



## Feasibility of anaerobic co-digestion of poultry blood with maize residues



M.J. Cuetos<sup>a</sup>, X. Gómez<sup>a,\*</sup>, E.J. Martínez<sup>a</sup>, J. Fierro<sup>a</sup>, M. Otero<sup>b,c</sup>

<sup>a</sup> Chemical and Environmental Bioprocess Engineering Department, Natural Resources Institute (IRENA), University of León, Avda Portugal 41, 24009 León, Spain

<sup>b</sup> Centre for Environmental and Marine Studies (CESAM), Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

<sup>c</sup> Department of Applied Chemistry and Physics, University of León, Campus de Vegazana, 24071 León, Spain

### HIGHLIGHTS

- Poultry blood and maize were digested under batch and semi-continuous conditions.
- TG, SEM and FTIR analyses were useful in evaluating digestion performance.
- Methane yields of up to  $188 \pm 21$  L CH<sub>4</sub>/kg VS were obtained under batch tests.
- Accumulation of organic material was observed under semi-continuous operation.

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### ABSTRACT

The potential of anaerobic digestion for the treatment of poultry blood was evaluated in batch assays at laboratory scale and in a mesophilic semi-continuously fed digester. The biodegradability test performed on poultry blood waste showed a strong inhibition. Maize residues were used as co-substrate to overcome inhibition thanks to nitrogen dilution. Under batch operation, increasing the maize concentration from 15% to 70% (volatile solids (VS) basis) provided an increase of biogas from  $130 \pm 31$  to  $188 \pm 21$  L CH<sub>4</sub>/kg VS. In the semi-continuous mesophilic anaerobic digester, the biogas yield was  $165 \pm 17$  L CH<sub>4</sub>/kg VS fed, as a result of strong volatile fatty acid (VFA) accumulation. Although physical modifications of maize particles were observed by Scanning Electron Microscopy (SEM), an incomplete degradation was confirmed from analysis of digestates. Furthermore, Fourier Transform Infrared (FTIR) spectroscopy analysis demonstrated that along with VFA build-up, an accumulation of non-degraded materials took place.

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### 1. Introduction

Anaerobic co-digestion of slaughterhouse wastes has been considered a feasible alternative for increasing biogas potential in conventional digesters. However, the use of slaughterhouse wastes as co-substrates must fulfil requirements established by Animal By-Products Regulations (ABPR, EC 1069/2009; 142/2011). Slaughterhouse wastes are characterized by presenting high nitrogen concentrations. This fact makes imperative the addition of a co-substrate in order to achieve mixtures with balanced C/N ratio that allows a decrease in nitrogen concentration and enhance biogas yields as consequence (Cueto et al., 2009; Lehtomäki et al., 2007). Although nitrogen is an essential nutrient for anaerobic microorganisms, ammonia inhibition has been frequently reported when treating wastes with high nitrogen content and inhibitory levels are accepted to be around 4 g N/L (Lobato et al., 2010; Resch et al., 2011).

Few studies in literature deal with the digestion of residual blood (Salminen et al., 2000, 2001; Wang and Banks, 2003). Different mixtures containing blood and slaughterhouse wastes as co-substrates have been recently studied due to their high biochemical methane potential. In this line, Alvarez and Lidén (2008) studied the digestion of mixed fractions of cattle rumen, stomach content and gut fill of swine and blood (with blood cow and blood swine being 34% by total weight) with fruit-vegetable wastes and manure. Recently Marcos et al. (2010) determined the optimal operational conditions when digesting mixtures of wastewater with blood and solid wastes from the meat industry (98% wastewater plus 2% blood and 97% wastewater and blood plus 3% solid offal, respectively (v/v)). In a similar way, Zhang and Banks (2012) reported operating limits for the organic loading rates when studying the co-digestion of sheep blood, mechanically recovered organic fraction of municipal solid wastes and pig intestine with flotation fat.

The combination of energy crops and slaughterhouse wastes offers the possibility of increasing biogas production of existing facilities not only by the effect of balancing nutrients but also by the increase in organic loading rate (Nges et al., 2012). Maize,

\* Corresponding author. Tel.: +34 660736200.

E-mail addresses: [xagomb@unileon.es](mailto:xagomb@unileon.es), [marta.otero@ua.pt](mailto:marta.otero@ua.pt) (X. Gómez).

sunflower, grass and sudan grass are the most commonly used energy crops (Amon et al., 2007a), with maize being the most dominating crop for biogas production (Amon et al., 2007b). Although there are several studies about conversion of energy crops as mono-substrates to biogas (Demirel, 2009; Klocke et al., 2007), the problems associated with the lack of micro and macro nutrients and buffering capacity lead to obtaining more stable systems when co-digesting crop biomasses with manures (Cuetos et al., 2011; Lehtomäki et al., 2007).

On the other hand, biochemical methane potential (BMP) tests that are performed to evaluate the biogas potential of wastes are time consuming. The use of fast and reliable methods that may indicate the adequacy of mixing certain substrates may be a useful tool when deciding the selection of co-substrates and preventing problems associated to degradation of complex compounds and the time needed for complete digestion of substrates in order to reduce post-digestion emissions. This is the case of the use of thermal analysis and Fourier Transform Infrared (FTIR) spectroscopy. These methods have been widely used as a way for evaluating the quality of organic material in an attempt to predict the maturity of biologically stabilised samples (Cuetos et al., 2009; Tintner et al., 2012).

This paper focus, for the very first time, on the anaerobic digestion of poultry blood and mixtures of blood with leaves of maize plants in combination with the proposal of using thermogravimetric kinetic analysis as a tool for the characterisation of substrates prior to digestion experiments. The anaerobic degradation of maize was studied along with the assessment of increments in biogas yield with the addition of blood as co-substrate.

## 2. Methods

### 2.1. Inoculum and substrate sources and characterisation

The inoculum used was obtained from an anaerobic slaughterhouse waste digester adapted to an ammonium-rich environment (Cuetos et al., 2009). This laboratory digester treated a mixture of poultry blood and OFMSW and was operated during 75 days at an HRT of 36 days. The total solid (TS) content of the inoculum was 41 g/L and volatile solid (VS) content was 29 g/L. The poultry blood was obtained from a local poultry slaughterhouse in León (Spain) and then pasteurised (60 min, 70 °C) prior to its use in digestion experiments. The maize used (*Zea mays* L.) was harvested and dried at room temperature. Leaves of maize plants were ground to a particle size around 3 mm to increase the superficial area and the accessibility for microbial action (Palmowski and Müller, 2000).

#### 2.1.1. Analytical techniques used in the characterisation of substrates

Kjeldahl nitrogen and organic carbon analysis were performed according to MAPA (1994). Protein content was calculated from the Kjeldahl-N content using a conversion factor of 6.25. Lipid content was determined using a Standard Soxhlet method (APHA, 1998). TS, VS and ammonia were determined in accordance with Standard Methods (APHA, 1998). Fibre characterisation was carried out by determination of cellulose, hemicellulose and lignin analysis as described by Van Soest et al. (1991). Acid detergent fibre (ADF), neutral detergent fibre (NDF) and crude fibre were determined using an ANKOM<sup>200</sup> fibre analyser.

#### 2.1.2. Thermogravimetric kinetic analysis

Thermal analysis was carried out in a TA Instruments SDT2960 equipment. Samples were further crushed using a 200MM ball mill Retch. The heating rates ( $\beta$ ) applied were of 5, 10, 25 and 50 K/min up to 1000 K under inert atmosphere. These dynamic runs were

carried out on a pan containing approximately 5 mg of the sample and a reference crucible containing the same mass of calcined calcium oxide. Three replicates were run to calculate mean values. During temperature-programmed runs, a continuous flow of 100 mL/min of Nitrogen (purity  $\geq 99.9994\%$ ) at a manometric gauge pressure of 1 atm (101 kPa) was fed into the furnace.

The description of the rate of heterogeneous solid-state reactions can be found in Otero et al. (2011). In this work, two different isoconversional models were applied to non-isothermal thermogravimetric data from the temperature-programmed combustion of the samples. Using dynamic integral TG curves obtained at four different  $\beta$ , in the same way as described elsewhere (Otero et al., 2008), and applying these models, the activation energy values and reaction order associated to the pyrolysis of maize and blood samples were determined. In addition, the use of the independent parallel first-order reactions (IPR) model (Sørum et al., 2001) was applied to DTG curves obtained from substrates. In this model, the decomposition of biomass is described by three independent parallel reactions that can be associated to the decomposition of the constituent components.

### 2.2. Anaerobic digestion tests

#### 2.2.1. Batch digestion experiments

Batch tests were performed at different proportions of maize (15%, 40% and 70% VS) in the mixture with blood. Experiments were performed in 100 mL Erlenmeyer flasks incubated at  $34 \pm 1$  °C in a water bath under stirring conditions (200 rpm). The inoculum to substrate ratio was kept in the range of 1–2 to avoid the addition of alkali solution for pH correction and volatile fatty acids (VFAs) overloading. Reactors were denoted as M\_15, M\_40 and M\_70 based on the proportion of maize added to the mixture. For each assay 20 replicates were initially set and two replicates were withdrawn from the water bath at days 1, 3, 5, 8, 11, 15, 18, 22, 25 and 30. The volume of biogas produced was measured by means of liquid displacement bottles. Values obtained were corrected to standard temperature and pressure.

The digestion of blood was also carried out in batch assays. In this case, 16 replicates were run during 20 days. Two replicates were withdrawn from the bath for liquid-phase analysis at days 1, 3, 7, 9, 11, 15 and 20.

In addition, control assays were run in parallel to measure the background methane production from the inoculum and the biogas production from maize. The residual biogas was subtracted from the total production in each case. The biogas production obtained from maize was used for comparing results obtained from thermogravimetric analysis and to evaluate results from co-digestion assays. Table 1 shows a description of the batch and semi-continuous experiments carried out.

Cumulative biogas curves were fitted to a modified Gompertz Eq. (1), which is a suitable model for describing the process of cumulative biogas production in batch experiments (Nopharatana et al., 2006):

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

where  $P_{(t)}$  is the cumulative biogas production (l);  $P_{max}$  is the biogas production potential (l),  $R_{max}$  is the maximum biogas production rates (l/d) y  $\lambda$  is lag-phase time (d) and  $e$  is 2.71. The software Origin 6.0 was used to fit the equation and determine  $P_{max}$ ,  $R_{max}$  and  $\lambda$ . The biogas yield (expressed as L/kg VS) and the maximum specific biogas production (SBP) rate (expressed as L/kg VS d) were obtained by dividing  $P$  and  $R_{max}$  by the VS content of the substrate.

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