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What is This?
Effect of moisture on disintegration kinetics during anaerobic digestion of complex organic substrates

Flavia Liotta¹, Giuseppe d’Antonio², Giovanni Esposito¹, Massimiliano Fabbricino², Luigi Frunzo³, Eric D van Hullebusch⁴, Piet NL Lens⁵ and Francesco Pirozzi²

Abstract
The role of the moisture content and particle size (PS) on the disintegration of complex organic matter during the wet anaerobic digestion (AD) process was investigated. A range of total solids (TS) from 5% to 11.3% and PS from 0.25 to 15 mm was evaluated using carrot waste as model complex organic matter. The experimental results showed that the methane production rate decreased with higher TS and PS. A modified version of the AD model no.1 for complex organic substrates was used to model the experimental data. The simulations showed a decrease of the disintegration rate constants with increasing TS and PS. The results of the biomethanation tests were used to calibrate and validate the applied model. In particular, the values of the disintegration constant for various TS and PS were determined. The simulations showed good agreement between the numerical and observed data.

Keywords
Anaerobic digestion, wet digestion, moisture content, mathematical modelling, particle size, ADM1

Introduction
The use of anaerobic digestion (AD) for solid wastes treatment has increased rapidly in Europe in the last few decades (De Baere et al., 2010). AD can be considered as the major development process in the field of solid waste treatment in Europe over the last two decades (Le Hylaric et al., 2011). Among biological treatments, AD of the organic fraction of municipal solid waste (OFMSW) is the most cost-effective owing to the high energy recovery linked to the process, and it has only limited environmental impact (Mata Alvarez et al., 2000). In fact, through AD it is possible to reduce the chemical oxygen demand of waste, obtaining the conversion of organic matter to biogas, which can be used as bio-fuel in power generation systems to produce heat and energy. Moreover, AD with energy recovery generates fewer greenhouse gases than incineration or landfilling because digested solid by-products can be composted (Edelmann, 2003).

In order to accurately predict the process efficiency at varying operational conditions and to optimize the reactor design, it is necessary to have mathematical models that can simulate the processes inside the reactor. Historically, AD is applied on wastewater treatment sludge and modelled as a multi-step process, through the combined action of four types of anaerobic micro-organisms: hydrolytic, fermentative, acetogenic and methanogenic bacteria. During this process the organic matter is progressively converted into simpler and smaller-sized organic compounds, obtaining biogas and digestate as final products. The resulting digestate is rich in nutrients and microelements, thus suitable for its utilization in agricultural contexts (Esposito et al., 2012a, 2012b).

The limiting step of the AD process cannot be unequivocally defined. Acetogenesis (Bryers, 1985; Costello et al., 1991a, 1991b; Hill, 1982; Siegrist et al., 1993) and methanogenesis (Graef and Andrews, 1974; Moletta et al., 1986; Smith et al., 1988), as well as hydrolysis (Vavilin et al., 2001) and disintegration (Batstone et al., 2002; Esposito et al., 2008, 2011a, 2011b) can constitute the rate-determining steps depending on the substrate to be digested and the AD reactor configuration used.

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When considering complex organic matter, the hydrolysis of complex polymeric substances becomes the rate-limiting step and modelling of this process has to be improved (Batstone et al., 2002; Pavlostathis and Giraldo-Gomez, 1991; Vavilin et al., 1996, 1997, 1999). In particular, several models have shown that the presence of OFMSW particles can be better described with the introduction of a disintegration step. This step individuates the physical breakdown and transformation of the complex organic matter into soluble organic particles, and represents the rate-limiting step of the process (Batstone et al., 2002; Esposito et al., 2008, 2011a, 2011b; Hills and Nakano, 1984; Sharma et al., 1988).

Several authors investigated the rate of hydrolysis and disintegration as a function of different parameters such as pH, temperature, hydrolytic biomass concentration, type of particulate organic matter and particle size (PS) (Esposito et al., 2008; Hill and Nakano, 1984; Pavlostathis and Giraldo-Gomez, 1991; Sanders et al., 2000; Sharma et al. 1988; Veeken and Hamelers, 1999; ). However, it is less understood how the total solid (TS) content can affect the hydrolysis and, in particular, the disintegration step of complex organic substrates. There have been several attempts made to model the effect of the moisture content on dry and semi-dry AD processes. In particular, Abbassi-Guendouz et al. (2012), by application of the AD model no. 1 (ADM1) model, found a decreasing first-order hydrolysis rate constant for carbohydrates by increasing the TS content. This constant was calibrated using batch experimental data with cardboard as initial substrate and imposing the TS content in the range of 15–30%. This finding is in agreement with results presented by Bollon et al. (2011).

There have also been several attempts made to investigate the effect of the TS content on the methane production by operating specific methanogenic activity tests and simulating experimental data using the Gompertz model (Lay et al., 1997a, 1997b, 1998; Le Hyaric et al., 2011, 2012). These authors also suggested that a high TS content could reduce substrate degradation, resulting in less methanogenic activity. These results are consistent with several studies performed by Qu et al. (2009), Fernandez et al. (2010), Forster-Carneiro et al. (2008) and Pommier et al. (2007), who found a reduction in methane production with higher TS concentration. All these studies showed that the moisture content plays an essential role in biogas formation as the nutrients and substrates for the microorganisms must dissolve in the water phase before they can be assimilated. Furthermore, the moisture content is also an important factor in low solids (wet, with 5–10% TS) anaerobic digestion because it supports bacterial movement and helps substrate and product diffusion through the porous medium (solid waste) to bacterial sites (Lay et al. 1997a, 1997b; Le Hyaric et al., 2012; Mora-Naranjo et al., 2004; Pommier et al., 2007).

The aim of this work is, therefore, to assess the impact of the moisture content on wet anaerobic digestion of carrot waste. To better evaluate the impact of the water content on the AD performance, computer simulations using a new version of the ADM1 of complex organic substrates, proposed by Esposito et al. (2008, 2011b, 2012b), was applied. The model was used to describe the experimental data and to define the dependence of the disintegration kinetic parameters on the PS and moisture content.

In more detail, the objectives of this work include:

- proposing an experimental procedure for obtaining an inoculum at different moisture contents;
- investigating the effect of the PS content on the disintegration step of the AD process of carrot waste;
- investigating the effect of TS on methane production;
- proposing a new mathematical modelling approach to describe the effect of TS on the disintegration step of AD using a new version of the ADM1 model proposed by Esposito et al. (2008);
- determining the surface-based kinetic constant for the cited selected substrate, using the model proposed by Esposito et al. (2008).

Materials and methods

Digester set-up and analytical measurements

Biomethanation Tests (BMT) were performed on a small scale under controlled and reproducible conditions in a 1000-ml glass bottle GL 45 (Schott Duran, Wertheim am Mein, Germany). Small amounts of Na2CO3 powder were also added to control the pH value. Each bottle was sealed with a 5-mm silicone disc that was held tightly to the bottle head by a plastic screw cap punched in the middle (Schott Duran). All digesters were immersed up to half of their height in hot water kept at a constant temperature of 308.15 K by 200 W A-763 submersible heaters (Hagen, Tokyo, Japan).

Once a day, each digester was connected by a capillary tube to an inverted 1000-ml glass bottle containing an alkaline solution (2% NaOH). The inverted 1000-ml glass bottle was sealed in the same way as the digesters. To enable gas transfer through the two connected bottles, the capillary tube was equipped on both ends with a needle sharp enough to pierce the silicone disc.

The weight, TS and volatile solids (VS) concentration of the anaerobic sludge, as well as the dry matter, moisture organic matter and ash content of substrate, were determined according to standard methods (APHA/AWWA/WEF, 1998). Temperature and pH of all mixtures investigated were monitored at least once a day with a TFK 325 thermometer (WTW, Weilheim, Germany) and a pH meter (Carlo Erba, Pomezia, Italy) respectively (Esposito et al., 2011b).

Preliminary tests: Drying procedure

In order to evaluate the effect of different moisture contents during AD, experiments at different TS contents are necessary. With the objective to evaluate only the effect of the moisture content, these experiments must be conducted using the same inoculum, at the same operational conditions, varying only the TS content. Therefore, fresh digestate was collected from a mesophilic AD of
a buffalo farm and stored in 10-l buckets at 4°C and used as inoculum source. The initial inoculum characteristics in terms of TS, VS, carbohydrates fraction, proteins fraction and lipids fraction are listed in Table 1.

The inoculum was dried by testing three different procedures: overnight drying of fresh digestate at 50°C until constant weight, centrifugation at 5040 G for 10 mins and membrane filtration with a Kubota 203 microfiltration module. The selected drying procedures were aimed at removing water from the inoculum, obtaining a final value of 4% TS. In order to evaluate the effects of different drying treatments, the concentrated inoculum was reported at the initial TS content of 2% adding distilled water and was compared with the untreated inoculum in terms of biomethane potential. The aim of these tests was to individuate the drying procedure that does not modify the inoculum characteristics in terms of biomass activity and methane production. Therefore, the inoculum obtained from each adopted drying procedure was used to carry out BMTs. These experiments were performed using 2.63 g of pasta and 5.24 g of cheese with known carbohydrate, protein and lipid concentrations. The selected substrate, carrot waste, allows the development of all microbial species involved in degradation of carbohydrates, proteins and lipids in order to evaluate the pre-treatment effect on all these species. The methane production is expressed under standard conditions and considers the gas content variation in the headspace of the reactor. The calculated methane production accounts for the global methane production without the residual endogenous methane production measured with the blank assay, which represent the reactor filled only with digestate without substrate addition (Figure 1).

### Effect of PS on AD

Biomethanation experiments were performed using a selected green grocery waste (i.e. carrot waste) as initial substrate with the chemical composition in terms of TS, VS and concentrations of carbohydrates, proteins and lipids reported in Table 1. This substrate was selected for modelling purposes owing to the ease to obtain a cylindrical shape (Figure 2). That shape was obtained using a cylindrical steel tube with a selected diameter. For each particle, the same diameter and height was imposed in order to obtain a ratio between area and mass equal to a particle with cylindrical potential.

The tests were conducted using four different PS: 0.25 mm, 4 mm, 9 mm and 15 mm (Table 2). The selected ratio between organic matter and anaerobic sludge was 0.5 organic matter/anaerobic sludge (i.e. food/mass ratio). The selected digestate was collected from a mesophilic AD of a farm treating buffalo manure. The mass composition adopted for all tests is described in Table 2. BMTs were operated in triplicate and a blank assay was also carried out. In total, 15 BMTs were performed.

### Effect of moisture content on AD

BMTs were performed using carrot waste with a cylindrical shape and buffalo manure anaerobic digestate. A specific value of

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### Table 1. Main characteristics of anaerobic sludge and carrot waste.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Initial total solid (%)</th>
<th>Initial volatile solid (%)</th>
<th>Carbohydrate (%)</th>
<th>Protein (%)</th>
<th>Lipids (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet anaerobic sludge</td>
<td>2</td>
<td>1.2</td>
<td>2.1</td>
<td>56</td>
<td>41.9</td>
</tr>
<tr>
<td>Carrot waste</td>
<td>12.7</td>
<td>11.4</td>
<td>0.121a</td>
<td>0.025a</td>
<td>0.006a</td>
</tr>
</tbody>
</table>

Buffière et al. (2006).
Table 2. Composition of the organic mixture in terms of food/mass [F/M] ratio, particle size, input substrate and inoculum for the experiments T1–T4.

<table>
<thead>
<tr>
<th>Tests</th>
<th>F/M</th>
<th>Initial radius (mm)</th>
<th>Carrots (g)</th>
<th>Anaerobic sludge (g)</th>
<th>Na₂CO₃ (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>0.5</td>
<td>15</td>
<td>48.2 ± 0.5</td>
<td>500 ± 1</td>
<td>0.30–0.40 ± 0.01</td>
</tr>
<tr>
<td>T2</td>
<td>0.5</td>
<td>9.0</td>
<td>48.2 ± 0.5</td>
<td>500 ± 1</td>
<td>0.30–0.40 ± 0.01</td>
</tr>
<tr>
<td>T3</td>
<td>0.5</td>
<td>4.0</td>
<td>48.2 ± 0.5</td>
<td>500 ± 1</td>
<td>0.30–0.40 ± 0.01</td>
</tr>
<tr>
<td>T4</td>
<td>0.5</td>
<td>0.25</td>
<td>48.2 ± 0.5</td>
<td>500 ± 1</td>
<td>0.30–0.40 ± 0.01</td>
</tr>
</tbody>
</table>

Table 3. Mixture composition of tests T5, T6 and T7 in terms of total solid (TS), volatile solid (VS) content, carrot waste and dried anaerobic inoculum amount.

<table>
<thead>
<tr>
<th>Test</th>
<th>TS mixture (%)</th>
<th>VS mixture (%)</th>
<th>Carrot waste amount (g)</th>
<th>Dried anaerobic sludge (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T5</td>
<td>11.3</td>
<td>8.57</td>
<td>40</td>
<td>120</td>
</tr>
<tr>
<td>T6</td>
<td>7.5</td>
<td>4.6</td>
<td>40</td>
<td>245</td>
</tr>
<tr>
<td>T7</td>
<td>4.98</td>
<td>3.7</td>
<td>40</td>
<td>320</td>
</tr>
</tbody>
</table>

A PS of 15 mm was selected in order to get the disintegration step as the rate-limiting step.

The initial TS content of the fresh digestate was 2%, which was dried by centrifugation in order to obtain the desired moisture contents. A fixed amount of substrate was defined, and only the digestate volume was modified in order to obtain different moisture contents. All the tests were performed imposing a selected ratio between organic matter and anaerobic sludge of 0.5 organic matter/inoculum. All the tests were conducted in triplicate. Nine bottles were operated with three TS contents: 4.98%, 7.5% and 11.3%. The mixture composition of each BMT test is reported in Table 3.

A further nine tests were conducted using only anaerobic sludge as a substrate to estimate the volume of methane resulting from the fermentation of the organics contained in the anaerobic sludge. In total, 18 tests were performed.

Mathematical model

For a better understanding of the effect of TS and PS on the anaerobic degradation of complex organic substrates, the anaerobic co-digestion model for complex organic substrates proposed by Esposito et al. (2011b) was used. The model was calibrated with the experimental data of the batch experiments to estimate the kinetic constant of the surface based disintegration process, $K_{sbk}$ (ML⁻²T⁻¹). The differential mass balance equations and the process kinetics and stoichiometry, described in detail in Esposito et al. (2011b), are based on the ADM1 approach.

The disintegration kinetic is based on the surface-based kinetic expression proposed by Sanders et al. (2000) and reformulated by Esposito et al. (2008, 2011b) by including $a^*$, which characterizes the disintegration process:

$$a^* = \frac{A}{M}$$

The $a^*$ coefficient is different than the one proposed by Esposito et al. (2011b, 2012b) as the solid particles have a cylindrical, instead of a spherical, shape.

Integration of the differential algebraic equations is performed using a multi-step solution algorithm based on the numerical differentiation formulas in the software tool MATLAB.

Several simulations were performed to identify the best fitting between experimental and modelling data. In particular, the disintegration kinetic constant $K_{sbk}$ (ML⁻²T⁻¹) was changed several times in the model to estimate the value that permits fitting of experimental data. The calibration was performed by comparing model results with experimental data of cumulative methane production for a selected particle size and to define the unknown parameter by fitting experimental data with model results. The calibration procedure proposed by Esposito et al. (2011b) was used. The comparison between experimental data and model results was performed by applying the root mean square error (RMSE) (Esposito et al., 2011b; Janssen and Heuberger, 1995).
Results and discussion

Effect of drying procedure

Figure 1 shows the cumulative methane production obtained using the different inocula resulting from the different drying procedures and the untreated inoculum. The biomethanation potential is the same for all tests, but by only adopting the centrifugation procedure it is possible to observe a similar trend as for the untreated digestate. These results indicate that all the tested methods are suitable drying procedures that do not alter the inoculum characteristics. For the following experiments, centrifugation was selected as the drying procedure because it gives the minimum alteration of the inoculum and it is the most simple and cheap method to apply in the laboratory.

Figure 3. Effect of particle size on the cumulative methane production of carrot waste at a total solid content of 3%.

Figure 4. Influence of particle size on initial methane production rate of carrot waste.

Effect of PS on AD performance

Figure 3 shows the cumulative methane production obtained using the different inocula resulting from the different drying procedures and the untreated inoculum. The biomethanation potential is the same for all tests, but by only adopting the centrifugation procedure it is possible to observe a similar trend as for the untreated digestate. These results indicate that all the tested methods are suitable drying procedures that do not alter the inoculum characteristics. For the following experiments, centrifugation was selected as the drying procedure because it gives the minimum alteration of the inoculum and it is the most simple and cheap method to apply in the laboratory.

Figure 5. Effect of total solids on the cumulated methane production from anaerobic digestion of carrot waste (particle size = 15 mm).

Effect of TS content on AD performances

Figure 5 shows the cumulated methane production for the reactors operated at three different TS contents during the whole experiments. Each curve represents the average of three replicates with an SD equal to 0.045. Lag-phase and the initial methane production rate were inversely proportional to the TS content. These results are consistent with previous studies performed by Lay et al. (1997a, 1997b), who made batch tests in mesophilic digesters at different pH values by testing the effect of the moisture content in the range of wet digestion. The final methane yield, measured at the end of each experiment can be assumed for all tests coincident and equal to the mean value of 450 ml gVS⁻¹ with an SD of 14.23 (Table 4). This is apparently not in agreement with the findings of Abbassi-Guendouz et al. (2012), Fernandez et al. (2008) and Dong et al. (2010), who found higher methane yields with lower TS in the range of dry and semidry AD. The difference is due to the different moisture content range investigated, as the present experiments were carried out in wet conditions. The conversion of acids to methane by methanogenic bacteria can thus be influenced by the lack of water (Ghosh, 1985; Lay et al., 1997b) that can occur at a higher TS content in the range of dry and semidry digestion (Abbassi-Guendouz et al., 2012; Dong et al., 2010; Fernandez et al., 2008).
Figure 6 indicates a linear relationship between the TS content and the initial methane production rate. Such a linear relationship was also observed for AD of selected dry organic wastes, for example sludge cake, meat, carrot, rice, potato and cabbage (Lay et al., 1997b), cellulose (Le Hyaric et al., 2012), cardboard (Abbassi-Guendouz et al., 2012), for waste samples excavated from landfill (Mora-Naranjo et al., 2004) and paper waste (Pommier et al., 2007). The presented results confirm that the TS content, also in wet AD, has a strong effect on the kinetic rates. In particular, at lower TS, owing to the increasing water content and better transport and mass transfer conditions, it seems plausible that the microorganisms are better provided with soluble substrates (Mora-Naranjo et al., 2004).

Modelling results

**Modelling the effect of particle size on AD.** Model calibration was used to estimate the kinetic constant of the surface-based disintegration process, $K_{\text{dis}}$ (kg m$^{-2}$s$^{-1}$). Calibration was performed by comparing model results with experimental measurements of methane production and adjusting the unknown parameters until the model results adequately fit the experimental observations. The measured data of experiment T1 (Table 5) were used, and a calibration procedure introduced by Esposito et al. (2011b) was applied. Using the previously calibrated $K_{\text{sbk}}$ model, validation was performed by calculating the RMSE for T2, T3 and T4 experiments.

The model calibration performed resulted in setting the kinetic constant $K_{\text{sbk}}$ equal to 0.28 kg m$^{-2}$ s$^{-1}$. $K_{\text{sbk}}$ was the value that maximizes RMSE (Figure 7), which shows a single monotone reversal trend, confirming the existence of one and only one solution to the specific optimization problem.

In Figure 8(a, b), a good overlap between the simulated and model data is shown. A small shift between experimental data and model results was observed.

The results of experiments T2, T3 and T4 were used to validate the mathematical model, assessing the agreement between simulated and observed data for the cumulative methane production with the parameter RMSE. Figure 8(c–h) shows very good agreement between the simulated and experimental data. This agreement is confirmed in Table 5, where the values of $a^*$ constant evaluated for the different PS ranges investigated are also reported.

**Modelling the effect of TS on AD.** The mathematical model proposed by Esposito et al. (2008, 2011b) was calibrated to set different values of the kinetic disintegration constant $K_{\text{dis}}$ ($T^*$) = $K_{\text{sh}}$ a$^*$ for different TS contents. For a selected PS of 15 mm, the value of a$^*$ constant was 0.561 m$^2$ kg$^{-1}$. The measured data of the experiment were used, a calibration procedure introduced by

| Table 6. Disintegration constant and index of agreement for different total solids of carrot waste (particle size = 15 mm). |
|---|---|---|---|
| Test | $a^*$ [m$^2$ kg$^{-1}$] | $K_{\text{sh}}$ [kg m$^{-2}$ s$^{-1}$] | RMSE |
| T5 | 0.561 | 0.1 | 0.0084 |
| T6 | 0.561 | 0.3 | 0.0088 |
| T7 | 0.561 | 0.55 | 0.0087 |

Figure 6. Influence of the total solids on initial methane production rate of carrot waste.
Esposito et al. (2011b) was applied, and the RMSE for T5, T6 and T7 experiments were evaluated.

The results (Figure 8i–p) show good agreement between the simulated and experimental data. This agreement is confirmed in Table 6, where the values of the $K_{dil}$ constant, evaluated for different TS, are also reported. In particular, the good fit between simulated and experimental concentrations shows the capability of the model to simulate the AD process of substrates with different initial TS.

Figure 9 indicates a linear relationship between TS and the disintegration kinetic constant obtained with the model proposed by Esposito et al. (2008, 2011b).

The linear correlation represented in Figure 6 can be expressed using the following linear equation:
equation (6): the methane production rate can be expressed by one equation. If first-order kinetics are assumed for the disintegration process, the rate of the overall disintegration (kg m⁻³).

By considering the presence of a limiting step (i.e. disintegration process), the rate of the overall AD process can be modeled by one equation. If first-order kinetics are assumed for the disintegration process, the methane production rate can be expressed by equation (6):

\[ \frac{d[C]}{dt} = K_{\text{dis}} [C] \tag{6} \]

where \( [C] \) is the substrate concentration (ML⁻¹).

By including the parameters \( f \) [angular coefficient of the interpolation line (−740.3)] and \( f \) [intercept value of the interpolation line on the axis of the initial methane production rate (84.34)], and integrating and making simplifications, it is possible to obtain the following equation:

\[ K_{\text{dis}} = \frac{\ln(I(\text{TS}%) + f) \cdot t}{C_0} \tag{7} \]

where \( t \) is the integration time for the initial biomethane production rate evaluation (d) and \( C_0 \) is the initial substrate concentration (kg m⁻³).

In Table 7 the values of the disintegration constant, obtained with equation (7) and with the mathematical model proposed by Esposito et al. (2008, 2011b), are reported, showing a good agreement of the results of the two methods. This confirms that a simplified model (i.e. a one-equation model) can approximate the results of a full model in case a rate-limiting step of the biological process is clearly present.

**Conclusions**

This study focused on the effect of TS content and PS on anaerobic digestion of complex organic substrates. A linear correlation between the initial methane production rate and TS content was also individuated. With regard to the particle size, an inverse correlation between this parameter and the specific methane production was found, and a linear relationship between 1/PS and the initial methane production rate for the substrate added. This result underlines a strong impact of the PS on the kinetic rates and individuating the disintegration process as the rate-limiting step for methane production.

The surface-based kinetic constant \( K_{\text{dis}} \) for the disintegration equation of carrot waste was determined. Also, the values of the disintegration constant for different TS content were assessed. Finally, a simple equation correlating TS and the disintegration constant was proposed, that showed a good agreement with the results of new version of the ADM1 of complex organic substrate proposed by Esposito et al. (2008, 2011b).

**Declaration of conflicting interests**

The authors do not have any potential conflicts of interest to declare.

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