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*Article*

# Assessing Digestate at Different Stabilization Stages: Application of Thermal Analysis and FTIR Spectroscopy

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**Abstract:** Anaerobic digestion is a biological process that transforms high-strength organic effluents into biogas with multiple benefits. However, concurrent with organics' biological transformation, a liquid phase with a high solid content is also derived from this process. Valorizing this fraction is not an easy task if an agronomic application cannot be considered as a suitable option. The thermal valorization of this fraction allows for energy extraction, but also gives rise to additional capital investment and increases the energy demand of the global process. In addition, the thermal treatment of digestate has to deal with a mineralized material. The changes in organic matter due to anaerobic stabilization were studied in the present manuscript, by evaluating the thermal behavior of samples, activation energy and organic transformation using FTIR spectroscopy. Digested samples of a mixture composed of manure and glycerin (5% v/v) were studied. The stabilization caused a dramatic decrease in aliphatic compounds, greatly increasing the mineral content of the sample. Thermal valorization of digestates must consider the lower energy content and more complex structure of these materials, with higher content of lignin and protein-type compounds.

**Keywords:** anaerobic stabilization; thermal valorization; mineralization; activation energy

## 1. Introduction

Anaerobic digestion is a biological process that has several benefits when considering the conversion of organic effluents into biogas and its capacity to recycle nutrients. Biogas is a gaseous stream mainly composed of methane and carbon dioxide, which can serve directly as a fuel in multiple energy production systems or upgraded to reach a quality similar to that of natural gas [1]. However, along with organics' biological degradation, a liquid slurry is also derived from this process, containing microbial biomass, undigested components, and a high proportion of minerals. The resulting slurry is known as digestate. Traditionally, application to crops is the final disposal option, bringing environmental and agronomic benefits [2]. This practice allows for nutrient recycling and improves soil quality, as demonstrated by several authors [3–5]. Management methods must take into account soil nutrient conditions and regional environmental regulations to establish fertilization rates [6].

The characteristics of digestate mainly depend on the process performance (operating parameters) and intrinsic characteristics of the feed. A detailed review performed by Fernández-Domínguez et al. [7] aggregates information regarding digestate characterization and factors affecting digestion outcomes. In a subsequent work, the same authors provided insight regarding the chemical structure of digestate based on inoculum type, digestion time, and substrate composition, reporting significant differences in digestates based on these parameters [8]. The biological anaerobic process occurs in a series of reactions where hydrolysis of complex substrates is the first stage, solubilizing particles and making them accessible to the microflora. Conversion into organic acids and subsequent degradation into methane follow a diversity of microbial pathways [9,10], ending with biogas as the product of interest and digestate as a residual by-product. Several digestion configurations have been proposed, including centralized and decentralized centers, partial decentralization by performing the initial digestion stage in small units and centralizing the subsequent digestion phase, or centralizing only the valorization of biogas after the initial digestion stage [11–14]. Regardless of the configuration option selected, a practical solution for the final disposal of digestate is necessary.

The biological degradation of organic materials starts with the preferential conversion of readily degradable compounds (polysaccharides) and the accumulation of complex components with longer degradation time, thus leading to the build-up of recalcitrant structures in the anaerobic slurry [15,16]. Therefore, the quality of the organic material suffers changes during the biological conversion, increasing mineralization and accumulating recalcitrant molecules. Any modification in reactor operating parameters will affect digestate characteristics, thus influencing its energetic content and subsequent thermal valorization.

The deployment of digestion plants has given rise to a secondary problem associated with the great amount of digestate requiring a final disposal option. If land spreading is not feasible due to regulation constraints, thermal valorization such as pyrolysis, gasification and incineration become suitable alternatives [17–20]. Other new technological options involve the production of proteins or the use of digestate as a carbon or nutrient source in different biological processes, as it may be the production of ethanol, polyhydroxyalkanoates, biopesticides, microalgae cultivation, among others [21–24]. However, these processes are in an incipient state of research; thus, practical application is yet to be found. When considering digestate as a raw material, the application of the final product and the simultaneous avoidance of cross-contamination are relevant aspects. Digestates are usually derived from manures. Therefore, the risk of creating a public health problem associated with the presence of viruses and bacteria should be carefully examined. The use and valorization of digestates need to be performed safely and in compliance with environmental, health and legal requirements [25].

Research activities currently focus on integrating digestion by-products to increase the cycling of nutrients in compliance with circular economy principles [26,27]. However, the energy demand of the value chain should also be carefully evaluated. Otherwise, the increase in energy requirements may offset any benefit associated with the cycling of materials. In the case of thermal treatment of digestates, the energy demand associated with the drying stage may be excessive, making the whole process unfeasible. Composting and bio-drying are two ways to reduce water content and improve the stability of organics [28]. Although considered a low-energy demanding process compared to thermal drying, the requirement of an extensive area and the careful control of emissions may act as a disincentive for this type of installation.

Solar dryers are a type of drying equipment characterized by low energy demand (20-30 kWh/t sludge [29]). These drying methods have not been as widely installed as they should have been expected, based on all the benefits associated with their performance, mainly because of the need for an extensive area for installing greenhouses where halls of sludge drying beds will be placed. The drying time is already high in summer periods, where high drying efficiencies and pathogen abatement are attained. However, during the winter period, drying time is further increased along with a decrease in drying efficiency, thus limiting the application of the technology to regions with high solar radiation or needing the aid of a boiler to supply additional heat [30,31].

Other aspects requiring careful analysis are the changes suffered by the organic material during anaerobic stabilization and, therefore, their implications in a subsequent thermal treatment. Therefore, thermal valorization of digestates must confront two main problems: the energy associated with dewatering and drying and the handling of the mineralized material. The present

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manuscript aimed to analyze changes experienced by the digestate during an extended stabilization phase and the implications of the mineralization attained when considering the conversion of the anaerobic slurry by thermal processing.

## 2. Materials and Methods

Swine manure was obtained from a farm located near León (Spain). The inoculum used was digested sludge from the wastewater treatment plant of León. Collection point and conservation were carried out as described in Fierro et al. [32]. Residual glycerin was used to boost biogas production at a 5% volumetric ratio. This ratio was selected based on previous work performed by the authors [33,34]. The glycerin was obtained from a local biodiesel plant located in San Cristobal de Entreviñas (Zamora, Spain). The characteristics of the inoculum and substrate are shown in Table 1.

**Table 1.** Characteristics of materials used in the digestion process

| Parameter [1] | Inoculum | Swine manure | Glycerin |
|---|---|---|---|
| TS (g/L) | 22.6 ± 0.3 | 46.2 ± 0.6 | 990.8 ± 5.3 |
| VS (g/L) | 14.0 ± 0.4 | 32.1 ± 0.7 | 961.4 ± 5.7 |
| COD (g/L) | 30.5 ± 1.3 | 58.7 ± 1.9 | - |
| pH | 7.6 ± 0.2 | 6.8 ± 0.2 | 10.0 ± 0.2 |

[1] TS: total solid, VS: volatile solid, COD: chemical oxygen demand

The co-digestion of swine manure and residual glycerin was carried out in continuously stirred tank reactors with a working volume of 3 L, operating at 34 ± 1 °C. The hydraulic retention time (HRT) was set constant at 30 d. Initially, the reactor was fed at a low load during the first 30 days, considering this period the acclimation stage. Subsequently, the desired HRT was established, and the system worked at a constant load of 2.4 g VS/L d for the remaining period (70 days). The stop of feeding was considered the beginning of the stabilization period; thus, a zero value was given to this day. Only total gas production and solid evolution were registered. Process parameters were followed for a 300-day period under room conditions (temperatures between 20 – 22 °C).

### *2.1. Analytical Techniques*

Total solids (TS), volatile solids (VS), ammonium and chemical oxygen demand (COD) measurements were carried out following standard methods [35]. pH was measured using a Crison pH meter. The feeding sample (feed) and three digested samples were analyzed. One collected right at the end of the working period (dig_0d), then after 30 days of stabilization (dig_30d) and at the end of the period (dig_300d).

Thermal analysis was carried out using methodology described by González et al. [36] using a TA Instruments equipment, model Q600. 5 mg of sample was used and submitted under a 100 mL/min of air flow. Heating ramp was 10 °C/min until reaching 700 °C. The mass loss (thermogravimetric curves, TG), derivative thermogravimetric curves (DTG) and differential scanning calorimetry (DSC) were obtained from this analysis. Two replicates were analyzed and mean values were used for representing the thermal profiles. Thermal profiles obtained from DTG curves were deconvoluted using OriginPro 2015 Software. Estimation of activation energy was carried out using methodology described by Gómez et al. [37] using the linearization method proposed by Dodampola et al. [38].

FTIR spectra was recorded based on methodology described by Arenas et al. [39]. An FTIR Thermo Scientific Nicolet iS5 (ID7 ATR accessory, a monolithic diamond ATR crystal with high efficiency) spectrophotometer was used (4000 – 650 $cm^{-1}$ range at a rate of 0.5 cm/s). Sixteen scans were collected with 0.482 $cm^{-1}$ spacing. Each spectrum was averaged and corrected against ambient air as background. Hierarchical cluster analysis (HCA) and principal component analysis (PCA) were used for evaluating FTIR spectra using OriginPro 2015 software.

## 3. Results and Discussion

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*3.1. Digestion and Stabilization Period*

Figure 1 shows results derived from reactor performance. The zero value was assigned to the cease of reactor feeding. There is an initial increase in the solid content of the reactor, reaching a steady behavior under the operation at an HRT of 30 days. The specific biogas production was 547.2 ± 68.4 mL gas/g VS. Once feeding stopped, a clear reduction in solid content was observed till reaching a value of 32.5 g VS/L at the end of the evaluation. The period corresponding to the stabilization of digestate was characterized by low gas production, with an increase in ammonia values after the removal of the feeding procedure, although no significant changes were observed in the solid content. The startup of the reactor and the working period, at an HRT of 30 days and 34 °C of temperature, were also represented in the graph.

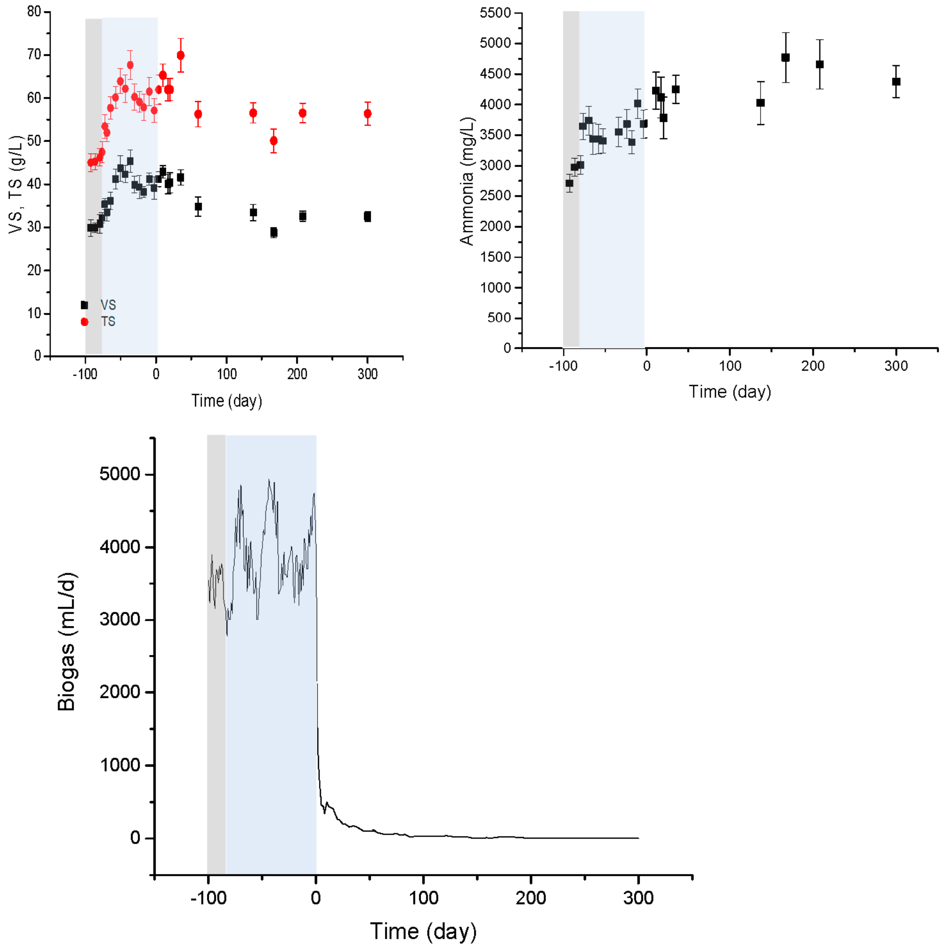


**Figure 1.** Evolution of solids (VS, TS), ammonia and biogas production. Gray: acclimation period, Blue: working period. Stabilization period corresponds to days 0 to 300.

*3.2. Thermal Analysis*

Figure 2 shows the thermal profiles derived from the analysis of the feeding material. The mixture of swine manure and glycerin shows two characteristics zones where thermal degradation of highly biodegradable material commonly takes place. In a previous work [36], the authors studied

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the thermal profiles of swine manure and glycerin as single substrates. In the present case, the interest was to focus on the evolution of organic materials when submitted to long-term degradation.

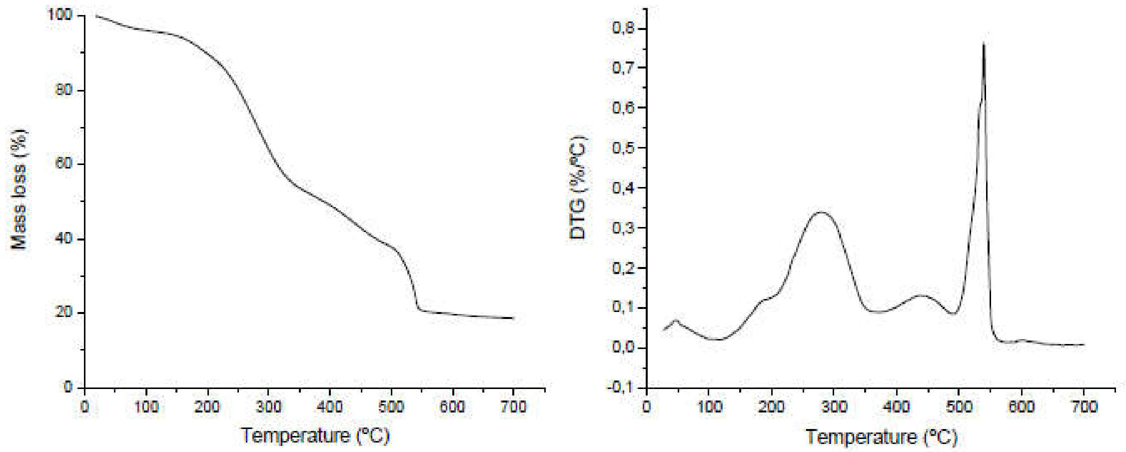


**Figure 2.** Thermal profiles of the feeding mixture (glycerin and swine manure) a) TG expressed as percentage (%) and b) DTG curves obtained from the feed sample.

The degradation of labile compounds takes place first with mass loss peaking at around 300 °C. The presence of glycerin can be observed by the early initiation of mass loss peaking around 200 °C [36,40]. The degradation of cellulose and hemicellulose then takes place, along with simple lipids and amino acids [37,41], followed by a partial pyrolysis process usually due to mass transfer limitations during the analysis. The second main process takes place around 550 °C, evidencing the presence of lignin-type complex components along with combustion of previous material partially degraded at a lower temperature [42,43]. Thermal analysis has been widely applied for characterizing biomass, particularly manure of different origins [16,44], due to the short time required for the analysis, easiness of sample preparation and results interpretation.

The thermal degradation process is associated with a release of energy when the sample shows an increment in temperature. In the present case, although an endothermic degradation characterizes glycerin, the mixture analyzed shows exothermic peaks during the evaluated temperature range. Figure 3 shows the DSC profile obtained, where a distinctive peak releasing a high amount of energy is identified at around 550 °C.

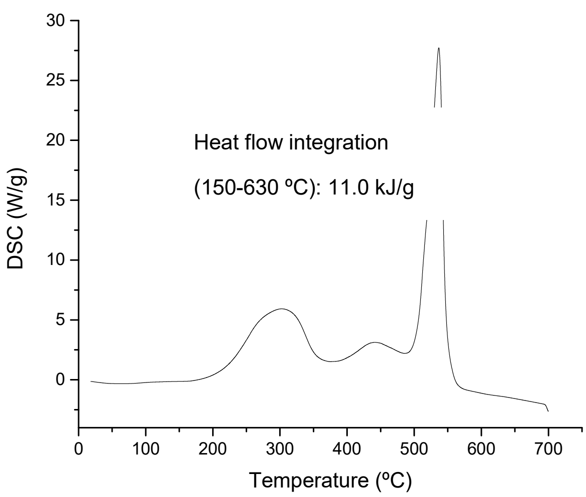


**Figure 3.** DSC profile obtained from the feeding sample (mixture of swine manure and glycerin).

The profile resembles those analyzed under oxidizing conditions for waste biomass with high lignocellulosic content [45]. The atmosphere (inert or oxidizing conditions) under which the analysis is performed affects the structure of the profile because conversion and minimum activation energy are highly affected by the presence or lack of oxygen [46]. However, kinetic analysis can be performed in either case, although careful comparison of results must be made, keeping in mind experimental conditions.

Figure 4 shows the results from the stabilization period. Mass loss curves and their corresponding derivative are represented (Figure 4a and 4b). Significant changes were observed, although the analysis of solid content did not reveal any relevant difference. A high mineralization was observed at the end of the stabilization period which was discernible by the higher amount remaining once thermal degradation finished. The mass remaining at the end of the thermal analysis accounts for 18.6% in the case of the feed. Digestion increased this value, and further stabilization raised this amount to 34%. This feature was previously observed by Martínez et al. [47], but in this case, analyzing the thermal behavior of digestate derived from fruit and vegetable wastes (fourth-range wastes).

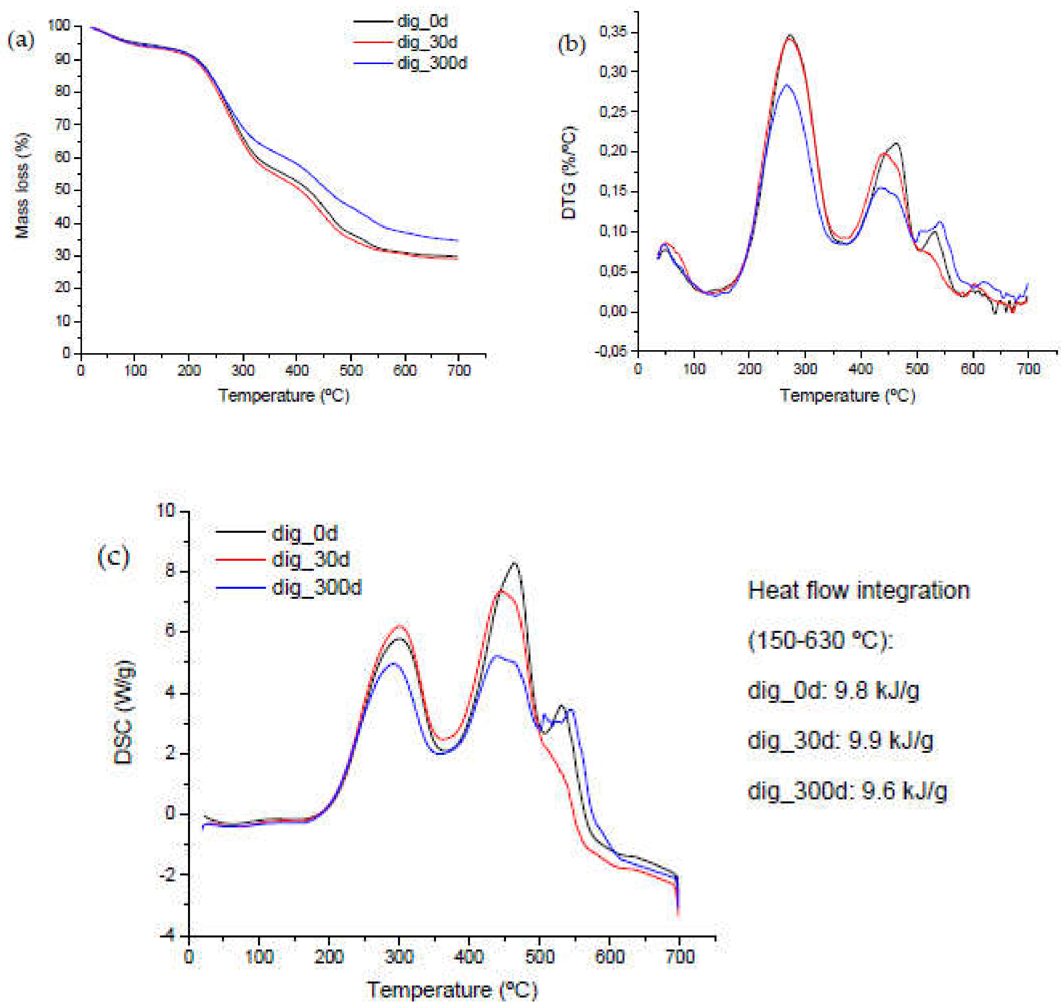


**Figure 4.** (a) TG and (b) DTG curves from stabilized samples of swine manure and glycerin mixture. (c) DSC curves derived from digested samples at different stabilization times.

The profile of the different peaks represented in the DTG curve indicates changes in the amount of material suffering degradation at each temperature range. Digestion transforms organic matter into biogas, concentrating mineral components in digestate and accumulating those of difficult degradation. Therefore, the greater size of the peak at 550 °C in the stabilized sample at day 300. The energy associated with each degradation stage can be observed in Figure 4c, where DSC profiles are represented. Samples showed greater intensity of the peak located at around 450 °C, with this behavior being an indication of the higher energy content of these complex materials.

The stabilization resulted in a significant decrease in the amount of organic components along with changes in their structure, demonstrating that the degradation of these structures by microbial microflora takes longer, as it is observed by the decrease in intensity of the 450 °C peak. The decrease in the energy content of the sample was also evidenced from the experiment. The fresh material showed a value of 11 kJ/g, whereas any of the digested samples reported values below 10 kJ/g. Therefore, if thermal valorization is intended, the thermal process will have to deal with a higher mineral content material with lower energy, thus increasing fouling and tar formation problems during thermal treatment.

*3.3. Stimation of activation energy*

The transformation process can be separated into individual stages by deconvoluting peaks observed visually in the profile (see Figure 5). In the case of the sample representing the feed, a small peak associated with labile organics can be inferred, and it was denoted in the present case as peak 1. The remaining peaks had a recurrent presence in digested samples, with significant modifications in intensity and size.

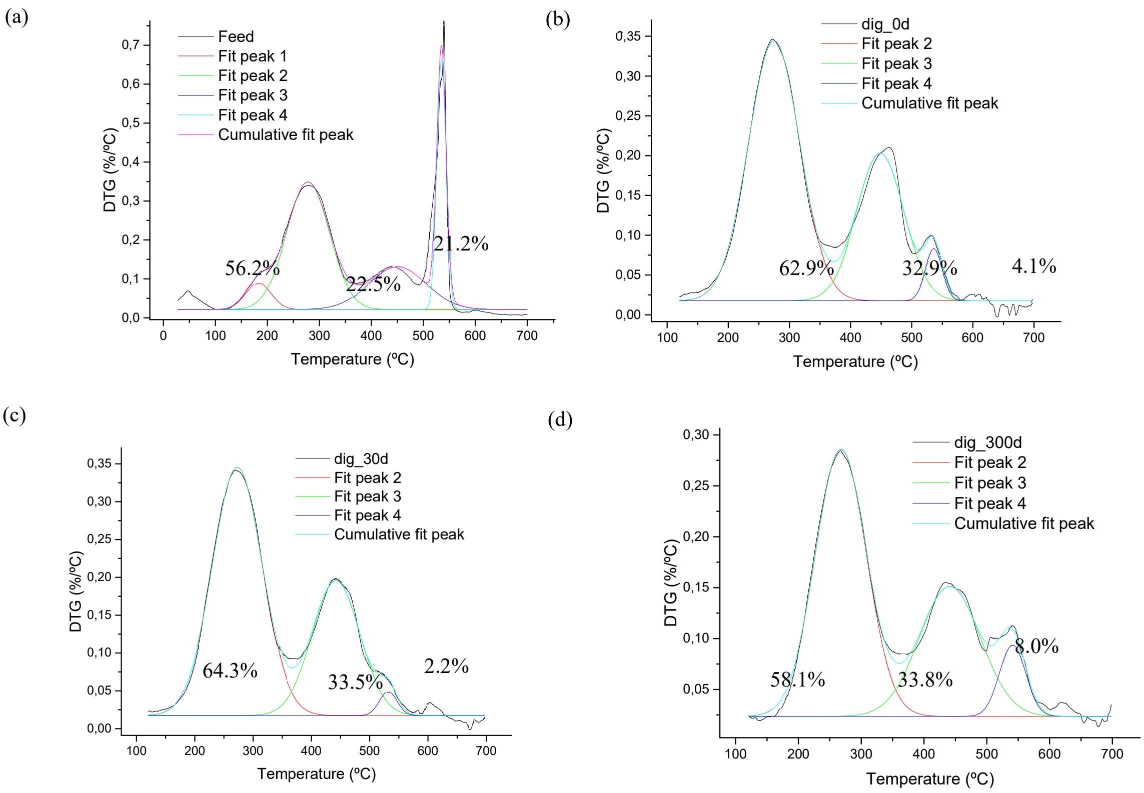


**Figure 5.** Deconvolution procedure applied to DTG curves obtained from the (a) feeding material (Feed), (b) digested at day 0 (dig_0d) and (c) stabilized at days 30 (dig_30d) and (d) 300 (dig_300d). Percentages correspond to values derived after normalizing DTG curves, representing the material loss associated with each peak.

The main striking difference is the almost complete removal of the 550 °C peak and increase of that one located at 450 °C as observed in Figure 5b. These peaks experienced significant changes associated with stabilization. Figure 5 also shows the normalized values for the area of each

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deconvoluted peak. In the case of the feed, the percentage of labile compounds is the sum of both peaks (denoted as Peak 1 and Peak 2) found in the temperature range of 150 – 350 °C.

There are no apparent changes in the proportion of labile compounds, but regarding the combustion stage taking place at around 440 – 450 °C, the feed sample shows a content of 22.5% of materials, whereas on average, digested samples have a content of 33.4%. The peak at high temperature observed in the feeding sample was greatly degraded, resulting in a small peak being discernible in digested samples, which increases to 8.0% in the stabilized sample at day 300, probably as a result of being composed of microbial complex compounds.

The activation energy (Ea) was estimated based on the previous deconvolution procedure. Table 2 reports values derived from single peaks and weighted averages estimated for the sample. There is a significant decrease in the average value of Ea for digested samples. Results are in the range of those reported by different authors when evaluating manures. Under an inert atmosphere, Chen et al. [48] reported a value of 123.8 – 126 kJ/mol for fresh cattle manure. Similar results were also reported by Yuan et al. [49] when evaluating different isoconversional methods, with an Ea value ranging from 170.3 to 195.5 kJ/mol (average values of Ea obtained at different conversion stages), and slightly higher values were reported by Gu et al. [44] (200 – 232 kJ/mol). Sharara and Sadaka [50] studied swine manure samples of different origins, reporting lower values, in the range of 98 – 116 kJ/mol, also under inert atmospheres. Estimations of Ea derived from oxidizing atmospheres are usually lower than those derived from pyrolysis [51,52], thus explaining the lower values derived from the present study.

**Table 2.** Activation Energy (Ea) and peak location (peak maximum location, Pmax) obtained from the different samples using the deconvolution procedure.

| Sample | Peak 1 | | Peak 2 | | Peak 3 | | Peak 4 | | Global |
|---|---|---|---|---|---|---|---|---|---|
| | Pmax (°C) | Ea (kJ/mol) | Pmax (°C) | Ea (kJ/mol) | Pmax (°C) | Ea (kJ/mol) | Pmax (°C) | Ea (kJ/mol) | Ea (kJ/mol) |
| feed [1] | 183 | 63.0 | 278 | 54.0 | 450 | 69.5 | 535 | 955 | 249.5 |
| dig_0d | - | - | 275 | 52.3 | 448 | 99.5 | 536 | 356.8 | 80.43 |
| dig_30d | - | - | 273 | 50.8 | 442 | 92.0 | 532 | 322.7 | 70.6 |
| dig_300d | - | - | 267 | 52.5 | 441 | 76.9 | 541 | 241.0 | 75.9 |

[1] The feed sample was the only one characterized by the presence of labile compounds with volatilization starting at temperatures close to 150 °C.

Fernandez-Lopez et al. [53] reported that fresh and digested swine manure samples showed similar Ea values, thus indicating a negligible effect of anaerobic digestion. However, different authors have described changes in the thermal profiles, where a reduction in labile compounds and increased structural complexity were demonstrated using different spectroscopic techniques [8,11,50]. Results obtained in this study align with those of Otero et al. [55], who also reported a decrease in Ea after digesting cattle manure.

### *3.4. FTIR*

Spectroscopic analysis aids in understanding changes in organic material suffered during microbial degradation. This technique has provided useful information when evaluating the composting of lignocellulosic material and wastes [56–58] and studying the digestion process of different substrates [8,59–61]. Figure 6 shows the spectra obtained from the feed and digested samples.

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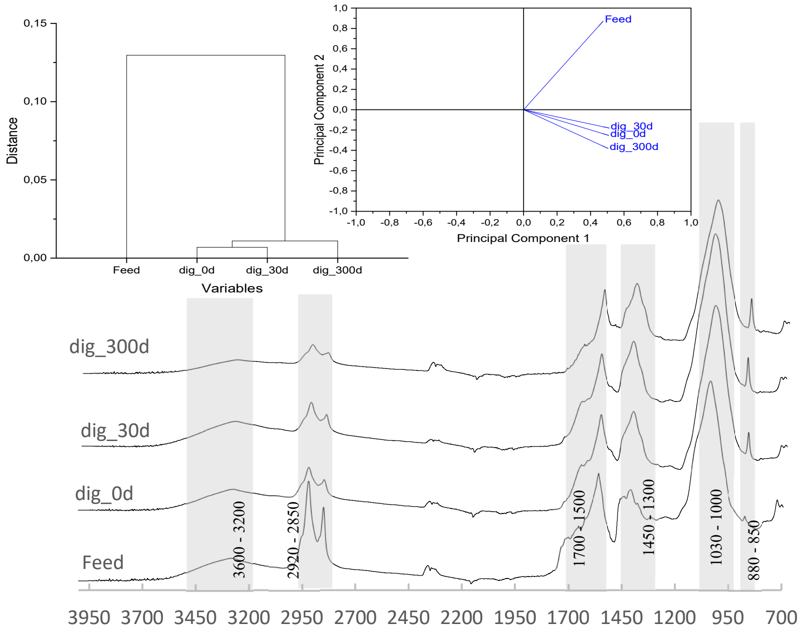


**Figure 6.** FTIR spectra obtained from the feed and digested samples. Results from PCA and dendogram are also shown.

The digestion process was characterized by the degradation of simple aliphatic structures, as observed from the intensity in signals of the two peaks located at the 2900 – 2800 $cm^{-1}$ range, and the accumulation of aromatic and lignin type structures, which is evident from the peaks located at 1560 and 1400 $cm^{-1}$. A similar trend was reported by Provenzano et al. [54] and Fernández et al. [62], indicating the recurrent presence of a signal at around 1420 $cm^{-1}$ representing microbial material. HCA and PCA indicated a strong correlation between all digested samples, clearly differentiating from the feed, finding a greater distance in the correlation between the feed and the sample stabilized at 300 days.

Table 3 shows the identification of FTIR signals. Changes experienced by the organic material are associated with the thermal behavior previously evaluated. A decrease in the aliphatic fraction is observed here by a decrease in signals ascribed to the region of 2950 – 2820 $cm^{-1}$ and those located at approximately 1440 – 1410 $cm^{-1}$. This decrease in aliphatic content coincided with results from thermal analysis by the lower magnitude of the mass loss peak observed in DTG curves at the low-temperature range (150 – 350 °C). Aromatization of the stabilized samples is observed by the clear peak around 1600 – 1520 $cm^{-1}$, explained by the accumulation of lignin-type materials. Increased mineralization of the digested samples is also observed in the FTIR spectra by the higher intensity associated with the signal at around 880 – 870 $cm^{-1}$ [56].

**Table 3.** Identification of main signals found in FTIR spectra derived from the feed sample (swine manure and glycerin mixture) and digested samples (at different stabilization periods).

| Spectrum signal ($cm^{-1}$) | Main components | Reference |
|---|---|---|
| 3600 - 3200 | OH groups in phenol, carbohydrate and carboxylic acid. NH- of amines and amides | [63] |
| 2920 and 2850 | $CH_3$- and -$CH_2$- stretching in aliphatic structure | [64] |
| 1720 | C=O functional group of carboxylic acids | [59,65] |

| | | |
|---|---|---|
| 1650 - 1620 | Amides groups and carboxylates (C=O stretching) | [66,67] |
| 1550 - 1520 | H-N and C-N in amides II<br>Lignin-type structures (-$CH_2$- vibrations) | [7] |
| 1440 - 1410 | CH bending in aliphatic structures.<br>O–H deformation.<br>C=O stretching of phenols and anti-symmetric COO– stretching | [68] |
| 1240 | C-N vibration (amide III), phospholipids ($PO_2$) asymmetric stretching | [69,70] |
| 1030 | Polysaccharides (C-O stretching) | [7] |
| 870 | Carbonate ions | [71] |

## 4. Conclusions

Clear changes in the structure of organic matter were observed during the extended stabilization of swine manure by anaerobic digestion. A decrease in the total amount of organic material was observed with time, along with the aliphatic nature of the sample and an increase in carbonates. An accumulation of recalcitrant structures was observed from FTIR spectra. The thermal valorization of digestion must consider changes in the thermal behavior of the organic material. Therefore, any technology considered for treating digestates (pyrolysis, gasification, hydrothermal carbonization, among others) must take into account the different nature of these raw materials and the effect the high mineral content would have in tar formation and the presence of polycyclic aromatic hydrocarbons. The integration of anaerobic digestion and thermal valorization of digestates should consider the energy demand associated with digestate drying and its thermal characteristics. A critical assessment of the two parameters is necessary, along with evaluating their effect on the global energy balance of the process. Future research will consider these aspects.

**Author Contributions:** Conceptualization, X.G. and R.G.; methodology, R.G.; software, D.C.P; validation, X.G. and R.G.; formal analysis, X.G.; investigation, S.G.R; resources, X.G.; data curation, S.G.R; writing—original draft preparation, D.C.P; writing—review and editing, S.G.R; visualization, X.G.; supervision, X.G. All authors have read and agreed to the published version of the manuscript.

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