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# THE EFFECT OF SOLID–LIQUID EFFLUENTS FROM ANAEROBIC DIGESTERS ON SOIL MICROBIAL ACTIVITY
## A calorimetric study

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A calorimetric procedure is developed to study the effect on the soil of the effluents resulting for the anaerobic digestion of slaughtering houses residues. DSC was used to study the pyrolysis properties of the effluent and the soil while isothermal calorimetry is applied to study the microbial activity in the effluent and to assess on its effect on the microbial activity of the soil where the industrial digester will be situated. The calorimetric data were studied together with the chemical and biological properties of that residue. Results showed that effluent is constituted by low levels of carbon and high levels of nitrogen. The power–time curves of the effluent have the typical shape of microbial growth yielding microbial growth rate constants between 0.37 and 0.53 $h^{-1}$ for about 4 and 11 h. The addition of the effluent to the soil decreases the heat of pyrolysis with time and stimulates the heat flow rate of the microbial metabolism.

***Keywords:*** *anaerobic digestion, calorimetry, soil*

## Introduction

The transition to a sustainable energy economy is one of the most important issues facing us in the 21st century. It involves the introduction of new energy sources for economic reasons dealing with the arising prices of a barrel of oil and for ecological reasons dealing with the sustainable use of our energy resources to minimize the impact of $CO_2$ in the atmosphere. One of the alternatives for synthesizing renewed energies is the conversion of biomass to biogas via anaerobic digestion. Biomass is a renewable energy resource which includes a wide variety of organic supplies. One of them are the slaughtering houses wastes. The economic activity linked to that sector generates 30000 tons of residues in Spain that can be processed and converted to biogas through anaerobic biodigestion. That process can be performed under controlled bioreactors by different methanogenic bacteria [1, 2]. The reaction yields a gas constituted by a mixture of methane and $CO_2$ but it also generates a solid–liquid effluent that is rich in nitrogen, phosphorous and potassium that could be used as soil fertilizer [3, 4]. The carbon content of this product has been reduced during the digestion and for that reason the effluent may favour the carbon to nitrogen ratio in soils that are nitrogen limited. This kind of product has been poorly investigated and there is very few information about its effect on soil. For that reason, calorimetry together with some chemical analysis is applied here to assess about the properties and the effect of these compounds on soil microbial activity. Calorimetry has the advantage to give information about two of the most important indicators of the soil quality: organic matter and microbial activity. The quantity and nature of the organic matter can be studied by differential scanning calorimetry (DSC) while the microbial activity can the continuously monitored by isothermal calorimetry [5, 6]. It is very easy to evaluate how a certain compound affects the heat rate of the soil microbial metabolism since every metabolic change can be continuously detected and registered through the power–time curves [7]. Here a calorimetric procedure is showed to assess about the effect of the effluents resulted of the anaerobic digestion of slaughtering houses residues on the quality and nature of the soil organic matter through DSC experiments and on the soil microbial activity via isothermal calorimetry.

## Experimental

### *Soil sampling and chemical analysis*

The soil sample was collected in the place where the industrial bioreactor is going to be settled. The carbon, C, and nitrogen, N, of the soil was determined by a Perkin-Elmer 2400B elemental analyzer. Both yield the C to N ratio, C/N of the sample. The same analysis was applied to the effluent to give the C and N composition. The analysis was done to the solid and liquid part of the effluent. It was also determined the fat content, the gas, the $H_2S$ and the volatile acids of the product of the anaerobic digestion.

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*DSC of the soil and effluent*

Curves of the soil and the effluent were obtained with a differential scanning calorimeter (Mettler TA4000-DSC822). DSC experiments were conducted with a heating rate of 10°C $min^{-1}$ under a flux of air and nitrogen (20 $cm^3$ $s^{-1}$) [8]. The effluent was analyzed by DSC as a solid-liquid mixture. Then it was dried at 105°C for 12 h and the solid part obtained was analyzed by DSC too. The same experimental procedure was applied to the soil samples and to a mixture of the soil and the effluent. Soil and effluent are mixed and kept for 5 days. Each day subsamples of the mixture are taken for DSC measurements in order to see the effect of the effluent on the soil curves using that of the soil as reference.

*Isothermal calorimetry*

The microbial activity of the effluent and that of the soil was recorded with a Calvet MS 70. A 9 mL of capacity calorimetric ampoule was filled with the effluent and hermetically closed. Then it is introduced in the calorimeter together with the reference ampoule filled with an inert substance ($Al_2O_3$). This procedure was performed with 5 samples named as cc1, cc3, cc6, cc7 and cc9 in order to check the reproducibility of the results. The measurements were done at 25°C. The same calorimeter was used to measure the heat rate of the soil basal metabolism. For those measurements 3 g of soil is introduced in the 9 mL calorimetric ampoule and 3 g of $Al_2O_3$ is used as reference. The measurements were done at 25°C too. The microbial activity is showed as power–time curves in both cases (effluent and soil). Then, 1 mL of the effluent is added to 3 g of soil and introduced in the calorimeter ampoule to compare the power–time curves under these conditions to that obtained from the soil without the effluent.

## Results and discussion

The C and N of the effluent and the soil are shown in Table 1. The solid part of the effluent represents the 1.86% of the whole product. It has the 40% of the total C and 0.044% of the total N. The liquid part of the effluent contains most of the N of the effluent. The C/N ratio of the solid part in 16.80 and that of the total effluent 1.65. It is not allowed to use animal compounds as fertilizers with a C/N ratio higher than 10. Thus it would not be used as compost but as liquid fertilizer. None of the solid or liquid part of the effluent exceeds the 6% limit of nitrogen given for these compounds.

**Table 1** Percentages of carbon, nitrogen, and hydrogen, of the liquid and solid part of the effluent and that of the soil sample

| Effluent | C/% | N/% | H/% |
|---|---|---|---|
| Liquid | 0.36±0.03 | 0.28±0.04 | 4.26±0.98 |
| Solid | 40.35±0.39 | 2.40±0.06 | 5.00±0.13 |
| Total | 0.71±0.08 | 0.43±0.06 | 5.48±1.29 |
| Soil | 6.52±0.36 | 0.36±0.03 | 0.50±0.10 |

Average±SD, *n*=3

The soil has a C/N ratio of 18. High C/N ratios are linked to immobilization of N [9, 10]. The N content of the effluent would increase up to 0.64% that of the soil, and the C/N ratio would decrease to a more favourable 10.75 value.

The curves obtained from the total effluent are shown in Fig. 1. The area limited by these curves was related to the C content of these samples to give the heat of pyrolysis of the effluent. Those values were –125 kJ $mol^{-1}$ C for the total effluent and –42.55 kJ $mol^{-1}$ C for the solid part of the effluent that contains the C. These values are very low and far from those values associated to the combustion enthalpy for carbohydrates and organic matter [11]. The nature of the C in the effluent could be inorganic. Both curves show two well defined exothermic peaks at about 293 and 443°C indicating the combustion of the organic compounds of the total effluent (solid and liquid). Figure 2 shows the curve obtained for the soil sample. The heat of pyrolysis calculated for the soil is –346 kJ $mol^{-1}$ C. This value is closer to the enthalpy values for organic compounds than those of the effluent. The curve of the soil has also two exothermic peaks well refereed by the literature. Those peaks are usually named as LER and HER [12, 13]. The temperature of the peaks in Fig. 2 fit well with those temperatures given by other authors. The first peak is caused by the combustion of aliphatic and carboxylic groups while the second peak is due to the combustion of aromatic compounds [14].

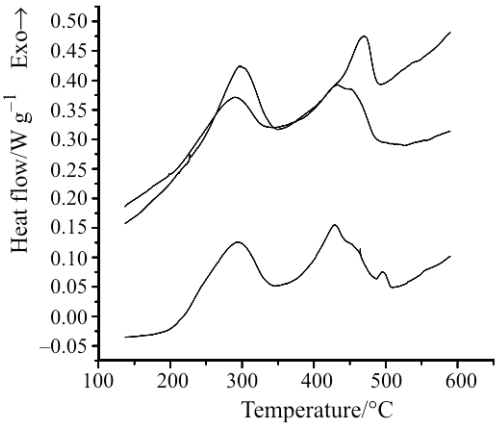


**Fig. 1** Curves of the solid–liquid effluent resulting from anaerobic digestion of slaughtering houses residues

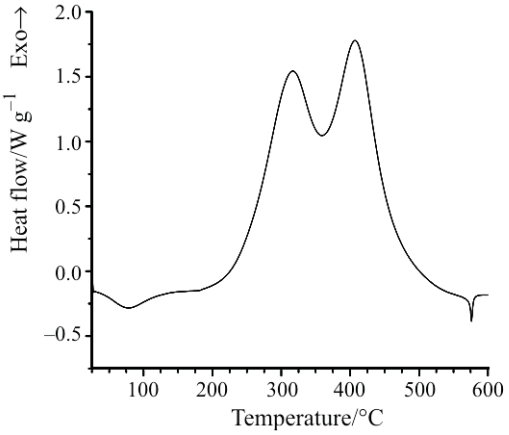


**Fig. 2** Curve of the soil where the effluent is tested

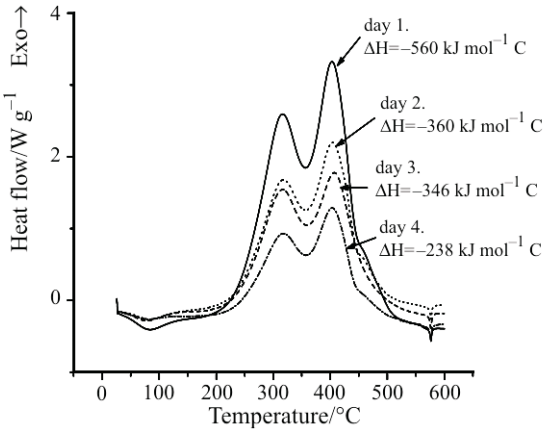


**Fig. 3** Curves of the mixture effluent–soil incubated for 4 days

Figure 3 shows the curves obtained when the soil sample is incubated with the effluent for four days. It is observed a clear decrease of the area limited by the curves as the incubation time increases. This is reflected in a fall of the heat of pyrolysis of that mixture with time. The heat values are also shown in Fig. 3. That decrease could be attributed to enhancement of soil microbial activity and degradation of the soil organic matter. For this reason the soil microbial activity was followed by isothermal calorimetry. Figure 4 shows the power–time curves of the soil sample together with that obtained when the effluent is added to the soil. There is a clear enhancement of the heat flow rate in samples with the effluent that can be related to stimulation by N sources. This increment can be given in quantitative terms by an increase in the soil mass specific heat rate [15] from –0.76±0.07 J g$^{-1}$ of soil and day, J g$^{-1}$ d$^{-1}$, registered for the soil basal metabolism, to –2.15±0.74 J g$^{-1}$ d$^{-1}$ when the effluent is added to the soil. The modification of the C/N ratio in soil by external N sources enhances the degradation of the soil C that probably modifies the quantity and nature of the organic matter. This process could

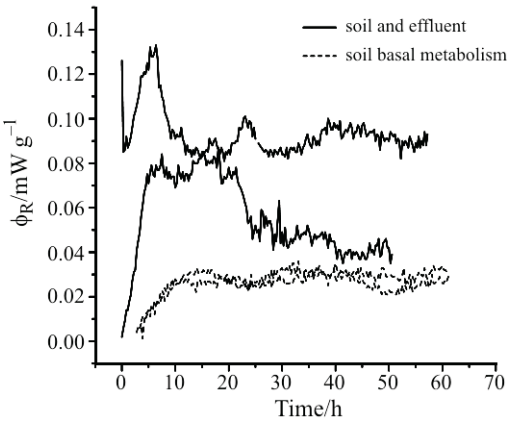


**Fig. 4** Power–time curves of the soil basal metabolism and those of the soil metabolism after amendment with the effluent. There is a clear increase of the heat flow rate when the effluent is added to the soil

be responsible for the observed changes of the heat of pyrolysis of the mixture soil-effluent given in Fig. 3.

The stimulation of the organic matter degradation is a very common process associated to the use of N fertilizers [16]. For this reason the effluent should be added to the soil together with an external C source to avoid losses of organic matter.

Another feature to take into account to test possible fertilizers is the microbial population that the fertilizer introduces in soil. The digestion of the slaughtering houses residues is done by anaerobic bacteria that are theoretically inhibited by the presence of oxygen. Therefore they would be inactivated when added to the soil. To introduce more knowledge about the microbial activity of this residue, it was studied by isothermal calorimetry also in order to register any possible microbial activity. The power–time curves obtained are shown in Fig. 5. It can be observed that the power–time curves of the effluent show a clear

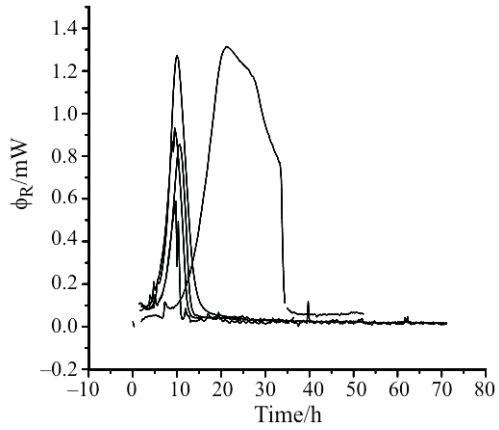


**Fig. 5** Power–time curves recorded from 5 samples of the effluent of the digestor. All of them show the typical shape associated to microbial growth reactions

**Table 2** Values of the total heat dissipated, $Q_T$, by the microbial growth reaction, the microbial growth rate constant, μ, the values of the maximum heat flow rate dissipated by the microbial growth reaction, $P_{max}$ and the latency time, τ, which is the duration of the microbial lag phase before exponential growth

| Sample | $Q_T$/J g$^{-1}$ | μ/h$^{-1}$ | $P_{max}$/mW g$^{-1}$ | τ/h |
|---|---|---|---|---|
| cc1 | 4.16 | 0.526 | 0.135 | 8.9 |
| cc3 | 2.63 | 0.534 | 0.137 | 4.5 |
| cc6 | 1.82 | 0.420 | 0.109 | 4.4 |
| cc7 | 1.45 | 0.480 | 1.577 | 5.4 |
| cc9 | 0.79 | 0.376 | 0.907 | 5.3 |

**Table 3** Analysis of the products of the anaerobic digestion studied by isothermal calorimetry

| Sample | Fats/ mg L$^{-1}$ | $H_2S$/ ppm | Gas/ L dial | Volatile acids/ mg L$^{-1}$ |
|---|---|---|---|---|
| cc1 | 110 | 430 | 7063 | 3840 |
| cc3 | 120 | 450 | 9070 | 2980 |
| cc6 | 600 | 470 | nd | 2590 |
| cc7 | 690 | nd | 8468 | 2590 |
| cc9 | 750 | 450 | 9498 | 3680 |

shape associated to microbial growth reactions. The shape of these curves follows a gaussian distribution that permits to compare the curves by ANOVA. The result yielded significant differences among plots.

All of them have an exponential phase that permits the calculation of the microbial growth rate, μ, [17, 18]. They also have a lag phase that give the latency time, τ, of the reaction and the quantity of microbial biomass through the Sparling´s equation [19]. The integral of these curves give the total heat dissipated by the microbial reaction, $Q_T$. These data associated to microbial kinetics are shown in Table 2. They permit to study the plots in quantitative terms and to associate the differences found among the plots to different products of the residue under digestion given in Table 3. Results show that the effluent has an active biomass that ranges from 533 to 744 μg of biomass C that grows exponentially at a rate that varies from 0.37 to 0.53 h$^{-1}$ for a period of time from 4 to 11 h. After that time, the heat flow rate stabilizes due to a metabolic change from a growth reaction to a maintenance metabolism characterized by values of heat flow rate of 0.026–0.061 mW. The inhibition of the microbial growth reaction is caused by the fat content of the residue. This is given by a significant correlation found between the values of $Q_T$, $P_{max}$ and μ given in Table 2 and the fat content determined for the residue given in Table 3. The equation resulting from the correlation existing between the μ values and the fat content give a value of μ=0.57 h$^{-1}$ if the quantity of fats is zero. This value is close to that reported for aerobic microbial growth with acetate as C source ($\mu_{max}$=0.7 h$^{-1}$). It is far for the value given for anaerobic reactions linked to the methanogenic bacteria (μ=0.015 h$^{-1}$) [20]. The aerobic degradation of acetate or acetic acid is also limited by the quantity of fats that act as alosteric inhibitors of the oxidative reaction linked to the microbial growth [21]. The μ values directly calculated from the power–time curves suggest an efficient metabolism compatible with the use of low energy substrates [22]. These results are very striking since the process under study is supposed to take place by anaerobic bacteria. The fact that the manipulation of the residue to be studied by calorimetry causes the registered metabolism suggest the existence of another type of bacteria like facultative anaerobic since the conditions of the bioreactor avoid the existence of strict aerobic bacteria. A strain of bacteria that fits well with these features is *E. coli*. The quantity of *E. coli* that can be in the fertilizers is under control, for that reason we suggest a more detailed microbiological analysis to confirm the existence of these bacteria in the effluent.

## Conclusions

The proposed procedure shows that the effluent stimulates the microbial activity in the soil. That is given by changes in the heat of pyrolysis of the organic matter of the soil sample calculated by DSC and by a clear enhancement of the heat flow rate of the soil after adding the effluent. The calorimetric study of the effluent detects the existence of microbial population and defines the nature of the detected metabolism. It permits to warn easily and fast about the quantity and the possible strain of the bacteria growing in order to avoid intoxications of the medium where the effluent is going to be used.

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