogenic sludges reinforces the importance of the role of these molecules in methanogenesis. Moreover, the variation of $\delta^{13}C$ value in the methanogenic sludges suggests that the residual organic C is chemically very stable and it is not utilized by bacteria for methane production.

The differences in relative concentrations of organic substances were also detected by the thermal profiles of sludges. The combustion heat of acetogenesis was higher than that of methanogenesis. This seems to be related to a higher content of acid functional groups (probably fatty acids) in acetogenesis than in methanogenesis, as revealed by DRIFT spectra.

A more detailed study will surely provide further information about the role of lipids and single molecular species involved in the biogas production, a basic issue in renewable energy.

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