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Biogas Production from Vegetable and Fruit Markets Waste—Compositional and Batch Characterizations

Carlos Morales-Polo *, **María del Mar Cledera-Castro** and **B. Yolanda Moratilla Soria**

ICAI School of Engineering, Comillas Pontifical University, Alberto Aguilera 25, 28015 Madrid, Spain; mcledera@comillas.edu (M.d.M.C.-C.); ymoratilla@comillas.edu (B.Y.M.S.)

* Correspondence: cmorales@comillas.edu

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Abstract: This study presents a complete characterization of the residual materials found in fruit and vegetable markets and their adaptability to be treated by anaerobic digestion with the aim of generating biogas as a new and renewable energy source. It has been determined that these substrates are perfectly suitable to be treated by anaerobic digestion, being rich in simple carbohydrates, with a high content of moisture and solids (total and volatile), which makes it a substrate of easy solubilization and with a great amount of matter directly accessible to the microorganisms responsible for anaerobic degradation. The process develops smoothly, with a slight release of acidic elements, but without impact by the development of the buffer effect by ammonia. In addition, a phenomenon of digestion is observed in two phases, indicating that despite the particulateing of the substrate, it manages to digest the organic matter directly accessible and the inaccessible. In numerical terms, 100 g of residue V produce 913.282 NmL of biogas, of which 289.333 NmL correspond to methane. The disintegration constant is 0.200 days^{-1}, with 16,045% of the substrate degraded. As an innovation, the hydrogen generated in the process has been used as an indicator of the stability and development of the process. Accompanied by a statistical analysis and mathematical adjustments, it is possible to characterize in depth the process and its evolution, determining that the degradation is fast, with a rapid and stable hydrolysis.

Keywords: biowaste; BMP; biomethane; biofuel; anaerobic digestion

1. Introduction

The agri-food industry is one of the fundamental pillars of the world economy. For example, at the European level, this industry exported 27.8 billion euros in the first quarter of 2019 and imported 18.6 billion euros [1]. This sector is closely linked to economic and social growth and therefore, becomes a fundamental pillar.

However, all productive activity generates a large amount of waste. Any material, whether intended or not for human consumption, that are lost, degraded, contaminated or disposed of from the food supply chain (FSC) is considered food waste (FW) or food loss (FL) [2].

Nowadays, the definition of FW also includes the losses incurred in the FSC stages or any other step of the agri-food industry, therefore, it is defined as “any food and inedible parts of food, removed from (lost to or diverted form) the food supply chain to be recovered or disposed (including composted, crops ploughed in/not harvested, anaerobic digestion, bio-energy production, co-generation, incineration, disposal to sewer, landfill or discarded to sea” [3,4].

The agri-food industry comprises activities from all economic sectors. As can be seen in Figure 1, FSC begins with stages of the primary sector (agriculture and livestock), which produces by-products (i.e., manure, waffle, cornstalk) and FW and FL in the form of low-quality products, damaged production, or products with no commercial value [3,5]. The FSC continues with the stages of product transformation, characteristic of the secondary sector, where FL and FW are mainly generated within the entire process chain due to problems in storage, damages during transport, contamination along the process, or in separation stages that create by-products not intended for human consumption (i.e., feathers, skins, fruit peels . . .). The end of FSC comes with the sales and distribution stage, typical of the tertiary sector [3,6]. Losses and waste are generated in food markets and retail systems in association with problems in storage, conservation, or unsold perishable products [3]. At the final stage of the product life cycle (end consumer), the FW is generated by a purchasing excess, over preparation processes, bad storage conditions, and other consumption behaviour patterns [7].

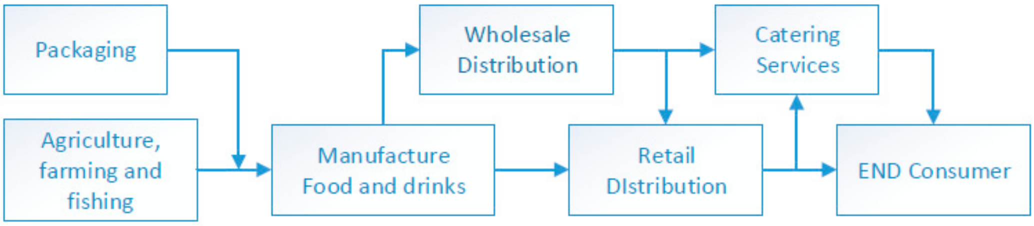


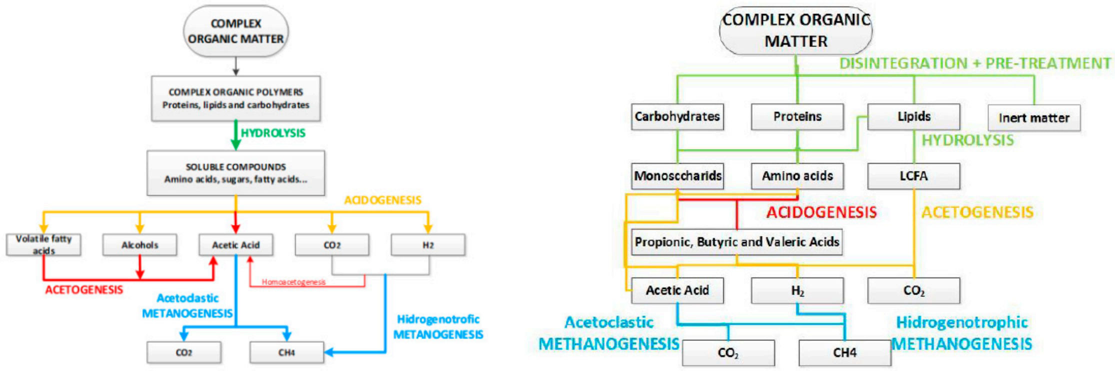
Figure 1. Food supply chain in the agri-food industry.

It is estimated that 33% of all agri-food production is lost in the form of waste [8]. Particularly in the European Union (EU), 90 million tons of this waste are generated annually [9,10]. This kind of waste production represents 12% of all the food entering a home, and a 25% of all the food of the FSC [7]. This implies that within the complete FSC, 40% of FW, and FL occurs during postharvest and processing stages and another 40% during the retail and consumer levels [7,11].

In this context, the generation of waste and materials to be disposed of in the agri-food industry is one of the major environmental problems [12,13], with an impact in the social, economic and political spheres [4]. Given the link to the growth of society, it is impossible to completely eliminate waste emissions. This is why they must be treated as appropriately as possible, achieving mutual and sustainable economic, energy and environmental benefit.

There are many ways to treat agri-food waste [4,12]: seeking an integrative solution [14], such as for energy production and biofuels [15,16] or biopolymer production [17,18]. Anaerobic Digestion (AD) is an optimal solution [19]. This technology is a microbiological process that in the absence of air, allows the transformation of organic materials, such as FW, into biogas, a mixture rich in methane that can be used as combustible gas [20]. The result of anaerobic digestion, apart from biogas, is digestate, the rest of the process that comprises everything that has not been measured, and that can be used as fertilizer for agricultural uses [21]. This is why energy benefits are achieved by obtaining a new energy source in the form of biogas, economic benefits from the savings of building an alternative energy source generated in-situ, and environmental benefits by reducing the amount of residues disposed in landfills, reducing energy consumption and promote the circular economy [22,23].

During the anaerobic degradation process, a series of chained processes occur [24], each producing the substrates necessary for the next stage to develop (Figure 2). Organic matter (OM) must first undergo a hydrolysis process [25], responsible for transforming the initial OM, presented in the form of lipids, carbohydrates and proteins, into simpler and more soluble organic compounds, such as amino acids, saccharides and long-chain fatty acids (LCFA) [26]. This first process is an extracellular reaction. The generated substrates then go through a phase of acidogenesis in which they are converted into volatile fatty acids (VFA) (propyonic, butyric, valeric . . .) and other by-products, such as alcohols, NH_3, CO_2 and H_2. These are treated in the acetogenesis phase, which transforms volatile fatty acids and other by-products into acetic acid. Finally, acetic acid is transformed by acetoclastic methanogenesis into methane, and hydrogenotrophic by hydrogen methanogenesis also into methane [20].



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

Figure 2. Anaerobic digestion process and reactions. (**a**) Simplified method and (**b**) adapted from [4] for ADM1 Simulation.

AD is an extremely complex process that should simultaneously digest all substrates in order to produce the substrate for the next phase reaction. This requires a stable environment for the different microbial growth. It is therefore important to maintain the key parameters of AD process within the correct range, to ensure the appropriate operation of AD [25].

These key parameters are, among others, temperature [27–29] and pH [30], which should be kept as stable as possible; the VFA content directly linked to pH and an indicator of a correct development of acidogenesis or an out-of-control of this stage the carbon content, nitrogen and the ratio between them (C/N ratio) [31,32]; content and training ammonia which can play a positive role [33] if it accumulates below a 2 g/L limit [34] acting as a pH buffer, or acting with negative effect [35,36] if it accumulates above the previous limit, causing a drastic increase in pH and process stop.

In addition, it is important to know the characterization of the substrate in terms of content in lipids, proteins and carbohydrates, which are the substrates that become the hydrolysis stage [37].

Carbohydrates have a very high conversion rate in methane, although they generate light amounts of methane, in the order of 750 Nl of biogas for each kilogram of total solids digested [35]. Anaerobic degradation of these substrates can result in excessive formation and accumulation of VFA, leading to a decrease in pH and process shutdown. Proteins have a high conversion rate with a theoretical biogas generation of 800 Nl for each kilogram of total solid digested [37]. When compounded with nitrogen, their degradation can lead to the formation and accumulation of ammonia, leading to an increase in pH and inhibition [38]. Lipids, on the other hand, are the slowest compounds to transform [39,40], but due to their high carbon content, they have the highest rate of biogas generation, in the order of 1390 Nl of biogas for each kilogram of total solid digested [41].

In short, it follows that in order to achieve that the transformation of waste into biogas by anaerobic digestion, the process must be known and the materials likely to be treated must be deeply characterized.

In the EU, AD is presented as a joint method for the successful implementation of waste management and energy supply strategies [42,43]. One consists of a correct separation and classification of the waste, so that the treatment plants minimize the rejection in the classification, and the management can be correct. The European waste strategy always prioritizes recycling, recovery and energy recovery over landfill ingesting [19]. In order to implement these measures, AD is presented as an optimal solution as it is a form of energy recovery [44], in addition to reducing the amount of waste disposed of in landfill.

In order to optimize the technology, as well as to predict methane production and possible inhibitions when treating a specific residue [45], a characterization in laboratory terms is necessary. Knowing the characterization of the AD process for a particular residue, in this case fruit and vegetable markets, not only helps to understand the evolution of the process and its applicability, but, by knowing the characterization of a separate residue, the behavior when digesting a mixture of wastes can be estimated, which would be a more predictable case, since there are always failures in the classification process [46].

The most important thing when treating this type of waste, according to different authors, is its high content of lignocellulose fibers [47], which are complex structures of plant materials. As other authors have studied, lignocellulose biofibers have a low biodegradability, as the cellulose is packaged inside the lignin [48], thus causing the degradation to be non-existent, causing a drastic reduction in the potential for anaerobic degradation [49,50]. This study determined whether the effect of digesting substrates with a lignocellulose content causes the methanogenic potential and the development of anaerobic degradation to be limited and reduced.

The objectives of this study were, on the one hand, to provide a deep characterization of the substrates to be treated, in terms of composition and the potential for anaerobic digestion. Secondly, to characterize the AD process of the substrates in terms of biogas generation, methane content and quality, and the correct process development. To check if the process has been developed without inhibitions and detect them if they exist, the generation and evolution of hydrogen in the process was used as an indicator. This is one of the main innovations introduced: the use of hydrogen content as an indicator of the stability and development of the process, being an intermediate gas that is generated and subsequently transformed into methane.

With all the results obtained, together with a thorough statistical analysis of the results, and a mathematical treatment of the data obtained, it is possible to determine the adaptability of the substrate for use as a source of methane generation, and thus meet the objectives described in the European strategies [9,42,43,51].

2. Materials and Methods

2.1. Test Samples

Given the variability of substrates (waste), whose composition depends on factors such as level of sale, consumer taste and seasonality, a constant laboratory-prepared residue was chosen to be used with similar characteristics depending on the bibliography, and named V. The source of microorganisms comes from sewage treatment plant UASB (Upflow Anaerobic Sludge Blanket) sludge (F), which is one of the sources recommended by the UNE [52] and VDI [53] standards mentioned above.

2.2. Analytical Methods Forcompositional Racterization of Substrates and Mixtures

For the characterization of the substrates and the sewage sludge used, both individually and mixed, the Standard Methods for the Examination of Water and Wastewater of the APHA (2015) [54] were followed. Table 1 resumes all the methods used for the characterization of substrates and inoculum.

The materials were characterized:

- In terms of physicochemical composition to determine the content in total solids (TS), volatile solids (VS), humidity (Hum) and Chemical Oxygen Demand (COD) both total (COD_t) and soluble (COD_f), in addition to the solubility coefficient, which determines the amount of total COD found directly accessible to microorganisms.
- In terms of organic composition, performing a macronutritional analysis to determine the content in lipids, proteins and carbohydrates (LPCH) and to determine the influence of the input material in the process.
- In terms of elemental analysis, determining the content in C, N, H and S, and the C/N ratio, whose optimal for the correct digestion of a substrate is around 20.
- In terms of nitrogen content, determining total nitrogen Kejldahl (TKN), Organic Nitrogen (ON) and Ammoniacal Nitrogen (AN), the latter being responsible for the formation and accumulation of ammonia. The accumulation limit of AN determined in the study is 2 mg/mL or 2 mg/g, below which it acts as a buffer compensating the pH, and above which ends up accumulating, causing inhibition.

- In terms of pH and alkalinity analysis, pH is used as a clear indicator of process development because if it decreases it, is indicative of an accumulation of acidic elements, and if increased, it is indicative of an accumulation of ammonia. Alkalinity is determined in terms of total alkalinity (TA), partial alkalinity (PA) (due to bicarbonates) and intermediate alkalinity (IA) (due to VFA). A decrease in IA is indicative of excessive generation of VFA, the accumulation of which can be verified with pH variations.

Table 1. Standardized procedures used for compositional characterization of substrates and mixtures.

	Method Procedure
Physical Parameters	
Hum [$\%_{hb}$]	APHA 2540-G
TS [$\%_{hb}$]	
VS [$\%_{hb}$]	
Macronutritional Analysis (LPCH Content)	
Lipids (L) [$\%_{hb}$]	UNE-EN-ISO 13804:2013
Proteins (P) [$\%_{hb}$]	
Carbohydrates (CH) [$\%_{hb}$]	
Organic Content Analysis (COD)	
COD_t [mg O_2/g - mL]	APHA 5220-B
COD_f [mg O_2/g - mL]	
Solubility [%]	
Nitrogen Content Analysis	
TKN [mg N/g - mL]	APHA 4500-N
AN [mg N/g - mL]	APHA 4500-NH_3
ON [mg N/g - mL]	APHA 4500-N_{org}
pH and Alkalinity Analysis	
pH	APHA 2320-B
TA [mg $CaCO_3$/g - mL]	
PA [mg $CaCO_3$/g - mL]	
IA [mg $CaCO_3$/g - mL]	
Elemental Analysis	
C [$\%_{db}$]	
H [$\%_{db}$]	
N [$\%_{db}$]	
S [$\%_{db}$]	
C/N Ratio	

2.3. Biochemical Methane Potential Tests to Determine de Anaerobic Degradation

The Biochemical Methane Potential (BMP) test procedure was developed in accordance with UNE-EN ISO 11,734 [52], including a gas chromatograph for the measurement of biogas composition. This standard uses manometric methods to quantify the amount of gas generated. To transform the pressure measurements into generated gas flow, the procedure described in VDI-4630 [53] was followed.

The test conditions were those usually used at full-scale level. Digestion occurs under mesophilic conditions 37 ± 1 °C, with a ratio between substrate and inoculum (i.e., between residue and UASB sludge of sewage treatment) 1:3 m/v, which means that for every 300 mL of UASB sludge, 100 g of fruit and vegetable residue have been digested.

The amount of gas generated and its composition, in terms of methane and hydrogen, were measured daily by gas chromatography, as described in the Standard Methods for the Examination of Water and Wastewater of the APHA (2015) [54]. This results in the curves of biodegradability of the substrate, of generation of methane and hydrogen, as well as the content of the latter two in biogas.

By analysing both curves of biodegradability, methane generation and hydrogen generation, together with the characterization of the substrate before and after the BMP test, the process and the variables that affect it can be inferred.

Particularly important is hydrogen, as this is an intermediate gas that begins to be generated in the early phases and that ends up disappearing to transform into methane. The rate of hydrogen formation is directly related to the development of hydrolysis and the transformation of hydrogen into methane is related to the ability of the process to complete successive stages.

2.4. Statistical Analysis of Results

The tests were repeated in 21-test runs, so that a significant number of curves are obtained. To determine whether all equal curves can be considered so, or if the dispersion between them is so large that there is no relationship, an ANOVA statistical analysis with a 95% confidence level was performed, accompanied by their respective DMS and Tukey contrasts.

In addition, the results of the obtained curves can be treated mathematically. If methane generation curves are treated as a first-order kinetics, according to the process described by Veeken and Hamlers [26] the disintegration constant (k_{dis}) can be obtained directly related to the ability to be treated by digestion anaerobia and the speed of the process. Similarly, by adjusting by least squares the methane generation curves with the reduction experienced in the COD, according to the [55] procedure, the maximum amount of methane expected for the substrate can be obtained. In addition, by reducing the COD content, as set out in [55], the percentage of substrate that has been biodegraded can be determined, directly related to the scope of the process.

3. Results and Discussion

3.1. Characterization of Materals and Samples

Before analysing the biodegradability and the obtained biogas during digestion, an analysis should be performed to determine the composition of the substrates before the digestion process, and when the process has concluded, in order to analyse the compositional differences created by the AD process.

Table 2 shows the results of substrate characterization and how the composition of the digestion mixture (waste + sludge) has changed when treating vegetable residue, at the time before starting the BMP test, and at 20 days, that is, when this one has finished.

V residue is a substrate with high humidity, so in principle, it is a substrate with easy solubilization and a fast and deep process is to be expected. It is a very rich residue in carbohydrates, compared to the content of lipids and proteins. These carbohydrates are especially simple cellulose-type carbohydrates. The digestion of these compounds is stable and fast, although they are susceptible to generating VFA during their digestion. In terms of COD, this is relatively high, with a solubility of 24.09%, indicating that of all the COD available to microorganisms, 24.09% is already solubilized and directly accessible without the need to release it from encapsulation. As for the nitrogen content, it is not very high, especially under the AN, indicating that an excessive release of ammonia and its accumulation is not foreseeable, even transforming the entire TKN into ammonia. The pH of the substrate is slightly acidic; there is no PA, as all the alkalinity was due to IA, that is, to VFAs, indicating, once again, that the substrate is susceptible to releasing and accumulating VFA. The C/N ratio of material V is in the optimal range, around 20, which demonstrates that there is an adequate balance between carbon and nitrogen, so that it develops the process correctly, generating methane and without inconvenience of ammonia accumulation.

In terms of the change in the characterization of the mixture introduced into the reactor (V+F) before and after being digested by AD, as can be seen, after 20 days of testing, the moisture of the mixture substrate+sludge was reduced slightly but not by large quantity, only 2%, which is logical when it is airtight reactors to ensure anaerobic conditions. The vs. reduction of almost 40% and ST of

21.59% is noteworthy. This gives a first indication that the digestion process developed correctly since the OM associated with The SV and ST degraded, causing the amount of these to drop.

Table 2. Characterization results for Biochemical Methane Potential (BMP) tests of residue V, at the start and after completion of the test.

	Substrate	**Inoculum**		**Initial Reactor Mix**		**Final Reactor Mix**
	Material V	**Material F**		**V+F**		**V+F**
Physical Parameters						
Hum [$\%_{hb}$]	87.90	94.30	→	92.10	⇝	90.25
TS [$\%_{hb}$]	12.10	5.70		7.41		5.81
VS [$\%_{hb}$]	10.91	4.92		6.52		3.99
Macronutritional Analysis (LPCH Content)						
Lipids (L) [$\%_{hb}$]	0.48	0.47	→	0.47	⇝	
Proteins (P) [$\%_{hb}$]	1.52	0.53		0.78		
Carbohydrates (CH) [$\%_{hb}$]	8.90	0.56		2.65		
Organic Content Analysis (COD)						
COD_t [mg O_2/g - mL]	173.64	101.65	→	120.33	⇝	117.97
COD_f [mg O_2/g - mL]	41.83	37.08		40.52		12.05
Solubility [%]	24.09	36.48		33.67		10.21
Nitrogen Content Analysis						
TKN [mg N/g - mL]	2.46	2.00	→	2.11	⇝	2.28
AN [mg N/g - mL]	0.03	1.15		0.87		1.15
ON [mg N/g - mL]	2.43	0.85		1.23		1.12
pH and Alkalinity Analysis						
pH	4.96	7.46	→	7.02	⇝	7.12
TA [mg $CaCO_3$/g - mL]	5.83	8.88		8.32		19.70
PA [mg $CaCO_3$/g - mL]	-	5.22		4.22		1.34
IA [mg $CaCO_3$/g - mL]	5.83	3.65		4.10		9.36
Elemental Analysis						
C [$\%_{db}$]	34.52	11.19	→	17.43	⇝	
H [$\%_{db}$]	6.43	9.00		8.41		
N [$\%_{db}$]	1.69	2.22		2.09		
S [$\%_{db}$]	0.09	0.18		0.14		
C/N Ratio	20.43	5.04		8.34		

As for the analysis of COD, the reduction was poor, as COD_f directly accessible by microorganisms was degraded by 70%, that is, most of the soluble components. In this case, monosaccharides and cellulose were eliminated after digestion. The digestion of these makes the process, in principle, more stable. Only 1.96% was reduced from the COD_t. That is, of the whole OM that was degraded, only 1.96%, and within that 1.96%, 70% corresponds to that directly accessible without hydrolyzing. This indicates that most of the directly accessible OM was digested during the AD process. However, the encapsulation was not digested, as it is not directly accessible to microorganisms. This makes the process uncomplete and indicates a failure of the disintegration stage + hydrolysis and will hopefully lead to a low methane content within the generated biogas.

Based on nitrogen content, TKN likely increased by the release of some of the nitrogen encapsulated in proteins (ON). Similarly, in NA, it increased by 31.8% to the value of 1.15 mg/mL of mixture, placing it in the buffer effect zone (<2 g/L), providing resistance against sudden changes in pH and making the process stable. For its part, NO decreased by about 9%, corroborating with the hypothesis of the release of nitrogen encapsulated in proteins and became part of the AN.

The initial and final pH were very similar, although this may have changed during the process. The most stable part of this analysis is the great increase in alkalinity, 136% TA, 145% alkalinity due to bicarbonates and 128.3 alkalinity due to VFA. This demonstrates once again the stability of the anaerobic digestion process, which becomes more resistant to sudden changes in pH, mainly motivated by the increase in AN that exerts, in this case, a buffer effect in the accumulation of VFA and bicarbonates.

3.2. Biogas Production

To determine biogas generation, internal pressure inside the digester was measured during BMP tests and then translated into the amount of gas generated.

The results are presented in two ways:

- GROSS production of biogas: Biogas produced by 100 g of waste material.
- SPECIFIC production of biogas: Biogas generated by each gram of SV of waste material introduced into the digester.

The most comfortable measure to characterize the generation in broad terms is the gross production of biogas. However, several authors have recommended expressing it as a specific production, as it allows the comparison of the substrates more agilely with each other. The content in solids, especially volatile ones, differs greatly between substrates, and degradability depends on them, so that when comparing results, specific production is more beneficial.

3.2.1. Gross Biogas Production

Figure 3 represents the gross biogas generation curves for 100 g of V residue.

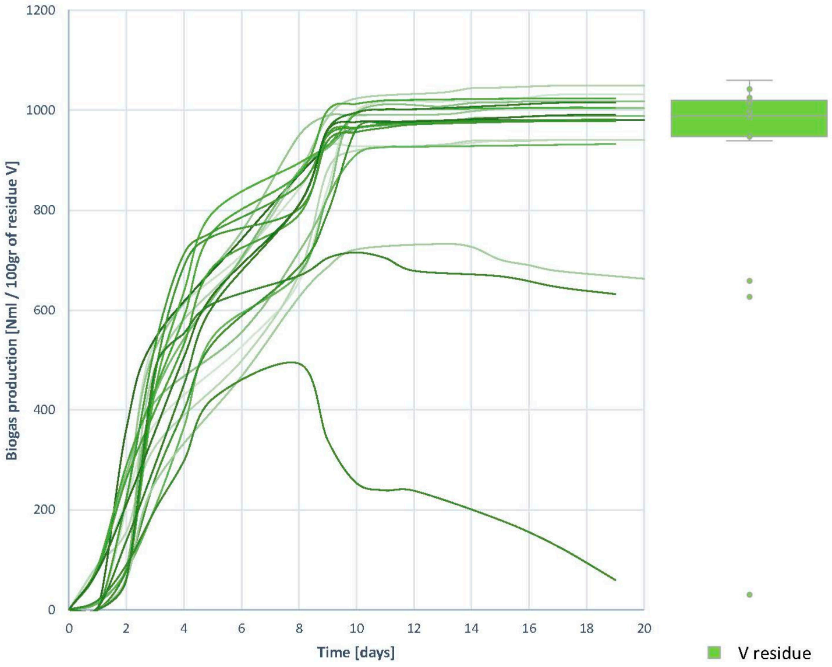


Figure 3. Gross biogas production curves from the anaerobic digestion of 100 g of residue V.

As can be seen, gas production was completed on day 10, producing an average of 913.28 (±14.433%) mL of biogas measured under normal conditions (Table 3). Although three of the trials failed, the curves seem to converge with sufficient precision. This is also demonstrated by the statistical analysis of the results obtained with the ANOVA analysis. This convergence of results in the curves was to be expected due to, as has been previously introduced, the characteristics of the substrate with easily soluble carbohydrates such as monosaccharides and cellulose.

Table 3. Gross production of biogas, methane and hydrogen, and descriptive statistics.

	Production	Standard Deviation	Coefficient of Variation	Relative Error
	[NmL/100 g of residue V]	**σ**	**CV**	**ε**
Biogas	913.282 NmL	222.904	0.244	14.436%
Methane	289,333 NmL	94,723	0.327	21.421%
Hydrogen	0.456 NmL + 0.200 NmL	0.298 + 0.200	0.655 + 1.000	57.583% + 90.609%

As seen in Figures 3 and 4, in the initial part of the curve, there are two clearly differentiated slopes. During the first day, a delayed phase occurred and between days 1 and 4–8, there is a very steep slope for the generation of gas; it stabilizes for a few days and then becomes more pronounced again between days 8 and 10. This means that the previous phases of digestion occurred in two stages. In the first stage (between days 1 and 4–8), the digestion of all soluble COD was produced, that is, around 70% were digested, and in the second stage, the rest of the COD began to solubilize over time.

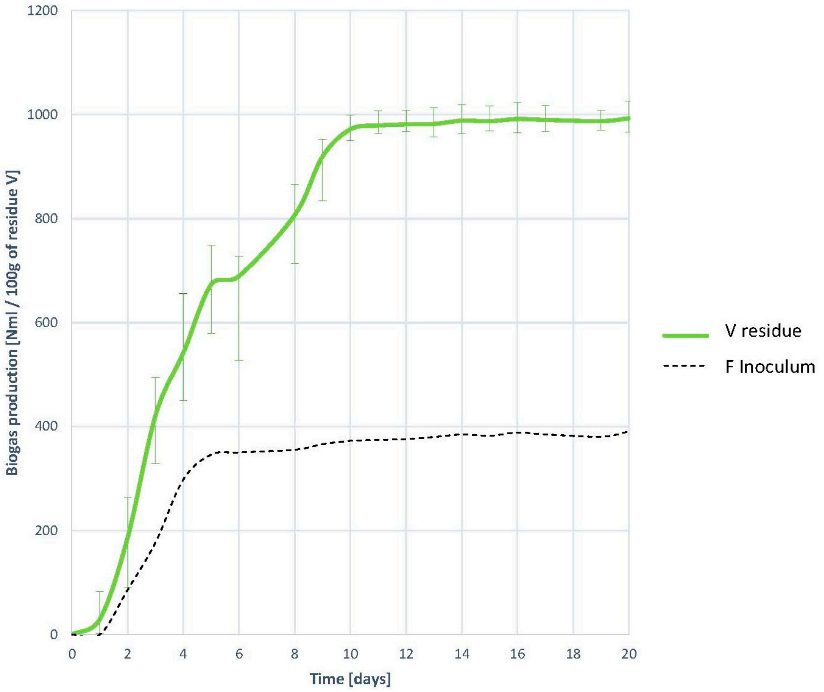


Figure 4. Gross biogas production mean curve from the anaerobic digestion of 100 g of residue V.

All this two-stage solubilization information can be justified to use a pre-treatment that will pre-solubilise the OM so that it is directly available and the initial part of the curve is as linear as possible, reaching the state before stationary gas generation and boosting methane generation.

Figure 4 also depicts the mean curve of biodegradability or gross biogas production for all the tests performed with residue V and compares this curve with that of the inoculum, that is, with that of UASB sludge alone.

In addition to being clearer ed all described about gas generation, it was found that the effect of adding V waste was positive from the point of view of biogas generation. Not only did it start to be generated earlier, the gas produced by adding waste also resulted in an increase of 134% from 390 mL under normal conditions generated by the sludge, to the normal 913,282 mL generated by working F and residue V together.

It is also appreciated that the variation between curves was more pronounced in the initial phase than in the stable phase. This may have been due to changes in the substrate solubility, which would be improved with pre-treatment.

To determine whether all the biodegradability curves of residue V resemble each other, i.e., if they are correlated and statistically can be assumed by a single, an ANOVA analysis was carried out. This statistical analysis shows that in all the curves, except for the three in which the trial failed, the significance level is 1000. This means that the null hypothesis of equality between curves is accepted, and it can be assumed that they all follow the same pattern and that statistically, they are all the same.

3.2.2. Specific Biogas Production

To obtain the specific production curves of biogas, it should be taken into account that as described in Table 2, in 100 g of residue V, there is 10.91 g of VS.

Figure 5 shows the specific generation of biogas of all the tests and the specific mean generation, estimated at 82.86 (±15.269%) ml of biogas (Table 4), measured under normal conditions, for each gram of vs. entered the digester. Logically, the shape of the curves and the conclusions drawn are analogous to those of gross biogas production.

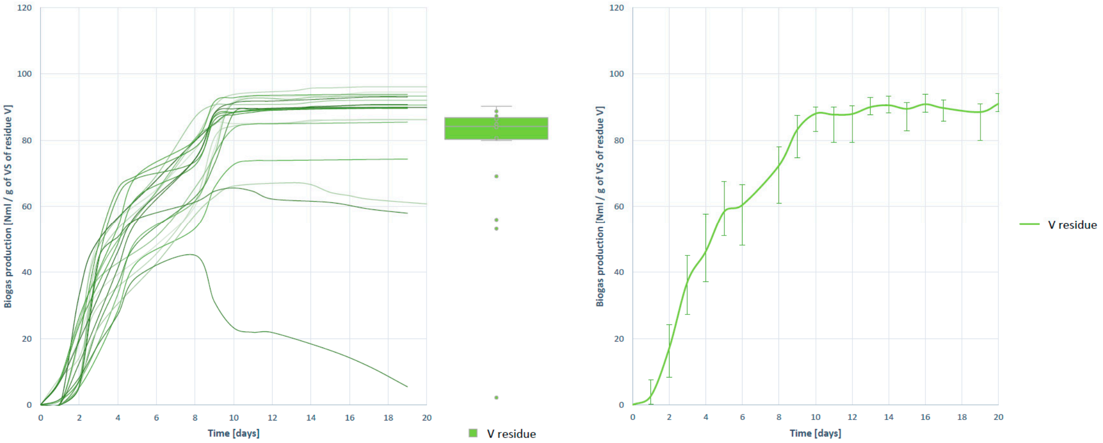


Figure 5. Specific biogas production curves from the anaerobic digestion of 100 g of residue V.

Table 4. Specific production of biogas, methane and hydrogen, and descriptive statistics.

	Specific Production	**Standard Deviation**	**Coefficient of Variation**	**Relative Error**
	[NmL/g of vs. of residue V]	**σ**	**CV**	**ε**
Biogas	82.862 NmL	20.432	0.246	15.269%
Methane	26.351 NmL	8.654	0.328	21.376%
Hydrogen	0.041 NmL + 0.018 NmL	0.108 + 0.188	0.584 + 0.841	59.365% + 94.2365%

3.3. Methane Production

Figure 6 represents the gross methane generation curves for 100 g of V residue, and the average curve of all of them is represented in Figure 7.

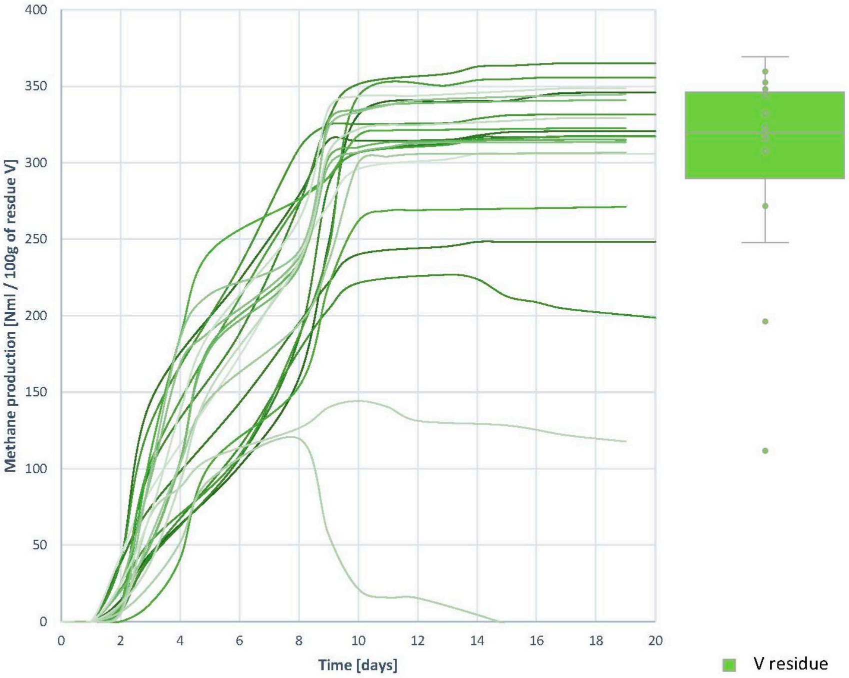


Figure 6. Gross methane production curves from the anaerobic digestion of 100 g of residue V.

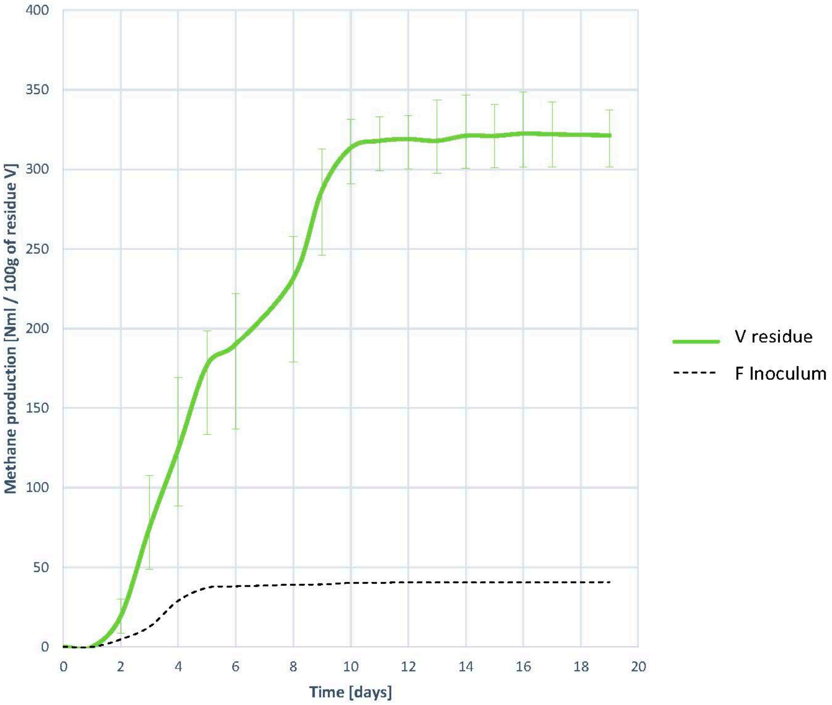


Figure 7. Gross methane production mean curve from the anaerobic digestion of 100 g of residue V.

The shape of the curves and the information that is drawn from them is similar to that of the biogas generation curves. Digestion is clearly observed in two phases, and that stability in methane generation and therefore, completion of the process is reached at 10 days. In particular, the generation of methane is 289,333 (±21,421%) methane measured under normal conditions, per 100 g of V waste (Table 3).

Thanks to the middle curve, it can be clearly seen that the generation of methane begins on day 1, and the clear step by the division of hydrolysis in two phases between days 6 and 8. It is also possible to compare the generation of methane between the white or mud UASB without any addition, and the test when adding a substrate. The effect of adding V residue generates a large increase in methane generation, from 611.767%, from generating 60.5 NmL of methane to 289.333 NmL with the addition of waste V.

There were three failed trials, and although the dispersion between the curves is greater than in the biogas generation curves, the descriptive statistics in Table 3 show that variability is not as pronounced as expected. In fact, the deviation between methane generation curves is less than that of biogas generation curves, despite the greater coefficient of variation, deviation, and error between curves.

These changes and variability between curves are due to the proportion of methane in biogas, which are an indicator that process stability and development develops into a single section.

Similarly, an ANOVA analysis was carried out to determine whether there is a correlation between methane production curves and the results indicate that statistically, all the curves can be resemble a single one, with a level of 1000, except for those in which the trial failed.

Figure 8 show the specific generation of methane of all tests and the specific mean generation, estimated at 26.351 (±21.376%) ml of methane, measured under normal conditions, for each gram of SV introduced into the digester. Again, the curves are proportional to the gross generation curves.

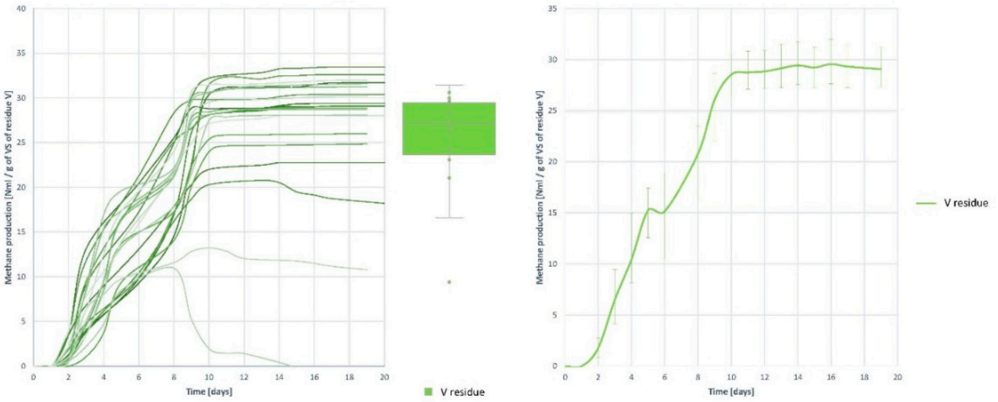


Figure 8. Specific methane production curves from the anaerobic digestion of 100 g of residue V.

3.4. Methane Content of the Produced Biogas

It can be observed in Figures 9 and 10 that the proportion of methane begins to be detected on day 1 and grows to stabilize on day 10. The curves converge to about 30% of methane content. The proportion grows with a very steep slope until day 4, drastically moderating the growth of methane content. This is logical since between days 4 and 10, the phenomenon of double digestion occurs; intermediate gases are generated and the increase in methane content slows. This fact reconfirms that digestion occurs in two phases, a first in which soluble and directly accessible OM is digested, and another phase in which the OM that solubilizes over time is digested, although it must be checked with another more accurate indicator, for example, the generation and content in H_2.

As indicated, and appreciated in the middle curve, the mean content in biogas methane is at 32,252 (±15.146%), resulting in an increase of 167.65% on target, from a methane content of 12% to 32.252% today (Table 5).

The methane content falls below 60%, and this may be due to some type of inhibition (unlikely as studied from the characterization of the before and after waste), to a process failure, or to the residue not being completely hydrolysed and biodegraded. To find out the reason, a joint analysis of all the process variables studied and a study of the level of biodegradation of the residue should be used, detailed in later sections.

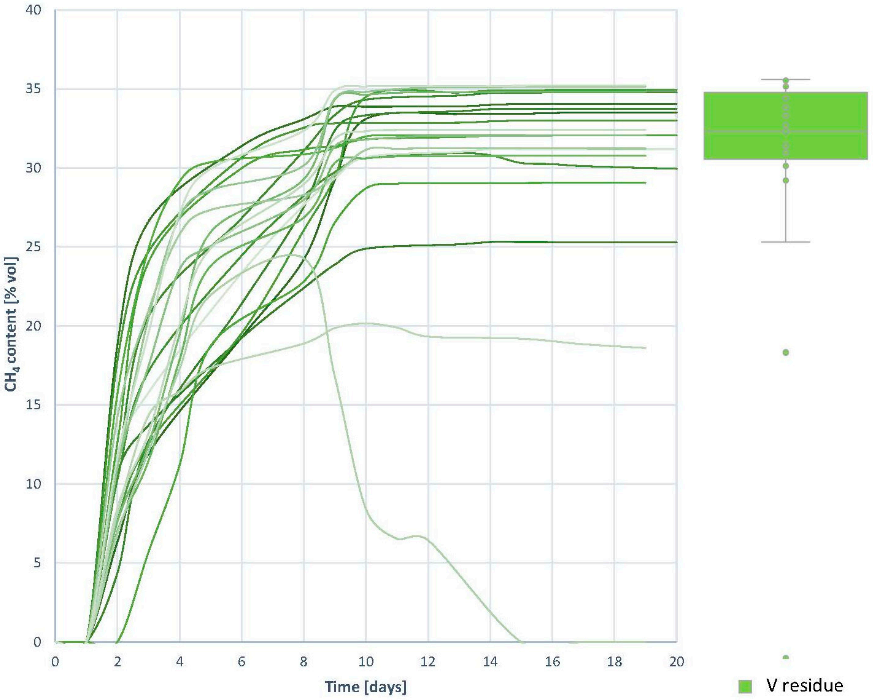


Figure 9. Methane content curves from the biogas produced in the anaerobic digestion of 100 g of residue V.

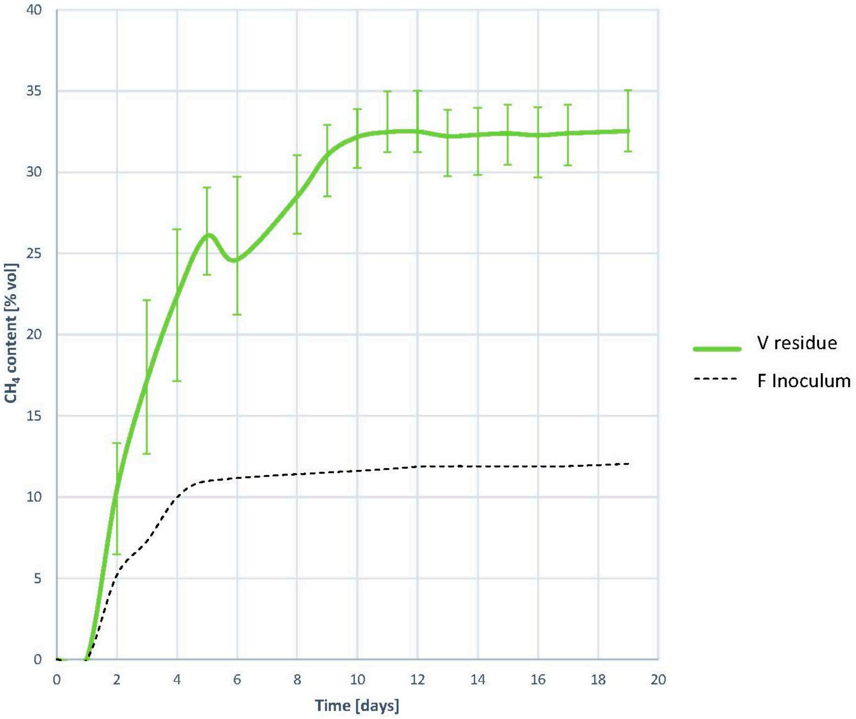


Figure 10. Methane content mean curve from the biogas produced in the anaerobic digestion of 100 g of residue V.

Table 5. Methane and hydrogen content of the produced biogas, and descriptive statistics.

	Content [% vol]	**Standard Deviation σ**	**Coefficient of Variation CV**	**Relative Error ε**
Methane	32.252%	7.906	0.245	12.051%
Hydrogen	0.265% + 0.017%	0.128 + 0.019	0.484 + 1.124	42.311% + 92.375%

3.5. Hydrogen Production

Hydrogen production occurs once hydrolysis has been completed, during the acidic stages (acidogenesis and acetogenesis) and subsequently become part of CH_4. Therefore, it can be used as an indicator of the evolution of the process and the correct development of hydrolysis.

It is to be expected that hydrogen will form during the first few days, and the higher its concentration and before it occurs, the more indicative that hydrolysis has developed successfully.

As soon as hydrogen begins to disappear, the process of methanogenesis occurs, so if it is past the peak of generation of H_2, this begins to descend, which is indicative of the beginning of methanogenesis. If, on the contrary, the content in H_2 remains, it means that inhibition by accumulation of acids has occurred during acid icing phases, which have not been transformed into methane during methane.

A peak production of H_2 should be accompanied by a decrease in pH, synonymous with the acidification and the development of acidogenesis and acetogenesis. As the H_2 level is reduced, the pH value should increase, which causes a destruction of acetic acids and VFA to be transformed into methane. If the peak of H_2 remains along with a constant decrease of pH, this means that acid accumulation has occurred and therefore, the process has been inhibited without becoming methane.

Figure 11 represents the gross hydrogen generation curves for 100 g of V residue obtained from each biodegradation test. Figure 12 also shows the average gross hydrogen production.

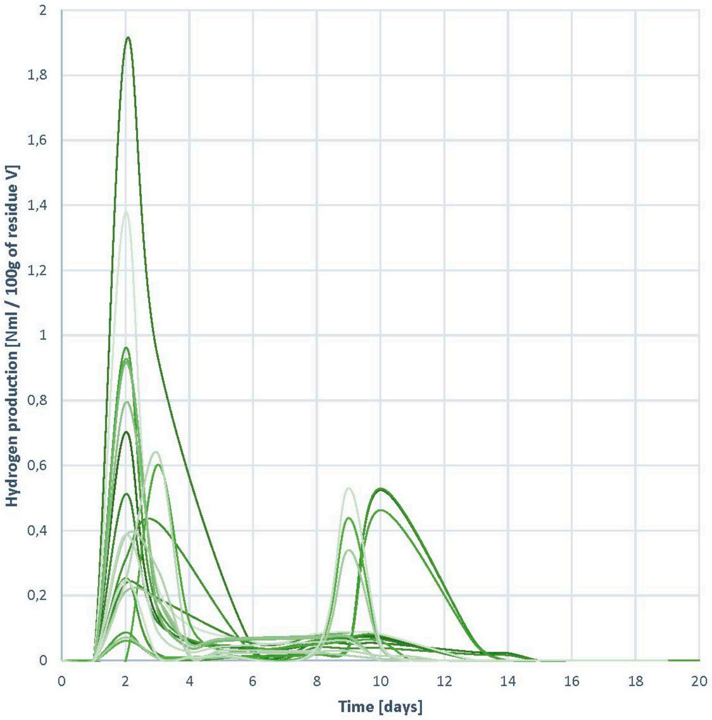


Figure 11. Gross hydrogen production curves from the anaerobic digestion of 100 g of residue V.

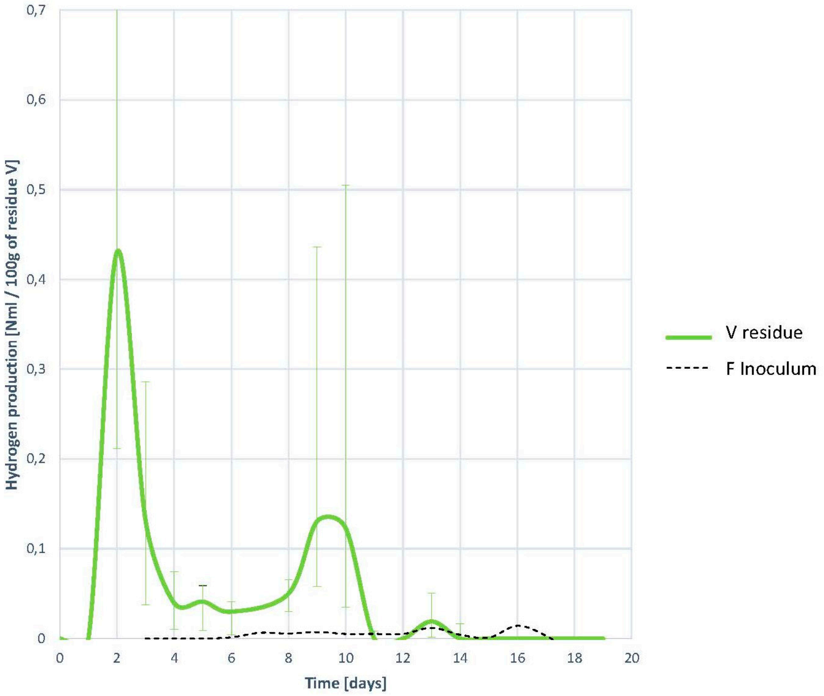


Figure 12. Gross hydrogen production mean curve from the anaerobic digestion of 100 g of residue V.

It is clearly appreciated that there is a first major peak of hydrogen generation on days 2 and 3. This shows that hydrolysis ends on the first day and acidogenesis and acetogenesis begin to occur after day 1. The generation of methane at this first peak is quantified at 0.456 (±57.583%) NmL per 100 g of V residue (Table 3). The variability of the data is very large, which is logical since when production occurs within a period of less than one day, some measures are performed before or after. In any case, the presence and generation of H_2 during day 2 is clear.

There is a second peak of H_2 generation at days 9 and 10, although of a lower value (0.200 (±90.609%) milliliters per 100 g of V waste). This may be due to a second hydrolysis of OM that is more difficult to access, in such a case, it should be checked with a joint analysis of the complete development of the process, or this may even be due to some form of inhibition of methanogenesis, which causes hydrogen to appear and the accumulation of associated acids.

Both options seem likely since residue V, as described by its characteristics:

- Is prone to the accumulation of VFA (although according to the analysis data after the BMP test the alkalinity due to VFA is increased, and the pH is maintained at efficient levels for methanogenesis).
- Given its strongly particulate nature with resistant external membranes, digestion can be given in two phases by first measured soluble MA, and secondly the non-soluble after particle disintegration and subsequent hydrolysis.

Likewise, to check if there is a relationship between the curves of hydrogen generation, an ANOVA analysis was developed with their corresponding contrasts, demonstrating that there is a clear relationship between the curves, being able to assume by the middle curve.

In view of the specific hydrogen production curves, represented in Figure 13, the two generation peaks are again observed on day 2 and day 9: 0.0418 (±57,701%) for the first peak on day 2, and 0.013 (±97.965%) for the second peak on day 9.

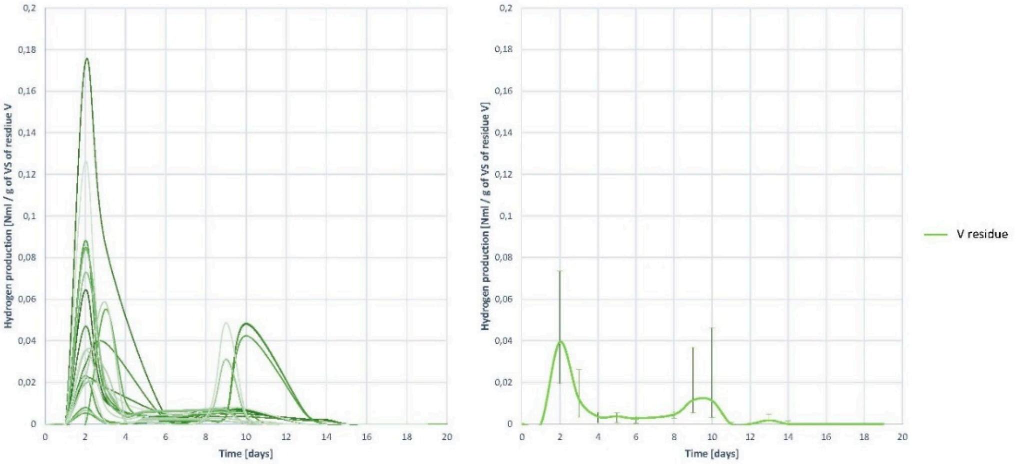


Figure 13. Specific hydrogen production curves from the anaerobic digestion of 100 g of residue V.

3.6. Hydrogen Content of the Produced Biogas

The evolution of the hydrogen content of biogas (Figures 14 and 15) can also be used as an indicator of process development. Although methane is one of the final gases of DA and is therefore cumulative, H_2 is an intermediate gas that must be transformed later. The proportion of hydrogen in biogas should be increased during the first few days, in which hydrolysis, acidogenesis and acetogenesis occur, peaking when the latter concludes. By the time methanogenesis begins, the proportion of H_2 should decline, as it is transformed into methane by hydrogenotrophic.

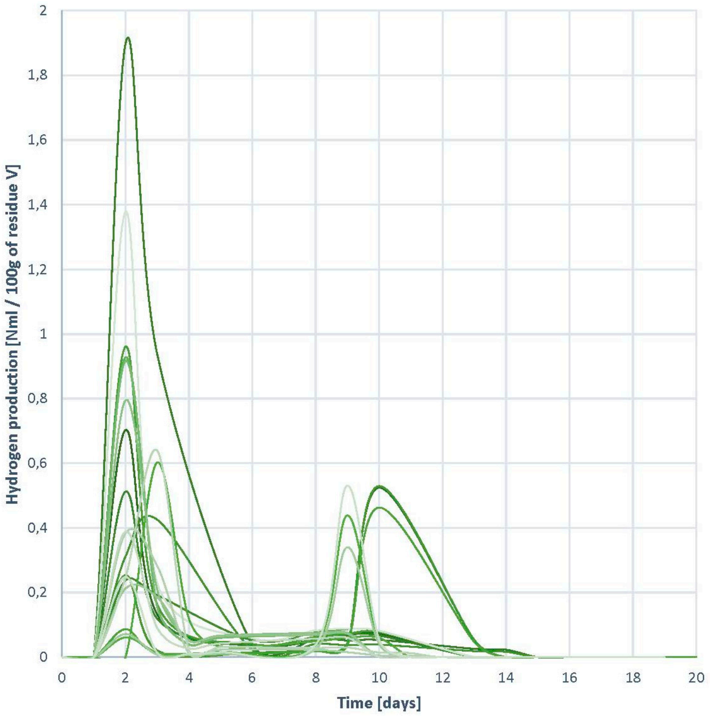


Figure 14. Hydrogen content curves from the biogas produced in the anaerobic digestion of 100 g of residue V.

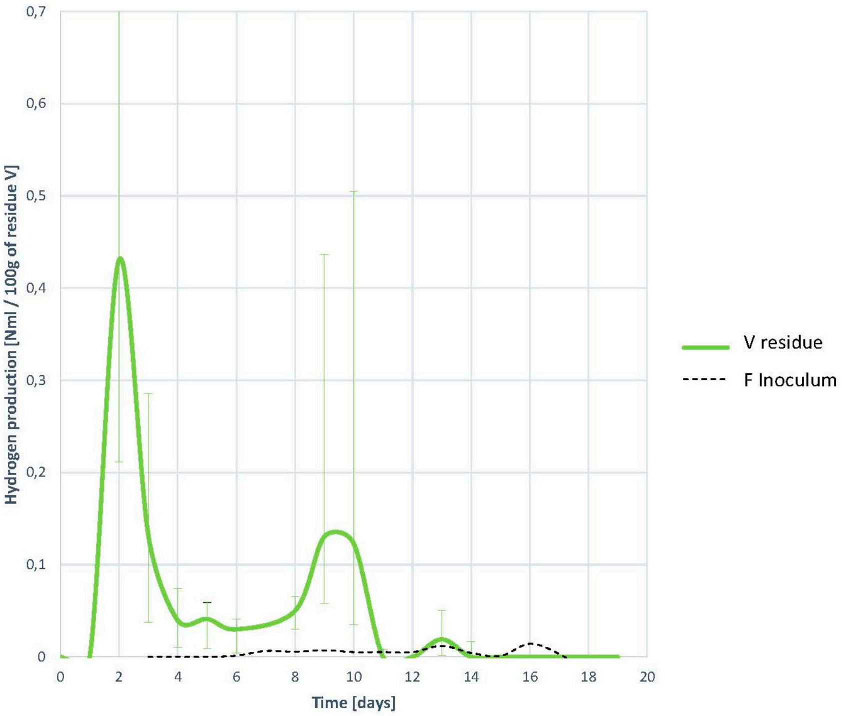


Figure 15. Hydrogen content mean curve from the biogas produced in the anaerobic digestion of 100 g of residue V.

Once again, two high-hydrogen peaks are re-found, the first and most pronounced during day 2, and the second most moderate during day 9. The first peak indicates that the H_2 content of the biogas was 0.265 for day 2 (±42,311%)% of H_2, on the other hand, it is indicative of probable digestion in two phases, which is more moderate, pointing to a hydrogen content for day 9 of 0.017 (±92.375%)%.

The ANOVA statistical analysis carried out shows that all hydrogen content curves resemble a single, thus denoting the uniformity of the process.

3.7. Evaluation of the Evolution of the Anaerobic Process

After analysing the curves represented in Figure 16, the development of the process can be analysed and studied:

- During the first day (day 0–day 1) there is a delay, not appreciating the formation of methane or hydrogen, so it is assumed that the disintegration +hydrolysis stage occurs throughout the first day.
- Between days 1 and 2, hydrogen formation begins, peaking on the same day 2. That is, during day 2 the acidogenic and acetogenic phase develops). At this moment, biogas and methane contained in this begins to appear. Methane growth is slower as it occurs only in an acetoclastic way. In addition, acidification occurs by lowering the pH by the formation of acetic acid and other VFAs.
- There is no inhibition by acidification or accumulation of VFA, as hydrogen evolves disappearing and transforming into methane.
- From day 2 to day 4, hydrogen transformation occurs, and methane growth is more pronounced as it is produced in acetoclastic and hydrogenotrophic ways.
- From day 5, nitrogen levels remain constant and virtually zero. The generation of biogas and methane stops, maintaining constant levels; with the disappearance of acetic acid and VFA to transform into methane, pH is increased to stable neutral values.

- On day 8, a second peak of hydrogen appears, so it is assumed that a new stage of digestion occurs when the generation of methane resumes and acidification (indicative of generation of acetic and other acids) occurs.
- The process ends on the 11th day with the disappearance of the H_2 and acids (pH increment to neutral values) and the end of methane generation.

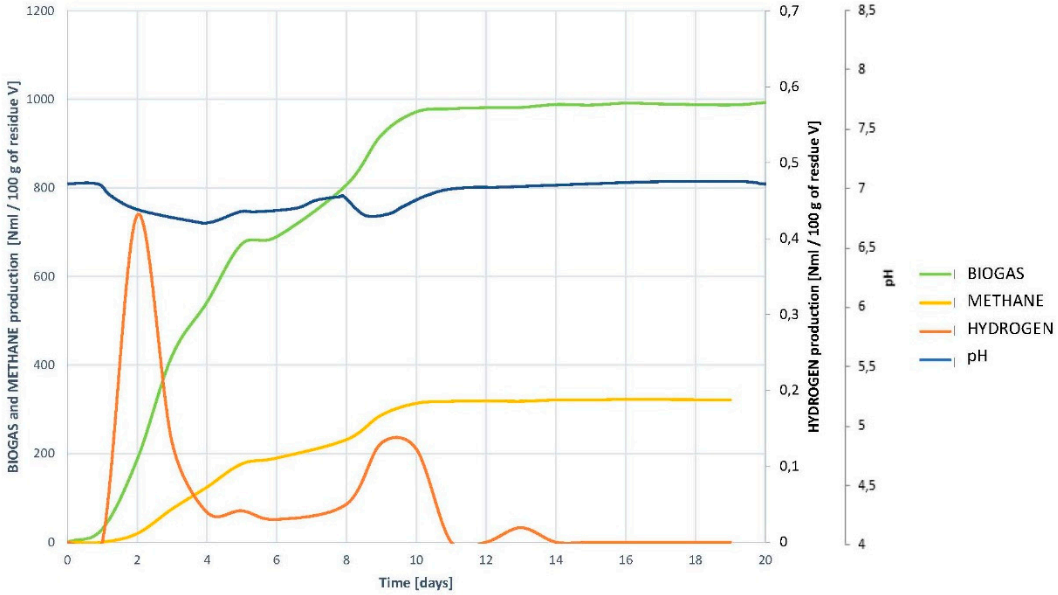


Figure 16. Evolution of the digestion process of waste V. Comparison of the generation of biogas, methane and hydrogen together with the evolution of pH.

In conclusion, the process was developed correctly without acid accumulation inhibitions.

There is two-phase digestion, clearly identified by the resumption of hydrogen generation, methane and pH changes. It is to be assumed that in the first stage of digestion, the OM is digested directly accessibly, and in the second stage, part of the non-soluble OM begins to hydrolyse.

3.8. Mathematical Analysis and Adjustment of the Anaerobic Process

Depending on the initial COD levels and after the trial is completed, together with the data obtained from the degradation in terms of methane generation, the process can be mathematically adjusted to then extrapolate and compare in other scenarios (Table 6).

Table 6. Results obtained in mathematical processing of the parameters of waste V biodegradation.

		Standard Deviation σ	Relative Error ε
Theoretical methane generation	292.808 NmL	91.809	22.260%
Maximum methane generation	323.000 NmL	90.961	16.786%
Disintegration constant	0.200 days^{-1}	0.044	17.920%
Substrate biodegradation	16.045%	1.677	7.422%

3.8.1. Theoretical Production of Methane and Kinetic Parameters

Taking into account the initial and final levels of COD in the test mixture, and the test volume, the theoretical production of methane is obtained, which is estimated at 292,808 (±22.260%) NmL, i.e., with the degradation that has taken place, the expected CH_4 volume resembles the level of CH_4 obtained (289,333 (±21.421%) NmL), so it follows that everything that was biodegraded was transformed into methane as expected, without inhibition of the process.

By mathematically adjusting methane generation curves by least squares to a first-order reaction, one can obtain the disintegration constants and the maximum methane generation. For residue V, taking into account the biodegradability studied, a maximum methane generation of 323,000 (±16.786%) was expected. NmL is the disintegration constant of 0.200 (±17.920%) days^{-1}, a relatively high speed, taking into account the values set by [56] for the digestion of solids. Due to this speed, the level of degradation is not deep, and therefore, the low methane content of biogas is explained.

3.8.2. Level of the Degradation of the Substrate or Material

The biodegradation level of residue V when subjected to DA with the selected F sludge was 16,045 (±7422%)%, a fairly low value taking into account the solubility of the substrate. This indicates that the entire digestion process is not being completed, and since there are no inhibitions, it must be improved in some way, for example, through pre-treatments.

4. Conclusions

In the present study, it was determined that, the composition of residue V is presented as a residue formed, mostly, by carbohydrates. It is the one that has the best solubility and is also relatively resistant to sudden changes in pH. Despite the positive elements, the presence of simple carbohydrates such as lignocellulose can compromise the development of anaerobic digestion. However, it was determined that during its digestion, it is not affected by any type of inhibition and its digestion is stable from the point of view of biogas generation, although incomplete by not reaching a high methane content. Because of its high solubility, digestion occurs in two phases, a first in which solubilized OM is digested, and a second, in a smaller proportion, in which the rest of the COD is digested. In accordance with mathematical adjustments, this is the residue that has a rapid degradation, with a fast and stable hydrolysis. Because of this speed, the level of degradation is not deep, and therefore, the methane content is not high.

In numerical terms, 100 g of residue V produces 913.282 NmL of biogas, of which 289.333 NmL corresponds to methane. The disintegration constant is 0.200 days^{-1}, with 16,045% of the substrate having degraded. This level of degradation opens the door to process improvements through techniques that allow better access of microorganisms to the substrate, for example, through pretreatments.

In short, the AD of fruit and vegetable waste is presented as an optimal solution for waste management, by reducing the volume of waste destined for landfill, being valued in energy as biogas, and also generating an economic benefit if it is used as an internal source instead of acquiring energy from external distributors, also saving on the expenses of a waste management technology provider.

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Abbreviations

AD	Anaerobic Digestion
AN	Ammoniacal Nitrogen
BMP	Biochemical Methane Potential
COD	Chemical Oxygen Demand
COD_f	Chemical Oxygen Demand (filtered)
COD_t	Chemical Oxygen Demand (total)
EU	European Union
F	Inoculum - Sludge

FL	Food Loos
FSC	Food Supply Chain
FW	Food Waste
Hum	Humidity
IA	Intermediate Alkalinity
KDIS	Disintegration Constant
LCFA	Long Chain Fatty Acids
LPCH	Lipid, protein and carbohydrate content
OM	Organic Matter
ON	Organic Nitrogen
PA	Partial Alkalinity
TA	Total Alkalinity
TKN	Total Kjeldahl Nitrogen
TS	Total Solids
UASB	Upflow Anaerobic Sludge Blanket Reactor
VFA	Volatile Fatty Acid
VS	Volatile Solids

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