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ORIGINAL PAPER

Anaerobic digestion of solid slaughterhouse waste: study of biological stabilization by Fourier Transform infrared spectroscopy and thermogravimetry combined with mass spectrometry

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Abstract In this paper, Fourier Transform infrared spectroscopy (FTIR) along with thermogravimetric analysis together with mass spectrometry (TG–MS analysis) were employed to study the organic matter transformation attained under anaerobic digestion of slaughterhouse waste and to establish the stability of the digestates obtained when compared with fresh wastes. Digestate samples studied were obtained from successful digestion and failed systems treating slaughterhouse waste and the organic fraction of municipal solid wastes. The FTIR spectra and TG profiles from well stabilized products (from successful digestion systems) showed an increase in the aromaticity degree and the reduction of volatile content and aliphatic structures as stabilization proceeded. On the other hand, the FTIR spectra of non-stable reactors showed a high aliphaticity degree and fat content. When comparing differential thermogravimetry (DTG) profiles of the feed and digestate samples obtained from all successful anaerobic systems, a reduction in the intensity of the low-temperature range (≈300°C) peak was observed, while the weight loss experienced at high-temperature (450–550°C) was variable for the different systems. Compared to the original waste, the intensity of the weight loss peak in the high-temperature range decreased in the reactors with higher hydraulic retention time (HRT) whereas its intensity increased and the peak was displaced to higher temperatures for the digesters with lower HRT.

Keywords Anaerobic co-digestion · Biogas · FTIR spectroscopy · Poultry slaughterhouse waste · Thermogravimetric analysis · Waste stability

Introduction

Slaughterhouse waste mainly consists of the contents of rumen, stomachs and intestines. A considerable amount of these wastes are produced each year, especially from poultry slaughterhouses (around 550,000 tons/year in Spain) due to the important consumer demand of poultry meat (MAFF 2007). Untreated slaughterhouses waste entering into municipal sewage purification systems may cause severe problems, due to the very high chemical oxygen demand (COD) (Edelman et al. 2000). Anaerobic digestion of this sort of wastes may be a way to decrease the related environmental problems, at the same time that provides biogas, which may satisfy some of the local energy needs.

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Anaerobic digestion is a process in which microorganisms degrade biodegradable material to methane in the absence of oxygen. It is widely used for organic waste minimization and stabilization, due to their benefices as a waste management system and a source of a renewable energy produced from the biogas formed during the process. Among the main components of waste, carbohydrates are rapidly converted via hydrolysis to simple sugars and alcohols and subsequently fermented to volatile fatty acids (VFA). Proteins are hydrolyzed to polypeptides and amino acids while lipids are hydrolyzed to long chain fatty acids (LCFA) and glycerol. Glycerol is converted to acetate by acidogenesis and LCFA are converted to acetate or propionate and hydrogen via β-oxidation (Weng and Jeris 1976). After that, fermentative bacteria convert the intermediates to VFA, hydrogen and carbon dioxide. Finally, methanogens ultimately produce methane and carbon dioxide (main components of biogas). LCFA accumulation and ammonia produced in protein degradation may inhibit the anaerobic degradation of solid slaughterhouse waste (Broughton et al. 1998; Salminen et al. 2000). In spite of that, anaerobic digestion of solid poultry slaughterhouse waste has been successfully carried out (Cuetos et al. 2008; Edström et al. 2003; Salminen and Rintala 2002; Salminen et al. 2000) producing very high methane yields as a consequence of the high theoretical methane potential of lipids (Pereira et al. 2003; Salminen and Rintala 2002).

Analyzing anaerobic process stability of the end product and assessment of organic matter of waste during the process is necessary for obtaining the best information about the stabilization process carried out. In this way, Fourier Transform infrared spectroscopy (FTIR) and thermal analysis are among the most promising tools for characterizing the heterogeneous organic matter, providing important information on the chemical characteristics of the samples.

Infrared spectra illustrate the plot of absorbed infrared radiation dependent on the wave-numbers (wavelengths) caused by the interactions of the infrared radiation with matter (Meissl et al. 2007). FTIR is based on the functional group interconversions via chemical reaction or by appearance or disappearance of functional groups carried by building blocks or protecting group introduced or removed during the reaction. The direct functional group analysis permits qualitative analysis of each reaction step throughout the synthesis (Dal Cin et al. 2002). Infrared spectroscopy has proven to be a valuable tool widely used to investigate the content of the main biochemical components such as carbohydrates, proteins, fats, lignin and cellulose of soil organic matter (Chen 2003; Provenzano et al. 1998), to describe the transformation of organic matter during a composting process or compost maturity (Ouatmane et al. 2000; Smidt et al. 2002), to analyze compost extracts (Carballo et al. 2008), humic and fluvis acids (Amir et al. 2005; González-Vila et al. 1999), to study microbial and fungal biomass (Grube et al. 1999) and relative amounts of proteins, fats, lignins, carbohydrates in organic matter (Mermut and Eswaran 2001; Orhan and Büyükgüngör 2000) and in wastes degradation (Calderón et al. 2006; Fakharedine et al. 2006; Won et al. 2006).

On the other hand, thermal analysis has also been proposed to characterize organic matter on the basis of the loss of energy undergone by materials during biological stabilization. Thermogravimetric (TG) analysis is a technique based on the continuous measurement of weight loss by a sample during heating in a controlled atmosphere. The rate of the weight loss undergone by the sample is the basis of differential thermogravimetry (DTG). Usually the DTG profile during heating of organic matter present a first exothermic peak at low temperature (200–300°C) produced by biodegradable material and semi-volatile compounds decomposition (Gómez et al. 2007a; Smidt and Lechner 2005) and an exothermic reaction at higher temperatures (450–550°C) related to the combustion of aromatic structures and cleavage of C–C bonds (Peuravuori et al. 1999; Provenzano et al. 1998). An initial exothermic peak around 100°C is attributed to dehydration of residual water of the samples (Dell'Abate et al. 1998; Melis and Castaldi 2004). Otero et al. (2002) proposed the use of thermogravimetric analysis as a technique for evaluating the degree of stabilization of biosolids. A relationship between thermal behaviour of different sewage sludge and the stabilization in thermogravimetry was found by Gomez-Rico et al. (2005), Font et al. (2001) and Font et al. (2005). This technique has been used to assess compost stability and maturity (Baffi et al. 2007; Dell'Abate et al. 2000; Smidt and Lechner 2005) and characterization of soil organic matter (Lopez-Capel et al. 2005; Siewert 2004). Recently, it has been used as a tool for monitoring the stabilization of digestates from biowastes treated by anaerobic digestion (Gómez et al. 2005; Gómez et al. 2007a; Sánchez et al. 2008) with successful results.

In previous work (Cuetos et al. 2008) mesophilic anaerobic digestion of slaughterhouse waste (SHW) and co-digestion with the organic fraction of municipal solid waste (OFMSW) were evaluated. The aim of the present paper is to study the transformations undergone by the organic fraction of slaughterhouse waste during anaerobic digestion through FTIR and thermogravimetry combined with mass spectrometry (TG–MS), with the main objective of determining the stability level of the end product. Different compounds evolved during anaerobic digestion were followed and correlated with the organic matter compounds of original waste in order to obtain a better understanding of the processes involved.

Materials and methods

Experimental procedure

The results obtained from the different anaerobic digestion of SHW and co-digestion with the OFMSW processes have been previously published (Cuetos et al. 2008) showing the acclimatization of the bacterial consortium to an ammonia-rich medium, high fats and LCFA concentrations. A first set-up, with a hydraulic retention time (HRT) of 25 days and organic loading rate (OLR) of 1.70 kg VS_{fed}/m^3day for digestion, and 3.7 kg VS_{fed}/m^3day for co-digestion, was not successful. A second set-up was initiated with an HRT of 50 days and an OLR of 0.9 kg VS_{fed}/m^3day for digestion and 1.9 kg VS_{fed}/m^3day for co-digestion, as the performance of the reactor was stable, the HRT was initially decreased to 36 days (while progressively increasing the OLR to 1.2 kg VS_{fed}/m^3day and 2.6 kg VS_{fed}/m^3day for digestion and co-digestion) and finally to 25 days, (increasing OLR to 1.7 kg VS_{fed}/m^3day and 3.7 kg VS_{fed}/m^3day for digestion and co-digestion, respectively). These digesters showed a highly stable performance, VFA were not detected and LCFA were undetected or only trace levels were measured in the analyzed effluent. Anaerobic digestion was thus found to be a suitable technology for efficiently treating lipid and protein waste.

The original wastes (SHW and OFMSW) and the final effluents of reactors were dried at 105°C in a furnace for 48 h and then ground in laboratory ball mill (Retsch mill model MM200) prior to FTIR and TG analysis. Total nitrogen, total organic carbon and C/N ratios were determined as described in Cuetos et al. (2008) for the effluents obtained from the reactors studied.

FTIR spectroscopy

Two milligram of separate dried milled samples were ground up with 200 mg KBr (FTIR grade) and homogenized in an agate mortar. KBr pellets were compressed under vacuum in a standard device under pressure of 6,000 kg/cm for 10 min. Infrared spectra were recorded as described Cuetos et al. (2009). The original wastes and the digestates were analyzed and mean values of three replicates were estimated for each sample.

TG–MS analysis

TG experiments were carried out with a thermobalance coupled to a quadrupole mass spectrometer as described Gómez et al. (2007a). The heating rate applied to the dried and milled samples was 15°C/min from 20 to 650°C with a flow rate of 100 ml/min of synthetic air. Three replicates of each sample were analyzed and the mean values calculated.

A mass-spectrometry apparatus was used in line with the thermal analysis equipment to monitor the gas emissions obtained from the combustion process, connected through a capillary filament maintained at 200°C. Although a full quantitative analysis could not be performed, a comparison of the intensity of the peaks obtained from different samples was made (i.e. semi-quantitative analysis) using the normalization procedure described by Arenillas et al. (1999). This method is based on the use of the signal recorded by the mass-spectrometry apparatus as normalization factor.

Results and discussion

Anaerobic digestion results

The highly stable performance demonstrated by the successful reactors proved that the relative high specific gas production (SGP) (0.7–1.0 $m^3/kg\ VS_{feed}$) of these reactors were a result of the efficient

Table 1 Chemical analyses of final effluents from the anaerobic digestion processes studied (values correspond to samples taken at the moment of reactor dismantling)

Digester	Description	HRT (d)	Total organic carbon (%[a])	Total nitrogen (%[a])	C/N ratio
SHW failed-25	Failed digestion	25	18.3 ± 0.7	4.2 ± 0.3	4.3
SHW + OFMSW failed-25	Failed co-digestion	25	16.5 ± 0.6	3.6 ± 0.2	4.5
SHW-50	Digestion	50	33.8 ± 0.3	5.7 ± 0.2	5.9
SHW-36	Digestion	36	25.4 ± 0.2	6.0 ± 0.1	4.2
SHW-25	Digestion	25	27.8 ± 0.3	6.3 ± 0.3	4.4
SHW + OFMSW-50	Co-digestion	50	24.8 ± 0.3	5.0 ± 0.2	5.0
SHW + OFMSW-36	Co-digestion	36	26.0 ± 0.2	6.1 ± 0.1	4.2
SHW + OFMSW-25	Co-digestion	25	32.2 ± 0.4	7.4 ± 0.3	4.3

[a] Dry basis

degradation of the slaughterhouse waste (Cuetos et al. 2008). The chemical analyses of the digestates obtained from these reactors are presented in Table 1.

The digestates of reactors working successfully showed similar total organic carbon values after the stabilization process disregard of the substrate treated. The evolution of total nitrogen concentration of the digestates, must be pointed out; there was a slight increase of nitrogen content of samples as the HRT was reduced as consequence of a decrease of protein degradation. The chemical analysis from failed reactors (SHW failed-25 and SHW + OFMSW failed-25) greatly differed from their homologous. As was explained in Cuetos et al. (2008), in these systems, VFA and LCFA accumulation led to failure and collapse of anaerobic process with floating aggregates of biomass and foam formation on the top of the reactor as consequence of adhesion of fats. This fact provoked the washout of the reactors and a loss of active biomass (Broughton et al. 1998; Salminen et al. 2001) and it can clarify the severe reduction in the values of total organic carbon and total nitrogen of the final effluents of these reactors due to loss of materials.

FTIR spectroscopy

Figure 1a shows the FTIR spectra of fresh wastes and the FTIR spectra of the final effluents of the anaerobic digestion and co-digestion process. OFMSW spectrum was presented previously (Cuetos et al. 2009). This substrate was characterized by the presence of easily oxidized materials indicating that it is an important carbon source for anaerobic co-digestion process. On the other hand, SHW spectrum presented a broad absorption band at 2,500–3,800 cm^{-1} attributed to O–H vibration of carboxylic and alcoholic groups (Padmavathy et al. 2003; Smidt and Lechner 2005) with a strong absorption between 2,800 and 3,000 cm^{-1}, related to aliphatic C–H stretching (González-Vila et al. 1999; Réveillé et al. 2003). The shoulder between 3,300 and 3,500 cm^{-1} was ascribed to an N–H stretching band (Won et al. 2006), while the band centred on 2,930 cm^{-1}, that shows C–H stretching of aliphatic methylene (Hafidi et al. 2005; Stevenson 1994) and the additional band at 2,851 cm^{-1} assigned to the stretching of aliphatic C–H bonds (Castaldi et al. 2005; Hafidi et al. 2005; Smidt et al. 2005) are related to fats and lipids (Chefetz et al. 1998; Réveillé et al. 2003).

An intense band is observed in SHW between 1,500 and 1,790 cm^{-1}, which was related to nitrogen-containing compounds (Réveillé et al. 2003): the high absorbance intensity at 1,712 cm^{-1} is a peak assigned to non conjugated C=O carbonyl groups, at 1,641 cm^{-1}, a peak ascribed to aromatic C=C bonds, C=O in primary amides, ketone and quinone groups (Amir et al. 2005; Castaldi et al. 2005; Fakharedine et al. 2006; Jouraiphy et al. 2005) and at 1,569 cm^{-1} is due to C=N stretching in secondary amides.

The absorption bands of SHW at 1,460 cm^{-1} that can be attributed to aliphatic C–H deformation of structures (Miikki et al. 1997) such as fatty acids (Lguirati et al. 2005; Senesi et al. 1996) and at 1,400 cm^{-1}, peak attributable to O–H deformation, C=O stretching of phenols, anti-symmetric COO^{-}

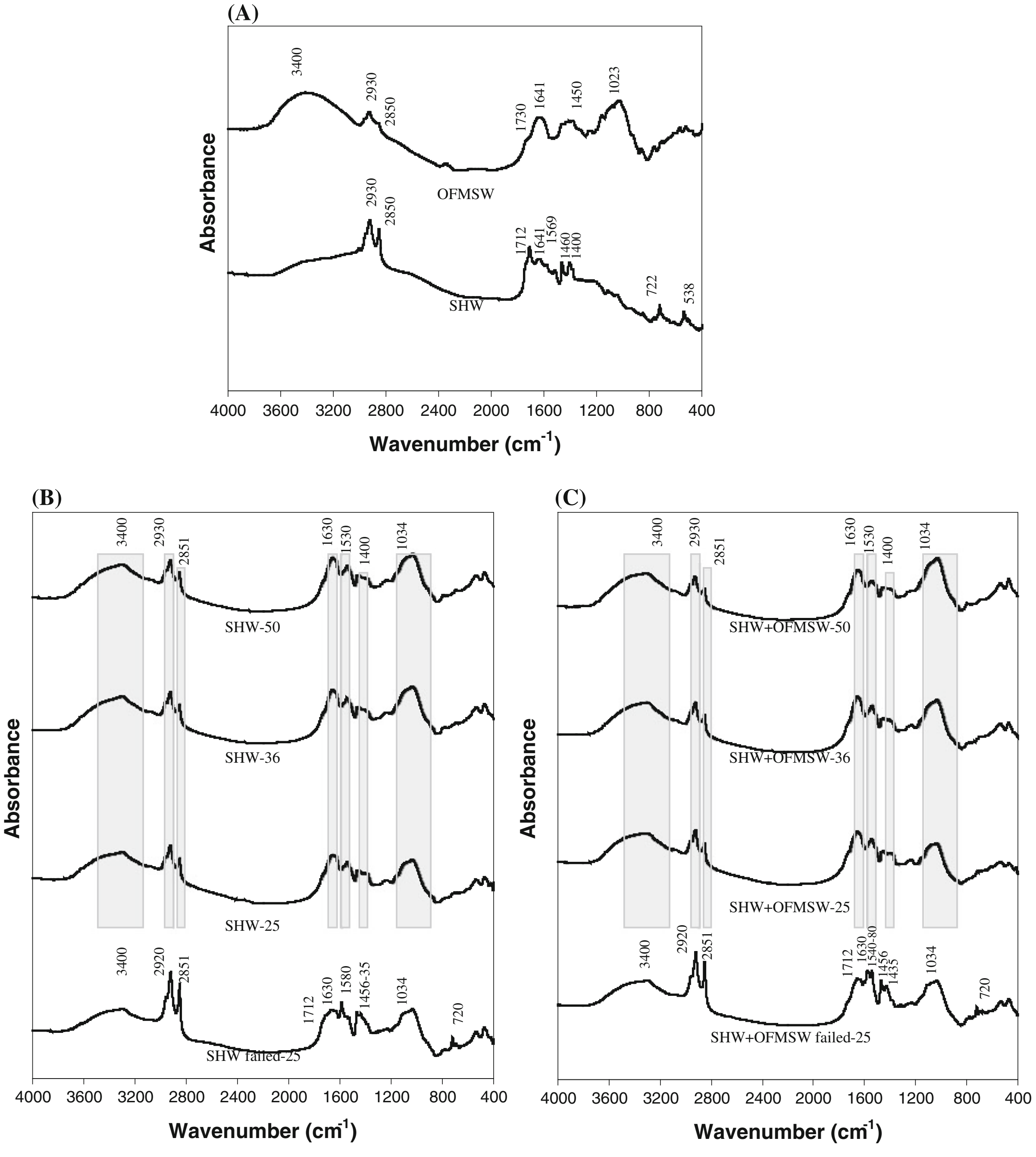

Fig. 1 FTIR spectra: **a** original wastes (slaughterhouse waste (SHW) and the organic fraction of municipal solid waste (OFMSW)) and **b**, **c** FTIR spectra of final digestates from anaerobic digestion obtained at the end of the stabilization processes (at the moment of reactor dismantling). *Note:* OFMSW spectrum has been published previously (Cuetos et al. 2009)

stretching and aliphatic C–H deformation (Hafidi et al. 2005; Senesi et al. 1996). Finally, the bands centred at 722 cm^{-1} and at 538 cm^{-1} are characteristic absorption peaks of amine groups (Pavia et al. 2001) and aromatic groups (Fakharedine et al. 2006), respectively.

As it may be seen in Fig. 1a, SHW spectrum reflected a high aliphaticity degree verifying a high content in nitrogen-containing compounds that agree with chemical characterization of fresh waste (Cuetos et al. 2008).

When compared with spectra obtained from fresh wastes, FTIR spectra of the final digestates uncovered some peaks representing different modifications of organic matter indicating that changes of functional groups and compounds have occurred during the anaerobic process (Fig. 1b, c). Digestates from reactors treating SHW and the co-digestion mixture of SHW and the OFMSW successfully presented higher absorbance at several diagnostic wavelengths. Bands at 3,300 cm^{-1} (related to H-bonded OH groups of alcohols, phenols and organic acids, as well as H-bonded N–H groups), at 1,630 cm^{-1} and at 1,530 cm^{-1} (related to primary and secondary amides) (Lguirati et al. 2005; Senesi et al. 1996) and at 1,034 cm^{-1} (related to vibration in carbohydrates, aromatic ethers and polysaccharides) increased markedly after the stabilization process. The enrichment in amide and aromatic structures may be explained by carbon lost (Calderón et al. 2006), microbial decomposition of alcoholic and acid structures (Amir et al. 2005) and digestion process, where an important part of carbon was emitted as CH_4 and CO_2. This, along with microbial growth has suggested the enrichment of the effluents in nitrogenous compounds in agreement with the high values of total nitrogen content of the digestates (Table 1).

On the other hand, bands associated with fats and lipids (such as C–H stretching at 2,920–2,930 cm^{-1} and at 2,851 cm^{-1}) in these digestates tended to decrease after anaerobic stabilization when compare to fresh SHW as a result of the drop in the levels of aliphatic structures. This fact confirms that fat removal was successfully carried out due to its utilization as C source, which was verified with the calculation of this parameter of experimental data reaching values of up to 61% (Cuetos et al. 2008).

There was likewise a loss of intensity and disappearance of some peaks in final digestates: at 720 cm^{-1} (amine groups), at 1,400 cm^{-1} (OH of phenols, –COO and –CH_3), at 1,712 cm^{-1} (non conjugated C=O carbonyl groups) and at 1,730 cm^{-1}, a characteristic band for early decomposed volatile compounds (Ouatmane et al. 2000; Smidt et al. 2005), and an increase of intensity at 1,630–1,650 cm^{-1}. The spectra followed the trend of loss of nitrogen-containing structures, aliphatic and carbohydrates chains together with volatile compounds reduction and an increase in the aromaticity degree, verifying anaerobic stability of organic matter (Gómez et al. 2007a).

In contrast, the failed reactors (SHW failed-25 and SHW + OFMSW failed-25) showed a high absorption band at 2,920 and at 2,851 cm^{-1}, along with the appearance of two relevant bands at 1,456 and 1,435 cm^{-1} (attributable to aliphatic C–H deformation of structures such as fats and lipids) confirming that the lipid content of reactors was elevated as consequence of the decrease of fats degradation due to a failed anaerobic process. These spectra likewise showed the existence of different absorption bands with a protein origin that were presented in spectra of original wastes but that were reduced in spectra of stable digestates: band at 1,712 cm^{-1} (forming part as a shoulder of the peak at 1,630–1,650 cm^{-1}), at 1,580 cm^{-1} and at 700 cm^{-1}, reflecting an increase of nitrogen-containing compounds and therefore a decrease of protein mineralization. Furthermore, when FTIR spectra of failed reactors are compared one from each other (SHW failed-25 and SHW + OFMSW failed-25), there is an increment in absorption band at 1,658 and at 1,580 cm^{-1} (bands with a protein origin) in the co-digestion rector, presenting a new absorption peak in this spectrum at 1,540 cm^{-1}, attributed to C=C or N–H stretching (Lguirati et al. 2005; Senesi et al. 1996), which was not presented in the SHW digestate sample. The results are in accordance with the poor performance results of the failed reactors.

Under different operational conditions the absorbance intensity was similar for the final digestates from anaerobic digestion of SHW and its co-digestion with the OFMSW, performed at HRT of 50, 36 and 25 days. The changes in FTIR spectra were evaluated by calculation of the ratio between the intensity of major peaks (Table 2) (Lguirati et al. 2005; Ouatmane et al. 2000). The peaks at 2,930, 2,851, 1,630, 1,530 and 1,034 cm^{-1} were chosen for these calculations. The 1630/2930, 1630/2851 and 1530/2930 ratios show the relationship between aromatic C/aliphatic C and aromatic C/carboxylic C, while the 2930/1034 ratio explain the correlation between aliphatic C and polysaccharide. Easily degradable organic matter constituents, such as aliphatic components, polysaccharides and alcohols are oxidized and the volatile content of wastes is reduced, increasing the aromaticity degree (Gómez et al. 2007a). In this sense, these ratios could be used as

Table 2 Ratios of selected FTIR peaks from digestate samples

Digester	1630/2851[a]	1630/2930[b]	1530/2930[c]	2930/1034[d]
SHW failed-25	0.43 ± 0.03	0.41 ± 0.03	0.26 ± 0.02	2.26 ± 0.20
SHW + OFMSW failed-25	0.57 ± 0.04	0.46 ± 0.03	0.36 ± 0.02	2.27 ± 0.20
SHW-50	1.70 ± 0.15	1.02 ± 0.09	0.70 ± 0.06	0.77 ± 0.06
SHW-36	1.70 ± 0.15	1.03 ± 0.09	0.71 ± 0.06	0.79 ± 0.07
SHW-25	1.06 ± 0.09	0.72 ± 0.06	0.44 ± 0.03	1.57 ± 0.13
SHW + OFMSW-50	3.05 ± 0.25	1.19 ± 0.09	0.72 ± 0.06	0.51 ± 0.04
SHW + OFMSW-36	2.04 ± 0.20	1.08 ± 0.09	0.77 ± 0.06	0.85 ± 0.07
SHW + OFMSW-25	1.50 ± 0.10	0.88 ± 0.07	0.63 ± 0.05	1.62 ± 0.14

[a] Relationship between aromatic C/aliphatic C

[b] Relationship between aromatic C/carboxylic C

[c] Relationship between aromatic C/aliphatic C

[d] Relationship between aliphatic C/polysaccharide

stability indexes since the changes in these ratios indicate changes in organic matter composition.

Ratio analysis allowed distinguishing between anaerobic systems at different HRT, a behaviour that could not be easily appreciated from FTIR spectra. The 1630/2930, 1630/2851 and 1530/2930 ratios decreased as HRT was decreased while 2930/1034 ratio increased with the decrease of HRT. This may rationalized by an increase in lipid content as consequence of a lower efficiency in the anaerobic process, originated from the decrease in fat degradation. An increase in the value of these ratios was expected with the increment in the HRT as consequence of the stabilization process.

Compared to the general trend, ratios of failed reactors presented the most important deviations. The results are in accordance with the collapse of anaerobic process suffered by these reactors. Successful anaerobic reactors with high HRT (SHW-50, SHW + OFMSW-50, SHW-36 and SHW + OFMSW-36) showed similar values (for anaerobic digestion and co-digestion samples, respectively), being the successful anaerobic reactors with short HRT (SHW-25 and SHW + OFMSW-25) the ones showing the most accentuated changes. These systems presented the highest values for parameters evaluated (alkalinity, ammonia and chemical oxygen demand) compared to those obtained from reactors working at an HRT of 50 and 36 days (Cuetos et al. 2008). The stable performance of these reactors (SHW-25 and SHW + OFMSW-25) was confirmed by the lack of VFA and only traces of LCFA detected in the effluent (Cuetos et al. 2008), but the stability level of the digestates was lower than that of their counterparts as was demonstrated by FTIR analysis.

TG–MS analysis

Thermogravimetric analysis

The above structural changes observed by FTIR spectroscopy were supported by TG–MS analysis. DTG and TG curves of the original wastes and the digestates of anaerobic digestion and co-digestion processes are presented in Figs. 2 and 3, respectively.

Disregarding the release of water (centred at around 80°C), DTG profiles showed two clearly differentiated zones: the first one around 300°C and the other one between 400 and 550°C; exception made for SHW sample, which presented a continuous weight loss from 500 to 650°C. This was probably due to the combustion of material which had suffer char formation at earlier temperatures (Font et al. 2001; Zhu et al. 2007) and that burns afterwards in the last stage of the process without allowing the decay of the curve represented. The wide intensity of the peaks observed at 200–375°C for the SHW waste sample (Fig. 2a), corresponding to readily oxidized materials, was the greatest, verifying that this substrate presents a high potential for valorization by means of anaerobic digestion process. On the other hand, the potential of OFMSW as a co-substrate for anaerobic digestion has been studied previously (Cuetos et al. 2009), and in the current work it was

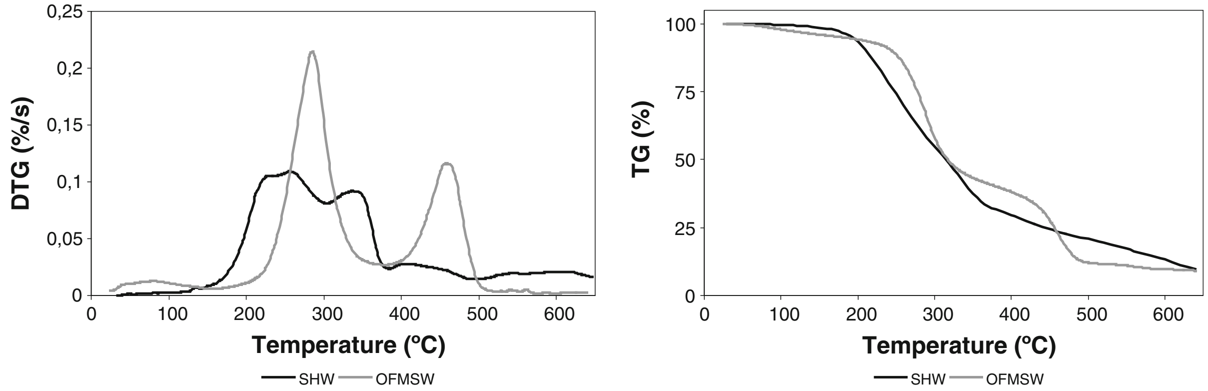

Fig. 2 Evolution of the weight-loss profiles (TG–DTG) of the original wastes. *Note*: OFMSW TG-DTG profiles has been published previously (Cuetos et al. 2009)

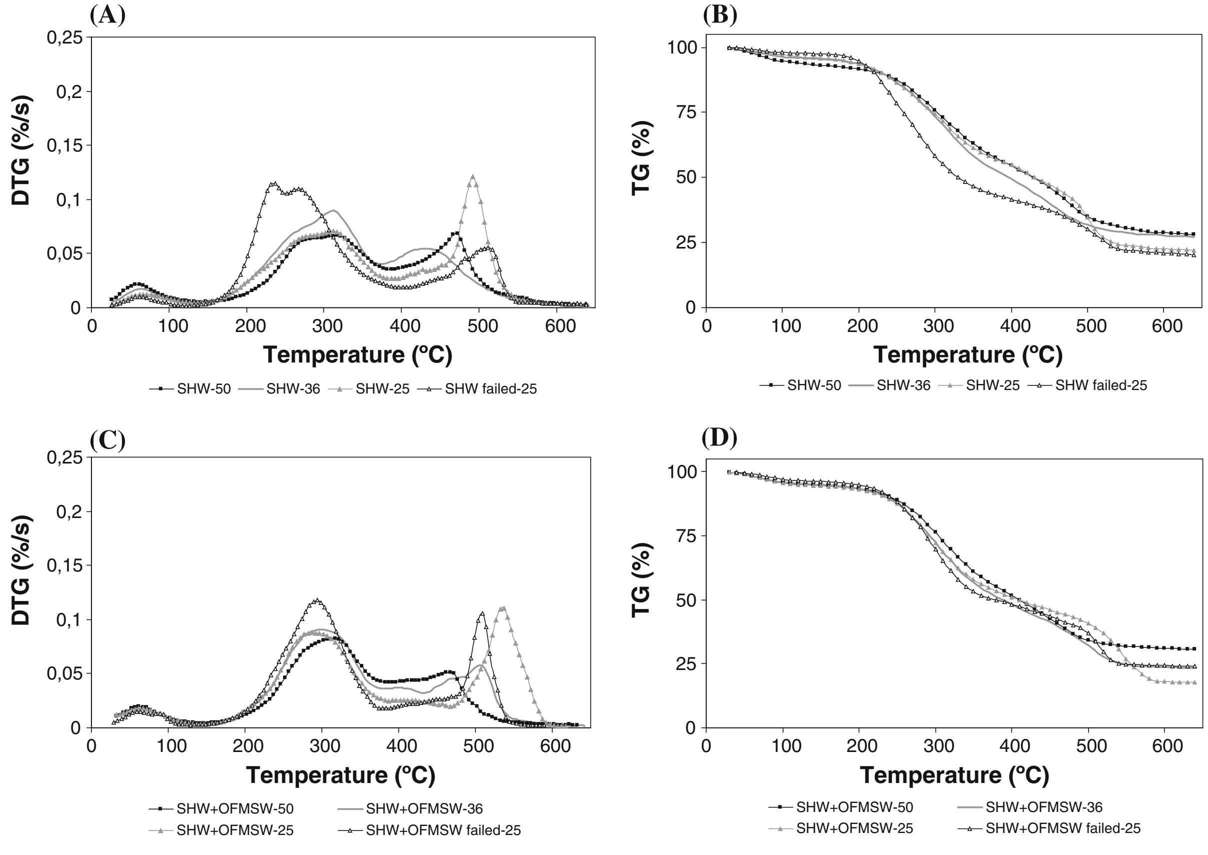

Fig. 3 Evolution of the weight-loss profiles (TG–DTG) of the obtained digestates from anaerobic digestion (**a**, **b**) and co-digestion process (**c**, **d**) under the different operational conditions considered

verified with the high intensity of the peak presented by the OFMSW DTG profile at low temperatures, being even greater than that of SHW waste.

The thermal degradation of readily oxidized compounds was shifted to the right on the temperature scale with the increment in the HRT (Fig. 3a).

Digestate SHW-50 presented thermal oxidation at a temperature close to 200°C, while digestates SHW-36 and SHW-25 initiated this oxidation at a lower temperature. The SHW failed-25 sample presented a peak with a comparable intensity to that of the SHW waste. In this case, it could be attributable to easily biodegradable materials that have not yet been consumed by microbial populations as consequence of the overloading and subsequent collapse of anaerobic digestion suffered by this system.

The degradation of organic matter by means of anaerobic digestion leads to an enrichment in aliphatic components (Gómez et al. 2007b, 2009). Francioso et al. (2009) in the study of the digestion of sludge in a two phase process reported the formation exclusively in the methanogenesis phase of alkanes (Cn-12 and Cn-13) by means of HS–SPME–GC–MS. These compounds usually derive from oils or fuels, but their presence in methanogenesis suggests a biological origin. The continuous mass loss experienced by the digestate sample SHW-50 and SHW-36 may be explained by the higher content of the aliphatic components.

In the high-temperature range, corresponding to the weight loss suffered by structurally more complex materials, the intensity of the peaks and the temperature at which they take place changed with the decrease in HRT. The intensity of this peak for reactors with higher HRT (SHW-50 and SHW-36) was lower, indicating that complex materials generated during the process are consumed subsequently, showing a preferential degradation during stabilization of the organic matter (in accordance with the increase in the aromatic structures in final effluents showed by FTIR). On the other hand, the DTG from sample of digestate SHW-25 presented a narrow peak with a greater intensity displaced to the right on the temperature scale compared with the other digestates from successful anaerobic process. This fact could be related to the formation of complex compounds during metabolic activity that have not yet been consumed by the microorganisms due to the smaller HRT applied to the digester.

On the contrary, the instability suffered by SHW failed-25 was verified by the trend of low-temperatures peak and the behaviour of the peak registered at high temperatures, which had an similar intensity to those of reactors samples SHW-50 and SHW-36 but showing a shift to the right on the temperature scale at a similar temperature to that of SHW-25. This indicated that the structurally more complex compounds have not been degraded and rather accumulate at low HRT, which was corroborated by the relative high content of aliphatic structures (fats and lipids) and nitrogen-containing compounds showed in FTIR spectrum (Fig. 1), demonstrating a low efficiency in fats removal and protein mineralization in the system. Although increments in the mineral content of digestates are known to redound in an increase of the burn out temperature of the samples (Rubiera et al. 2002; Vamvuka et al. 2006), in the present study samples with higher mineral content showed lower burn out temperatures (SHW-50 and SHW-36), which may be probably due to the higher content of lipids and N-compounds of digestates with the reduction in the HRT.

The overall mass loss undergone by the SHW waste was around 90% (as it may be seen in the TG profile in Fig. 2b), while SHW-50 and SHW-36 samples presented values around 72%, indicating a clear increment in the degree of mineralization (Fig. 3b). At lower HRT (SHW-25 and SHW failed-25 system) the overall mass loss was around 80%. Although the reduction in the HRT in the digestion process presented successful results for SHW-25, the quality of the digestate deteriorates in terms of the mineralization attained; however, it should be born in mind that the amount of material undergoing oxidation at lower temperatures is smaller for this sample than for SHW-36 sample. The thermal behaviour of digestate samples at low temperatures has to be taken into consideration, being the volatile removal of organic materials similar for successful reactors whatever HRT. Nevertheless, the failed reactor presented a high intensity peak centred approximately at 205°C in accordance with the inhibitory process suffered by this reactor.

The behaviour of DTG for digestates from co-digestion process with different HRT was similar to those obtained from the anaerobic digestion process (Fig. 3c) as the stabilization process took place, the weight loss in the low-temperature range was displaced to higher temperatures, with a decrease in its intensity, confirming that the availability of susceptible material for microorganisms was diminishing (Gómez et al. 2005), while the intensity of the peaks in the high-temperature range was lower (centred around 450°C) for the reactors with higher HRT's (SHW + OFMSW-50 and SHW + OFMSW-36).

This fact is related with the lost of carbohydrates and nitrogen-containing compounds and aliphatic structures degradation as stabilization proceeded, which was previously observed with FTIR spectroscopy (Fig. 1).

The DTG profile of the sample from co-digestion at an HRT of 25 days (SHW + OFMSW-25) presented a high weight loss in the high-temperature range. This peak was shifted to the right on the temperature scale in a similar way to that of the DTG profile from its homologous (digestate sample from the digestion carried out at the same HRT, but without co-substrate, SHW-25).

On the other hand, the differences among SHW + OFMSW failed-25 and the rest of digestates from the co-digestion process presented at low temperatures were minor than those among SHW failed-25 with the rest of digestates from the digestion process. The intensity of SHW + OFMSW failed-25 sample in the high-temperature range was in an intermediate position between the reactors with higher HRT and SHW + OFMSW-25 sample, possible as consequence of OFMSW contribution.

As it may be seen in the TG profile in Fig. 3d, the reduction in the HRT in the digestion process presented a more poor quality of the digestate in terms of the mineralization attained when it was compared with the DTG profile of SHW sample: the overall mass loss undergone by SHW + OFMSW-50 and SHW + OFMSW-36 samples was around 63 and 75%, while SHW + OFMSW-25 sample presented a value around 81%.

Mass-spectrometry analysis

Evolved gas analysis is a technique to determine the nature and amount of volatile products formed during thermal degradation of materials (Plante et al. 2009). Results from the present study are presented in Fig. 4 where the changes in the relative intensity of the species recorded by mass spectrometer can be seen. (Data of evolution of the emission profiles for mass/charge (m/z) signals of the obtained digestates from anaerobic digestion process are not shown since their tendency was the same than the signals of the obtained digestates from anaerobic co-digestion).

Sulphur compounds (signal m/z 64) were detected along the whole oxidation of feed sample for both feeds, starting at an initial temperature close to 150°C. Reactors treating SHW and the co-digestion mixtures presented a maximum emission registered around 300°C, associated to the weight loss experienced by the DTG profile. There is an increment in the emission signal with the digestion process which may be explained by the fact that the total amount of mass remaining at the end of stabilization process is lower, so the materials that are not turned into biogas are concentrated in the remaining solids. Although some sulphur compounds are lost with the biogas as hydrogen sulphide and volatile sulphur compounds, from signal m/z 64 it can be seen that some others do remain in the solids (Gómez et al. 2007a). The failed systems samples (SHW failed-25 and SHW + OFMSW failed-25) presented an initial increase in the emission at low temperature (~250°C) indicating that those components are associated to the formation of intermediary products in the early stage of the digestion process. The modifications registered by the signal m/z 64, evidence that the increase in the digestion time resulted in the accumulation of final products characterized by suffering combustion in the narrow range between 300 and 400°C.

Signal m/z 41, related to the release of light hydrocarbons products, has an important contribution for the mass loss experienced for different samples in the low-temperature range (200–400°C). The stabilization process results in the reduction of volatile emissions with a maximum peak ~300°C. Both feeds, SHW and OFMSW, presented two clear peaks correlated to the maxima recorded in the DTG profiles, with the second peak being reduced as the stabilization process takes place, being in accordance with previous studies (Gómez et al. 2007a). Consequently, the profile obtained from signal m/z 41 at high temperatures verifies the inhibitory effect causing the low performance of SHW-25, SHW + OFMSW-25 reactors and failed reactors as was already demonstrated by thermal analysis (Fig. 3) and FTIR spectra (Fig. 1) due to the lack of acclimation of the systems. Incomplete combustion of the organic matter reflected by the release of volatile components may be associated with the degree of stabilization attained by the organic matter. Dell'Abate et al. (2003) reported a continuous release of CO signal in the thermal degradation of humic substances under oxidizing atmosphere, indicating prevalence of hydroxyl and carboxylic groups. In the present study samples obtained either at a lower HRT

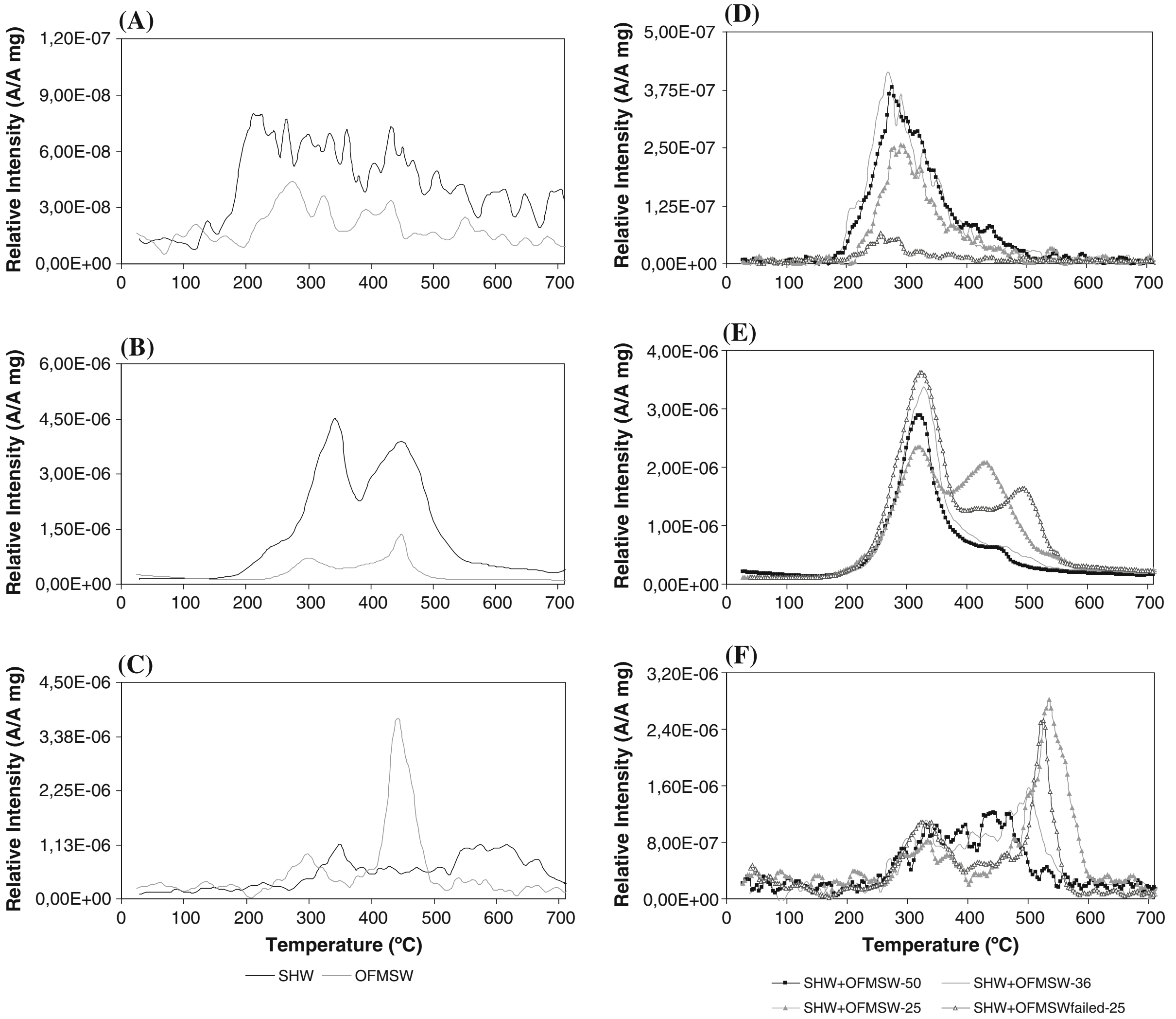

Fig. 4 Evolution of the emission profiles for mass/charge (m/z) signals in an oxidizing atmosphere of the original wastes: **a** m/z 64, **b** m/z 41, **c** m/z 46 and of the obtained digestates from anaerobic co-digestion under the different operational conditions considered: **d** m/z 64, **e** m/z 41, **f** m/z 46

or from the failed system present a higher emission of light hydrocarbons at higher temperatures, while successful digestion reported a high intensity centred at 300°C (samples SHW-50) which may be explained probably by the enrichment in long chain alkanes suffering pyrolysis at this temperature.

Finally, signal m/z 46 is related to NO_2 emission. Final effluents from SHW digestion and co-digestion systems, at high HRT, were able to degrade components responsible for emissions at high temperatures, nevertheless SHW-25, SHW + OFMSW-25 reactors and failed reactors, presented higher emissions shifted to the right on the temperature scale, representing accumulation of nitrogen-containing compounds in the digestate. Variations in relation to the position of the high temperature peak in the DTG profile were coincident with the modifications registered by signal m/z 46, indicating an important contribution of nitrogenous components reported from the FTIR spectra (primary and secondary amides and compounds with an amine group) in the mass loss experienced at high temperatures. The accumulation of nitrogen containing compounds may be rationalized as a preferential degradation of easily degradable components and subsequent degradation of complex organic matter which is only possible if an adequate HRT is set in the digestion process (Gómez et al. 2009). At a high HRT, complex materials are degraded and transformation and/or accumulation into condensate organic and nitrogen forms are the result of the stabilization process. In this sense results obtained by Lopez-Capel et al. (2006) reporting an increase in the emission of NO and CO_2 species at 530°C are in agreement with those obtained in the present study, although the prolonged retention time inside the digester (sample SWH-50) allowed the partial degradation of these materials. Calvo et al. (2004) in the study of sludge samples from a mesophilic digestion process, performed TG–MS analysis under atmospheres with varying oxygen content, reporting NO_2 emissions centred around 300–400°C under complete oxidizing conditions which may be indicative of the lack of aromatic structures containing nitrogen. From results obtained may be hypothesized that degradation of stable C and N forms may be attained under anaerobic digestion if sufficient time is provided.

Conclusions

Fourier Transform infrared spectroscopy (FTIR) and thermogravimetric analysis together with mass spectrometry (TG–MS analysis) aided in determining the stability differences of the end products obtained from anaerobic digestion of slaughterhouse waste (SHW).

A successful stabilization process of organic matter led to an increase in aromaticity degree and a reduction of volatile compounds as stabilization process was carried out. FTIR spectra obtained from successful anaerobic systems presented a high amount of unsaturated bands of digestates. These reactors also showed a reduction in the low-temperature range ($\approx$300°C) of derivate thermogravimetry (DTG) profiles, related to easily oxidized materials, while the weight loss in the high-temperature range (450–550°C) was variable for the different systems, so the intensity of the weight loss peak in the high-temperature range decreased in the reactors with higher hydraulic retention times (HRT) whereas its intensity increased and the peak was displaced to higher temperatures for the digesters with lower HRT.

The less stable products obtained from successful anaerobic digestion and co-digestion processes presented a slight increase of volatile compounds (signal emission intensity m/z 41) in the high-temperature range ($\approx$450°C) and a high emission of nitrogenous compounds (signal emission intensity m/z 46) at even higher temperatures ($\geq$500°C).

On the other hand, the FTIR spectra of non-stable reactors showed a high aliphaticity degree and fat content, in a similar way to that of fresh wastes, as well as an increase in nitrogen-containing compounds (signal emission intensity m/z 46) as consequence of the decrease in protein mineralization.

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