Sustainability **2014**, *6*, 8348-8363; doi:10.3390/su6128348

OPEN ACCESS

sustainability

ISSN 2071-1050

www.mdpi.com/journal/sustainability

Article

The Effect of Substrate-Bulk Interaction on Hydrolysis Modeling in Anaerobic Digestion Process

Antonio Panico [1,*], Giuseppe d'Antonio [2], Giovanni Esposito [3], Luigi Frunzo [4], Paola Iodice [1] and Francesco Pirozzi [2]

[1] Telematic University Pegaso, piazza Trieste e Trento 48, 80132 Naples, Italy; E-Mail: paola.iodice@unipegaso.it

[2] Department of Civil, Architectural and Environmental Engineering, University of Naples Federico II, via Claudio 21, 80125 Naples, Italy; E-Mails: giuseppe.dantonio@unina.it (G.D.); francesco.pirozzi@unina.it (F.P.)

[3] Department of Civil and Mechanical Engineering, University of Cassino and Southern Lazio, via Di Biasio 43, 03043 Cassino (FR), Italy; E-Mail: giovanni.esposito@unicas.it

[4] Department of Mathematics and Applications Renato Caccioppoli, University of Naples Federico II, via Cintia, Monte S. Angelo, I-80126 Naples, Italy; E-Mail: luigi.frunzo@unina.it

* Author to whom correspondence should be addressed; E-Mail: antonio.panico@unipegaso.it; Tel.: +39-081-768-3434; Fax: +39-081-593-8344.

External Editor: Vincenzo Torretta

Received: 9 September 2014; in revised form: 5 November 2014 / Accepted: 12 November 2014 / Published: 25 November 2014

Abstract: In an Anaerobic Digestion (AD) process treating particulate substrates, the size of solids is expected to negatively affect the rate of hydrolysis step and consequently influence the performance of the whole process. To avoid any disadvantage due to size of solids, expensive pre-treatments aimed at disintegrating and solubilizing substrates are commonly conducted prior to AD. This practice is doubtlessly successful, but not always necessary, since some organic substrates, although particulate, once immersed in water, tend to solubilize immediately. This aspect, if properly considered, could result in saving money and time in the AD process, as well as refining the development and calibration of AD mathematical models. The present study is actually aimed at demonstrating, through experiments and mathematical simulations, different results deriving from the AD process performed, under the same operating conditions, on two different substrates, *i.e.* homemade pasta and carrot batons, having the same particle size, but different chemical composition

and texture. Experimental outcomes highlighted the effect of particles size on bio-methane production only from the bio-methanation potential tests (BMP) conducted on carrot batons. Similar results were obtained by mathematical model calibration, *i.e.*, different kinetic constants for differently-sized carrot batons and same kinetic constant for differently-sized homemade pasta solids.

Keywords: bio-methane; anaerobic digestion; hydrolysis; mathematical modelling

1. Introduction

Anaerobic Digestion (AD) is a complex biological process resulting in the conversion of biodegradable organic matter into biogas (a mixture mainly composed of CH_4 and CO_2 and other gases in trace) and mineralized material.

Nowadays, treating waste technologies based on the AD process are largely and commonly used in the field of wastewater treatment as a pretreatment to reduce organic load in liquid waste or a complete treatment to stabilize sludge from wastewater treatment plants (WWTPs) [1], whereas these technologies have recently gained interest also in the field of solid waste disposal [2,3] as method coupled with aerobic composting processes aimed at reducing the harmful impact produced by untreated land-filled organic waste on the environment.

Moreover, applying AD to treat organic waste turns into the following further benefits:

(i) Recover a renewable energy vector (*i.e.*, biogas that has a 4000–5000 kcal/Nm^3) as well as a solid material rich in nitrogen and carbon (*i.e.*, digestate) that can be successfully used as fertilizer and a carbon soil-enrichment product in agriculture or furthermore processed to produce compost;
(ii) Reduce the land used to dispose waste as well as the emissions in the atmosphere of gases responsible for global warming.

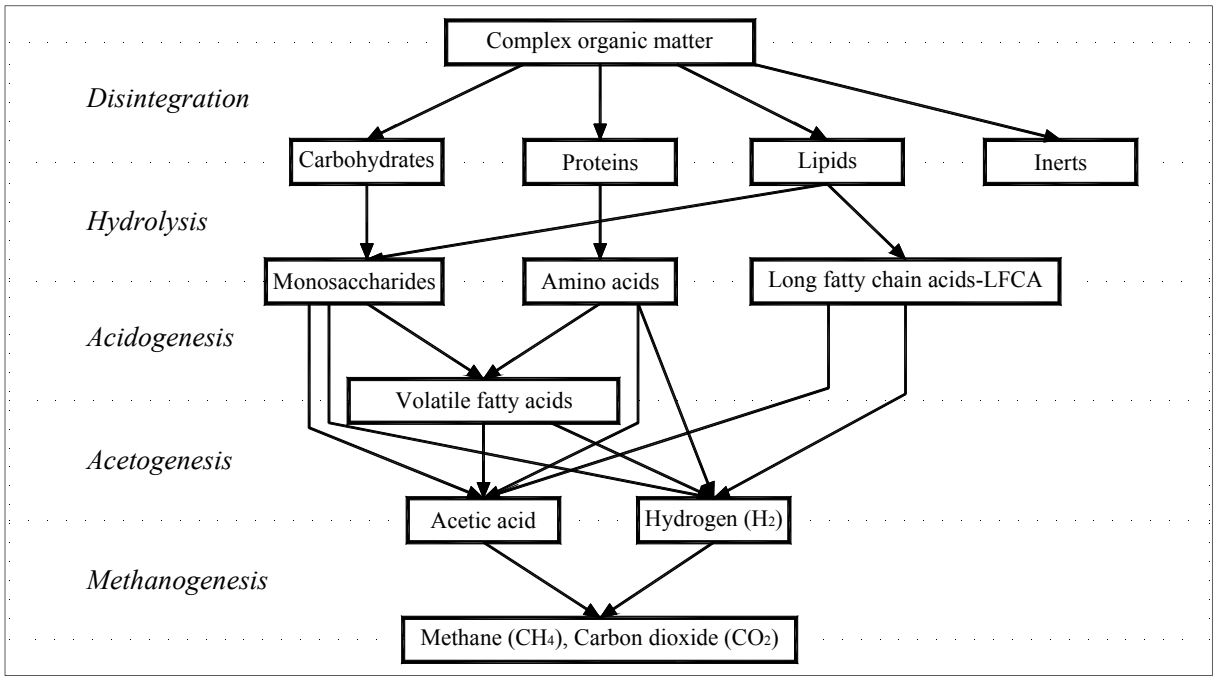
The AD process is the result of physical, chemical and biochemical interactions that involve different groups of microorganisms as well as the intrinsic characteristics and conditions of the medium (liquid bulk) where microorganisms thrive and the AD process takes place.

Like all biochemical processes, AD is actually catalyzed by extracellular and intracellular enzymes. Disintegration and depolymerization of the solid organic particles are extracellular processes, mainly catalyzed by enzymes (cellulase, protease and lipase) excreted from the hydrolytic and fermentative bacteria, whereas the subsequent digestion of the soluble materials by the microbial consortia is an intracellular process resulting in the growth of the microorganisms and production of liquid and gaseous metabolites, e.g., volatile fatty acids (VFAs), butyric acid, valeric acid, acetic acid, acetate, hydrogen, methane, carbon dioxide, *etc.* (Figure 1).

Usually the extracellular processes that govern the AD are commonly known with the basic name of hydrolysis [4]. This process is the first to take place in the pathway through which the AD process evolves and its proper development is essential for the success and performance of the whole process as it represents the starting point of the process and governs the lag-phase of the AD process during the start-up [5,6]. The rate of hydrolysis depends on substrate bioaccessibility [7–9], as well as

substrate bioaccessibility depends on size and nature of particulate solids constituting the organic substrate so much that hydrolysis can play the role of the limiting step [10] of the whole AD.

Figure 1. Anaerobic Digestion process flow chart.



The effect of the solid particles size on the rate of hydrolysis can be easily explained by considering that solid particles can offer to the action of enzymes only their external surface, whereas the inner part remains hidden and not degraded as long as the outer part is not removed [11], therefore anytime only a fraction of the total biodegradable mass present in the system can be effectively available to be degraded by microorganisms and the amount of this fraction depends directly and massively on the distribution size of solids constituting the substrate. Moreover, the effect of size on hydrolysis rate becomes negligible when the particulate substrate shows an ultra-fine granulometry as experimentally found by Silva *et al.* [12].

Instead the effect of solid particles nature, *i.e.*, their specific chemical composition and texture, on the rate of hydrolysis is a consequence of the interaction between solids and the liquid bulk where bacteria thrive, since organic solids are actually made of fractions, some of them are classified in water as soluble and others not soluble [13–16]. This distinction can explain why, for specific organic solid substances, hydrolysis can result faster than expected and consequently in an AD process fed with solid particles hydrolysis could not result, contrary to expectations, the limiting step of AD [17]. This role is actually played by methanogenesis, where are active methanogenic *archaea* that, among all microorganisms involved in AD process, show the slowest growth rate and are the most sensitive to environmental condition changes. When this condition occurs, all those pretreatments (thermal, chemical and physical) usually set prior AD process to accelerate the hydrolysis [18] represent unnecessary costs [19] to be avoided. This aspect, if adequately taken into account, can actually result in being very useful to save money, as well as implement reliable mathematical models used for designing new AD reactors or monitoring the efficiency of those already in service [20,21].

The study presented in this paper is actually focused on showing and discussing through experiments as well as numerical simulations the different evolution of an AD process fed with solid particles having approximately same size but different composition with the aim of demonstrating the relevant role played on the performance of the AD process by nature and texture of solid particles when they are immersed in a medium almost exclusively made of water.

2. Hydrolysis Concept and Its Modeling

Hydrolysis in the AD process of organic waste has been widely studied and modeled over recent years [22–35].

According to Batstone *et al.* [4] hydrolysis can de described by two different mechanisms:

(i) Into the bulk liquid microorganisms secrete enzymes than can be absorbed onto the solid particle or react with soluble substrates;
(ii) Microorganisms attach themselves to the particles of substrate, where they secrete enzymes and take benefits from soluble products derived by enzyme action.

And the effect of hydrolysis on solid particles can be conceptually divided into two models [28]: Shrinking Particle Model (SPM) and Particle Break-up Model (PBM).

SPM assumes that the process of hydrolysis does not break apart particles but continuously reduces the particles diameter, thus the surface area of a single particle decreases over time, as long as all substrate is solubilized, whereas PBM assumes that particles breakup into smaller fragments during hydrolysis. In this case the particle surface increases over time, as long as all substrate is solubilized.

On the base of these two models, authors have formulated different expressions (Table 1), each of them derived from a specific case study or adapted to it.

Table 1. Several expressions modeling the hydrolysis process.

Type	Equation, dS/dt =	Reference
Chemical first order	$-K_H \cdot S$	[29]
Biological n-order	$-K_H \cdot S \cdot B^n$	[32]
Michaelis-Menten	$-K_H \cdot S \cdot B/(K_s + S)$	[32]
Contois model	$-K_H \cdot S \cdot B/(K_s \cdot B + S)$	[33]
Chen-Hashimoto model	$-K_H \cdot S \cdot B/[K_s(S - S_0) + S]$	[34]
Two phase model	$-K_H \cdot S \cdot B/[(K_s + S)(K_b + B)]$	[20]
Shrinking core model	$-3K_H \cdot S_0 \cdot R^2 \cdot B,\ dR/dt = -K_H \cdot B$	[35]
Surface based kinetics	$-K_H \cdot A_s$	[22]
Surface based kinetics	$-K_H \cdot a^* \cdot S$	[6]

K_H = Hydrolysis rate constant (T^{-1}). S = Substrate concentration (M/L^3). B = Biomass concentration (M/L^3). n = exponent of equation [dimensionless]. K_s = Semi-saturation substrate concentration (M/L^3). K_b = Semi-saturation biomass concentration (M/L^3). S_0 = Initial substrate concentration (M/L^3). R = Particle radius (L). t = Time (T). A_s = Substrate surface area (L^2). a^* = Specific substrate surface area ($L^2 \cdot M^{-1}$).

For example, the chemical first order kinetics ($K_H \cdot S$) by Eastman and Ferguson [29] shows a process rate only depending on the amount of substrate (S) in the system. In this case, size and nature of

substrate are irrelevant in hydrolysis process evolution, as well as the role of bacteria, is not taken into account: their number and their sensitiveness to reaction byproducts actually do not affect the process. Such equation can find, therefore, its proper application when organic substrate is all soluble and the ratio between biomass and substrate is so high that neither biomass reaches a level of saturation in food demand nor is it affected by any inhibition factor. The situation described above corresponds to case rather wide studies, but is singular anyway. Therefore first order kinetics cannot be used as the only expression describing the hydrolysis. Vavilin *et al.* [27], starting from the results obtained by Fernandez [30], in a work aimed at studying the anaerobic biodegradability of solid wastes, actually formulated that the hydrolysis process cannot be dissociated from the growth of hydrolytic bacteria, as well as the substrate heterogeneity, and therefore other expressions coupled with the bacterial growth and physical characteristics of solid particles are as much relevant to describe hydrolysis as the first order kinetics. From this perspective, all those mathematical expressions where the rate of hydrolysis is related not only to substrate concentration but also to biomass concentration and physical characteristics of solids or to one of the previous two factors gain relevance.

To the first group of hydrolysis expressions (*i.e.*, those depending on substrate concentration and biomass concentration as well as physical characteristics of solids) belongs the expression formulated by Terashima [29] where the hydrolysis rate is considered to be dependent on the biomass concentration and surface area as well as density of substrate.

To the second group of hydrolysis expressions (*i.e.*, those depending on substrate concentration and biomass concentration or physical characteristics of solids, respectively) belong two different types of kinetics:

(i) The biological first order kinetics, the half order biomass kinetics, and n-order biomass kinetics, where a hydrolysis rate constant is considered and different weights are attributed to the biomass concentration [31,32];
(ii) The kinetics developed by Sanders *et al.* [25] and Esposito *et al.* [6], where the hydrolysis process rate is related to surface area of organic solid particles.

However, no expression, among all above-mentioned, takes into account that the AD process occurs in an environment where water content ranges between 80% (dry digestion) and 97% (wet digestion). Therefore the effect of water (e.g., its power to chemically dissolve substances) on solid particles cannot be neglected. For instance, solid particles of food are made of soluble as well insoluble fractions. Those soluble are almost immediately dissolved in water, causing the simultaneous disaggregation of particles, and therefore when the substrate is suspended, a hydrolysis rate modeled on the bases of a dependence on particles size (Sanders, Esposito and Terashima) can have unexpectedly inappropriate results, whereas a hydrolysis first order kinetics or kinetics only depending on biomass concentration can successfully simulate the hydrolytic process. The relevance of this aspect is proved with the experiments and mathematical simulations described in the following subsections.

3. Materials and Methods

The effect of solubilization process performed by water molecules on the hydrolysis rate in the AD process when organic solid particles are used as substrates have been proved by comparing

experimental and numerical results obtained by Liotta *et al.* [36] with those experimentally obtained in this work through Bio-Methane Potential (BMP) tests and successively modeled using a mathematical model [37] developed by the same authors as an extension of ADM1 [4].

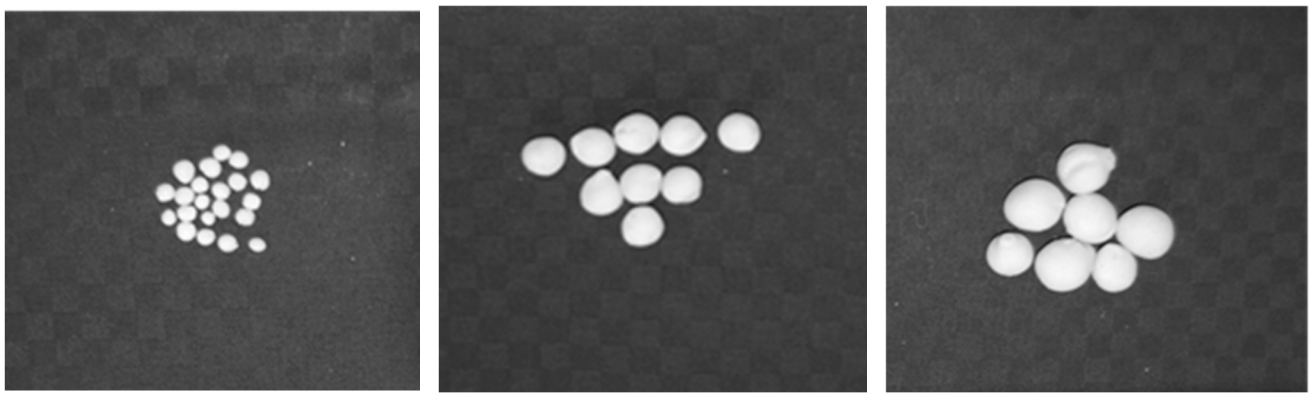
3.1. Results Previously Published by Liotta et al.

Liotta *et al.* [36] conducted BMP tests to study the effect of solid particles size distribution (PSD) on methane production rate in an AD process. For this aim, cylindrical shaped carrot batons were used as substrates with 4 different sizes (bases diameter of 0.25, 4, 9 and 15 mm), each of them with a height equal to its corresponding base diameter. Experimental results clearly showed an inverse dependence of methane production rate on solid particles size: the smaller the size was, the faster the methane was produced. Such results were used to calibrate and validate the mathematical model proposed by the authors (and also used in this work) where the hydrolysis process was considered the limiting step of the AD and modeled using a surface based kinetic. Calibration and validation showed a really good agreement between experimental and simulated data.

3.2. Experiments Design

BMP tests were conducted using homemade spherical shaped pasta with three different diameters, *i.e.*, 4, 9 and 15 mm (Figure 2) whose main characteristics in terms of Total Solids (TS), Volatile Solids (VS), Carbohydrates Fraction (F_{ch}), Proteins Fraction (F_{pr}) and Lipids Fraction (F_{li}) are shown in Table 2.

Figure 2. Homemade spherical shaped pasta used in BMP tests. (**a**) 4 mm; (**b**) 9 mm; (**c**) 15 mm.



(**a**) (**b**) (**c**)

A further BMP test was conducted on the inoculum to estimate the volume of methane resulting by the fermentation of the organic solids contained in the anaerobic sludge. In total, 4 BMP tests, named with the acronyms P_1 (4 mm diameter pasta), P_2 (9 mm diameter pasta), P_3 (15 mm diameter pasta) and I (inoculum) were conducted, each of them in triplicate (Table 3).

3.3. Substrates Collection and Preparation

The dough used to make pasta was bought from the pasta factory Divella located in Bari (Italy). The homemade spherical shaped pasta was successively dried for 24 h in a ventilated oven (model DR 435 CB, BOMANN, Germany) with the aim of reducing its moisture content and give it a solid

consistency. The size of pasta diameter was controlled by a commercial caliper. Inoculum was collected from a real scale anaerobic digester operating in Albanella (Italy) to treat dairy waste produced from mozzarella cheese factories.

Table 2. Characteristics of substrate used in BMP tests.

Substrate	Parameter				
	TS (%)	VS (%)	F_{ch} (%)	F_{pr} (%)	F_{li} (%)
	Wet Mass	Dry Mass	Dry Mass	Dry Mass	Dry Mass
Homemade pasta	91.2 ± 3.2	89.9 ± 2.7	76.7±3.4	12.5±2.8	1.4 ± 0.7

Table 3. Characteristics of the organic mixtures in terms of ratio between inoculums and organic matter, solid particles size and amounts of substrate, inoculums and soda, respectively, on wet mass.

BMP Test	$\left(\frac{\text{VS inoculum}}{\text{VS organic matter}}\right)$	Diameter (mm)	Substrate (g)	Inoculum (g)	Na_2CO_3 (g)
P_1	2	4	13.1 ± 0.6	400.5 ± 5.6	1.5 ± 0.1
P_2	2	9	13.0 ± 0.4	400.1 ± 3.8	1.5 ± 0.1
P_3	2	15	13.7 ± 0.3	412.2 ± 4.3	1.5 ± 0.2
I	---	---	---	400.0 ± 2.5	1.5 ± 0.1

3.4. BMP Tests Set up and Operation

Following the same procedure [38–40] used by Liotta *et al.* [36], each BMP test conducted on homemade pasta was performed under controlled and reproducible conditions in a 1000 mL glass bottle GL 45 (Schott Duran, Duran Group GmbH, Wertheim/Main, Germany). Each bottle was partially filled with inoculum and substrate, according to a ratio equal to 2 between their VS content; tap water was added up to a 500 mL total volume. Small amounts of Na_2CO_3 powder, around 1.5 g, were also added (Table 3) to prevent critical drops in pH. Each bottle was sealed with a 5 mm thick silicone disc that was held tightly to the bottle head by a plastic screw cap punched in the middle (Schott Duran, Germany). All bottles were shaken for 30 min at 80 rpm speed by KL-2bottle shakers (Edmund Bühler, Germany) and were immersed up to half of their height in hot water, kept at a constant temperature of 35 ± 1 °C by 200 W A-763 submersible heaters (Hagen, Germany). Once a day, each bottle was connected by a capillary tube to an inverted 1000 mL glass bottle containing an alkaline solution (2% NaOH) and sealed in the same way as done for the BMP bottle. To enable gas transfer through the two connected bottles, the capillary tube was equipped on both ends with needles sharp enough to pierce the silicone disc.

3.5. Measurements

TS, VS, COD, as well as F_{li}, of each substrate were measured according to Standard Methods [42]. F_{pr} was obtained multiplying 6.25 by the organic nitrogen content of each substrate (TKN minus NH4-N) measured according to Standard Methods [41], whereas, F_{ch} was evaluated by subtracting the sum of proteins and lipids from the total VS content [42].

Daily methane production was monitored measuring the volume of alkaline solution displaced from the measure bottle and collected in a graduated cylinder. The CO_2 contained in the biogas did not affect the volumetric methane measurements as it was dissolved in the alkaline solution. Temperature and pH (data not shown) in each BMP bottle were also monitored at least once a day with a TFK 325 thermometer (WTW, Germany) and a pH meter (Carlo Erba, Italy), respectively.

No significant drop in pH was noticed by the daily monitoring in any BMP bottle. The pH slightly fluctuated around 8.3 during each test.

3.6. Mathematical Modeling

A mathematical model capable of predicting the methane production has been presented elsewhere by the authors [37]. This model can consider different organic substrates (e.g., sewage sludge and organic fraction of municipal solid waste-OFMSW) and the AD process can be modeled using different hydrolysis kinetics:

(i) First order kinetics, according to the ADM1 [4], when the substrate is soluble:

$$\frac{dC}{dt} = -K_H \cdot C \tag{1}$$

(ii) Surface based kinetic expression, according to Esposito *et al.* [6], when the substrate is suspended and highly complex:

$$\frac{dC}{dt} = -K_{sbk} \cdot a^* \cdot C \tag{2}$$

with

$$a^* = \frac{A}{M} = \frac{\sum_{i=1}^{n} A_i}{\sum_{i=1}^{n} M_i} = \frac{nA_i}{nM_i} \tag{3}$$

where:

C = concentration of the complex organic substrate in the anaerobic reactor ($M \cdot L^{-3}$);
K_H = first order hydrolysis kinetic constant (T^{-1});
K_{sbk} = surface based hydrolysis kinetic constant ($M \cdot L^{-2} \cdot T^{-1}$);
A = solids surface total area (L^2);
M = suspended organic total mass (M);
A_i = single solid particle surface area (L^2)
M_i = single solid particle mass (M)
n = total number of organic solid particles (dimensionless)
According to Equation (3) a^* assumes the following expressions:

$$a^* = \frac{n \cdot 4\pi R_i^2}{n \cdot \delta \cdot \frac{4}{3}\pi R_i^3} = \frac{3}{\delta R} \tag{4}$$

$$a^* = \frac{n \cdot 6\pi R_i^2}{n \cdot \delta \cdot 2\pi R_i^3} = \frac{3}{\delta R} \tag{4bis}$$

for spherical shaped pasta and cylindrical shaped carrot batons, respectively, used by Liotta *et al.* [36], with

δ = organic solid particle density ($M \cdot L^{-3}$)

R = organic solid particles radius (L), assumed time dependent in according to the following expression proposed by Sanders *et al.* [25]:

$$R = R_0 - K_{sbk} \frac{t}{\delta} \tag{5}$$

with:

R_0 = initial organic solid particles radius (L).

Observing Equations (4) and (4bis) it can be easily noticed that the expression of a^* is the same for spherical shaped pasta and cylindrical shaped carrot batons.

Substituting Equation (4) or (4bis) in Equation (2), the latter results in the following Equation:

$$\frac{dC}{dt} = -\left(\frac{3 \cdot K_{sbk}}{\delta}\right) \cdot \frac{C}{R} \tag{6}$$

3.7. Model Calibration and Validation

The same model calibration procedure described in Liotta *et al.* [36] was performed on test P_1 (characteristic size of 4 mm). Model results were actually compared with experimental measurements of methane production and the unknown parameters were iteratively adjusted until the model adequately resulted in fitting the experimental data.

To model hydrolysis, the first order kinetics as well as the surface based kinetic expressions were used. After the calibration process was complete and the values of the hydrolysis constants were known, the model was validated using the results of the BMP tests from P_2 (*i.e.*, characteristic size of 9 mm), and P_3 (*i.e.*, characteristic size of 15 mm).

According to Liotta *et al*. [36] and Esposito *et al.* [43], model validation was then evaluated graphically as well as numerically [44] by calculating Model Efficiency (*ME*), the Index of Agreement (*IoA*) and the Root Mean Square Error (*RMSE*).

The expression used to calculate *ME*, *IoA* and *RMSE* are as follow:

$$ME = 1 - \frac{\sum_{i=1}^{K} (y_i - y'_i)^2}{\sum_{i=1}^{K} (y_i - y_M)^2} \tag{7}$$

$$IoA = 1 - \frac{\sum_{i=1}^{K} (y_i - y'_i)^2}{\sum_{i=1}^{K} \left(|y'_i - y_M| + |y_i - y_M|\right)^2} \tag{8}$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{K}(y_i - y'_i)^2}{K}} \quad (9)$$

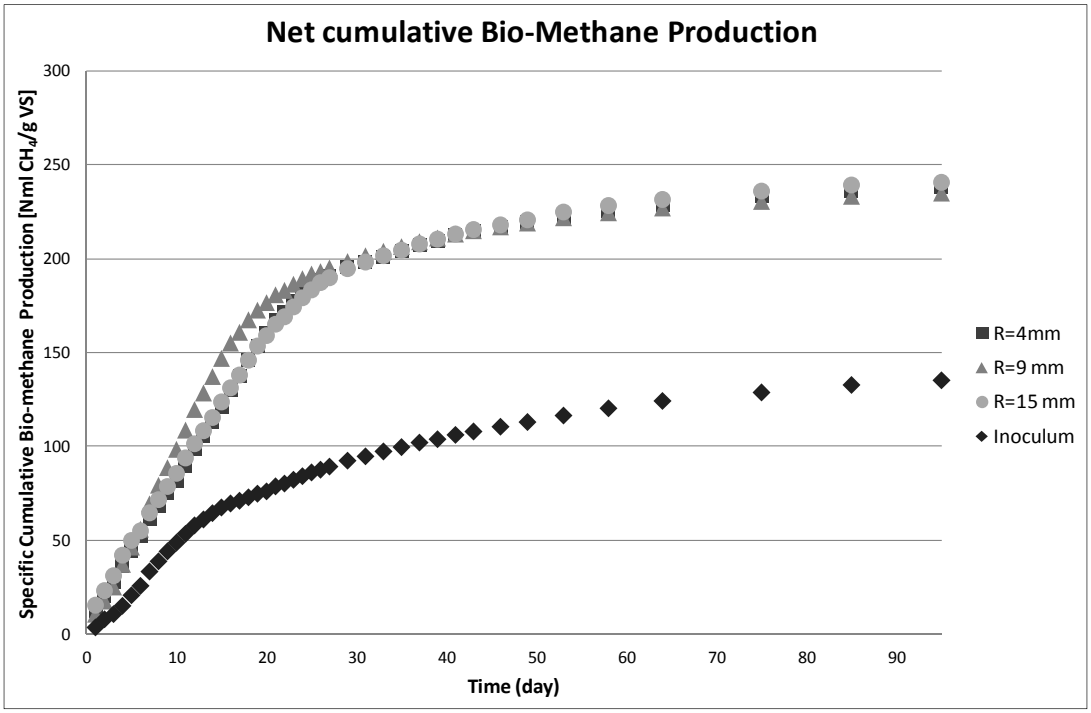
where K is the number of observed values, y_i is the single numerically simulated value, y'_i is the corresponding experimentally observed value, y_M is the average of the numerically simulated values.

4. Results and Discussion

4.1. Effect of Particle Size on Methane Production

Observing Figure 3, where the net cumulative methane production from the BMP tests conducted using homemade spherical shaped pasta is shown, it can be easily noticed that, contrary to results obtained by Liotta *et al.* [36], although experimental tests were conducted using substrates composed of differently sized particles, the effect of size is completely absent, as the three curves overlap and show the same slope.

Figure 3. Net cumulative methane production obtained from BMP tests.



A little gap is only noticeable between days 10 and 25 and it could have been reasonably caused by the heterogeneity of the mixture used in the tests. The gap actually disappears after the 25th day and the three curves remain overlapped until the end of the test.

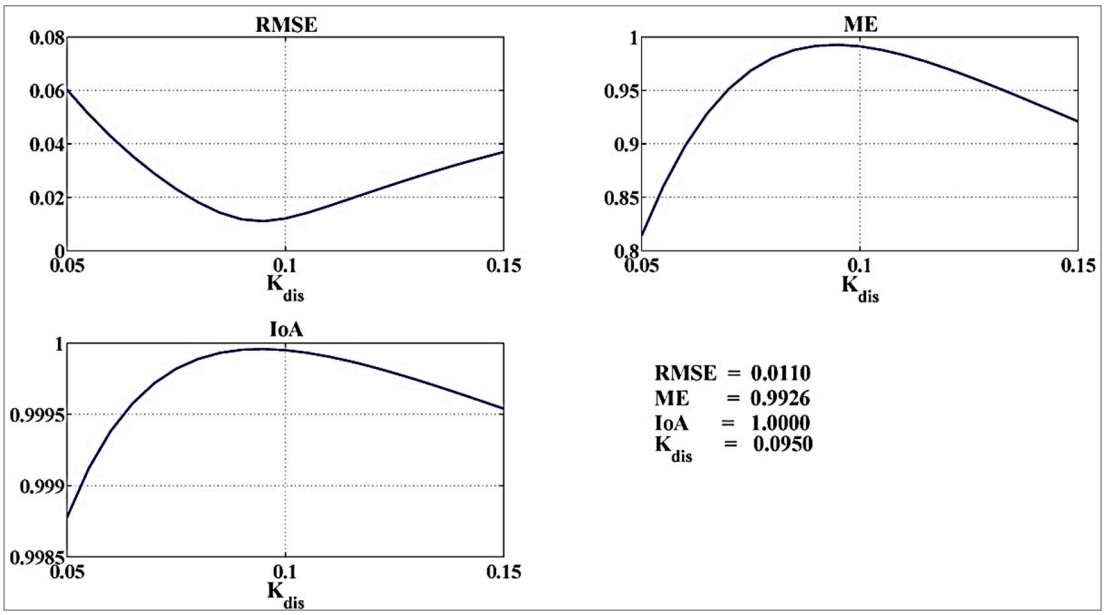
This unexpected result can be explained considering that the chemical composition and texture of homemade pasta are, contrary to those characterizing the carrot batons, suitable to be fast dissolved in water according to Van Soest fractionation method [14–16]. To prove this aspect, samples of both homemade pasta and carrot batons with the same sizes used in BMP tests were immersed in sterilized water and after 10 min it was noticed that while samples of carrots kept their form and size, all samples

of pasta went completely mashed, thus, making negligible any effect on the AD process deriving by the initial different size. Therefore a porous texture and the presence of soluble fractions in chemical composition of solids represent two elements that can favor their almost instantaneous physical disintegration in water with no need of any enzymatic activity performed by bacteria.

4.2. Effect of Particle Size on Hydrolysis Process Modeling

The model calibration performed on data obtained from the test P_1 resulted in setting the hydrolysis kinetic constant, K_H, to 0.095 s^{-1}. This value maximizes both *ME* and *IoA* and minimizes *RMSE*, making the gap between the simulated model and experimental data as small as possible (Figures 4 and 5A).

Figure 4. Model calibration results.



Since K_H, according to Equations (1) and (2), can be split in the product between K_{sbk} and a^*, and a^* for the test P_1, according to Equation (4), was algebraically calculated resulting in 0.566 $m^2 \cdot kg^{-1}$, K_{sbk} was evaluated to be equal to 0.168 $kg \cdot m^{-2} \cdot s^{-1}$.

The values of the kinetic constants, K_H and K_{sbk}, obtained by performing the model calibration, were separately used to validate the model through the experimental data from tests P_2 and P_3.

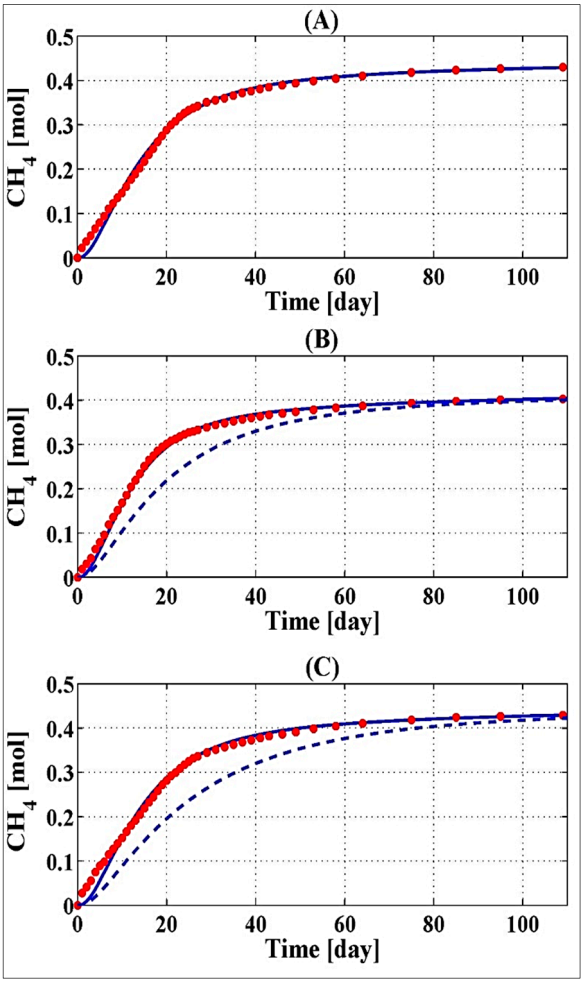
The results of the validation process were evaluated by calculating the same indexes *ME*, *IoA* and *RMSE*, previously used for the calibration process.

In Figure 5B,C experimental data are compared with those simulated by the model. From the figures, it can be clearly noticed that where a first order kinetics, K_H, was used for modeling the hydrolysis process, a high level of agreement between the two sets of data (*i.e.*, experimental and simulated) was found. This good outcome is also confirmed numerically by the values of the fitting parameters reported in Table 4, whereas, where a surface based kinetics, K_{sbk}, was used, the validation process resulted in a low agreement between experimental and simulated data. Such result is furthermore confirmed by the values of the fitting parameters reported in Table 5.

Therefore, although the substrate made of homemade pasta initially showed a mixture of solid particles, the hydrolysis process, contrary to expectations, was not affected by particles size and

consequently, a first order kinetics rather than the surface based kinetics, was suitable for modeling the AD process.

Figure 5. (**A**) Comparison between experimental (points) and simulated methane production (continuous line) for test P_1; (**B**) comparison between experimental (points) and simulated methane production for test P_2 using a first order hydrolysis kinetics (continuous line) and surface based hydrolysis kinetics (dotted line); (**C**) comparison between experimental (points) and simulated methane production for test P_3 using a first order hydrolysis kinetics (continuous line) and surface based hydrolysis kinetics (dotted line).



An opposite outcome was obtained by Liotta *et al.* [36] when the same experiments described in this paper, but utilizing carrot batons as substrate, were conducted. In that case, the hydrolysis process results were dependent on particles size, and also the modeling gave excellent results when surface based kinetics was used to model the hydrolysis process. This is a further proof of the relevance of texture and chemical composition of solid particles when they are used to feed an AD process. The obtained results are in agreement with the Van Soest biochemical fractionation method and its modified versions [15] that, although have been developed to evaluate forage digestibility, are helpful to evaluate the biodegradability of a substrate by fractionating it in different components, from more easily bioaccesible and, thus, readily biodegradable (water soluble fractions mainly made of simple carbohydrates and proteins) to less easily bioccessible and, thus, slowly biodegradable (cellulose,

hemicellulose, lignin, *etc*.), where the fraction extractable through a hot and cold water bath actually represents the most easily bioaccessible and therefore, a substrate mainly composed of water soluble fractions, as pasta is, are expected to be hydrolyzed in a shorter time than others, mainly composed of cellulose based fractions, such as carrots.

Table 4. Results of the validation process modeled with a first order hydrolysis kinetics.

Tests	Diameter (mm)	K_H (s^{-1})	*ME*	*IoA*	*RMSE*
P_2	9	0.095	0.964	0.999	0.023
P_3	15	0.095	0.989	0.999	0.013

Table 5. Results of the validation process modeled with a surface based hydrolysis kinetics.

Tests	Diameter (mm)	*a** ($m^2 \cdot kg^{-1}$)	K_{sbk} ($kg \cdot m^{-2} \cdot s^{-1}$)	*ME*	*IoA*	*RMSE*
P_2	9	0.503	0.168	0.845	0.998	0.055
P_3	15	0.302	0.168	0.790	0.998	0.063

5. Conclusions

This paper proves that in an AD process fed with organic solid particles, the hydrolysis can be faster than expected and not represent the limiting step of the whole process.

Organic solids with a porous texture and a chemical composition mainly made of simple carbohydrates can be easily disintegrated in water, thus resulting in immediately availability to microorganisms that utilize them for their metabolism. In this specific condition any expensive pretreatment set to accelerate the disintegration and solubilization of solid particle prior to AD can be reasonably avoided, resulting in economical benefits. Moreover if the rate of hydrolysis is underestimated, the AD process can experience a failure due to acid accumulation in the system, as the hydrolysis rate cannot limit the volatile acid production occurring during the acidogenesis step that is usually much faster than the following methanogenesis step, where acids are consumed to produce methane. Therefore, an evaluation of the substrate solubility and bioaccessibility prior to the AD process by a Van Soest fractionation method and its modified versions could help to take decisions on how to best run the process.

Finally, the effect of interactions between solid particles and water also has to be carefully taken into account when mathematical models are implemented, as the choice of expressions to simulate the processes massively affects the efficiency and reliability of the model and its results.

Acknowledgments

This research is in the framework of the project *Integrated system to treat buffalo slurry, aimed to recover water and safe energy—STABULUM*, funded, in agreement with the Decision of the European Commission No C(2010) 1261, 2 March 2010, by the Agriculture Department of the Campania Region in the context of the Programme of Rural Development 2007–2013, Measure 124 Cooperation for development of new products, processes and technologies in the agriculture and food sectors.

Author Contributions

Antonio Panico designed the study, participated in performing the experiments as well as in writing the mathematical model code, interpreted the data, wrote the manuscript and revised it until its final version. Giuseppe d'Antonio supervised the whole work, edited the manuscript and revised it critically giving an important intellectual contribute. Giuseppe Esposito participated in designing the study as well as in writing the manuscript and revised it critically. Luigi Frunzo participated in designing the study as well as in writing the mathematical model code, ran the model and analyzed the output data. Paola Iodice participated in writing the manuscript, performed the experiments and interpreted the data. Francesco Pirozzi participated in writing the manuscript revising it critically.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Stillwell, A.; Hoppock, D.; Webber, M. Energy Recovery from Wastewater Treatment Plants in the United States: A Case Study of the Energy-Water Nexus. *Sustainability* **2010**, *2*, 945–962.
2. Glatz, P.; Miao, Z.; Rodda, B. Handling and Treatment of Poultry Hatchery Waste: A Review. *Sustainability* **2011**, *3*, 216–237.
3. Cossu, R.; Masi, S. Re-thinking incentives and penalties: Economic aspects of waste management in Italy. *Waste Manag.* **2013**, *33*, 2541–2547.
4. Batstone, D.J.; Keller, J.; Angelidaki, I.; Kalyuzhnyi, S.V.; Pavlostathis, S.G.; Rozzi, A.; Sanders, W.T.M.; Siegrist, H.; Vavilin, V.A. *Anaerobic Digestion Model No.1, Rep. No. 13*; IWA Publishing: London, UK, 2002.
5. Noike, T.; Endo, G.; Chang, J.; Matsumoto, J. Characteristics of carbohydrate degradation and the rate-limiting step in anaerobic digestion. *Biotechnol. Bioeng.* **1985**, *27*, 1482–1489.
6. Esposito, G.; Frunzo, L.; Panico, A.; d'Antonio, G. Mathematical modelling of disintegration-limited co-digestion of OFMSW and sewage sludge. *Water Sci. Technol.* **2008**, *58*, 1513–1519.
7. Aquino, S.F.; Chernicharo, C.A.L.; Soares, H.; Takemoto, S.Y.; Vazoller, R.F. Methodologies for determining the bioavailability and biodegradability of sludges. *Environ. Technol.* **2008**, *29*, 855–862.
8. Mottet, A.; Ramirez, I.; Carrère, H.; Déléris, S.; Vedrenne, F.; Jimenez, J.; Steyer, J.P. New fractionation for a better bioaccessibility description of particulate organic matter in a modified ADM1 model. *Chem. Eng. J.* **2013**, *228*, 871–881.
9. Jimenez, J.; Gonidec, E.; Cacho Rivero, J.A.; Latrille, E.; Vedrenne, F.; Steyer, J.P. Prediction of anaerobic biodegradability and bioaccessibility of municipal sludge by coupling sequential extractions with fluorescence spectroscopy: Towards ADM1 variables characterization. *Water Res.* **2014**, *50*, 359–372.
10. Vavilin, V.A.; Rytov, S.V.; Lokshina, L.Y. A description of hydrolysis kinetics in anaerobic degradation of particulate organic matter. *Bioresour. Technol.* **1996**, *56*, 229–237.
11. Sanders, W.T.M. Anaerobic Hydrolysis during Digestion of Complex Substrates. Ph.D. Thesis, Wageningen University, Wageningen, The Netherlands, 2001.

12. Silva, G.G.D.; Couturier, M.; Berrin, J.G.; Buléon, A.; Rouau, X. Effects of grinding processes on enzymatic degradation of wheat straw. *Bioresour. Technol.* **2012**, *103*, 192–200.
13. López, G.; Ros, G.; Rincón, F.; Periago, M.J.; Martínez, M.C.; Ortuño, J. Relationship between Physical and Hydration Properties of Soluble and Insoluble Fiber of Artichoke. *J. Agric. Food Chem.* **1996**, *44*, 2773–2778.
14. Lashermes, G.; Nicolardot, G.; Parnaudeau, V.; Thuriès, L.; Chaussod, R.; Guillotin, M.L.; Linères, M.; Mary, B.; Metzger, L.; Morvan, T.; *et al*. Typology of exogenous organic matters based on chemical and biochemical composition to predict potential nitrogen mineralization. *Bioresour. Technol.* **2010**, *101*, 157–164.
15. Peltre, C.; Dignac, M.F.; Derenne, S.; Houot, S. Change of the chemical composition and biodegradability of the Van Soest soluble fraction during composting: A study using a novel extraction method. *Waste Manag.* **2010**, *30*, 2448–2460.
16. Zhang, Y.; Lashermes, G.; Houot, S.; Doublet, J.; Steyer, J.P.; Zhu, Y.G.; Barriuso, E.; Garnier, P. Modelling of organic matter dynamics during the composting process. *Waste Manag.* **2012**, *32*, 19–30.
17. Pavlostathis, S.G.; Giraldo-Gomez, E. Kinetics of anaerobic treatment: A critical review. *Crit. Rev. Environ. Control* **1991**, *21*, 411–490.
18. Ariunbaatar, J.; Panico, A.; Esposito, G.; Pirozzi, F.; Lens, P.N.L. Pretreatment methods to enhance anaerobic digestion of organic solid waste. *Appl. Energy* **2014**, *123*, 143–156.
19. Ariunbaatar, J.; Panico, A.; Frunzo, L.; Esposito, G.; Lens, P.N.L.; Pirozzi, F. Enhanced anaerobic digestion of food waste by thermal and ozonation pretreatment methods. *J. Environ. Manag.* **2014**, *146*, 142–149.
20. D'Acunto, B.; Esposito, G.; Frunzo, L.; Pirozzi F. Dynamic modeling of sulfate reducing biofilms. *Comput. Math. Appl.* **2011**, *62*, 2601–2608.
21. D'Acunto, B.; Frunzo, F. Free boundary problem for an initial cell layer in multispecies biofilm formation. *Appl. Math. Lett.* **2012**, *25*, 20–26.
22. Aldin, S. The Effect of Particle Size on Hydrolysis and Modelling of Anaerobic Digestion. Ph.D. Thesis, School of Graduate and Postdoctoral Studies, The University of Western Ontario London, Ontario, ON, Canada, 2010.
23. Vavilin, V.A.; Rytov, S.V.; Lokshina, L.Y.; Rintala, J.A.; Lyberatos, G. Simplified hydrolysis models for the optimal design of two-stage anaerobic digestion. *Water Res.* **2001**, *35*, 4247–4251.
24. Morgenroth, E.; Kommedal, R.; Harremoës, P. Processes and modeling of hydrolysis of particulate organic matter in aerobic wastewater treatment—A review. *Water Sci. Technol.* **2002**, *45*, 25–40.
25. Sanders, W.T.M.; Geerink, M.; Zeeman, G.; Lettinga, G. Anaerobic hydrolysis kinetics of particulate substrates. *Water Sci. Technol.* **2000**, *41*, 17–24.
26. Myint, M.; Nirmalakhandan, N. Evaluation of first-order, second-order, and surface-limiting reactions in anaerobic hydrolysis of cattle manure. *Environ. Eng. Sci.* **2006**, *23*, 966–976.
27. Vavilin, V.A.; Fernandez, B.; Palatsi, J.; Flotats, X. Hydrolysis kinetics in anaerobic degradation of particulate organic material: An overview. *Waste Manag.* **2008**, *28*, 939–951.
28. Dimock, R.; Morgenroth, E. The influence of particle size on microbial hydrolysis of protein particles in activated sludge. *Water Res.* **2006**, *40*, 2064–2074.

29. Eastman, J.A.; Ferguson, J.F. Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. *J. WPCF* **1981**, *53*, 352–366.
30. Fernandez, B.; Porrier, P.; Chamy, R. Effect of inoculum-substrate ratio on the start-up of solid waste anaerobic digesters. *Water Sci. Technol.* **2001**, *44*, 25–40.
31. Rozzi, A.; Merlini, S.; Passino, R. Development of a four population model of the anaerobic degradation of carbohydrates. *Environ. Technol. Lett.* **1985**, *6*, 610–619.
32. Valentini, A.; Garruti, G.; Rozzi, A.; Tilche, A. Anaerobic degradation kinetics of particulate organic matter: A new approach. *Water Sci. Technol.* **1997**, *36*, 239–246.
33. Henze, M.H.; Jonsen, J.C.; Arven, E. *Waste Water Treatment*; Springer-Verlog: Berlin, Germany, 1997.
34. Hashimoto, A.G.; Chen, Y.R.; Verel, V.H. Theoretical aspects of methane production: State of the art. In *Livestock Waste: A Renewable Resources*; Proceedings of the Fourth International Symposium of Livestock Wastes; Transaction of ASAE: St. Joseph, MI, USA, 1981; pp. 86–91.
35. Negri, E.D.; Mata-Alvarez, J.; Sans, C.; Cecchi, F. A mathematical model of volatile fatty acids (VFA) production in a plug flow reactors treating the organic fraction of municipal solid waste (MSW). *Water Sci. Technol.* **1993**, *27*, 201–208.
36. Liotta, F.; d'Antonio, G.; Esposito G.; Fabbricino, M.; Frunzo, L.; van Hullebusch, E.D.; Lens, P.N.L.; Pirozzi, F. Effect of moisture on disintegration kinetics during anaerobic digestion of complex organic substrates. *Waste Manag. Res.* **2014**, *32*, 40–48.
37. Esposito, G.; Frunzo, L.; Panico, A.; Pirozzi, F. Model calibration and validation for OMSW and sewage sludge co-digestion reactors. *Waste Manag.* **2011**, *31*, 2527–2535.
38. Esposito, G.; Frunzo, L.; Liotta, F.; Panico, A.; Pirozzi, F. Bio-methane potential tests to measure the biogas production from the digestion and co-digestion of complex organic substrates. *Open Environ. Eng. J.* **2012**, *5*, 1–8.
39. Esposito, G.; Frunzo, L.; Giordano, A.; Liotta, F.; Panico, A.; Pirozzi, F. Anaerobic co-digestion of organic wastes. *Rev. Environ. Sci. Biotechnol.* **2012**, *11*, 325–341.
40. Esposito, G.; Frunzo, L.; Panico, A.; Pirozzi F. Enhanced bio-methane production from co-digestion of different organic wastes. *Environ. Technol.* **2012**, *33*, 2733–2740.
41. APHA/AWWA/WEF. *Standards Methods for the Examination of Water and Wastewater*, 20th ed.; United Book Press Inc.: Baltimore, MD, USA, 1998.
42. Galí, A.; Benabdallah, T.; Astals, V.; Mata-Alvarez, J. Modified version of ADM1 model for agro-waste application. *Bioresour. Technol.* **2009**, *100*, 2783–2790.
43. Esposito, G.; Frunzo, L.; Panico, A.; Pirozzi F. Modelling the effect of the OLR and OFMSW particle size on the performances of an anaerobic co-digestion reactor. *Process Biochem.* **2011**, *46*, 557–565.
44. Janssen, P.H.M.; Heuberger, P.S.C. Calibration of process-oriented models. *Ecol. Model.* **1995**, *83*, 55–66.