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Anaerobic Digestion of the Residue (Combination of Wastewater and Solid Waste) from a New Olive-Oil Manufacturing Process Based on an Olive Cold-Pressing System: Kinetic Approach and Process Performance

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Citation: Fernández-Rodríguez, M.J.; Cubero-Cardoso, J.; de la Lama-Calvente, D.; Fernández-Prior, Á.; Rodríguez-Gutiérrez, G.; Borja, R. Anaerobic Digestion of the Residue (Combination of Wastewater and Solid Waste) from a New Olive-Oil Manufacturing Process Based on an Olive Cold-Pressing System: Kinetic Approach and Process Performance. *Processes* **2022**, *10*, 2552. https://doi.org/10.3390/pr10122552

Academic Editor: Sebastián Sánchez Villasclaras

Received: 28 October 2022
Accepted: 28 November 2022
Published: 1 December 2022

Abstract: This research evaluates the anaerobic digestion (AD) process of the residue generated in a new olive-oil manufacturing process for cold-pressed olive, a residue consisting of a mixture of the wastewater and solid waste obtained from this process. Additionally, in order to assess the possible influence of the level of ripening of the olives on the performance of anaerobic processing, olives of the Picual variety were collected at two stages, i.e., green olives and olives in veraison. The AD processes of the residues obtained from the cold-pressing process and the process without pressure (control) were comparatively assessed by means of biochemical methane potential (BMP) assays conducted at mesophilic temperature (35 ± 1 °C). Maximum values for methane yield (390 ± 1 NL CH_4/kg VS_{added}) and biodegradability (84.5%) were obtained from the cold-pressed green olive residues. For the rest of the wastes studied, biodegradability also reached high values, ranging from 79.1 to 79.6%. The logistic model adequately fit the experimental data and allowed for the assessment of the anaerobic biodegradation of these wastes and for obtaining the kinetic parameters for each case studied. The theoretical values for ultimate methane production predicted from this model showed less than a 1% deviation from the experimental values. A decrease was detected for both types of olives tested in the rate of maximum methane production, R_m, during the cold-pressing process, from 44.3 ± 0.1 to 30.1 ± 1.3 L CH_4/(kg VS·d) (green olives) and from 43.9 ± 1.5 to 38.7 ± 1.6 L CH_4/(kg VS·d) (olives in veraison). Finally, the highest energy output result was detected in the waste from cold-pressed green olives (15.7 kJ/g $VS_{removed}$), which coincided with its high methane yield.

Keywords: anaerobic digestion; mixture waste; wastewater and solid waste; cold-pressing olive-oil manufacturing process; kinetics; process performance

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1. Introduction

The olive oil industry is among the most important sectors in the food industry in Spain, where 80% is concentrated in the southern region of the country, called Andalusia. Spain is responsible for about 45% of olive oil production worldwide, which means that an average of 7 million tons per year of waste, or by-product, is generated [1]. In Spain, this by-product is mainly generated through the two-phase, continuous extraction method and is called alperujo. In spite of the difficulty of its treatment, technologies have already been implemented that serve to use all of its components. One such technology is thermal treatment [2]. Thermal treatment improves the management of the by-product but does not have a direct impact on the quality of the oil, which is obtained in previous stages in the mill. One of the main changes being made to improve the quality of the oil is to process decreasingly ripe olives, which promotes the production of oils with high organoleptic and functional quality [3]. Gone are the years when in Spain the priority was to obtain a greater quantity of oil by searching for olives with a high degree of ripeness, which in most cases meant harvesting olives that were damaged, fermented, or susceptible to fermentation. Nowadays, the aim is to harvest green olives to obtain a high-quality oil with the detriment of quantity, since fat recovery is much lower than in the case of riper olives. For this reason, technologies are being sought to increase the percentage of oil recovery from green olives without altering the quality of the main product. In this sense, the application of new systems for beating the olive paste once it has been milled, or the application of vacuum, ultrasound-assisted extraction [4], among others, are being studied. More recently, the use of cold pressure has been employed as a novel system that allows for increasing oil recovery yield while improving the use of its by-products, mainly alperujo (olive pomace). Previous studies have shown that the application of a cold-pressure treatment does not improve the subsequent application of anaerobic digestion (AD) of the olive pomace for the integral utilization of the by-product, although it is also possible [5]. These studies were carried out using the solid fraction obtained after olive-oil extraction with or without cold-press treatment.

As already indicated, the evolution of the sector is focused on the application of thermal treatments such as thermo-malaxation in the pomace extractors for better utilization of the olive pomace. To this end, the use of a beating at temperatures between 50 and 60 °C [2] prior to a three-stage centrifugation has been implemented. An oily phase, a liquid phase, and a solid phase with 55–60% moisture are obtained. The oily phase is the so-called crude pomace oil that must be refined to be marketed as olive pomace oil. The liquid phase is usually concentrated and its final destination is as a source of bioactive components, mainly phenols, or for the formation of fertilizers. The solid fraction can be further extracted from the pomace oil, or used as fuel. This work presents a novel study on how a combination of cold-pressure treatment with thermo-malaxation and three-phase centrifugation influences the subsequent AD of the mixture of the wastewater and solid waste generated. This work studies the application of AD as a final step for the integral utilization of mixture residue through the union of the solid and liquid phases obtained after applying a first step of cold pressure to improve the extractability of the olive oil and a second step of extraction in three phases after thermo-malaxation. The addition of the liquid fraction provides a more accessible source of organic matter in addition to phenols, which are potentially toxic for digestion—factors that depend on previous processing, such as cold pressing.

2. Materials and Methods

2.1. Olive Processing by Cold-Pressing Reactor

The cold pressure treatment was applied in the experimental plants of the Instituto de la Grasa-CSIC. Specifically, a 100 L stainless steel reactor working at a maximum pressure of 1.2 MPa and manual top closure was used. Between 5 and 10 kg of both green and olives in veraison samples were introduced into the reactor. Pressure was applied through an air compressor at up to 7 kg/cm^2 for 10 min. Then, in order to depressurize the reactor, the upper valve was opened and the olives were collected through the lower valve. The treated olives and the untreated control olives were subjected to an oil extraction process using the Abencor equipment [2]. The olives were ground in a hammer mill below 4 mm and the resulting paste beaten at 29 °C for 45 min, adding 100 mL of water and talcum powder halfway through the beating process. In this way, two fractions were obtained, one liquid and one solid. The Abencor system with water addition resembles a three-phase system in the industry. The liquid, which included the oil and water, was separated by decantation. The aqueous and the solid fractions were then stored at −20 °C until further use. Finally, both fractions (liquid and solid) were mixed and subjected to the AD process in the present research.

In order to assess the possible influence of the level of ripening of the olives on the AD performance of the produced wastes, olives of the Picual variety were collected at two stages, i.e., green olives and olives in veraison. The olives were harvested from the local area "Valle de los Pedroches, Pozoblanco" (Cordoba, Spain). The harvest date for both varieties was 5 December 2020.

Therefore, four mixed residues (mixtures of wastewaters and solid waste) were tested: residue from cold-pressed green olives, residue from olives that were not cold-pressed (control), residue from cold-pressed olives in veraison, and their residue without pressing (control).

The main characteristics pertaining to the four residues are presented in Table 1. In addition, Table 2 shows the individual phenolic composition contained in the wastewater fractions and solid wastes, both components (at 50%) of the final residues studied in this research.

Table 1. Principal characteristics of the four residues (mixtures of the wastewaters and solid wastes) subjected to AD experiments. Values represent means ± standard deviations. Different superscripted letters (a, b) mean values are significantly different.

Parameters	Cold-Pressed Green Olives	Green Olives Control	Cold-Pressed Olives in Veraison	Olives in Veraison Control
TS (g/kg)	147 ± 2 ^{a}	186 ± 2 ^{b}	143 ± 2 ^{a}	146 ± 1 ^{a}
VS (g/kg)	171 ± 1 ^{a}	132.2 ± 0.3 ^{b}	130.6 ± 0.5 ^{b}	132 ± 1 ^{b}
VS/TS	0.90	0.92	0.91	0.90
Moisture content (%)	85.6	82.7	85.5	85.3
Total phenols (g gallic acid/L)	10.00	10.97	10.99	11.27
tCOD (g O_2/L)	240 ± 30 ^{a}	200 ± 50 ^{a}	200 ± 80 ^{a}	190 ± 50 ^{a}
sCOD (g O_2/L)	140 ± 50 ^{a}	140 ± 40 ^{a}	160 ± 50 ^{a}	150 ± 30 ^{a}

TS: total solids; VS: volatile solids; tCOD: total chemical oxygen demand; sCOD: soluble chemical oxygen demand.

Table 2. Composition of the main individual phenolics in the liquid fractions (wastewaters) and the solid waste fractions as determined by HLPC. n.d.: not determined.

Phenolics Composition	Wastewaters (mg/L)				Solid Wastes (mg/kg)			
	Green Olives Control	Cold-Pressed Green Olives	Olives in Veraison Control	Cold-Pressed Olives in Veraison	Green Olives Control	Cold-Pressed Green Olives	Olives in Veraison Control	Cold-Pressed Olives in Veraison
3,4-Dihydroxyphenylglycol	107 ± 1	100 ± 3	114 ± 7	172 ± 8	43 ± 2	37 ± 2	69 ± 2	41 ± 3
Hydroxytyrosol glucoside	59 ± 1	5 ± 0	108 ± 6	7 ± 1	10 ± 0	29 ± 1	34 ± 0	41 ± 2
Hydroxytyrosol	440 ± 10	810 ± 10	840 ± 15	510 ± 20	62 ± 2	128 ± 8	209 ± 1	210 ± 10
Tyrosol	127 ± 8	112 ± 5	149 ± 3	154 ± 5	10 ± 1	17 ± 0	24 ± 3	23 ± 1
Syringic acid	19 ± 0	26 ± 0	27 ± 1	29 ± 1	2.6 ± 0.1	31 ± 1	2 ± 0	2 ± 0
Apigenin	n.d.	n.d.	n.d.	n.d.	6.3 ± 0.1	9.2 ± 0.7	7.6 ± 0.8	7.9 ± 0.6
Luteolin	n.d.	n.d.	n.d.	n.d.	25 ± 1	31 ± 1	26 ± 1	30 ± 1
p-coumaric acid	35 ± 2	32 ± 1	31 ± 3	36 ± 2	11 ± 0	15 ± 0	13 ± 1	10 ± 1
Oleuropein	60 ± 1	56 ± 3	53 ± 1	78 ± 1	n.d.	n.d.	n.d.	n.d.
Feluric acid	6.4 ± 0.8	10.3 ± 0.1	9.4 ± 0.7	7.3 ± 0.1	n.d.	n.d.	n.d.	n.d.
Comsegoloside	610 ± 10	610 ± 10	620 ± 10	680 ± 10	244 ± 6	257 ± 9	224 ± 9	261 ± 8

2.2. *Biochemical Methane Potential Assays*

A mesophilic granular sludge from a full-scale UASB reactor treating brewery wastewaters was used as anaerobic inoculum. The main characteristics of the inoculum were as follows: pH: 7.5 ± 0.2, total solids (TS): 25.0 ± 1.1 g/kg and volatile solids (VS): 19.9 ± 1.2 g/kg. Reactors of 250 mL with a 210 ± 2 mL working volume were used for the AD tests carried out in batch mode. A mesophilic temperature of 35 ± 2 °C was selected for the experiments, which was controlled by placing the reactors in thermostatic baths under constant stirring (400 rpm). The reactors were then filled with an inoculum to a substrate (ISR) ratio of 2 (VS), reaching a final concentration of 24 g VS/L, along with a micronutrient solution [5,6]. At the outset of the experiment, nitrogen gas was flushed through the reactors for two minutes (40 mL headspace volume) with the aim of maintaining anaerobic conditions. The reactors were activated for three days before the addition of each substrate. The reactors were replicated three times for each substrate. In addition, three blanks, with no substrate, were placed in order to obtain the inoculum's endogenous methane production, which will be subtracted from the final yield of each test. The resultant biogas was then passed through a 2N NaOH solution in order to retain the CO_2. The displacement volume was determined as methane, which was expressed according to standard or normalized (N) conditions of pressure and temperature (N: 0 °C, 1 atm).

2.3. Analytical Methods

The different mixtures tested were analyzed by determining the following parameters: total chemical oxygen demand (tCOD), soluble chemical oxygen demand (sCOD), TS, VS, and total phenol concentration. After 26 days of operation, sCOD, TS and VS, pH, total alkalinity, and volatile fatty acids (VFA) were measured in the resultant anaerobic digestates. The analyses were carried out in accordance with the methods previously described elsewhere [6].

2.4. Extraction and Analysis of Individual Phenolics by HPLC-DAD

The preparation of the aqueous samples for the determination of individual phenols was carried out by filtration through a 0.45-micron syringe filter. In the case of the solid phase samples, three sequential extractions were performed using an Ultra Turrax IKA T25 digital blender for 60 s at 1000 rpm, with a methanol:water solution (4:1 (v/v)) at a 1:1 ratio of solid:methanol and water mixture (w/v). The three extractions were combined and brought to dryness under vacuum at 40 °C and dissolved in a ten-times smaller volume of the hydroalcoholic mixture. Identification and quantification of the main individual phenols were made by HPLC with a UV Diode array detector (DAD). The equipment used was a Hewlett-Packard 1100 liquid chromatograph, and the individual phenols were quantified at wavelengths of 254, 280, and 340 nm. The column used was a C-18 Teknokroma Mediterranea Sea 18, 250 mm × 4.6 mm, i.d. 5 µm. The mobile phase was Milli-Q water acidified with trichloroacetic acid (0.01%) (A) and acetonitrile (B). The process was performed in gradient mode described as follows: 95% A, 75% A (30 min), 50% A (45 min), 0% A (47 min), 75% A (50 min), and 95% A (52 min) until completion of the run (55 min). Identification was made according to retention times and absorption spectra for each compound, and quantification was carried out by calibration of a curve with external standards.

2.5. Kinetic Evaluation

The substrate degradation during the AD process was determined by the mathematical modeling of the kinetics derived from experimental methane production. The obtained parameters aid in designing and optimizing full-scale anaerobic plants [7]. Kinetic parameters serve to determine the necessary time for microorganisms to acclimate to their new environment, the length of the digestion period, and the ability of the substrate to biodegrade. Therefore, these kinetic parameters serve as indicators for assessing the performance of the anaerobic reactor.

The logistic function model (LM) was used to estimate performance parameters and kinetic constants in the anaerobic digestion of the four residues tested. This model proved the fit of the experimental data shape of methane production kinetics [8]: an initial exponential increase after a small lag stage with final stabilization at the maximal level of production.

The logistic model is provided by the following equation:

$$B = P/[1 + \exp(4R_m(\lambda - t)/P + 2] \quad (1)$$

where B is the cumulative specific methane production (L CH_4/kg VS_{added}), P is the ultimate methane production (L CH_4/kg VS_{added}), R_m is the maximum methane production rate (L CH_4/(kg $VS_{added}\cdot$d), t (days) is the digestion time, and λ is the lag time (days).

This model presupposes that the rate of methane production will be influenced by the amount of gas previously produced and that the R_m and maximum capacity for methane production will also affect the process [9]. The Logistic model also estimates the delay in λ and the R_m together with the potential for methane production of the substrates tested. This model has already been used for the anaerobic digestion of different organic substrates, and for estimating methane production in leachate from landfills [9].

2.6. Energy Output

The heat energy output corresponding to the BMP tests was determined by using the experimental data according to Equation (2) [5,10]:

$$E_0 = (P_{CH4} \times \mathcal{E} \times \lambda_m)/VS_{removed} \quad (2)$$

where

E_0 is the energy output in (kJ/g $VS_{removed}$);
P_{CH4} is the cumulative methane production after digestion time (m^3);
$\mathcal{E}$ is the lowest heating value for methane (35,800 kJ/m^3 CH_4);
λ_m is the energy conversion factor of methane (0.9);
$VS_{removed}$ is the grams of VS removed at the end of the BMP test (g/L).

2.7. Statistical Analysis

All analyses and tests were performed in triplicate. The statistical analyses were carried out using the SigmaStat software (Palo Alto, CA, USA). A one-way analysis of variance (ANOVA) test was used to determine levels of confidence among various results. The kinetic mathematical models were adjusted from the experimental data using the SigmaPlot software (version 11). All the results were expressed as means ± standard deviations.

3. Results and Discussion

3.1. Substrate Characterization

Table 1 presents the main characteristics of the four residues used in this experiment (wastewaters and solid-waste mixture from cold-pressed green olives, residue from olives without cold-pressing (control), residue from cold-pressed olives in veraison, and residue from olives without pressing (control)). The VS contents in the residues from the green olive control, cold-pressed olives in veraison, and olives in veraison control were around 132 ± 1 g/kg, with no significant differences among them (Table 1). In contrast, the residues from cold-pressed green olives presented a VS content of 171 ± 1 g/kg, which is higher and statistically different from the other mixtures studied. The TS/VS ratio was similar for the four residues studied (0.90, 0.92, 0.91, and 0.90 for residues from cold-pressed green olives, residue from green olive control, residue from cold-pressed olives in veraison, and residue from olives in veraison control, respectively). The high values for the VS/TS ratio denote the marked organic character of the residues studied and were considered optimal for the AD process.

One of the main drawbacks of the olive-oil processing residues regarding anaerobic digestion performance is their phenols content, which can be decisive and even inhibit methane production [11]. Table 2 shows the phenolic compounds found in each residue used in this experiment.

Alperujo is a wet–solid waste, which is mainly composed of polysaccharides, proteins, fatty acids, pigments, and polyphenols [12]. Literature shows that alperujo is rich in polyphenols such as hydroxytyrosol but also contains important amounts of other compounds with high added value (e.g., vanillic acid, rutin, caffeic acid, oleuropein, tyrosol, p-coumaric acid, elenolic acid, catechol, and verbascoside) [12]. The main polyphenol found in the studied solid fractions was comsegoloside (Table 2) in concentrations between 224 and 261 mg/kg, followed by hydroxytyrosol, with a lower concentration in the green olive residues. The solid fraction from the control green olives presented a hydroxytyrosol concentration of 62 mg/kg, which increased to 128 mg/kg when the samples were treated (Table 2). In contrast, the solid residues of the olives in veraison presented a hydroxytyrosol concentration of between 209 and 210 mg/kg (Table 2).

The process wastewater or liquid residue consists of lipids, polyphenols, pectins, soluble sugars, and polyalcohols, among other minor compounds [13]. As in the solid fractions, the wastewater or liquid fraction main phenolic compound is hydroxytyrosol but it is also rich in others (e.g., gallic acid, tyrosol, vanillic acid, oleuropein, luteolin, verbascoside, and caffeic acid, among others) [13].

In the olive wastewater studied it is worth highlighting the presence of hydroxytyrosol with 440 ± 10–840 ± 15 mg/L, comsegoloside with concentrations between 610 ± 10–680 ± 10 mg/L, and tyrosol with a significantly higher concentration in the liquid residue from olives in veraison (112 ± 5–154 ± 5 mg/L) (Table 2).

Similar results were reported by Nunes et al. and Mallamices et al. [14,15], where hydroxytyrosol and comsegoloside represented around 79% of the total phenolic compounds present in the olive residues.

3.2. Organic Matter Removal and Methane Yield Coefficients

Graphs showing the production of biogas versus time for the four mixtures assayed (residue from cold-pressed green olives, residue from the green olives control, residue from cold-pressed olives in veraison, and residue from olives in veraison control) are shown in Figure 1. The methane yield of three of the four samples studied was very similar until day 12, except for the residues from the control of the green olive, which produced up to 10% more methane on day 10 after the start-up of the assays. Finally, from day 12, the methane production stabilized at around 300 NL CH_4/kg VS in the residues from olives in veraison, both control and cold-pressed, and the residues from the green olive control. The residues from cold-pressed green olives continued to produce methane until day 23, obtaining the highest methane yield (390 NL CH_4/kg VS). The overall methane production of the other three residues was between 18 and 23% lower (residue from the green olive control, residue from cold-pressed olives in veraison, and residue from olives in veraison control at 320, 302, and 320 NL CH_4/kg VS, respectively). The methane production from the residue of the cold-pressed green olives was significantly higher than those obtained for the other three residues tested. These results are in accordance with the higher biodegradability value found for the residue from cold-pressed green olives, i.e., 84.5%, compared with the others.

Tsigkou et al. [16] reported higher methane yield for raw three-phase olive mill wastewater (OMW) (472 mL CH_4/g VS_{added}). On the other hand, they obtained similar values to those obtained in the present work for the centrifuged OMW (391 NL CH_4/kg VS). The biodegradability reported by Tsigkou et al., 2019, was greater than 90% in all cases. The presence of alperujo in the mixtures studied in the present work could provide more difficult-to-degrade compounds, which was confirmed by the obtained biodegradability (82–86%) as well as by the methane yield (302–390 NL CH_4/kg VS). In another study carried out by Donoso-Bravo et al. [17], lower values for final methane yield were reported (274 NL CH_4/kg VS) after subjecting the olive pomace (OP) to an enzymatic maceration pre-treatment under mesophilic conditions with an ISR of 2.

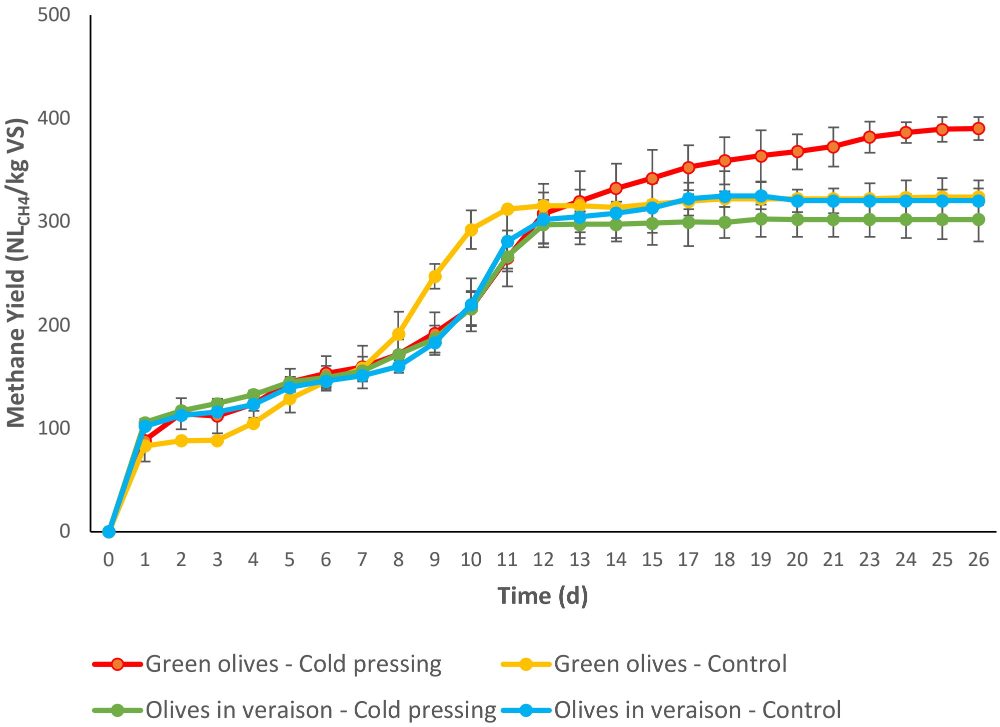

Figure 1. Curve of methane production against time for each test.

3.3. Characterization of the Anaerobic Digestates

The final pH of the different tests carried out was within the established optimal values for methane production by the methanogenic Archea [18] (7.91 ± 0.04, 8.07 ± 0.01, 7.91 ± 0.03 and 7.85 ± 0.03 for residue from cold-pressed green olives, residue from the green olive control, residue from cold-pressed olives in veraison, and residue from olives in veraison control, respectively; Table 3). Another very important parameter for the development of methanogenic microorganisms is the buffer capacity system. Total alkalinity values between 2500 and 5000 mg $CaCO_3$/L are considered optimal values for AD. Total alkalinity values ranged from 5900 ± 400 mg $CaCO_3$/L to 7490 ± 20 mg $CaCO_3$/L at the end of the experiment (residue from olives in veraison control and residue from cold-pressed green olives, respectively). Volatile fatty acids (VFAs) are good indicators of process stability in the AD system [19]. The main chemical equilibrium that controls alkalinity is carbonic acid-bicarbonate when pH values are between 6 and 8. The VFAs were measured in each reactor at the end of the experiment in order to avoid acidification inside the reactor. In all the residues studied, the presence of acetic acid was detected with values between 82 ± 2 and 150 ± 10 mg/L. The propionic acid concentration found was <15 mg/L and of isobutyric acid < 25 mg/L. Butyric and isovaleric acid were only found in residues from cold-pressed green olives and the control, at 74.2 ± 0.5 and 27.2 ± 0.1 mg/L, respectively (Table 3). No volatile fatty acids with longer chain accumulation were found. These results indicated that acidification processes did not occur during the anaerobic processes, which is indicative of the high stability of the four systems investigated in this work.

3.4. Kinetic Modeling

Table 4 presents a summary of the different parameters determined by the application of LM to the methane production experimental data against time, as can be seen in Figure 1. Errors are defined as the difference between predicted and measured methane yield, and they were <1% for all tests. The high determination coefficients ($R^2 > 0.99$ across the board) and the low standard errors of estimates show the remarkable fit of the model to the experimental results. As an example, Figure 2 shows the superposition of the experimental points of methane production against time with the theoretical curves of the model applied to the residues from green olives with cold-pressing and the controls.

Table 3. Principal characteristics of the four digestates or anaerobic effluents resulting from the BMP experiments of the mixture residues tested. Values represent means ± standard deviations. Different superscripted letters (a, b) mean values are significantly different. n.d.: not determined.

Parameters	Cold-Pressed Green Olives	Green Olives Control	Cold-Pressed Olives in Veraison	Olives in Veraison Control
pH	7.91 ± 0.04^{a}	8.07 ± 0.01^{b}	7.91 ± 0.03^{a}	7.85 ± 0.03^{a}
TA (g $CaCO_3$/kg)	7490 ± 20^{a}	6900 ± 700^{a}	6500 ± 300^{b}	6000 ± 400^{b}
Acetic acid (mg/L)	150 ± 10^{a}	86 ± 1^{b}	90 ± 10^{b}	82 ± 2^{b}
Propionic acid (mg/L)	n.d.	14.9 ± 0.1^{a}	n.d.	14.1 ± 0.1^{a}
Isobutyric acid (mg/L)	n.d.	25.3 ± 0.1^{a}	n.d.	23 ± 1^{b}
Butyric acid (mg/L)	74.2 ± 0.5^{a}	n.d.	n.d.	n.d.
Isovaleric acid (mg/L)	27.2 ± 0.1^{a}	n.d.	n.d.	n.d.
SCOD (mg O_2/L)	2800 ± 400^{a}	1700 ± 100^{b}	2400 ± 200^{a}	2500 ± 700^{a}
TS (g/kg)	35.3 ± 0.4^{a}	35.9 ± 0.4^{a}	36.4 ± 0.7^{a}	36 ± 1^{a}
VS (g/kg)	26.4 ± 0.4^{a}	27 ± 1^{a}	27.2 ± 0.6^{a}	27.7 ± 0.9^{a}

Table 4. Values corresponding to the parameters obtained from the logistic model (Sigmoidal 4 parameters) for the four mixture residues studied. Values represent means ± standard deviations. Different superscripted letters (a–c) mean values are significantly different.

Residue	P (NL CH_4/kg VS)	R_m (LCH_4/(kg VS·d))	λ (d)	R^2	S.E.E.	Error (%)
Residue from cold-pressed green olives	387 ± 9^{a}	30 ± 1^{a}	10.3 ± 0.2	0.9958	10.32	0.8
Residue from green olives–control	323 ± 5^{b}	44.3 ± 0.1^{b}	7.8 ± 0.1	0.9965	8.53	0.3
Residue from cold-pressed veraison olives	303 ± 5^{c}	39 ± 2^{c}	9.5 ± 0.1	0.9954	7.65	0.3
Residue from veraison olives–control	320 ± 4^{b}	44 ± 2^{b}	9.6 ± 0.1	0.9961	8.12	0.2

P: ultimate or maximum methane production; R_m: maximum methane production rate; λ: lag time. S.E.E.: standard error of estimate; R^2: determination coefficient; Error (%): difference (in percentage) between the experimental and calculated ultimate methane production.

Regarding the ripening stage of the olives, the values of R_m were significantly higher for the residues from green olives than for olives in veraison. Parallelly, the pressing process caused a decrease in the R_m parameters of the green olives from 44.3 ± 0.1 to 30 ± 1 L CH_4/(kg VS·d); similarly, although less severe, the R_m from the residues from olives in veraison also decreased (from 44 ± 2 to 39 ± 2 L CH_4/(kg VS·d)). This fact may be attributed to the lower phenolic compound content observed in the residues from cold-pressed olives compared with those obtained from the controls, especially in the case of green olives, for which a decrease of 8.8% in the total phenolic compound concentration was observed.

A similar trend in the R_m values was observed when only the solid waste derived from a similar cold-pressing was subjected to batch anaerobic digestion [5]; although, in this case, higher R_m values were found compared with those obtained in the present research. This fact is attributed to the higher phenolic compound concentration found in the mixture residue as a consequence of the addition of wastewater to the solid waste. The previously mentioned research in which single olive pomace was digested also revealed a reduction in the R_m value from 87 ± 7 to 73 ± 6 L CH_4/(kg VS·d) when the green olives were cold-pressed compared with the control (without cold-pressing).

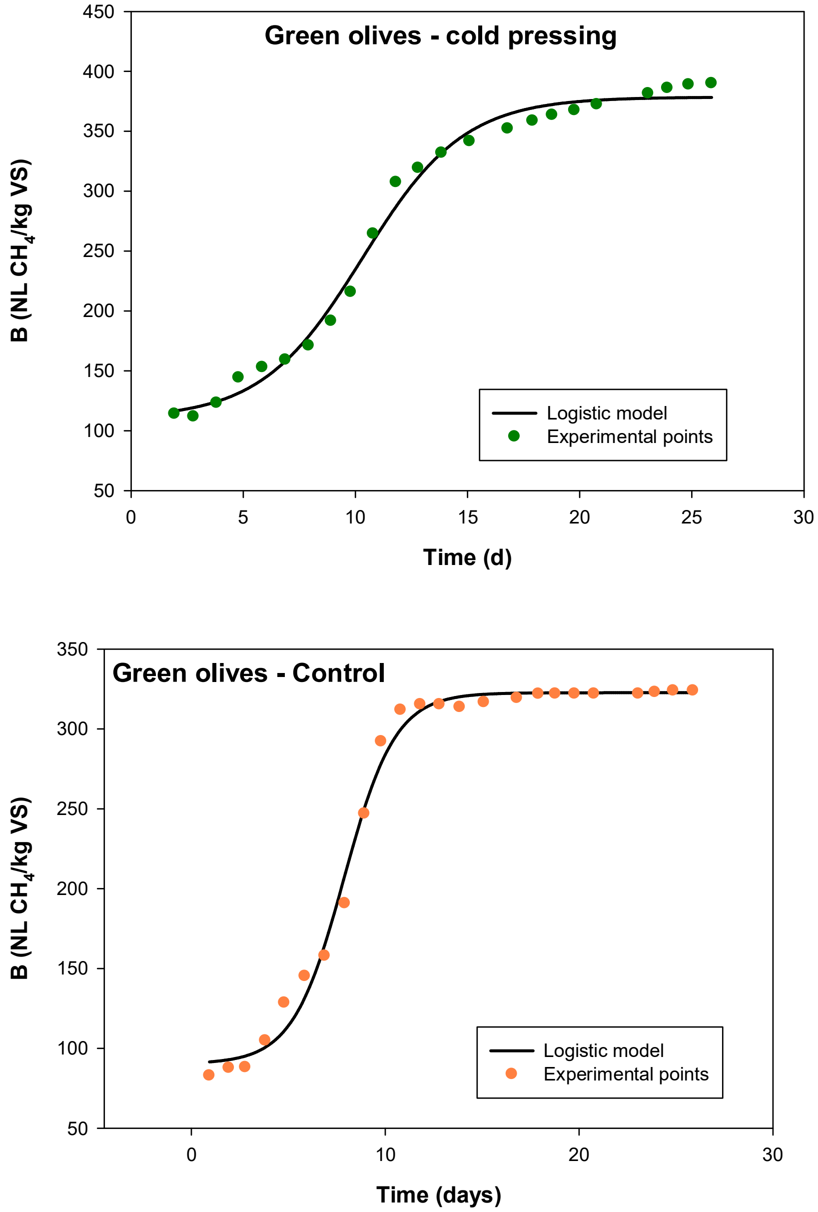

Figure 2. Variation in the experimental values for methane production and the theoretical values obtained from the logistic model (solid lines) for the residues derived from the cold-pressed green olives and the control.

A decrease in the R_m was also observed for two-phase olive mill solid waste (TPOMSW) subjected to a BMP test after pre-treatment by steam explosion at temperatures ranging from 138 to 171 °C and times varying between 5 and 30 min [17]. In this case, the maximum R_m (24.2 ± 0.7 L $CH_4/(kg\ VS \cdot d)$), obtained from the pre-treatment conditions of 141 °C and 30 min, is much lower than the results reported in this study, especially in the case of both green and veraison olives subjected to cold-pressing. In addition, Donoso-Bravo et al. [18] also revealed that the direct enzyme addition pre-treatment did not enhance either the rate or the maximum methane production. Similarly, steam explosion showed no increment in the biodegradability of TPOMSW; however, thermal hydrolysis performed at 148 °C for 30 min without rapid depressurization notably enhanced both R_m (50%) and the methane yield (70%) [17].

Considerably lower R_m values than those found in this study were reported when olive agro-food by-products (composed basically of olive-pomace and small proportions of straw) were co-digested with animal manure at ratios of 1:1, 1:2, and 2:1 [20]. In this case, the R_m values ranged from 1.06 to 1.83 L $CH_4/(kg\ VS \cdot d)$, and these authors determined that biogas production was slower and lower when the agro-food by-product load increased. However, a higher animal manure ratio increased the process kinetics [20].

In contrast, R_m values of 35 ± 3 and 31 ± 2 L $CH_4/(kg\ VS \cdot d)$ were found in BMP tests of raw olive mill wastewater (OMW) and centrifuged OMW (at 4000 rpm for 15 min), respectively [16], using an anaerobic inoculum derived from an anaerobic digester that treats urban wastewater. This difference may be due to the compositions of VS in the raw and centrifuged OMW, which contribute to a higher R_m. Moreover, the raw OMW presented more highly biodegradable carbohydrates in addition to oils and grease, which resulted in a higher R_m value.

Furthermore, the maximum P value was observed for the residue derived from cold-pressed green olives (387 ± 9 NL CH_4/kg VS), in contrast with that observed for R_m. This may be because of the higher organic matter proportion (VS: 171 ± 1 g/L; tCOD: 236 ± 33 g O_2/L) detected in the residue when compared with others (VS ranged from 130.6 to 132.2 g/L and tCOD from 190 to 202 g O_2/L). The highest P value reached for this residue coincided with the maximum biodegradability (VS removed) value obtained (84.5%) compared with the others, whose VS removal values were between 79.0 and 79.6%.

On the other hand, when only the single solid waste derived from cold-pressed olives or not subjected to this process was anaerobically digested, the maximum value for ultimate methane production was found for the solid waste from green olives that were not cold-pressed (319 ± 6 NL CH_4/kg VS). This result may be due to the soluble matter (sCOD: 113 g/L) detected in the single solid waste, which was higher in comparison with the others (sCOD: 105–107 g/L). In this case, the highest maximum methane yield reached for this solid waste coincided with the highest biodegradability obtained (90.8%) in comparison with the other sole solid wastes, with values within 74.5 and 86.4% [5].

It is also worth pointing out that the P achieved for the mixture residue from the cold-pressed green olives (387 ± 9 NL CH_4/kg VS) was 31.6% higher than that obtained for an olive pomace previously subjected to steam explosion treatment (200 °C for 5 min with rapid decompression) (294 NL CH_4/kg VS) [11]. This behavior may be explained by the fact that the steam explosion process generated undesirable compounds such as furan and complex phenols, which could inhibit the AD process [17,21].

Maximum methane yield values of 320 and 325 L CH_4/kg VS were reported for the anaerobic co-digestion processes of agro-food by-products, composed basically of olive pomace with pre-treated waste sludge and animal manure, respectively, [20]. As seen in Table 4, these values were very similar to the values obtained in the present work when the olives (both green and in veraison) were not subjected to cold-pressing.

The maximum methane yields obtained in batch anaerobic digestion experiments of raw and centrifuged OMW using different types of anaerobic inoculum were higher in every case for the untreated OMW (325–472 L CH_4/kg VS) in comparison with the same OMW after a subsequent centrifugation step (219–391 L CH_4/kg VS [16]. This result may be due to the higher biodegradable total carbohydrates, oils, fats, and soluble COD contents in the raw OMW compared with the centrifuged wastewater [16].

The lag periods found for the four mixture residues used in this work were much higher (between 7.8 and 10.3 days) than the lag periods found in the anaerobic digestion of single solid residues, whose values varied between 0.20 and 0.23 days [5]. This difference may be attributed to the higher phenolic compound content present in the mixture residues compared with those contained in the single solid waste. The shape of the curves of methane production–time observed in the present research clearly indicates a fast increase in methane generation after an initial lag period of 6–7 days, during which the hydrolysis of the slowly digestible substrate components took place for the four mixture residues. Higher lag period values (11.9 days) than those obtained in the present work were reported for the anaerobic co-digestion processes of the mixtures of agro-food by-products, composed mainly of olive pomace, with animal manure at a ratio of 3:1 [20]. These authors demonstrated that a higher proportion of olive residue in the co-digested mixture led to higher lag periods. On the contrary, similar lag period values (7.5–9.0 days) to those obtained in the present work were revealed in the BMP tests of two-phase olive mill solid wastes previously subjected to steam-explosion processes at temperatures ranging from 140 to 170 °C for 5–30 min [17].

3.5. Energy Assessment

Despite being a resource-efficient process, anaerobic digestion reduces the organic matter and considerably decreases the contamination power of wastes. The high amount of methane produced along with the energy output generated during the process are remarkable. Equation (2) was applied to determine [5,10] the energy output from the BMP data from the experiments. The energy yield from the process, or viability, is a key factor in the scaling up of any AD process run at the lab scale. Additionally, it should be considered that the inoculum was not acclimatized to the new residues before the anaerobic experiments; hence, the generated methane had been, presumably, underestimated. The energy output values for mixture residues from cold-pressed green olives were 15.7 kJ/g VS, and 12.3 kJ/g $VS_{removed}$ for the control; meanwhile, for the mixture residues from cold-pressed olives in veraison the values were 12.2 kJ/g VS and 13.0 kJ/g $VS_{removed}$ for the control.

The highest energy output found for the cold-pressed green olives mixture was 27.6% higher than that obtained for the residue from control green olives. By contrast, the values observed for the wastes from olives in veraison were very similar. The highest energy output value found for the residue from cold-pressed green olives is in accordance with the highest methane production and biodegradability values determined in this residue compared with the others.

A previous study [22] revealed that the energy output of a mixture containing 16% olive pomace with corn silage (17%), citrus pulp (25%), whey (18%), cattle manure (4%), and poultry litter (8%) was 1.2 kWh-e/kg dry feedstock mixture. This fact allows for valorizing the most important agricultural wastes and by-products from southern Italy (Sicily), including olive pomace.

Pasalari et al. [10] reported a range of energy output values from 9.4 to 25.5 kJ/g $VS_{removed}$ for the AD process applied to pretreated landfill leachate by an electrochemical oxidation process. The values obtained in this study were of the same order of magnitude as the values reported in the present research. High energy output results (76.25 kJ/g fed VS) were also observed for the co-digestion of sewage sludge and food waste after a microwave pre-treatment [23]. The study concluded that the use of microwave as a pre-treatment enhanced the solubilization of organic compounds and the hydrolysis of protein to NH_4^+-N, and also increased the methane yield as well as the R_m during the co-digestion process [23].

Author Contributions: M.J.F.-R.: conceptualization, investigation, validation, writing—review and editing; J.C.-C.: investigation conceptualization, validation; D.d.l.L.-C.: investigation, conceptualization, validation; Á.F.-P.: investigation, conceptualization, validation; G.R.-G.: supervision, resources, funding acquisition, writing—original draft; R.B.: supervision, writing—original draft, writing—review and editing, resources, funding acquisition. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the regional government of Andalucía, Junta de Andalucía, Project 18-616, “Consejería de Economía, Conocimiento, Empresas y Universidad”, Andalucía, Spain, and Project PID2020-114975RB-100/AEI/10.13039/501100011033 was financed by the Spanish Ministry of Science and Innovation. The project FEDER UPO-380782 was financed by the regional government of Andalucía, Junta de Andalucía, “Consejería de Trasformación Económica e Industria”, providing financial support to Dr. Fernández-Rodríguez.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data will be made available on request.

Conflicts of Interest: The authors declare no conflict of interest.

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