

ORIGINAL ARTICLE

# Enhancing anaerobic digestion of poultry blood using activated carbon

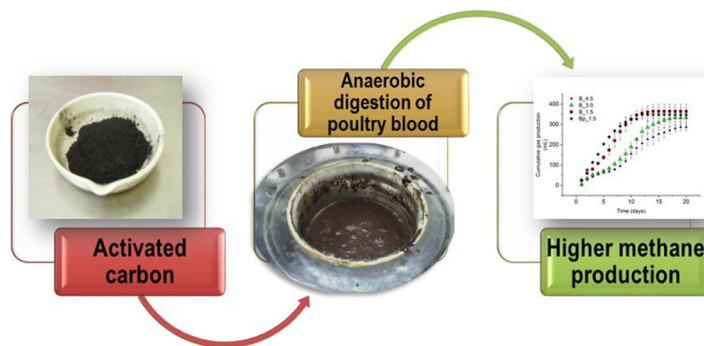


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GRAPHICAL ABSTRACT



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ABSTRACT

The potential of using anaerobic digestion for the treatment of poultry blood has been evaluated in batch assays at the laboratory scale and in a mesophilic semi-continuous reactor. The biodegradability test performed on residual poultry blood was carried out in spite of high inhibitory levels of acid intermediaries. The use of activated carbon as a way to prevent inhibitory conditions demonstrated the feasibility of attaining anaerobic digestion under extreme ammonium and acid conditions. Batch assays with higher carbon content presented higher methane

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production rates, although the difference in the final cumulative biogas production was not as sharp. The digestion of residual blood was also studied under semi-continuous operation using granular and powdered activated carbon. The average specific methane production was  $216 \pm 12$  mL CH<sub>4</sub>/g VS. This result was obtained in spite of a strong volatile fatty acid (VFA) accumulation, reaching values around 6 g/L, along with high ammonium concentrations (in the range of 6–8 g/L). The use of powdered activated carbon resulted in a better assimilation of C3-C5 acid forms, indicating that an enhancement in syntrophic metabolism may have taken place. Thermal analysis and scanning electron microscopy (SEM) were applied as analytical tools for measuring the presence of organic material in the final digestate and evidencing modifications on the carbon surface. The addition of activated carbon for the digestion of residual blood highly improved the digestion process. The adsorption capacity of ammonium, the protection this carrier may offer by limiting mass transfer of toxic compounds, and its capacity to act as a conductive material may explain the successful digestion of residual blood as the sole substrate.

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**Introduction**

Anaerobic digestion is a well-known process for the production of biogas, a mixture of methane and carbon dioxide, which is currently used and exploited at a local level in an efficient way. However, when considering large scale usage of biogas, the capital investment and upgrade costs associated with methane valorisation make it unfeasible in some cases [1]. Furthermore, the accumulation of toxic compounds and anaerobic intermediaries may cause a severe decrease in biogas yields, therefore compromising plant feasibility.

The use of adsorbents in anaerobic digestion has been widely studied to avoid inhibitory stages during the processes associated with high ammonia levels or to prevent odour emissions from the treatment of livestock wastes [2,3]. Many studies have focused on the addition of natural zeolites and clays for treating nitrogen-rich wastes [4] or their post-treatment to remove phenolic compounds [5]. Recently, the combination of anaerobic digestion and adsorption processes has led to using industrial clay residues [6], zeolites synthesised from coal fly ash [7], and low-cost adsorbents such as biochar [8] in an attempt to reduce the cost of the process.

Traditionally, slaughterhouse wastes have been considered a suitable co-substrate in digestion systems, with several authors reporting a marked increase in biogas production and stable performance of digesters as long as certain operational constraints are taken into account [9–11]. High amounts of solid organic by-products are generated from poultry slaughterhouses. These wastes usually comprise poultry manure, feathers, blood, and intestinal wastes [12]. Slaughterhouse wastes present a high potential for energy valorisation; this is particularly true for gastrointestinal residues characterised by high-fat content [13]. However, the main problems that arise when digesting this type of waste are associated with foaming and flotation of sludge, along with ammonium inhibition due to the high protein content [14,15].

The number of studies dealing with the digestion of residual blood has increased in the recent years [10,16,17]. However, residual blood is a complex substrate with high nitrogen content; therefore, its use as co-substrate has been widely studied, but attempting its individual digestion can lead to various difficulties due to the accumulation of ammonium in the reactor. Nitrogen is an essential nutrient in biological processes, but excess nitrogen can cause ammonia inhibition, as frequently

reported, with inhibitory levels noted to be around 4–6 g N/L expressed as total ammonia nitrogen. It should also be taken into account, however, that particular characteristics of the process and substrate, such as pH condition, temperature, and type of seed sludge, among others, have a major effect on the degree of inhibition [18,19].

The digestion of nitrogen-rich wastes has been attempted with the aid of a carbon-rich substrate in order to increase the carbon to nitrogen (C:N) ratio. The digestion of abattoir wastes with mixtures of food wastes and cheese whey was evaluated by Allen et al. [20], who reported an increase in digestion performance based on the higher capacity of the reactor to treat the organic matter, which was associated with an increase in carbon content. The treatment of slaughterhouse wastes containing residual blood and grease was also investigated by Ortner et al. [21]. These authors reported volatile fatty acid (VFA) build-up (> 8.0 g/L) and high free ammonia levels. The decrease in the organic loading rate that was achieved in an attempt to lower the ammonium content in the reactor to values below 6 g/L resulted in a successful alternative for the recovery of the digestion process and gas yields. Similar results were also reported by Alvarez and Lidén [22], who studied the co-digestion of slaughterhouse wastes containing residual blood from cattle and swine with food wastes. These authors reported on a decrease in biogas yield due to the accumulation of ammonia in the reactor.

To the author's knowledge, this paper is the first work focused on the anaerobic digestion of poultry blood as the sole substrate. The aim of the present study was to evaluate the digestion of residual blood under semi-continuous conditions. The effect on gas production and performance of the digester was evaluated when using granular and powder activated carbon as way to prevent ammonium and VFA inhibitory conditions. The digestion process was assessed with the aid of thermal analysis and scanning electron microscopy (SEM) for evaluating changes in the carbon surface and organic material.

**Material and methods***Inoculum and substrate sources*

The inoculum was obtained from a laboratory digester treating slaughterhouse waste adapted to an environment rich in ammonia. The acclimation procedure was performed based

on the one described by Fierro and co-workers [23]. The reactor was fed with slaughterhouse waste with a hydraulic retention time (HRT) of 50 d, and then the feeding volume was increased to reach a 36 d HRT. The inoculum thus obtained was stored under ambient conditions to allow for the further release of biogas. The residual poultry blood was obtained from a local poultry slaughterhouse in León (Spain) and pasteurised (60 min, 70 °C) prior to its use in digestion experiments. Characteristics of the inoculum and substrate are presented in Table 1.

Granular and powdered activated carbon (from Sigma-Aldrich) was used in batch and continuous digestion experiments. The granular activated carbon had a particle size of 12–20 mesh and a mean surface area of 600 m<sup>2</sup>/g. The powdered activated carbon had a particle size of 200–325 mesh, with an approximate surface area of 750 m<sup>2</sup>/g.

#### Batch digestion experiments

The batch digestion of blood was carried out in batch assays. Sixteen replicates were run over the course of 20 days. Two replicates were removed from the bath for liquid-phase analysis on days 1, 3, 7, 9, 11, 15, and 20.

Digestion experiments for evaluating the effect of adding activated carbon were also performed under batch conditions. These experiments were carried out using different proportions of poultry blood and activated carbon. The mixtures were made using ratios of blood (total solids (TS)) to mass of activated carbon added, of 4.5, 3.0, and 1.5 [4]. This ratio expresses the amount of organic blood material added to the reactor (measured in terms of TS) and the mass of activated carbon added in terms of a proportion; in other words, for every 4.5 g of TS of residual blood, 1.0 g of activated carbon is added in the first case, 1.5 g in the second case, and 3.0 g, in the third case. Experiments were performed in 100 mL Erlenmeyer flasks incubated at 37 ± 1 °C in a water bath under stirring conditions (200 rpm). The inoculum to substrate (I:S) ratio was kept constant for all batch experiments with a value of 2.0 to avoid adding an alkali solution for pH correction and prevent VFA overloading. Reactors were denoted as B\_4.5, B\_3.0, and B\_1.5 based on the ratio of activated carbon added in the mixture. For each assay, 20 replicates were initially set and two replicates were withdrawn from the water bath on days 1, 3, 5, 8, 11, 15, 18, 22, 25, and 30. The volume of biogas produced was measured using liquid displacement bottles. Values obtained were corrected to standard temperature and pressure.

**Table 1** Characteristics of residual blood and inoculum used in the study.

Chemical parameters	Residual blood	Inoculum
Total organic carbon (%) <sup>a</sup>	31.9 ± 1.2	32.2 ± 0.7
Organic matter (%) <sup>a</sup>	54.8 ± 2.0	55.4 ± 1.3
Nitrogen Kjeldahl (%) <sup>a</sup>	12.3 ± 1.6	5.7 ± 0.8
C/N	2.7 ± 0.4	5.6 ± 0.8
Ammonia (mg/L)	8360 ± 175	3400 ± 68
TS (g/L)	54.0 ± 1.3	12.0 ± 0.3
VS (g/L)	46.2 ± 1.6	7.5 ± 0.2

<sup>a</sup> Dry basis.

An additional batch experiment was performed using powdered activated carbon as an adsorbent at a ratio of 1.5 in order to evaluate any improvement in biogas production. This experiment was denoted Bp\_1.5. In addition, three control assays were run in parallel to measure the background methane production from the inoculum. The residual biogas was subtracted from the total production in each case.

Cumulative biogas curves were fitted to a modified Gompertz equation (1). This model has been successfully tested for adjusting biogas data obtained from batch digestion assays using residual blood and co-substrates [10]:

$$P_{(t)} = P_{max} \cdot \exp \left[ -\exp \left[ \frac{R_{max}}{P_{max}} e^{-(\lambda - t) + 1} \right] \right] \quad (1)$$

where  $P_{(t)}$  is the cumulative biogas production (l),  $P_{max}$  is the maximum biogas value obtained (mL),  $R_{max}$  is the maximum biogas production rate (mL/d),  $\lambda$  is the lag-phase time (d), and  $e$  is 2.71. The software Origin 6.0 was used for fitting data to the equation and obtaining the model parameters  $P_{max}$ ,  $R_{max}$ , and  $\lambda$ .

#### Adsorption assay

Adsorption experiments were carried out using 100 mL Erlenmeyer flasks with magnetic stirrers at 37 °C. These flasks contained 100 mL of a solution with a 5 g/L concentration of a single component. Adsorption tests were performed on acetic, propionic, butyric, and ammonia chloride solutions (reagents purchased from Merck), adding to each Erlenmeyer flask 0.5 g of granular activated carbon. The amount of activated carbon added was the same as that added to test B\_1.5. The concentration of the different species was regularly measured during a 24 h period.

#### Semi-continuous anaerobic digestion

Semi-continuous digestion was carried out in reactors with a working volume of 900 mL. Reactors worked under static conditions using granular activated carbon in one case and powdered in the other. Reactors were denoted as RG when using granular carbon and RP when powdered activated carbon was added. Manual agitation was performed once a day before and after the feeding procedure. Reactors were kept at 37 ± 1 °C and worked at an HRT of 36 d with an organic loading rate (OLR) of 1.15 g VS/L d. Reactors were manually fed every day using a ratio of poultry blood (TS content) and mass of activated carbon of 3.0. Reactors were evaluated for a 75 d period. Daily gas production was measured using a reversible device with liquid displacement and a wet-tip counter. Gas composition was analysed by gas chromatography. TS, VS, pH, alkalinity, ammonia, chemical oxygen demand (COD), and VFAs were routinely analysed.

#### Analytical techniques

Kjeldahl nitrogen (KN), TS, volatile solids (VS), COD, alkalinity, ammonium, and pH were measured in accordance with standard methods [24]. Free ammonia (FA) was calculated based on the equilibrium equation (2) based on Bonmati and Flotats [25]. Total ammonia (TAN) values were measured using the ion selective electrode.

$$[\text{NH}_3] = \frac{[\text{NH}_3 + \text{NH}_4^+]}{1 + 10^{\text{p}K_a - \text{pH}}} \quad (2)$$

Organic matter was measured using the Walkley–Black method [26], and the total organic carbon (TOC) content was calculated from the organic matter value, using a correlation factor of 1.72. Biogas composition was analysed using a gas chromatograph (Varian CP 3800 GC) equipped with a thermal conductivity detector. A column 4 m long, packed with HayeSepQ80/100, followed by a molecular sieve column 1 m long, was used to separate CH<sub>4</sub>, CO<sub>2</sub>, N<sub>2</sub>, H<sub>2</sub>, and O<sub>2</sub>. The carrier gas was helium, and the columns were operated at 331 kPa at a temperature of 50 °C. VFAs were analysed using a gas chromatograph (Varian CP 3800 GC) equipped with a Nukol capillary column (30 m × 0.25 mm × 0.25 μm) from Supelco (Bellefonte, PA, USA) and a flame ionisation detector. The carrier gas was helium. The temperature of the injector was 250 °C, and the temperature of the oven was initially set at 150 °C for 3 min and thereafter increased to 180 °C. Samples were previously centrifuged (20 min, 3500g) and the supernatant was cleaned using the procedure described by Cuetos et al. [27].

Inoculum, activated carbon, and digestate samples from the semi-continuous reactors were collected for thermal analysis. Thermogravimetric analysis was performed using a Setaram TGA92 analyser. Five milligrams of sample was used in each experiment. Analyses were carried out under an air flow of 100 mL/min at a heating rate of 15 °C/min from room temperature (~22 °C) to 850 °C. The mass loss (TG) and derivative curves (DTG) were represented as a function of temperature.

The surface of activated carbon and solids obtained after dismantling the reactors was analysed by SEM. Digestates samples were obtained after sedimentation (3 d) of the reactor liquor. Solids were dried at 105 °C and ground using a ball mill Retsch MM200. Samples were sputter-coated with gold in high vacuum (0.05–0.07 mbar) conditions with a coater Blazers SCD 004. The samples were examined using a JOEL JSM 6840 LV scanning electron microscope.

## Results and discussion

### Batch digestion experiments

Residual blood used in batch digestion experiments as a substrate presented a low C:N ratio (as shown in Table 1). The production of biogas obtained from the digestion assay of poultry blood was 46.5 L/kg VS. This system was characterised by high pH values throughout the digestion assay (around 8.8). Furthermore, the concentration of ammonium was about 4500 mg/L, resulting in the presence of high levels of free ammonia (with an average value of 1813 mg/L). These conditions translated into a severe inhibitory stage, thus explaining the low biogas yield. VFA build-up was attained on the fourth day of the experiment, with the acetic content reaching a value of 2184 mg/L, while acid species corresponding to C3–C5 forms reached an average value of 350 mg/L. The high and rapidly attained ammonia and acid concentrations probably prevented further degradation of the organic material.

On the other hand, the addition of granular activated carbon resulted in successful digestion of the residual blood. Experiments presented similar values of final cumulative biogas production (see Fig. 1(a)), with the difference in the pro-

cess being associated with initial stages of the batch experiment. A clear improvement is easily observed when comparing gas data with those of the control experiment (Blood).

Experiments with lower contents of activated carbon demonstrated a lower rate at the beginning of the cumulative curve (B\_4.5 and B\_3.0). The lower gas production rate of these experiments is related to the VFA build-up, which adds to the negative effect caused by the high ammonia content in these reactors. During the first 10 days, VFAs presented high values, which in turn explain the low degradation rate of the substrate (Fig. 1(b) and (c)). However, the anaerobic microflora could circumvent this stage, assimilating the whole amount of VFA initially produced. The addition of a larger amount of activated carbon resulted in a higher biogas production rate during the first five days of the batch experiment, which was in line with the lower amount of VFAs measured. The biochemical methane production value obtained from experiment B\_1.5 was 317.4 ± 31.8 mL CH<sub>4</sub>/g VS. A similar value was obtained when using powdered activated carbon. From the curves presented in Fig. 1(a), it is clear that the form of carbon used (either granular or powder) does not greatly affect the gas production rate.

The presence of a larger amount of granular activated carbon not only affected the initial amount of VFA accumulated in the system but also affected the concentration of ammonium in the reactor. Although the initial value was similar in the three experiments, the average value obtained during the experimental period was much lower for the B\_1.5 system (Fig. 1(e)). This behaviour aided in reducing the inhibitory stage associated with protein conversion and therefore results in a better digestion performance. The B\_1.5 system had a higher methane production rate during the first 10 days (34 mL biogas/d, calculated as the slope of the curve during the first 10 days) because of lower levels of accumulated inhibitory substances during this batch test. These inhibitory conditions were prevented by the increase in the amount of activated carbon added.

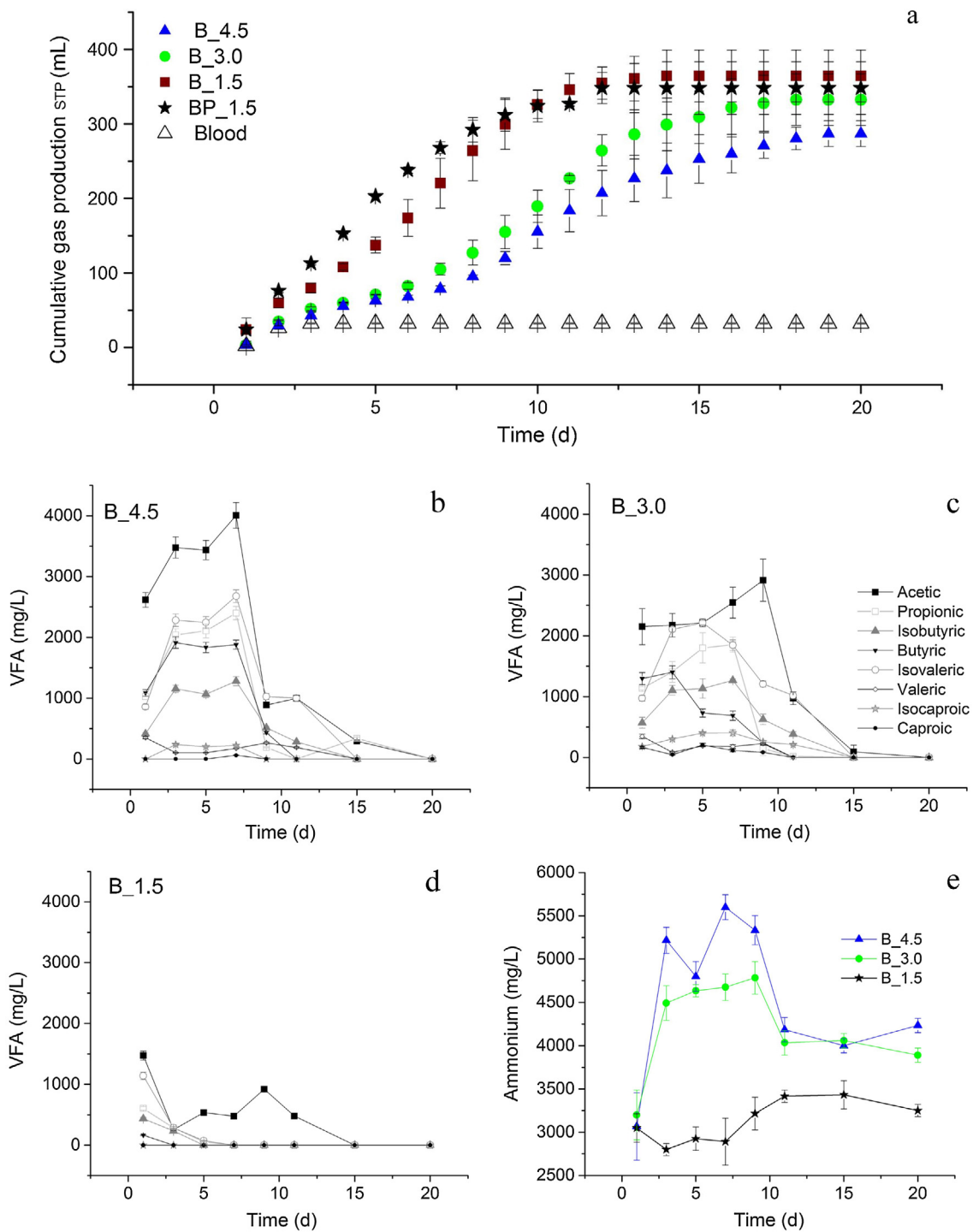
Results of the application of the modified Gompertz model to gas production curves obtained from the different experimental sets are presented in Table 2. All data sets presented a good fit to the model, as observed from the high *R*<sup>2</sup> values. There is a reduction in *λ* values due to the increase in the amount of activated carbon added to the reactor. This factor also affected the gas production rate; those experiments with higher amounts of activated carbon also presented higher *R*<sub>max</sub> values.

The pH of the three systems was about 8.0, with this value being lower than that obtained for the batch experiment digesting blood and having a strong effect on the ionic species present in the solution (e.g., ammonia form). The pH in the digestion system is affected by the equilibrium species of carbonates, ammonium, and VFA, with ammonia levels substantially influencing the buffer capacity of the solution [28]. In the present study, the adsorption capacity of activated carbon also plays a crucial role in the final pH value attained, thereby relieving the inhibitory conditions responsible for preventing the degradation of the substrate.

### Results from adsorption assays

Fig. 2 shows the results obtained from adsorption assays using the maximum amount of activated carbon tested in previous

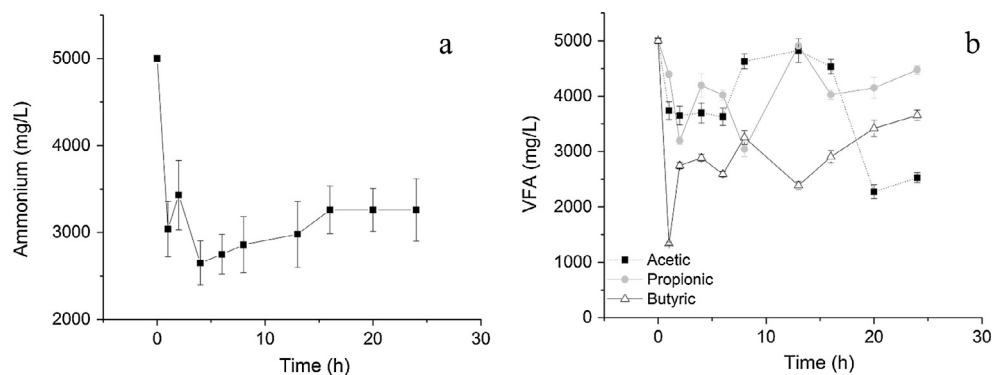




**Fig. 1** Cumulative gas production from batch experiments (a). Volatile fatty acid (VFA) evolution at blood:carbon ratio of 4.5 (B\_4.5) (b), ratio of 3.0 (B\_3.0) (c) and ratio of 1.5 (B\_1.5) (d), ammonium values for the three batch tests (e).

**Table 2** Results from biogas data fitted to the modified Gompertz equation.

Digestion system	Mass of activated carbon added (g)	$P_{max}$ (mL)	$R_{max}$ (mL/d)	$\lambda$ (d)	$R^2$
B_1.5	0.54	378.80 ± 5.94	43.24 ± 9.45	1.47 ± 0.13	0.991
Bp_1.5	0.54	352.50 ± 1.86	47.58 ± 4.21	0.67 ± 0.05	0.998
B_3.0	0.27	383.41 ± 17.31	27.79 ± 10.90	2.75 ± 0.34	0.987
B_4.5	0.18	346.21 ± 19.36	21.21 ± 9.01	2.59 ± 0.45	0.988



**Fig. 2** Ammonium (a) and VFA values (b), measured over the course of the adsorption experiments when adding 0.5 g of activated carbon.

experiments. Although the concentration curves represented in Fig. 2 could not be fitted to any particular adsorption model, the results indicate a great capacity for retaining ammonium, which is one of the major inhibitors when digesting residual blood. Ammonium levels could be reduced to around 3000 mg/L, and this value obtained after 24 h of the experiments was similar to that obtained at the end of the batch digestion process when using the highest addition of activated carbon (B\_1.5).

In the case of VFA, the effect of acetic and propionic acid was less pronounced, while butyric acid was highly retained by the adsorbent during the initial hours, finally reducing its concentration in solution after 24 h to about 3600 mg/L. However, in all cases, the concentration of any of VFA presents high variability during the time of the experiment. Just as in the previous case, VFA adsorption curves could not be fitted to any adsorption model, but the curves obtained were indicative of a mild retention of acids onto the activated carbon surface, which may have partially alleviated that at the inhibitory stages microorganisms are subject to when dealing with the digestion of residual blood.

The adsorption of water/organic mixtures is a complex phenomenon because of the nonuniformity of the adsorbent surfaces and specific interactions of polar molecules with oxygen-containing surface groups [29]. The adsorption equilibrium for organics on activated carbon is mainly dependent on the chemistry of the carbon surface. Heterogeneous oxygen groups play an important role in the adsorption process, as well as hydrogen bonding and the water adsorption effect [30,31]. Carboxylic functional groups of the organic acids are present in solution in their negative form ( $\text{COO}^-$ ), experiencing repulsive electrostatic interactions with the negative carbon surface. In contrast to these repulsive forces are the formation of H-bonds by carboxylic groups in organic acids. Gun'Ko et al. [29] proposed the formation of a chain or a cluster of organic acids associated with the H-bonding mechanism, which could lead to pore blockage, similar to water adsorption. The erratic behaviour observed in Fig. 2b is the result of the net effect of these two opposing mechanisms.

The addition of adsorbents like biochar has been demonstrated to enhance the digestion process by reducing the lag phase and improving the resistance of anaerobic microflora to highly acidic conditions [32]. In the present experiment, a similar effect was observed with the addition of activated car-

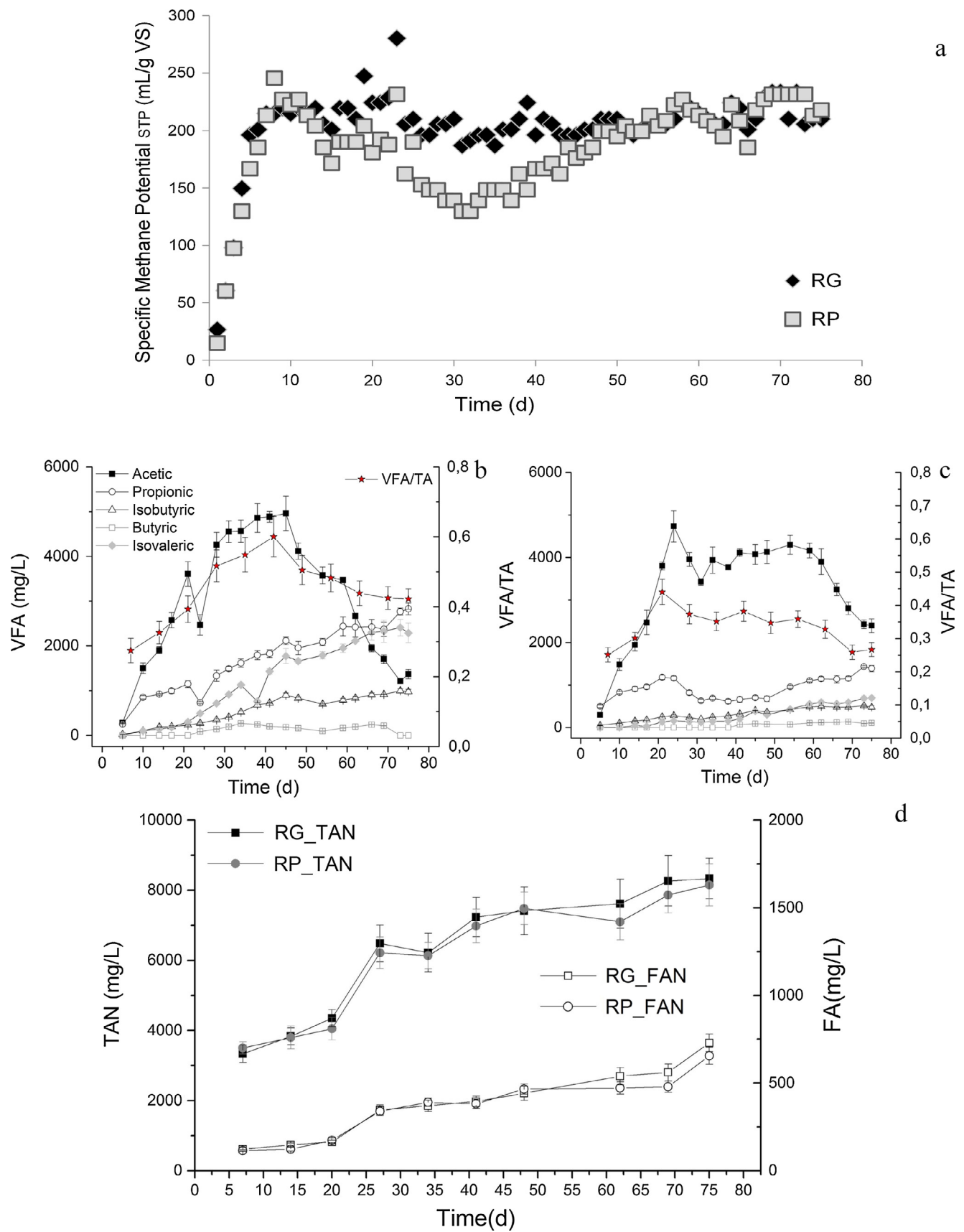
bon, yielding better performance even though a high VFA content was observed. Adding activated carbon to the digestion batch tests resulted in the complete digestion of the substrate, even with the lower dose of activated carbon. However, the economics of this approach are unfavourable because the addition of this adsorbent would increase operating costs of the plant by 50%, when considering calculations for an industrial digester ( $3500 \text{ m}^3$ ) based on the economic assumptions proposed by Fierro et al. [33] at a price of 2500 €/t of activated carbon.

#### *Semi-continuous digestion tests*

The reactors were operated under semi-continuous conditions, and the results are shown in Fig. 3. Systems demonstrated low biogas production at the beginning of the study, which increased progressively during the first eight days of operation. Once a period equivalent to an HRT had elapsed, the biogas profile became stable, with an average specific methane production (SMP) value of  $216 \pm 12 \text{ mL CH}_4/\text{g VS}$ . The methane content in biogas ranged from 52 to 56% for both reactors. Although the SMP was far below the value obtained from batch tests, this result is in any case remarkable taking into account the adverse conditions in which the digestion was taking place.

Reactors presented an initial accumulation of acetic acid, which caused a serious build-up of VFAs during most of the operating period (Fig. 3). Values between 4000 and 5000 mg/L were reached after 20 days of operation. However, a decreasing trend in the content of acetic acid was observed for both reactors when the experiment was near the end, with this trend starting at an earlier stage for the RG system (on day 45). In spite of this phenomenon, the reduction observed in acetic acid concentrations during the last days of the experiment was not associated with increased biogas production for any of the reactors.

The affinity of activated carbon for acetic acid was shown to be rather irregular, with a low adsorption capacity being obtained in particular hours of the experiment and higher adsorption levels being obtained at the end of the 24 h adsorption test. Therefore, it may be inferred that the adsorption of VFA may not play a main role in the improvement of digestion performance; other phenomena, such as favouring microbial metabolism, may be the reason behind the improved results.



**Fig. 3** Specific methane production (SMP) data obtained from semi-continuous operation of static reactors (a). Volatile fatty acid (VFA) measurements from RG (b) and RP (c) reactors. Ammonium measurements (d). *RG*: Reactor with addition of activated carbon in granular form. *RP*: Reactor with addition of activated carbon in powder form.

The addition of activated carbon to anaerobic digesters has been evaluated by Xu et al. [34]. These authors reported the use of this adsorbent with different particle sizes, reporting an enhancement of methane production associated with the benefits caused by syntrophic metabolism of alcohol and VFAs. Therefore, the adsorption and faster degradation of VFAs observed in the present experiments lead us to expect positive effects on methane production.

The decreasing trend of acetic acid observed for both reactors may then be related to the ability of microorganisms to find proper protective sites. In the case of the RP system, this takes place around day 60, which may be associated with the lower amount of protective sites and this carbon offers to the anaerobic microflora because it is unable to efficiently remove this acid from the liquid phase. This increased amount of acetic acid found in the RP systems explains the observed instabilities in daily gas production at the end of the first HRT period.

In general, the free acetic acid levels attained in any of the reactors were much higher than the levels reported as inhibitory by Fukuzaki and co-workers [35] when evaluating the methanogenic fermentation of acetate. The RG system presented a maximum free acetic acid value of 95  $\mu\text{M}$  on day 24, while the maximum value in the RP system was 69  $\mu\text{M}$  on day 21.

Total VFA values were higher than 6 g/L for most of the experimental period for reactor RG and were close to this value for reactor RP. An inhibitory threshold of 6 g/L was reported by Siegert and Banks [36]; therefore, the digestion of blood was attained in extreme conditions for the anaerobic microflora. The ratio of VFA to total alkalinity (VFA/TA) is also shown in Fig. 3b and c. This ratio is considered to give a good indication of the stability of the digestion process when its value is below 0.4 units [27]. In the present study, the reactor supplemented with granular activated carbon presented values above this limit throughout the operation period, giving a clear indication of the severity of the inhibitory conditions experienced. On the other hand, the addition of powdered activated carbon (Fig. 3c) helped in reducing this ratio and reaching stability levels. This better performance may be influenced by the size of carbon particles used which allowed for an enhancement in the assimilation of acids with more than two carbon atoms.

In the case of C3–C5 acid forms, these acids demonstrated an increasing trend that was more pronounced in the RG system. The propionic acid concentration continuously increased during the time of the experiment for this reactor, reaching final values around 3000 mg/L (Fig. 3b). Inhibitory effects of propionic acids have been reported, with a value of 900 mg/L being indicated as the threshold [37]. The presence of iso-forms has been associated with instabilities based on the different degradation rates of VFA and the inhibitory effects caused by high acetic and propionic levels on iso-form degradation [38]. However, different reports have also been published indicating that high levels of propionic acid do not necessarily affect methane production in an adverse way [39]. In the present experiments, high levels of propionic acid and iso-forms were reached, and still a stable biogas production was obtained. The addition of powdered activated carbon into the reactor affected the behaviour of iso-forms. For this latter system, the difference between the initial and final values for the C3–C5 acid forms (in any form) was less,

probably an indicator of better adsorption performance (Fig. 3c).

The presence of the solid phase in the reactor may offer protection to microorganisms against these harsh environmental conditions, allowing for the stable behaviour of biogas evolution reported in Fig. 3. However, the different behaviours observed for these C3–C5 forms when the addition of the activated carbon is carried out in the powder form are not reflected in an improved performance. Fig. 3c shows the evolution of VFA in the RP system. Propionic acid has a mean value of  $930 \pm 270$  mg/L, which is much lower than that obtained for reactor RG, while isovaleric shows a clear increasing trend with the final value being around 700 mg/L. The higher specific surface area of the powdered carbon probably offers a greater adsorption capacity for these acids; the SMP value obtained from this system, however, was not higher than that of the RG system, indicating that the lower VFA content obtained in this reactor was not enough to further improve the digestion process.

The values of pH measured during the working period for the two reactors were in the range of 7.0–7.5 with alkalinity values greater than 15 g/L. Values of VFA measured were those typical for start-up stages, acid phases, and systems subjected to overloading [40]. The ratio of VFA-to-alkalinity was close to 1.0 at the end of the study, which leads to considering the digestion as a failed anaerobic process. However, thanks to the buffering capacity provided by the high protein levels in the residual blood, stable pH values were obtained from the entire process.

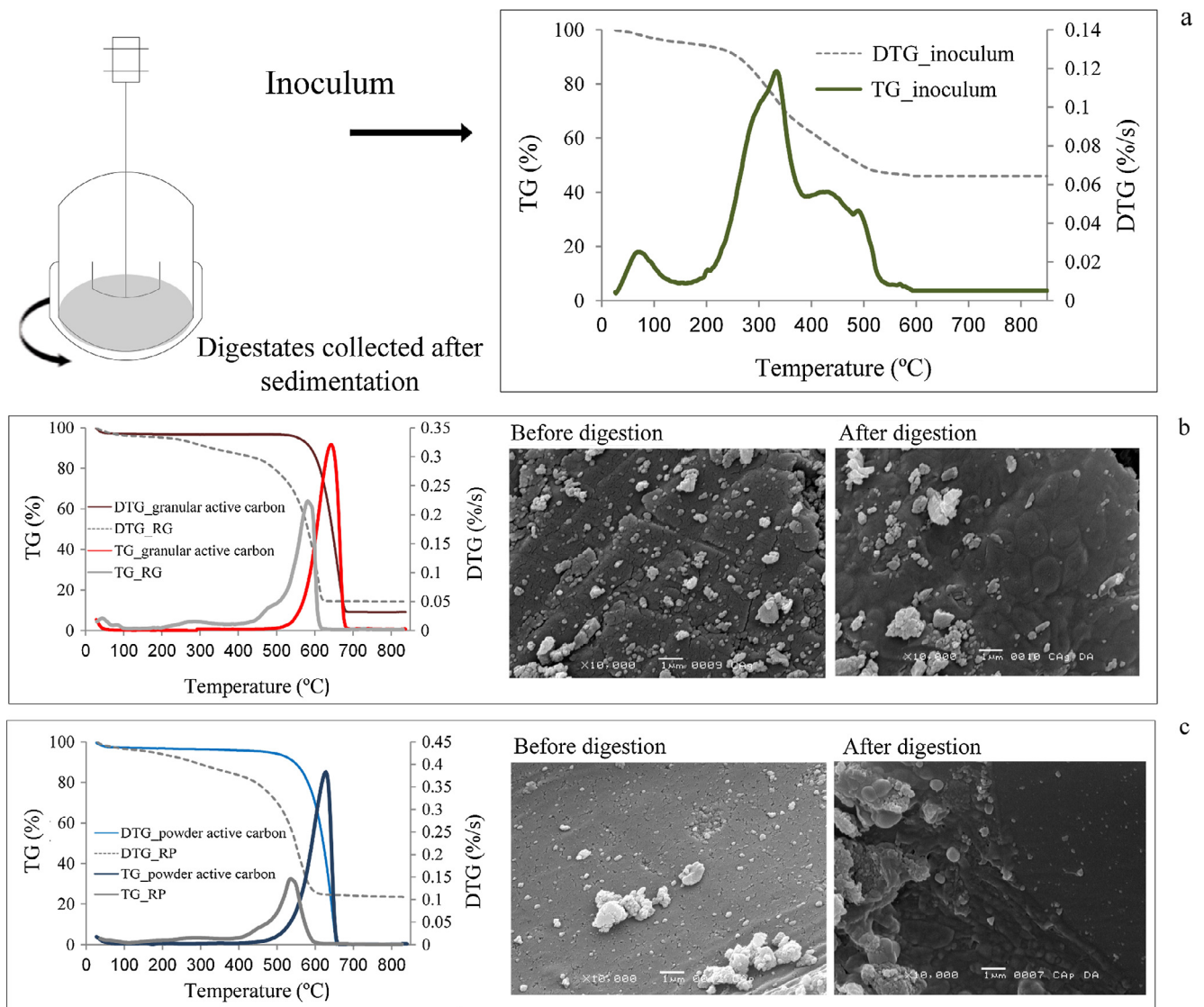
The high ammonia levels reached during the digestion process helped maintain high pH values despite the high VFA build-up (See Fig. 3d). However, these values can be considered inhibitory based on the results reported by Moestedt and co-workers [41] who set the threshold values as 1.0 g/L of  $\text{NH}_3$  for evidencing negative effects of methane production. The different physical properties of the activated carbon used had no significant effect on ammonium evolution. Ammonium content presented a similar profile in both studied reactors, reaching levels around 8000 mg/L at the end of the second retention time.

The aggregation of cells is a key factor for efficient methanisation as a direct result of an efficient electron transfer between obligate  $\text{H}_2$ -producing acetogens and methanogens. Direct interspecies electron transfer (DIET) is a syntrophic metabolism in which free electrons flow from one cell to another without being shuttled by reduced molecules such as molecular hydrogen or formate [42]. DIET has been suggested as the reason for obtaining better degradation rates of simple substrates and higher biogas yields in anaerobic systems when carbon-based conductive materials are added [43], as was demonstrated by Rotaru et al. [44] and Zhao et al. [45] when studying the use of activated carbon. In the present experiments, enhancement via DIET may be similarly relevant, as the improvement of digestion may not be completely explained by the adsorption phenomenon.

#### *Results from thermal analysis and SEM*

Because of the presence of carbon particles inside the reactor, the measurement of TS and VS was not useful. The digestate samples taken at the end of the process were analysed by means of thermal analysis and SEM. Fig. 4 shows the thermal





**Fig. 4** Results from thermal analysis of inoculum sample (a), and thermal analysis - SEM images for granular activated carbon/RG digestate (b) and powdered activated carbon/RP digestate (c).

profile of the inoculum sample and changes experienced by the original carbon sample and solids collected from the reactors at the end of the digestion. The mass losses experienced around 300 and 450 °C are associated with the presence of microbial biomass and the residual organic material obtained from the digestion process; in particular, these mass losses are associated with the organic carbon content of the sample [46]. The profiles in Fig. 4b and c show the TG curves for the original carbons and the mixture of digestate and activated carbon obtained after the biological transformation. There is a relevant increase in the ash content after the digestion process due to the inorganic material accumulated in the reactor. This increase is typically observed in waste digestion processes as the mineralisation of the organic matter takes place [47].

Fig. 4 also shows the differences in DTG profiles for the same samples. For both reactors, the residual organic material mixed with the activated carbon causes the early loss of mass at around 200 °C. It is also responsible for the interaction between digestate stable compounds and the activated carbon

particles, which is observed as early oxidation of these latter particles in the DTG curves. Digestates are usually characterised as experiencing an early mass loss associated with labile compounds and a high-temperature oxidation, which is normally associated with the thermal degradation of either recalcitrant compounds or organic molecules with complex structure. These compounds may have already been present in the original material or they may have been generated during the microbial decomposition [48].

The SEM images also show the changes experienced by the carbon surface due to the presence of microorganism inside the reactor. The image shows the carbon surface before the digestion process and the surface of solid material obtained at the end of the semi-continuous operation. An increase in roughness is noticeable, being more evident in the case of the powder activated carbon (Fig. 4b and c).

The study by Xu et al. [34] on the use of activated carbon in anaerobic digesters reported the development of a layered structure of the anaerobic sludge granule, where the outer

layer was dominated by *Bacteria* and the inner one by *Archaea*. These authors also attributed the improvement in digester performance to the increased microbial population of methanogenic bacteria and syntrophic metabolism bacteria. *Methanosarcina* and *Methanoculleus* were the predominant species, along with *Bacteroidales*, *Desulfuromonas*, and *Thermotogaceae*, which were also found to be more abundant in the reactor operating with powder activated carbon.

In a different study, Zhao et al. [45] reported a change in microbial populations when evaluating anaerobic reactors for propionate/butyrate degradation with the aid of activated carbon. *Methanosaeta* and *Methanosarcina* species constituted a dominant part (81.49%) of the communities in their initial seed sludge, which significantly decreased when propionate and/or butyrate was used as the sole carbon source. However, they described no effects on the syntrophic metabolism of the substrate. On the other hand, Dang et al. [43] reported the main role of *Methanosarcina* (which are capable of DIET) when conductive materials are incorporated in anaerobic digesters. These authors highlight the benefits of accepting electrons from conductive materials by *Methanosarcina*, because the conversion of acetate to methane yields little energy, and this type of organism typically grows slowly on acetate. Electrons obtained via DIET might enhance their metabolism and even increase their ability to produce methane by acetate decarboxylation.

In the present study, the use of activated carbon allowed for digestion of the substrate, which was in no other way possible. The growth of microorganisms on the carbon surface probably promoted DIET; this phenomenon, in addition to the protective effect associated with mass transfer limitation of inhibitory compounds and the adsorption capacity of the activated carbon, aided in the degradation of the organic material by anaerobic microflora.

## Conclusions

The addition of activated carbon to the digestion of residual blood greatly improved the digestion process due to its adsorption capacity for ammonium, resulting in lower levels of ammonium during batch digestion experiments. The presence of the solid phase (addition of granular and powdered activated carbon) probably acted as a protective layer for microorganisms, resulting in successful digestion under semi-continuous conditions. Although inhibitory levels of VFA and  $\text{NH}_4^+$  were reached, biogas production was maintained with low variations, and this behaviour may be explained by the presence of protective sites offered by the activated carbon particles.

Although specific methane productions were similar for the two semi-continuous reactors tested, the use of granular activated carbon resulted in higher accumulation of propionic and iso-forms. However, the use of powdered activated carbon resulted in better assimilation of C3-C5 species, probably indicating enhancement of syntrophic metabolism.

## Conflict of Interest

*The authors have declared no conflict of interest.*

## Compliance with Ethics Requirements

*This article does not contain any studies with human or animal subjects.*

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