**Evaluation of simultaneous incidence of head space and temperature on biochemical methane potential in food waste**

Miguel R. Casallas-Ojeda¹*, Luis Fernando Marmolejo-Rebellón¹ and Patricia Torres-Lozada¹

**Abstract:** The anaerobic digestion (AD) of food waste (FW) has been recognised as a low-cost and high-efficiency alternative that mitigates the impacts of the final disposal of FW, while facilitating the production of high value-added by-products, such as biogas and digestate. In this study, the simultaneous incidence of two significant variables in the AD of FW were evaluated: the temperature (20, 35 and 55 °C), which affects the kinetics of the process, and the head space (20, 35 and 50%), which can affect the pH and therefore the biochemical methane potential (BMP). A composite central design was used, and response surface methodology (RSM) and kinetic models (the first-order and Gompertz models) were used to analyse the process. At higher temperatures and smaller head spaces, inhibition phenomena were reduced by the consumption of bicarbonate alkalinity and decreasing the pH, resulting in a higher process performance. The optimal conditions were a temperature of 48.9°C and a head space of 20%. The Gompertz kinetic model fit the process dynamics better and with a lower error than the first-order model.

**Subjects:** Agriculture & Environmental Sciences; Environment & Resources; Environmental Change & Pollution

**Keywords:** anaerobic digestion; food waste; head space; process kinetics; temperature

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**PUBLIC INTEREST STATEMENT**

A consequence of growing population, is the production of Municipal Solid Waste and due to their composition predominantly organic (as Biowaste and Food Waste), the anaerobic digestion is a biological alternative that not only allows to increase the useful life of landfills and minimize the generation of leachate and GHG emissions, but also its valorization for the production of energies such as methane. Biochemical Potential of Methane (BMP) assay, is the main tool of prediction, on controlled scale of the potential energetic of the substrate. Both, the head space and the temperature have influence the process and the methan generation, and is necessary to study their joint influence, to stablish the optimal condition for the process.
1. Introduction

In developing countries, the predominant component of municipal solid wastes (MSW) is the organic fraction (50-70%), commonly known as OFMSW or MBW (municipal biowaste) (Oviedo, Marmolejo, & Torres, 2012; Thi, Kumar, & Lin, 2015; Kazo et al., 2018). The MBW consists mainly of food waste (FW: 40-60%) (Cabeza, Maria, Aura, Paola, & Marioc, 2016; Parra-Orobio et al., 2018). Unlike other solid wastes, such as industrial or agricultural waste (Wang & Nie, 2001), FW is considered a heterogeneous flow, mainly because of the difficulty of separating the FW stream from other waste streams, such as paper, plastics and inert waste (De Gioannis, Diaz, Muntoni, & Pisanu, 2008), in addition to the low frequency of source separation and selective collection of MSW (Parra-Orobio et al., 2018; Soto-Paz, Oviedo-Ocaña, Manyoma-Velásquez, Torres-Lozada, & Gea, 2019).

Final disposal (FD) in landfills (LF) is not an appropriate management strategy for MSW (Saer, Lansing, Davitt, & Graves, 2013; Schott, Wenzel, & la Cour Jansen, 2016) because the subsequent environmental impacts, such as the generation of greenhouse gases (GHG) and leachates, however, LF remains the most commonly used management alternative for MBW and FW in most developing countries, such as Colombia, which is covered by Decree 2820 (2010) from the Ministry of Environment, Housing and Territorial Development (MAVDT 2010). Silpa, Yao, Bhada-Tata, and Van Woerden (2016) have reported that the GHG produced worldwide from MSW disposal in LF are on the order of $1.6 \times 10^9$ tons of carbon dioxide (CO$_2$equivalent), representing 5% of global emissions, which deteriorate the ozone layer and negatively impact human health and the environment (Christensen, Manfredi, & Knox, 2011). This form of management also has other unfavourable effects, such as the depletion of the useful life of the FD sites, which are in increasingly limited supply (Manyoma, Orejuela, Torres, Marmolejo, & Vidal, 2015).

However, being predominantly organic, these residues have a high use potential through strategies such as anaerobic digestion (AD) and composting. AD is a biochemical process that occurs in the absence of molecular oxygen and consists of multiple stages involving different microorganisms; the process occurs via the transformation of complex compounds, such as carbohydrates, proteins and lipids, into simpler products such as 90% biogas (mainly methane -CH$_4$), which has a high energy potential and 10% biosolids or digestate (Foresti, Florêncio, Van Haandel, Zaiat, & Cavalcanti, 1999; Satchwell et al., 2018), which has a high potential for use in soil recovery and agricultural activities (Tampio, Salo, & Rintala, 2016). Thus, research on AD technology has increased in recent years (Lin, Xu, Ge, & Li, 2018).

The biochemical methane potential (BMP) assay is a traditional form that is widely used to evaluate the AD viability of a substrate. There is still no consensus on a methodology to determine BMP development, despite the efforts of researchers, such as Angelidaki et al. (2009), Raposo, De La Rubia, Fernández-Cegri, and Borja (2012), Holliger et al. (2016) and Cárdenas-Cleves, Parra-Orobio, Torres-Lozada, and Vásquez-Franco (2016). The temperature and the head space are two significant AD incidence variables (Komilis et al., 2017; Valero, Montes, Rico, & Rico, 2016; Sasa et al., 2019; Mirmohamadsadeghi, Karimi, Tabatabaei, & Aghbashlo, 2019; Ohemeng-Ntiamoah et al., 2019); the temperature affects the process kinetics (Boulalagui et al., 2004; Kim, Oh, Chun, & Kim, 2006; Labatut, Angenent, & Scott, 2014), being the mesophilic (20–45 °C) and thermophilic (46–60°C) ranges typically considered (with the former being most frequently used), and few studies have been performed over the psychrophilic range (< 20 °C) (Connaughton, Collins, & O’Flaherty, 2006; Kothari, Pandey, Kumar, Tyagi, & Tyagi, 2014). On the other hand, the head space can affect the pH of the medium and destabilise microbial consortia and thus the BMP quantification, and the solubilisation of gases, such as CO$_2$, can have a buffer effect on the process (Jankowska, Chwiałkowska, Stodolny, & Oleskowicz-Popiel, 2015; Valero et al., 2016). Ranges of head space between 20 and 50% of the total volume of the reactor are generally used (Li, Liu, Su, & Yan, 2016; Wei et al., 2014; Zhang & Jahng, 2012).

In this study, the simultaneous incidence of temperature and head space on the BMP of FW was evaluated. The FW simulated the characteristics of MBW from a locality that performs separation...
at the source and selective MSW collection, which are fundamental premises of the use of this type of substrate (Han & Zhang, 2017; Lakeh et al., 2019; Razavi, Koupaie, Azizi, Hafez, & Elbeshbishy, 2019; Soto-Paz et al., 2019).

2. Materials and methods

2.1. Substrate and inoculum

A sample was reconstituted with the characteristics of the MBW from a locality that performs separation at the source and selective collection of MSW. The substrate was formed from raw FW that was separated at the source of a university restaurant that serves an average 4338 lunches per day (Parra-Orobio et al., 2018) and a market place in the City of Cali, Colombia. The FW composition was as follows: carbohydrates (55.4%), citrus fruits (25.0%), fibres and minerals (8.2%), non-citrus fruits (8.2%), herbs and others (3.2%) (Parra-Orobio et al., 2018; Soto-Paz et al., 2019).

An inoculum was formed using a 75/25 (V/V) mixture of flocculent sludge and granular sludge, respectively. The first one was obtained from an anaerobic digester of a municipal wastewater treatment plant (WWTP) that serves a population of more than two million inhabitants, and the granular sludge was obtained from an anaerobic reactor of a slaughter plant for cattle and pigs (Parra-Orobio et al., 2018).

2.2. Analytical methods

The FW were characterised in terms of pH (units), total alkalinity (TA, mg CaCO$_3$/L) and bicarbonate alkalinity (BA, mg CaCO$_3$/L), volatile fatty acids (VFA, mg CaCO$_3$/L), chemical oxygen demand (COD, mg O$_2$/L), total organic carbon (TOC, mg/kg), total solids (TS, mg/L) and volatile solids (VS, mg/L), total nitrogen (TN, mg/kg), total phosphorus (TP, mg/kg), lignin (%), cellulose (%), hydrogen (H,%), oxygen (O,%), nitrogen (N,%), carbohydrates (%), proteins (%) and lipids (%), according to APHA et al., (2005), ICONTEC (2004) and ICONTEC, (2011).

The inoculum was characterised according to APHA and WEF (2005), Aquino et al., (2007), ICONTEC (2004), ICONTEC, (2011) and Hu, Wang, and Yu (2004) in terms of the pH, TA, BA, VFA, TS, VS, specific methanogenic activity (SMA, gCOD$_{CH_4}$/g VS·d), hydrolytic activity (HA, gCOD$_{Glucose}$/gVS·d) and specific acidogenic activity (SAA, gCOD$_{Glucose}$/gVS·d).

3. Calculation of theoretical methane potential

Different calculation schemes were used to compare the theoretical methane production with the experimental results. In the first calculation, the stoichiometry of the substrate degradation reaction was used with Equations 1 and 2 (Buswell & Muller, 1952 and Sosnowski et al., 2003). A second calculation (Equation 3) was performed using the percentage of carbohydrates, proteins and lipids in the sample (Lesteur et al., 2010; Raposo et al., 2012; Nielfa, Cano, & Fdz-Polanco, 2015).

$$C_nH_aO_bN_c + \left( n - \frac{a}{2} - \frac{b}{8} - \frac{3c}{4} \right)H_2O \rightarrow \left( \frac{n}{2} - \frac{a}{8} - \frac{b}{4} + \frac{3c}{8} \right)CO_2 + \left( \frac{n}{2} - \frac{a}{8} - \frac{b}{4} + \frac{3c}{8} \right)CH_4 + cNH_3 \quad (1)$$

$$BMP_{theoretical}^{(CH_4/gVS)} = \frac{22.4 \times \left( \frac{n}{2} - \frac{a}{8} - \frac{b}{4} + \frac{3c}{8} \right) \times 1000}{12n + a + 16b + 14c} \quad (2)$$

where the number of moles of carbon, hydrogen, oxygen and nitrogen are represented by n, a, b and c, respectively; 22.4 is the volume in litres (L) occupied by an ideal gas at standard conditions; 1000 corresponds to the conversion factor from L to mL; and 12, 1, 16 and 14 are the molecular weights of carbon, hydrogen, oxygen and nitrogen, respectively.

$$BMP_{theoretical}^{(CH_4/gSV)} \times 415 \times \%Carbohydrates + 496 \times \%proteins + 1014 \times \%lipids \quad (3)$$

where 415, 496 and 1014 are multiplicative constants for the percentages of carbohydrates, proteins and lipids from the FW sample (Lesteur et al., 2010; Raposo et al., 2011; Nielfa et al., 2015).
4. Experimental design
A composite central experimental design was performed in which the simultaneous incidence of the temperature ($X_1$) and the head space ($X_2$) were evaluated using response surface methodology (RSM) (Montgomery, Runger, & Hubele, 2009). In this study, each factor was evaluated at three levels corresponding to minimum, intermediate and maximum values (the normalised values were $-1$, $0$, $+1$, respectively), following studies by Zhang and Jahng (2012), Wei et al. (2014), Deepanraj, Sivasubramanian, and Jayaraj (2015), Li et al. (2016) and Parra-Orobio et al., (2018). Table 1 presents the values used in the design and the different experimental configurations. The BMP was the response variable.

5. Experimental setups and process monitoring
A CB15 industrial food processor was used to grind the substrate to a particle size of less than 30 mm (Izumi et al., 2010; Pearse et al., 2018), with which the PBM assays were developed. A substrate/inoculum (S/I) ratio of 1 gVS$_{\text{substrate}}$/gVS$_{\text{inoculum}}$, was maintained, according Parra-Orobio et al., (2018).

The BMP assays were performed in triplicate in 0.25 L amber glass reactors. To ensure that the manometric measurement of the biogas corresponded mainly to methane, a polypropylene cartridge containing four NaOH beads was placed inside each bottle under the lid to capture CO$_2$ (Holliger et al., 2016; Souto, Aquino, Silva, & Chernicharo, 2010). A manometer (WIKA, model CPG500) was used to record the pressure of the biogas that accumulated in the headspaces of the reactors. The biogas composition was confirmed by chromatography (Shimadzu GC-2014). Nutrient addition was performed, and a control reactor (target) containing only the inoculum and distilled water was used to subtract endogenous production (Cárdenas-Cleves et al., 2016).

The temperature was controlled by maintaining the lowest temperature ($20 \pm 1$ °C) in a WTW TS 606/G12-i incubator; the intermediate temperature ($35 \pm 0.5$ °C) in a WTW TS 606-G/2-i incubator and the high temperature ($55$ °C $ \pm 1.5$ °C) in a WTB binder E-28 oven. The BMP was determined using equations formulated by Aquino (2007), Giménez, Martí, Ferrer, and Seco (2012) and Cárdenas-Cleves et al. (2016), where the proportion of the dissolved methane was used to determine the volume of methane produced under standard conditions. The assays were manually agitated and monitored four times a day (Parra-Orobio et al., 2018), and biogas samples were withdrawn for chromatographic analysis. The monitoring was terminated upon the observation of asymptotic behaviour, following the recommendations of Holliger et al. (2016).

6. Data processing
The results of the characterisation of the substrate and inoculum were analysed using descriptive statistics tools. The production results obtained from each of the experimental configurations were

| Treatment | $X_1$ | $X_2$ | Temperature (°C) | Head space (%) |
|-----------|------|------|-----------------|----------------|
| A         | -1   | -1   | 20              | 20             |
| B         | -1   | 0    | 20              | 35             |
| C         | -1   | +1   | 20              | 50             |
| D         | 0    | -1   | 35              | 20             |
| E         | 0    | 0    | 35              | 35             |
| F         | 0    | +1   | 35              | 50             |
| G         | +1   | -1   | 55              | 20             |
| H         | +1   | 0    | 55              | 35             |
| I         | +1   | +1   | 55              | 50             |

Table 1. Experimental configurations
processed using MATLAB® 2017 software to develop a response surface, showing the optimal points at which the variable of interest (the BMP) was maximised. A two-way ANOVA model was similarly implemented, whereby a second-degree polynomial (Equation 4) was generated to reflect the interaction of the considered variables in the process (Zainudin et al., al., 2005). The data were normalised using the normal standard variation method, as described by Equation 5:

\[
Y = b_0 + \sum_{i=1}^{n} b_i x_i + \left( \sum_{i=1}^{n} b_{ij} x_i \right) + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} b_{ij} x_i x_j
\]  

(4)

\[
Xinar = \frac{X_i - \bar{x}}{\sigma_X}
\]  

(5)

where \(Y\) is the response variable; \(b_0\) is a constant coefficient; \(b_i\) is the linear coefficient; \(b_{ij}\) is the coefficient for the interaction of the variables; \(b_{ii}\) is the quadratic coefficient; \(x_i\) and \(x_j\) are the coded values of the variables; \(X_i\) is the intercept of each variable (Figure 2); \(\bar{x}\) is the average of the selected values; and \(\sigma\) is the deviation from the selected values (the selected values are presented in Table 1).

7. Process kinetics

The experimental data were fitted to the kinetic models presented in Table 2. The mean squared error (MSE) was determined for the model fits. The kinetic analysis was performed in the MATLAB® 2017 software using the Levenberg-Marquardt algorithm (Güçlü, Yılmaz, & Özkan-Yucel, 2011).

8. Results and discussion

8.1. Characterisation of substrate and inoculum

Table 3 presents the results of the physico-chemical characterisation of the substrate and the inoculum.

The pH of the FW is within the reported range for this type of substrate and is consistent with characteristic BA and VFA concentrations of easily acidifiable substrates, showing the need for an external alkalising source (Steinmetz et al., 2016; Parra-Orobio et al., 2018). Although the COD, TOC and VS/TS ratio indicate a high content of organic matter, the COD/filtered/COD_total ratio confirms the predominance of particulate organic matter, which can be a limiting condition in hydrolysis (Li et al., 2012; Aldine et al., 2011). The TN and TP values show that nutrients are provided for the requirements of the microorganisms involved in the biological process and reflect the presence of legumes in the FW (Elser, 2012). The values for lignin and cellulose (which are slowly degrading organic materials) are similar to those from other studies on AD (Parra-Orobio et al., 2018) and composting (Soto-Paz et al., 2019) of this type of waste, which was separated at the source and obtained by selective collection.

The pH and the BA/TA ratio (0.63) of the inoculum indicate a good buffer capacity, which prevents the accumulation of VFAs produced during AD. These values are similar to previously reported values, such as by de Raposo et al., (2012) and Torres-Lozada, Díaz-Granados, and Parra-Orobio (2015). Although the VS/TS ratio (0.53) confirms that the incorporation of granular sludge into flocculent sludge improves the characteristics of the inoculum, the SMA of 0.15 gCOD_C4H8/gVS is low, while being within the range reported by Angelidaki et al. (2009). The HA and SAA values of 0.26 and 3.77 gCOD_glucose/gVS.d, respectively, are close to reported values, such as by Regueiro et al. (2012) and Parra-Orobio et al., (2018).

9. Theoretical potential of methane

The elemental analysis and composition of the FW samples, in terms of carbohydrates, proteins and lipids (Table 3), was used to determine the theoretical production using Equations 1, 2 and 3. Considering the reaction stoichiometry in Equations 1 and 2 results in a theoretical production of 429 mLCH4/gVS; however, these are theoretical methods based on an elemental analysis, in which
| Model       | Equation                                                                 | Source                                      |
|-------------|--------------------------------------------------------------------------|---------------------------------------------|
| First-order | BMP = BMP<sub>0</sub> - e<sup>-kt</sup>                                   | Neef et al. (2015)                          |
| Gompertz    | BMP<sub>0</sub>e<sup>-(λt + 1)</sup> = BMP<sub>0</sub>e<sup>-eλ</sup> + R<sub>PBM</sub><sup>0</sup><sup>λ</sup>/C<sub>0</sub> - 1<sup>1</sup> | Lay, Li, and Nolke (1997)                   |

Table 2. Kinetic models

- **BMP**<sub>0</sub>: Biochemical potential of accumulated CH<sub>4</sub> (mL CH<sub>4</sub>/g VS)
- **BMP**: BMP substrate (theoretical) (FW) (mL CH<sub>4</sub>/g VS)
- **k**: Hydrolysis constant (d<sup>-1</sup>)
- **R<sub>PBM</sub><sup>0</sup>**: Production rate of CH<sub>4</sub> (mL CH<sub>4</sub>/g VS d<sup>-1</sup>)
- **λ**: Lag phase (d)
- **e** (Exponential value 1) = 2.7183
- **e<sup>-eλ</sup>**
100% degradation of the FW is assumed (Fernandez et al., 2008), which corresponds to an ideal theoretical condition. For example, with respect to carbon, the degraded fraction corresponds only to anaerobically biodegradable organic carbon, which is also affected by the presence of materials such as lignin and cellulose (Puyuelo, Ponsá, Gea, & Sánchez, 2011). Some components, while being organic in nature, are difficult to degrade and have limited solubility and as it was shown in the physico-chemical characterisation of the substrate, they represent an important fraction in these residues. Using Equation 3 resulted in a production of 127 mL CH₄/gVS, which is close to the maximum value of 107 mL CH₄/gVS obtained in the present study.

By contrast, Equation 3 only accounts for easily degradable monomers in the FW (Mahmoud et al., 2018). Nielfa et al. (2015) have reported an error of only 10% for the experimental production using Equation 3. Thus, different AD incidence variables need to be simultaneously evaluated for FW to better correspond the theoretical conditions, while remaining cognisant that not all of the substrate may be degraded or easily assimilated.

### 10. Response surface methodology (RSM) for determination of optimal temperature and head space

Figure 1 shows the response surfaces for the interaction between (a) the head space and time; (b) the temperature and time; and (c) the interaction of the two considered variables.

| Parameters | FW (n = 3) | Inoculum (n = 3) |
|------------|------------|-----------------|
| pH (units) | 4.92 ± 0.01 | 7.71 ± 0.10     |
| TA (mg CaCO₃/L) | 3940.23 ± 238.56 | 1718.26 ± 95.66 |
| BA (mg CaCO₃/L) | —          | 1166.58 ± 90.66 |
| BA/TA      | —          | 0.68            |
| VFAs (mg/L) | 34,102.70 ± 120.36 | 398.33 ± 83.11 |
| Total COD (mg/L) | 109,751.88 ± 198.32 | —            |
| Filtered COD (mg/L) | 241,097.69 ± 220.36 | —            |
| TOC (mg/kg) | 47,333.48 ± 23.97 | —              |
| TS (mg/L)  | 101,630.02 ± 2.40 | 44,850.00 ± 3.54 |
| VS (mg/L)  | 92,881.68 ± 2.27 | 25,566.66 ± 2.44 |
| VS/TS      | 0.91        | 0.57            |
| TN (mg/kg) | 10,860.00 ± 1.09 | —              |
| TP (mg/kg) | 240.36 ± 0.13  | —              |
| Lignin (%) | 2.20 ± 0.64   | —              |
| Cellulose (%) | 1.67 ± 0.57   | —              |
| Carbon (%) | 41.16 ± 0.81  | —              |
| Hydrogen (%) | 5.04 ± 0.36   | —              |
| Oxygen (%) | 40.61 ± 1.99  | —              |
| Nitrogen (%) | 1.76 ± 0.03   | —              |
| Carbohydrates (%) | 9.60 ± 0.6   | —              |
| Proteins (%) | 6.79 ± 0.8    | —              |
| Lipids (%)  | 5.30 ± 1.2    | —              |
| SMA (gCODCH₄/g VS·d) | —          | 0.15 ± 0.15    |
| HA (gDQOGlucose/gVS·d) | —         | 0.26 ± 0.02    |
| SAA (gDQOGlucose/gVS·d) | —          | 3.72 ± 0.25    |

*Elemental Analysis (n = 8)
Figure 1(a) shows an inverse relationship between the head space and methane production, which is in agreement with the results of Stumm and Morgan (2012) and Himanshu, Voelklein, Murphy, Grant, and O’Kiely (2017), wherein increasing the reactor head space was found to decrease the methane yield, because the pH and the partial pressure of CO₂ are related to the presence of CO₂ in the biogas and the inorganic carbon dissolved in the interior of the reactor.

Rittman and McCarty (2001) and Panigrahi and Dubey (2019) reported that increasing the partial pressure (the lower head space) increases the dissolution of inorganic carbon, which is a natural buffering agent and prevents microbial populations (i.e. methanogenic Archaea) from being affected by decreases in the pH. Microbes play a critical role in the degradation process and the transformation of intermediate products to methane. Some of these microbes are highly pH-sensitive, and some bacterial consortia require an almost neutral pH to perform well (Jankowska et al., 2015).

Figure 1(b) shows that thermophilic temperatures result in approximately 50% more methane production than mesophilic temperatures, which is in agreement with reports by Kim et al. (2006) and Mirmohamadsadeghi et al. (2019). High temperatures also favour the solubilisation of this type of compound, facilitating degradation by microorganisms (Frigon & Guiot, 2010; Shi, Guo, Zuo, Wang, & Zhang, 2018), which increases methane production, in contrast to mesophilic or psychrophilic conditions, which lower production.

Figure 1(c) shows the interaction between the temperature and the head space. The area delimited by temperatures between 50 and 55 °C and head spaces between 20 and 25% correspond to the conditions under which the BMP increased in this study. The optimal values for each variable were determined by using Equation 5 to denormalise the values and data from the intercepts in Figure 2. This analysis shows that the optimal conditions are a temperature of 48.9 °C and a head space of 20%, which is consistent with the aforementioned results and reports by Deepanraj et al. (2015) and Shi et al. (2018) of increased methane production under thermophilic temperature conditions (50–55°C).
The ANOVA analysis was fitted to a 2FI quadratic interaction model for the interaction of two variables, which has been widely used for authors as Li et al. (2015) and Oliveira, Alves, and Costa (2015). The analysis produced Equation 8 with a $R^2$ value of 0.98 and $p = 0.02$:

$$y = 65.05 + 25.15 \times T - 14.16 \times Hs - 5.42 \times T \times Hs$$  

where $y$ is the response variable (BMP), $T$ is the temperature, and $Hs$ is the head space.

Equation 8 shows that temperature variable has the most significant impact on the process, with a coefficient of 25.15 compared to the head space coefficient of $-14.16$. The coefficient of $-5.42$ for the interaction between the two variables is less significant. Thus, at higher temperatures, the BMP increases, and the greater the head space is, the lower the BMP. This result is consistent with the significance of each variable: $p < 0.0001$ (temperature), $p = 0.0002$ (head space) and $p = 0.0290$ (interaction of the two variables), where the interaction of the temperature and the head space has the $p$-value closest to 0.05.

Note that although the degradation rate over the thermophilic range is generally faster than over the psychrophilic or mesophilic ranges, there are disadvantages associated with the thermophilic range. i. Instabilities are introduced into the process from temperature fluctuations, along with the risk of accumulation of VFAs (i.e. propionic acid) from an enhanced high reaction rate, especially during acidogenesis (Mao, Feng, Wang, & Ren, 2015; Panigrahi & Dubey, 2019; Rajagopal, Belloavance, & Rahaman, 2017). ii. More energy must be expended to maintain the temperature above ambient (Shi et al., 2018). Thus, the cost/benefit ratio for production versus the required consumption should be evaluated to determine the feasibility of the proposed strategy. Most AD processes for FW, including industrial scale processes, are carried out under mesophilic conditions to ensure that the process remains stable under the high organic loads supplied (Guo et al., 2014).

Given the above-mentioned limitations of implementing thermophilic conditions and increasing the BMP without incurring cost overruns for reactor heating, it is advisable to explore alternatives, such as i. the use of insulating materials for temperature maintenance (Martí-Herrera, Soria-Castellón, Díaz-de-Basurto, Alvarez, & Chemisana, 2019); ii. a staged process or phase separation (Wu, Kobayashi, Li, & Xu, 2015; Xiao et al., 2018); iii. pre-treatment of the substrate and/or inoculum (Li et al., 2016; Parra-Orobio et al., 2017) and iv. innovative studies on the interaction between variables to optimise the process to achieve high biogas production at the lowest possible expense.

11. Kinetic analysis of process
Table 4 shows the kinetic parameters obtained using the first-order and Gompertz models, along with the VFAs obtained for each configuration at the end of the process.

Using the Gompertz model resulted in a maximum production of 133.45 mLCH4/gVS (G), which is far from the maximum production (I) calculated using the first-order model (348.36 mLCH4/gVS), showing that the first-order model overestimates production (Díaz et al., 2015). The theoretical value of 127 mLCH4/gVS obtained using the substrate composition (in terms of the carbohydrates,
| ID | Tem (°C) | HS (%) | Experimental Production | First-order Model | Gompertz Model | VFAs |
|----|----------|--------|-------------------------|------------------|---------------|------|
|    |          |        |                         | Pmax  k  R²  RMSE| Pmax  λ (d)  Rmax  R²  RMSE |
| A  | 20       | 20     | 47.96                   | 99.05  0.01  0.99  1.17 | 58.71  0.40  1.59  0.99  0.98 | 33,564.80 |
| B  | 20       | 35     | 37.30                   | 56.02  0.01  0.98  1.40 | 40.51  0.51  1.59  0.99  1.02 | 34,802.55 |
| C  | 20       | 50     | 29.65                   | 47.40  0.01  0.96  1.75 | 30.38  2.89  1.57  0.99  0.61 | 36,241.26 |
| D  | 35       | 20     | 81.47                   | 194.92  0.02  0.99  1.67 | 95.14  0.78  2.99  0.99  1.53 | 18,196.71 |
| A  | 35       | 35     | 68.33                   | 159.50  0.01  0.99  1.81 | 81.37  1.10  2.49  0.99  1.32 | 20,233.04 |
| F  | 35       | 50     | 54.91                   | 184.04  0.01  0.98  2.09 | 66.93  1.72  2.06  0.99  1.73 | 32,711.58 |
| G  | 55       | 20     | 107.06                  | 193.66  0.03  0.99  2.67 | 133.45  1.24  3.46  0.99  2.12 | 15,763.24 |
| H  | 55       | 35     | 91.74                   | 211.31  0.02  0.98  2.71 | 118.86  2.41  2.95  0.99  2.58 | 17,361.14 |
| I  | 55       | 50     | 67.05                   | 348.36  0.01  0.99  1.97 | 80.84  4.44  2.54  0.99  1.51 | 28,203.20 |

**Tem:** temperature; **HS:** head space; **units:** experimental production (mL CH₄/gVS); **Pmax** (mL CH₄/gVS); **λ** (d); **Rmax** (mL CH₄/gVS)
proteins and lipids in the FW, Equation 3) is near the experimental value of 107 mLCH4/gVS and the result from the Gompertz model. Thus, both the theoretical method and the Gompertz model can be successfully used to describe the AD of FW because of the small difference between these methods, especially considering that using Equation 3 results in production values with an estimation error below 10% (Nielfa et al., 2015).

Following Donoso-Bravo, Pérez-Elvira, and Fdz-Polanco (2010) and Parra-Orobio et al., (2018), $R^2$ should be maximised and the error (RMSE) should be minimised. The Gompertz model produced the best fit to the data ($R^2 = 0.99$ and RMSE < 2.12) and thus describes the process better than the first-order model. A comparison of the kinetic parameters shows that the first-order model, despite its widespread use, did not adequately estimate the methane production for all of the experimental configurations (Díaz, Reyes, Lundin, & Horváth, 2011) and that the Gompertz model better represented the data, in agreement with the results of Cárdenas-Cleves, Marmolejo-Rebellón, and Torres-Lozada (2018). The latency phase increased with the head space (Table 4), indicating the impact of this parameter on methane production via the accumulation of VFAs and the absence of CO$_2$ dilution because of insufficient pressure (Valero et al., 2016).

The VFA concentrations are consistent with the high presence of acidogenic bacteria (AAE, Table 3), which transform organic matter into VFAs such as valeric, propionic and butyric acids (Zhang et al., 2014). This bacterial presence, in conjunction with the bacterial growth rate ($R_{max}$), explains the high VFA concentrations at the end of the process. In addition, methanogenic Archaea are known to be very sensitive to changes in pH, requiring almost neutral conditions (Jankowska et al., 2015). The VFA concentrations at the end of the process showed that a neutral environment did not predominate in most of the assays, which is in agreement with the experimental methane production.

The results from Table 4 for the latency phase of the process (λ) show that a short latency does not always favour AD, because increasing the reaction rate or the degradation of VS also increases the accumulation of VFAs, which can inhibit the process if there is no buffer capacity (Lee et al., 2015). By contrast, a prolonged latency phase may allow the microbial consortia to better adapt to the substrate and increase methane production. These results were reflected in the D and G assays. These assemblies were subjected to different temperatures for the same head space value, and different yields were obtained for different latency phase values.

The first-order model does not take into account the latency phase and overestimates methane production data. However, this model is useful for describing hydrolysis in the process: the data showed that the reaction speed was generally not rapid, except in the G assay (Parra-Orobio et al., 2018). Inhibition phenomena may have occurred in the assays with head spaces above 20%, but at lower rates than at the mesophilic and thermophilic temperatures, especially given the reaction rates at elevated temperatures (Mirmohamadsadeghi et al., 2019). Thus, the incidence of the head space is reflected in $R_{max}$, which increases with the temperature.

In general, it can be stated that the Gompertz model is a non-linear model that can be used to evaluate the entire process easily, whereas the first-order model, as its name indicates, reflects the effect that the variable of interest has on hydrolysis. These models are not comparable but can be complementary: the Gompertz model can be used to provide data on the latency phase, whereas the first-order model can provide information on the critical stage of AD of FW (Rajagopal et al., 2017).

12. Conclusions

- FW contains lignocellulosic compounds that degrade slowly and can thus inhibit high AD yields. Equations have been developed to estimate the potential of methane production from this type of substrate.
- An ANOVA of the considered variables shows that the temperature is the highest incidence variable in the process. The optimal temperature was found to be 48.9 °C, and temperatures
above this value did not result in increased methane production. The optimal head space was found to be 20%, and head spaces greater than this value resulted in the inhibition of the process from the accumulation of VFAs.

- The result of the models implemented in the study showed that the Gompertz model best describes the AD of FW because the latency phase of the process is considered and does not overestimate methane production, unlike the first-order model. The two models are not comparable to each other.

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