







Article

Enhancing Biogas Production: An Assessment of Pasteurization Effects on Poultry, Swine, Bovine Manure and Food Waste Substrates

Ioanna Michailidou ¹, Ifigeneia Grigoriadou ², Themistoklis Sfetsas ^{2,*}, Christos Vlachokostas ³, Georgios Arsenos ⁴ and Aristotelis Lymperopoulos ^{1,*}

¹ Laboratory of Farm Animal Reproduction and Animal Breeding, Division of Animal Science, Department of Agriculture, International Hellenic University, 57400 Thessaloniki, Greece; drag202015@agro.ihu.gr

² Research & Development, Quality Control and Testing Services, QLAB Private Company, 57008 Thessaloniki, Greece; igrigoriadou@q-lab.gr

³ Sustainability Engineering Laboratory, School of Mechanical Engineering, Faculty of Engineering, Aristotle University Thessaloniki, 54124 Thessaloniki, Greece; vlahoco@meng.auth.gr

⁴ Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece; arsenosg@vet.auth.gr

* Correspondence: tsfetsas@q-lab.gr (T.S.); alymperopoulos@ihu.gr (A.L.); Tel.: +30-2310784712 (T.S.); +30-2310013753 (A.L.)

Abstract: Within the evolving regulatory landscape of the European Union concerning animal by-product (ABP) management within the circular economy framework, this study explores the concurrent objectives of safeguarding public health and environmental integrity and maximizing final product value. Anaerobic digestion (AD) emerges as a holistic solution for ABP management, addressing sanitation concerns while enhancing end-product quality. Through laboratory-scale experimentation, the AD process applied to four substrates—poultry manure, swine manure, cattle manure, and food waste—is scrutinized. Prior to AD, pasteurization at 70 °C for 60 min ensures microbial safety. Subsequently, four experimental AD cycles compare pasteurized and unpasteurized substrates. Results highlight the efficacy of pasteurization in sanitizing final products across all substrates, emphasizing its pivotal role in product safety. However, pasteurization's impact on system performance varies. While enhancing biogas yield from animal waste notably, its influence on food waste biogas production is less pronounced, indicating substrate-specific dynamics. This study offers insights into optimizing ABP management strategies, emphasizing the interplay between pasteurization, substrate characteristics, and AD performance. Such insights are crucial for advancing sustainable practices in the circular economy paradigm, balancing environmental stewardship with economic viability.

Keywords: anaerobic digestion; pasteurization; animal by product; sustainability; biogas yield



Citation: Michailidou, I.; Grigoriadou, I.; Sfetsas, T.; Vlachokostas, C.; Arsenos, G.; Lymperopoulos, A. Enhancing Biogas Production: An Assessment of Pasteurization Effects on Poultry, Swine, Bovine Manure and Food Waste Substrates. *Sustainability* **2024**, *16*, 7130. <https://doi.org/10.3390/su16167130>

Academic Editor: Francesco Ferella

Received: 8 July 2024

Revised: 8 August 2024

Accepted: 13 August 2024

Published: 20 August 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Sustainable development and the circular economy represent two overarching strategies directed towards establishing a harmonized and enduring framework for both humanity and the environment. Sustainable development advocates for a developmental paradigm that meticulously considers the requisites of present generations while safeguarding the capacity of future generations to fulfill their own needs. Central to this endeavor is the pursuit of social, economic, and environmental prosperity, facets that are intrinsically intertwined with the foundational principles underpinning the circular economy. Originating several decades ago, the concept of the circular economy emerged as a deliberate response to the myriad challenges engendered by the linear economic model.

On the contrary, the circular economy endeavors to minimize waste disposal and resource depletion through the advocacy of recycling, reusing, and renewing materials and

products by integrating both sustainable development and circular economy principles, and a more resilient and efficient system of economic and social development can be cultivated, one that prioritizes the conservation of natural resources and the protection of the environment. The application of the circular economy extends across various sectors of productive activity, exemplified prominently within the agri-food sector, specifically animal production. This sector, while yielding substantial financial gains on a global scale, also exerts a considerable environmental footprint. However, through the adoption of circular economy principles, opportunities emerge to mitigate environmental impact and enhance sustainability within this sector [1].

The strategic management of animal by-products in a comprehensive manner yields multifaceted benefits, whereby even the most minute waste is repurposed, thus augmenting its value and contributing to economic prosperity through valorization and the creation of derivative products. This integrated approach not only bolsters the economy but also ensures the preservation of public and animal health, alongside environmental conservation, thereby generating profits and advantages.

One approach for the management of animal by-products involves biogas production through anaerobic digestion, as illustrated in Figure 1. However, to uphold public health standards and safeguard all living organisms, the European Union has enacted regulatory directives [2,3] mandating the pasteurization of animal by-products prior to their utilization in anaerobic digestion processes. In compliance with EU Regulation (EC) 142/2011, pasteurization of animal by-products necessitates exposure to a core temperature of 70 °C for a duration of 60 min [4] ensuring that the resultant product from anaerobic digestion is sanitized and safe for subsequent use.

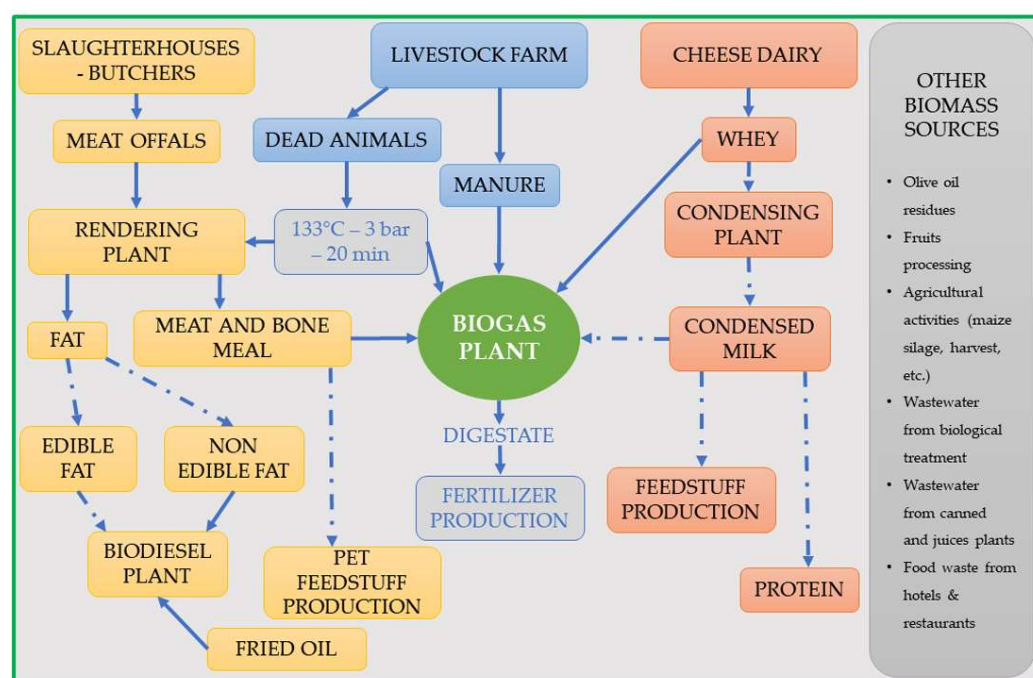


Figure 1. ABP's flowchart according to 142/2011 EU Regulation [3].

Beyond its primary role in ensuring sanitation, pasteurization serves as a pretreatment method aimed at enhancing the performance of methane production and the operational efficiency of the system [5], owing to the heightened temperature conditions.

The efficacy of pasteurization in ensuring the sanitization of the final product remains uncontested [6]. However, divergent perspectives persist regarding its influence on system performance. Numerous scholars posit that pasteurization, when employed as a pretreatment method in anaerobic digestion, notably enhances methane yield [7]. As articulated by Edstrom et al. [7] pasteurization augments methane production, possibly attributable to

enhanced biodegradation of organic matter, facilitated by microbial breakdown of lipids. Pasteurization reduces the size of solid organic constituents, thereby augmenting the solubility [8] of free molecules (such as ammonia, hydrogen, and VFAs), thereby fostering nutrient hydrolysis—the initial stage of anaerobic digestion.

Conversely, conflicting findings exist within the literature [9] with some studies indicating an increase in methane yield following pasteurization of certain substrates, while observing negligible effects on others, such as poultry and swine slaughterhouse by-products [10]. The minimal impact on poultry by-products contrasts with a significant elevation in biochemical methane potential (BMP) observed in swine by-products. Notably, pasteurization exerts negligible effects on substrates rich in lignin, lignocellulose, and other celluloses [11]. In a separate investigation [12] pasteurization yielded positive outcomes for blood but failed to elicit significant effects on bovine manure and food waste (FW). Similarly, investigations into slaughterhouse by-products [10] and FW revealed no considerable augmentation in methane yield.

The main aim of this study is to examine the influence of pasteurization on the anaerobic digestion efficacy of diverse animal by-product substrates. Specifically, the aim is to evaluate the effects of pasteurization on methane yield and overall system efficiency within the context of anaerobic digestion processes.

2. Materials and Methods

Within this context, the present study undertook a comparative examination of anaerobic digestion involving four distinct animal by-product (ABP) substrates on a laboratory scale, employing pasteurization. Four experimental batches were conducted, each comprising four untreated samples derived from initial raw materials, four pasteurized samples from the same substrates, and an inoculum sample serving as a control, with biogas production recorded for each batch.

2.1. Sampling

Within the framework of this study, the following raw materials were utilized for the experiments:

1. Poultry manure from a broiler farm (poultry manure—PM).
2. Swine manure from a pig farm (swine manure—SM).
3. Cattle manure from a bovine farm (cattle manure—CM).
4. Expired or unsuitable for human consumption foodstuffs (FW), including both liquid and solid foods. This category encompasses animal-origin liquids and plant-based foods (e.g., milk, juices, oil) as well as solid foods such as restaurant leftovers, fruits, vegetables, legumes, canned goods, deli meats, and dairy products.
5. Inoculum containing all the necessary anaerobic microorganisms (hydrolytic, acidogenic, acetogenic, and methanogenic) to initiate the chemical and enzymatic reactions of the raw materials within the digester.

This selection of raw materials ensures a comprehensive examination of various waste types and their potential for biogas production under anaerobic digestion conditions.

Sampling was conducted using plastic containers with a capacity of 15 L each. Four samples were collected, one from each raw material. During the experiments, one sample was taken from each type of material, and these specific samples were exclusively used in all experiments. This approach was employed to ensure the stability and uniformity of the physicochemical parameters across all samples, as variations in the composition of raw materials can affect and potentially alter the reliability of the final results.

Sample preparation: After sampling, each one of the raw materials was divided into smaller samples, stored at -18°C .

Grinding: The raw materials were ground to achieve a more homogeneous form, in accordance with the requirements of DIN 4630/2016 [13], which specifies standards for the homogenization and uniformity of samples intended for anaerobic digestion. Compliance with EU Regulation 142/2011 [3] was also ensured, which mandates that the particle size

of raw materials for anaerobic digestion should not exceed 12 mm. The grinding process was conducted using the PULVERISETTE 11 knife mill (Fritsch, Karnataka, India), with all components and dry ice utilized according to the manufacturer's specification [14].

Pasteurization: Pasteurization was conducted on approximately 100 g samples of each of the four raw materials. The samples were individually placed in closed glass containers to prevent evaporation during the process. A dedicated in-house isothermal water bath was used, maintained at a temperature of 70 ± 1 °C. The water bath was preheated to the desired temperature prior to sample immersion. Samples were submerged in the water bath and maintained at 70 ± 1 °C for a duration of 1 h. Continuous monitoring of the water bath temperature was ensured using a Sonof thermostat. Following the 1 h pasteurization period, samples were removed from the water bath and allowed to cool to room temperature.

2.2. Physicochemical Analysis

Total solids (TS): For the determination of total solids, a sample quantity of 2–5 g was placed in a predried and preweighed dish, and the weight of the sample was recorded. The dish containing the sample was placed in a drying oven at 105 °C overnight. After drying, the dish was cooled in desiccator to ambient temperature and weighed. The method followed the Total Solids Dried at 103–105 °C protocol as per APHA 2540-B [15].

Volatile solids (VS): For the determination of volatile solids, the sample was first dried before being placed in a muffle furnace. The dish containing the sample was weighed, then ignited for 4 h at 550 °C, cooled in a desiccator, and weighed again. This method was based on the Fixed and Volatile Solids Ignited at 550 °C protocol according to APHA 2540-E [15].

Determination of nutrients—trace elements—heavy metals: For metal determination, the sample is decomposed in acid at a high digestion vessel pressure with the help of a Milestone Ethos Up microwave oven (Milestone Srl, Sorisole, Italy) and the resulting solution is analyzed. First, 0.5–1.0 g sample was weighed and HNO₃ and H₂O₂ were added to the sample followed by gradual digestion up to 210 °C. Then, the sample was diluted and analyzed by ICP-MS. An Agilent 7850 ICP-MS (Agilent Technologies, Santa Clara, CA, USA) equipped with the ORS4 collision cell was used to analyze macro-elements and trace metals. Sampling was performed using an Agilent SPS 4 autosampler. The 7850 ICP-MS was configured with the standard ISIS 3 injection system. The samples were prepared for analysis according to the digestion procedure outlined in ISO 17294-2:2016 (ISO, 2016) [16] and APHA 3125 [15].

Determination of theoretical biomass yield according to Baserga 1998: The assessment of the potential for energy recovery from specific biomass substrates can be performed by various methods. The contents of fat, protein, and fibrous substances are used to calculate the theoretical biogas and methane content, according to the Baserga equation. All values were expressed for dry matter (e.g., crude protein % DM), and the biogas yield L/kg (OM) was calculated according to the following equation:

$$\text{Biogas Yield L/Kg (OM)} = [(0.57 \times \text{Crude Protein\%DM} \times 700) + (0.8 \times \text{Crude Fat\%DM} \times 1250) + (0.64 \times \text{Crude Fiber\%DM} \times 790) + (0.9 \times \text{NFE\%DM} \times 790) \times \text{DM\%}] / 10,000$$

2.3. Microbiological Analysis

The conducted microbiological tests focused on *E. coli*, *Enterococcus faecalis*, and *Salmonella*, were performed according to internationally certified standards:

- For the detection of *E. coli*, ISO CEN/TR 16193 [17] was applied.
- For the detection of *Enterococcus faecalis*, ISO 7899-2/2000 [18] was applied.
- For the detection of *Salmonella*, ISO 6579-1:2017 [19] was applied.

Detection of *Salmonella*. The method for detecting *Salmonella* was based on ISO 6579-1:2017 [19]. This protocol involves the use of solid selective substrates to identify colony-forming micro-organisms exhibiting defined biochemical and serological char-

acteristics. Initially, the sample is pre-enriched in buffered peptone water at ambient temperature, followed by incubation at 34–38 °C for 18 h. The culture obtained from this pre-enrichment stage is then inoculated into two selective substrates. The resulting cultures are subsequently transferred in two solid selective substrates: xylose lysine deoxycholate agar (XLD agar) and a supplement to XLD agar. Potential *Salmonella* colonies are finally sub-cultured on a non-selective substrate (nutrient agar) for identity confirmation using appropriate biochemical and serological tests.

Enumeration of bacteria of the Enterobacteriaceae. This method followed the methodology outlined in ISO 21528-2:2017 [20]. Initially, the sample was inoculated onto violet red bile glucose (VRBG) agar, followed by overlaying with an additional layer of agar to create semi-anaerobic conditions. Plates were also prepared using decimal dilutions of the sample. Incubation was carried out at 37 °C for 24 h. Presumptive colonies of Enterobacteriaceae were sub-cultured onto non-selective agar and confirmed through tests for glucose fermentation and the presence of oxidase. The count of Enterobacteriaceae per gram or milliliter of the sample was determined based on the number of confirmed typical colonies per plate.

2.4. Biogas Measurement

The biochemical methane potential (BMP) test is utilized to determine the methane or gas production from the anaerobic decomposition of organic substrates. The test was conducted following protocols established by Bioprocess Control. A Bioprocess Gas Endeavour AMPTS® III (S/N: 1100-2100-5100-1235) setup (BPC Instruments (Haining) Co. Ltd., Haining, China), was employed, consisting of 15 identical 500 mL Duran Schott bottles, each with a working capacity of 400 mL and a headspace of 100 mL. The setup also included a thermostatic water bath, a gas outlet connected to a measuring cylinder submerged in water for volume measurement, and Teflon caps for sealing the bottles. The quantity of substrate added to each bottle was determined based on:

$$m_{is} = ISR \frac{m_{tot.VS_s}}{VS_i + 2VS_s}$$

where:

m_{is} : The mass of inoculum in g.

m_{tot} : The total mass that is placed in the bottle that is 400 g.

VS_s : The quantity of volatile solids of substrate.

VS_i : The quantity of volatile solids of substrate inoculum $\kappa\alpha\iota$.

ISR: A constant expressing the ratio.

$$ISR = \frac{\text{quantity of inoculum VS}}{\text{quantity of substrate VS}} = 2$$

according to the manufacturer's manual [21].

The bioreactors were set up with both samples (batches containing inoculum and substrate) and blanks (batches containing only inoculum). To establish anaerobic conditions, the bioreactors were purged with nitrogen gas for 2 min. Subsequently, the bioreactors were placed in a thermostatic water bath set to a hydraulic retention time (HRT) of 30 days and maintained at a mesophilic temperature of 40 °C. The volume of biogas produced was monitored daily.

Biochemical methane potential (BMP) is defined as the volume of methane produced per unit amount of organic substrate material added to the reactor and can be expressed by the following equation:

$$BMP = (V_S - V_I) / m_{VS,SS}$$

where:

V_S : Accumulated volume of biomethane from the reactor containing the sample (substrate and inoculum).

V_I : Volume of biomethane produced by the inoculum present in the sample bottle.

$m_{VS,SS}$: Amount of organic material (substrate) contained in the sample bottle.

This equation quantifies the efficiency of methane production from the added organic substrate material under the given experimental conditions.

2.5. Statistical Analysis

Statistical analysis was conducted using the two-sample *t*-test assuming equal variances. This method was chosen to compare the means of two groups. A 95% confidence interval (CI) was used to estimate the range within which the true difference between the means likely lies. The analysis was performed in Microsoft Excel with an alpha (α) value set to 0.05.

The null hypothesis was rejected if the *p*-value was less than the alpha value. In other words, if the *p*-value was less than 0.05, we concluded that there was a statistically significant difference between the two groups. Conversely, if the *p*-value was greater than 0.05, we did not reject the null hypothesis, suggesting no significant difference between the groups.

3. Results

Physicochemical Analysis

The determination of theoretical biomass yield in this study involved analyzing substrates across four experimental cycles. The substrates included poultry manure, swine manure, bovine manure, and food waste. Table 1 presents the comprehensive results of these analyses. A close evaluation revealed no significant differences in biomass yield between the different types of substrates. Furthermore, the process of pasteurization, intended to eliminate pathogens and enhance the safety of the biomass, did not significantly impact the yield outcomes. This consistent result across all substrates and treatments suggests that the type of organic material and the application of pasteurization are not critical factors influencing the theoretical biomass yield under the conditions tested.

Table 1. Theoretical analysis of 4 unpasteurized and pasteurized substrates and inoculum in each batch.

Raw Material	Total Solids (TS) %	Volatile Solids (VS) %	Fat FM *	Fat DM **	Protein FM *	Protein DM **	Crude Fibers FM *	Crude Fibers DM **	Nitrogen Free Extracts (NFE) %	Biogas Yield mL/g FM *	Biogas Yield mL/g VS
1st batch											
Inoculum	3.3	1.9									
PM	63.1	54.6	3.8	6.1	19.7	31.8	11.4	18.3	30.0	211	394
SM	2.1	1.7	0.2	5.4	1.4	40.4	0.5	15.1	19.3	7.4	440
CM	6.8	5.5	0.4	4.7	1.9	23.2	1.9	22.8	31.4	24.0	436
FW	32.9	30.5	6.5	19.5	6.0	18.1	2.1	6.2	48.6	248	814
PM (p)	63.3	53.6	4.0	6.5	19.3	31.0	12.0	19.2	29.5	207	407
SM (p)	2.8	2.3	0.3	8.9	1.1	32.7	0.4	10.9	26.7	8.9	396
CM (p)	6.6	5.4	0.4	5.8	1.6	23.7	1.4	20.9	31.8	42.5	785
FW (p)	33.1	30.2	7.5	22.0	6.2	18.2	2.9	8.5	44.1	196	648
2nd batch											
Inoculum	3.5	2.2									
PM	62.0	53.4	4.0	6.5	18.7	30.1	11.9	19.1	30.5	214	400
SM	3.5	2.8	0.4	10.2	1.5	42.1	0.8	23.0	5.0	13.6	480
CM	8.4	6.9	0.4	4.2	2.7	32.2	4.6	54.7	0.0	21.6	315
FW	33.0	30.5	8.2	24.8	5.7	17.4	2.3	7.0	43.3	248	814
PM (p)	62.3	53.7	3.5	5.7	19.2	30.8	12.4	19.9	29.9	206	383
SM (p)	3.3	2.6	0.3	9.4	1.3	40.2	0.9	27.8	1.8	11.7	447
CM (p)	6.8	5.6	0.3	3.9	1.1	16.6	<0.30	0.7	60.9	41.8	753
FW (p)	34.0	31.6	7.6	22.4	5.1	14.9	2.4	7.2	48.3	245	775

Table 1. Cont.

Raw Material	Total Solids (TS) %	Volatile Solids (VS) %	Fat FM * %	Fat DM ** %	Protein FM * %	Protein DM ** %	Crude Fibers FM * %	Crude Fibers DM ** %	Nitrogen Free Extracts (NFE) %	Biogas Yield mL/g FM *	Biogas Yield mL/g VS
3rd batch											
Inoculum	4.1	2.7									
PM	57.1	48.8	3.6	6.3	19.4	33.9	12.1	21.2	24.1	185	379
SM	18.4	15.2	0.4	9.4	1.3	35.5	0.3	8.4	39.2	8.6	250
CM	9.3	7.5	0.6	6.6	2.7	28.7	2.6	28.1	17.5	22.3	298
FW	32.3	29.9	7.8	24.1	5.8	17.9	2.5	7.9	42.8	224	750
PM (p)	59.4	51.2	3.7	6.3	19.6	33.0	14.7	24.7	22.2	246	481
SM (p)	3.1	2.8	0.4	13.0	1.5	49.7	0.8	27.2	1.2	8.9	320
CM (p)	13.1	11.1	0.4	3.1	1.9	14.2	2.0	15.3	52.2	35.7	320
FW (p)	34.0	31.9	6.2	18.1	4.0	11.8	1.7	5.0	58.8	199	623
4th batch											
Inoculum	4.1	2.7									
PM	56.9	54.6	4.0	7.1	19.8	34.8	12.1	21.3	21.7	177	363
SM	3.5	3.4	0.4	11.2	1.5	42.4	0.8	22.8	0.9	7.3	217
CM	10.0	7.4	0.6	5.9	2.9	28.6	4.4	44.2	3.9	19.0	253
FW	32.7	30.7	8.4	25.7	6.0	18.2	2.5	7.8	40.6	189	633
PM (p)	58.4	54.4	4.0	6.9	19.6	33.6	14.5	24.8	20.0	201	393
SM (p)	3.7	2.8	0.4	10.6	1.5	41.3	0.8	21.1	5.5	9.5	341
CM (p)	13.7	10.6	0.4	3.2	1.9	13.9	2.1	15.5	53.5	24.0	216
FW (p)	33.2	31.9	7.9	23.7	6.2	18.6	2.9	8.8	41.2	169	530

* Fresh Matter; ** Dry Matter.

The subsequent phase in evaluating biogas yield involved subjecting the materials to the anaerobic digestion process. This procedure aimed to determine the efficiency of biogas production from the different substrates analyzed earlier.

Anaerobic digestion: Given the findings on the biogas yield of each batch, we proceeded with a comprehensive statistical analysis to draw our conclusions. This involved calculating the mean, standard deviation, and coefficient of variation for each raw material and batch (Tables 2 and 3). Additionally, data recorded from the AMPTS III system are depicted in two comparative diagrams (Figure 2), illustrating the performance of each sample across different batches. It is observed that pasteurization significantly enhances anaerobic digestion in all types of manure (poultry, swine, and cattle), but not in food waste. The enhanced biogas production in pasteurized manure can be attributed to the breakdown of complex organic compounds and reduction of pathogen load, which facilitate a more efficient digestion process. Conversely, the slight decrease in biogas yield from pasteurized food waste suggests that the diverse components of food waste, including both animal and plant materials, may respond differently to pasteurization. This complexity might inhibit certain microbial communities or alter substrate composition in ways that reduce its biodegradability under anaerobic conditions.

Table 2. Biogas production in mL/g FM from each different sample.

Batch Number	PM	PMp	SM	SMp	CM	CMp	FW	FWp
1st	211	207	7.4	8.9	24.0	42.5	248	196
2nd	214	206	13.6	11.7	21.6	41.8	248	245
3rd	185	246	8.6	8.9	22.3	35.7	224	199
4th	177	201	7.3	9.5	19.0	24.0	189	169
mean	197	215	9.2	9.7	21.7	36.0	228	202
stdev	15.9	16.2	2.6	1.2	1.8	7.4	24.3	24.3
p-value	0.12		0.07		0.02		0.11	
coefficient of variation	0.08	0.08	0.28	0.12	0.08	0.21	0.11	0.12

Table 3. Biogas production in mL/g VS from each different sample.

Batch Number	PM	PMp	SM	SMp	CM	CMp	FW	FWp
1st	394	407	440	396	436	378	814	648
2nd	400	383	480	447	315	423	814	775
3rd	379	481	250	320	298	320	750	623
4th	363	393	217	342	253	216	633	530
mean	384	416	347	376	325	334	753	644
stdev	14.4	38.6	115	49.4	67.5	77.8	73.9	87.4
<i>p</i> -value	0.12		0.35		0.44		0.04	
coefficient of variation	0.04	0.09	0.33	0.13	0.21	0.23	0.10	0.14

Despite the impact of pasteurization, food waste emerged as the best substrate in terms of biogas yield when compared to poultry manure, swine slurry, and cattle manure. This finding underscores the potential of food waste as a highly efficient substrate for biogas production, even though pasteurization does not enhance its yield. The superior performance of food waste can be attributed to its high organic content, which provides a rich source of biodegradable material for methane-producing micro-organisms.

Comparing the mean, standard deviation, and coefficient of variation among the manures across all batches, pasteurized poultry manure consistently showed the best performance. It exhibited the highest biogas yield and substrate stability, as evidenced by lower variability in yield across four batches. This suggests that pasteurized poultry manure is a particularly robust and reliable substrate for biogas production under the conditions tested. Furthermore, the application of inductively coupled plasma mass spectrometry (ICP-MS) to the four substrates allowed for the measurement of nutrients, heavy metals, and trace elements. As shown in Table 4, there were no significant changes in these parameters between pasteurized and non-pasteurized samples. This indicates that pasteurization does not adversely affect the nutrient profile or heavy metal content of the substrates, maintaining their quality and suitability for use in anaerobic digestion.

The statistical tables and comparative diagrams provide a systematic and detailed overview of the performance metrics for each substrate, confirming that pasteurization generally improves biogas production for manures while highlighting the unique response of food waste. These findings offer valuable insights into the optimization of anaerobic digestion processes. Specifically, they suggest that while pasteurization can enhance biogas yield and process stability for certain substrates, its application may need to be tailored depending on the specific characteristics of the substrate in question.

Pasteurization was evaluated and documented as a method of sanitizing animal by-products (ABPs) in terms of microbial agents, in accordance with the requirements of EU Regulation (EU) 142/2011, which mandates pasteurization at 70 °C for one hour or an alternative validated pasteurization method. The microbial load was measured for each substrate before and after anaerobic digestion using a laboratory-scale anaerobic digester. The results are presented in Table 5. These results demonstrate the critical role of pasteurization in ensuring the microbial safety of substrates used in biogas production. By meeting the requirements of EU Regulation (EU) 142/2011 [3], pasteurization ensures that ABPs are sufficiently sanitized, reducing the risk of pathogen transmission. Moreover, the findings highlight the importance of anaerobic digestion as a complementary process that not only aids in biogas production but also further mitigates microbial risks, particularly when initial pasteurization is applied.

Table 4. Nutrients, trace elements, and heavy metals in 4 batches.

Raw Material	Nutrients										Trace Elements				Heavy Metals						
	P g/kg	K g/kg	Ca g/kg	Mg g/kg	S g/kg	Na g/kg	Cu mg/kg	Zn mg/kg	Fe mg/kg	Se mg/kg	Co mg/kg	B mg/kg	Mo mg/kg	Mn mg/kg	Al mg/kg	Ni mg/kg	Cd mg/kg	Cr mg/kg	Pb mg/kg	As mg/kg	Hg mg/kg
1st batch																					
PM	4.4	18.7	8.7	3.9	3.3	1.2	41.9	251	467	0.8	0.5	45.7	3.6	385	364	4.9	0.12	3.39	0.30	0.21	0.05
SM	0.5	0.6	0.7	0.3	0.2	0.2	22.7	102	55.3	0.1	0.0	4.2	0.3	17.5	30.6	0.5	0.01	0.42	0.06	0.02	0.08
CM	0.5	2.1	1.7	0.8	0.2	0.7	7.3	22.7	173	0.1	0.3	5.6	0.3	20.6	77.4	3.9	0.01	1.96	0.06	0.03	0.05
FW	1.0	1.8	1.6	0.3	0.6	4.0	2.8	11.9	272	0.1	0.1	3.5	0.2	9.6	126	0.7	0.01	1.51	0.45	0.05	0.01
PM (p)	4.0	17.2	7.6	3.5	3.0	1.1	37.0	221	400	0.7	0.4	38.8	3.1	356	286	4.2	0.10	2.02	0.28	0.18	0.09
SM (p)	0.6	0.6	0.9	0.4	0.2	0.2	25.3	113	59.6	0.1	0.0	4.2	0.3	18.7	26.9	0.5	0.01	0.50	0.06	0.02	0.04
CM (p)	0.4	1.5	1.4	0.6	0.2	0.5	4.8	19.0	141	0.1	0.2	4.4	0.3	17.2	64.9	2.9	0.01	2.61	0.06	0.02	0.02
FW (p)	1.0	1.9	1.7	0.3	0.6	4.0	2.4	16.3	294	0.1	0.1	4.0	0.2	10.6	123	0.6	0.01	1.25	0.47	0.05	0.13
2nd batch																					
PM	3.7	15.5	6.7	3.3	2.7	1.0	35.7	187	400	0.6	0.4	32.7	3.2	320	289	4.0	0.10	1.57	0.19	0.21	0.01
SM	3.1	0.9	5.0	1.7	0.9	0.4	101	467	438	0.2	0.1	6.2	1.3	104	102	1.7	0.03	1.28	0.23	0.08	0.10
CM	0.7	1.9	2.4	1.1	0.4	0.7	10.2	31.3	277	0.1	0.4	6.3	0.5	32.1	104	5.9	0.02	2.67	0.22	0.03	0.01
FW	0.9	1.7	1.4	0.3	0.5	3.7	3.9	9.4	305	0.1	0.1	2.9	0.2	9.4	131	0.7	0.02	1.38	0.50	0.05	0.01
PM (p)	3.7	16.2	7.9	3.5	2.8	1.1	37.1	193	412	0.6	0.4	33.7	3.2	355	315	4.0	0.10	1.64	0.20	0.17	0.01
SM (p)	0.2	0.5	0.3	0.1	0.1	0.2	9.7	37.6	18.8	0.0	0.0	2.4	0.1	5.3	7.9	0.2	0.00	0.32	0.02	0.01	0.01
CM (p)	0.4	1.5	1.3	0.6	0.3	0.5	4.8	13.1	125	0.1	0.2	4.7	0.3	16.4	51.1	3.0	0.01	1.33	0.04	0.02	0.01
FW (p)	0.8	1.4	1.2	0.2	0.5	4.1	2.4	7.4	412	0.0	0.1	3.0	0.2	9.0	116	0.7	0.01	1.30	0.43	0.05	0.01
3rd batch																					
PM	3.9	16.4	7.7	3.6	2.9	1.1	42.9	204	457	0.6	0.5	36.6	3.6	362	327	4.5	0.14	1.72	0.20	0.20	0.01
SM	0.3	0.4	0.4	0.2	0.1	0.2	11.7	47.7	28.8	0.0	0.0	2.3	0.2	8.7	9.9	0.3	0.00	0.17	0.02	0.01	0.03
CM	0.6	2.4	2.1	1.1	0.4	0.9	9.6	22.6	225	0.1	0.4	5.2	0.4	26.7	92.1	5.1	0.02	2.40	0.06	0.03	0.01
FW	0.9	1.7	1.5	0.3	0.5	3.8	4.7	10.3	307	0.1	0.1	2.5	0.2	9.9	118	0.7	0.01	1.66	0.47	0.05	0.01
PM (p)	3.9	16.2	7.2	3.4	2.8	1.1	36.8	195	417	0.6	0.4	33.1	3.2	346	297	4.1	0.10	1.88	0.21	0.17	0.01
SM (p)	0.4	0.8	0.6	0.3	0.2	0.3	20.2	83.9	47.4	0.1	0.0	3.2	0.3	14.5	16.9	0.5	0.01	0.22	0.04	0.02	0.01
CM (p)	0.3	1.9	0.9	0.6	0.1	0.7	3.6	9.1	86.1	0.0	0.2	3.6	0.2	11.4	34.1	2.1	0.01	0.93	0.02	0.02	0.01
FW (p)	0.7	1.2	1.0	0.2	0.5	3.5	2.0	6.2	396	0.0	0.1	2.6	0.2	7.6	315	0.8	0.01	1.18	0.35	0.04	0.01
4th batch																					
PM	4.0	16.9	7.7	3.6	3.0	1.1	40.2	214	441	0.6	0.5	38.4	3.5	356	327	4.5	0.12	2.22	0.23	0.21	0.02
SM	1.3	0.6	2.0	0.7	0.4	0.3	45.1	205	174	0.1	0.1	4.2	0.6	43.4	47.5	0.8	0.01	0.62	0.10	0.03	0.07
CM	0.6	2.1	2.1	1.0	0.3	0.8	9.0	25.6	225	0.1	0.4	5.7	0.4	26.5	91.2	5.0	0.02	2.34	0.11	0.03	0.02
FW	1.0	1.8	1.5	0.3	0.6	3.8	3.8	10.5	294	0.1	0.1	3.0	0.2	9.6	125	0.7	0.01	1.51	0.47	0.05	0.01
PM (p)	3.9	16.5	7.6	3.5	2.9	1.1	36.9	203	409	0.6	0.4	35.2	3.2	352	299	4.1	0.10	1.85	0.23	0.17	0.04
SM (p)	0.4	0.6	0.6	0.2	0.2	0.2	18.4	78.2	41.9	0.1	0.0	3.2	0.3	12.8	17.2	0.4	0.01	0.35	0.04	0.02	0.02
CM (p)	0.4	1.6	1.2	0.6	0.2	0.6	4.4	13.7	118	0.1	0.2	4.2	0.3	15.0	50.1	2.7	0.01	1.62	0.04	0.02	0.01
FW (p)	0.9	1.5	1.3	0.2	0.5	3.9	2.3	10.0	367	0.1	0.1	3.2	0.2	9.0	184	0.7	0.01	1.24	0.42	0.05	0.05

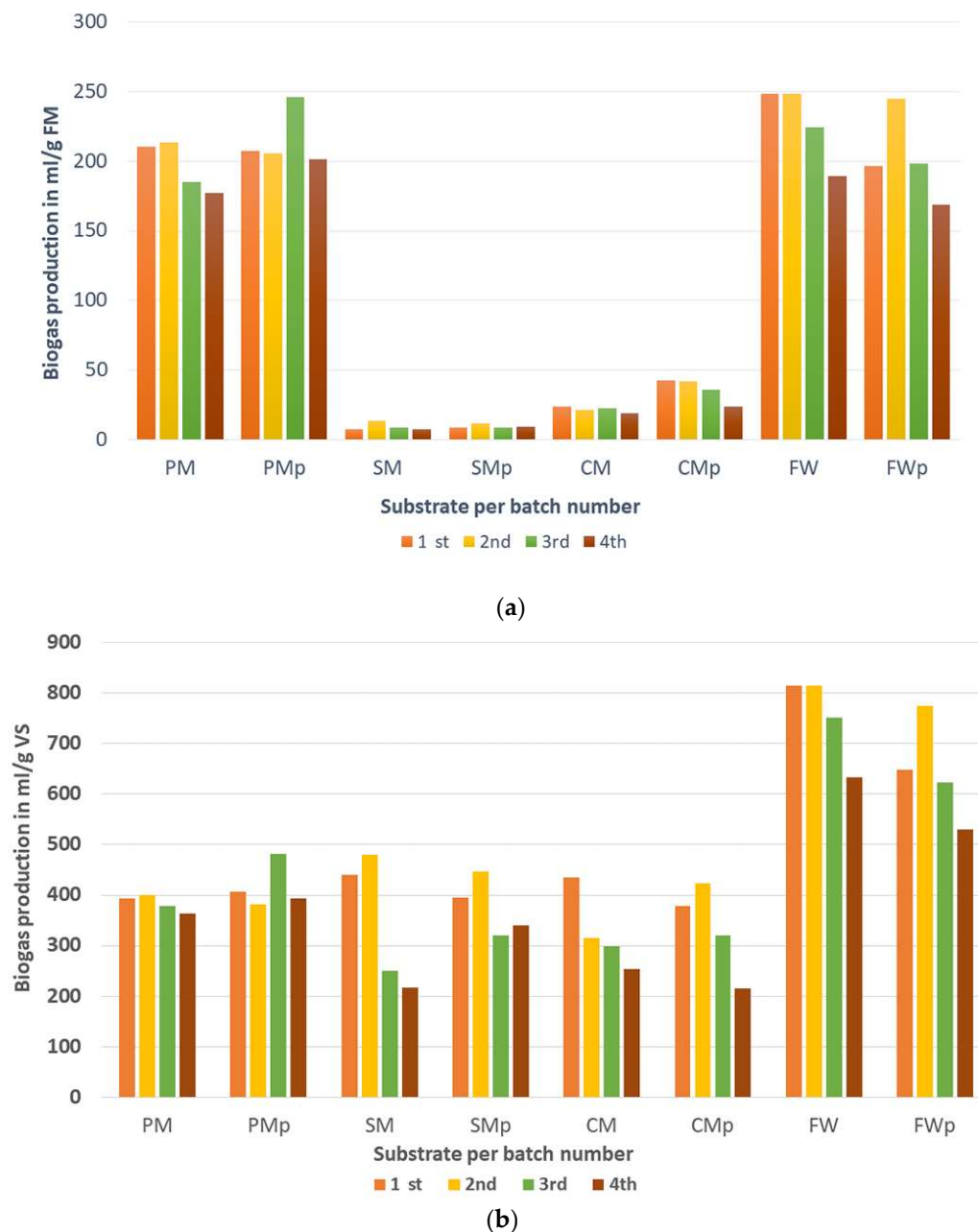


Figure 2. (a) Biogas production of fresh matter (FM) in mL/g FM from each different sample. (b) Biogas production of fresh matter in mL/g VS from each different sample.

Ensuring that ABPs are adequately pasteurized before anaerobic digestion can enhance the overall safety of the biogas production process. This is particularly important for facilities processing a variety of organic wastes, including manure and food waste, which may have different levels of initial microbial contamination.

Table 5 shows the conducted microbiological tests focused on *E. coli*, *Enterococcus faecalis*, and *Salmonella*.

From the microbiological results, it can be concluded that in the pasteurized samples, both before and after anaerobic digestion, the findings are within legislative limits in terms of safety and hygiene. This indicates that the pasteurization process effectively reduces the microbial load to acceptable levels, ensuring the substrates are safe for further processing and utilization.

Table 5. *E. coli*, *Salmonella*, and *Enterococcus faecalis* in four batches before and after anaerobic digestion.

Raw Material	<i>Salmonella</i> in 25 g	1st Batch		1st Batch after AD		
		<i>E. coli</i> cfu/g	<i>Enterococcus faecalis</i> cfu/g	<i>Salmonella</i> in 25 g	<i>E. coli</i> cfu/g	<i>Enterococcus faecalis</i> cfu/g
PM	ND **	<9.1	2.3×10^3	ND **	<9.1	6.2×10^3
SM	ND **	2.5×10^2	8.4×10^3	ND **	<9.1	<9.1
CM	ND **	<9.1	1.1×10^3	ND **	<9.1	<9.1
FW	ND **	<9.1	1.3×10^4	ND **	<9.1	1.0×10^3
PM (p)	ND **	<9.1	2.1×10^2	ND **	<9.1	<9.1
SM (p)	ND **	<9.1	<9.1	ND **	<9.1	<9.1
CM (p)	ND **	<9.1	<9.1	ND **	<9.1	64 est
FW (p)	ND **	<9.1	<9.1	ND **	<9.1	1.1×10^2
Raw material	<i>Salmonella</i> in 25 g	2nd batch		2nd batch after AD		
		<i>E. coli</i> cfu/g	<i>Enterococcus faecalis</i> cfu/g	<i>Salmonella</i> in 25 g	<i>E. coli</i> cfu/g	<i>Enterococcus faecalis</i> cfu/g
PM	ND **	<9.1	2.2×10^3	ND **	<9.1	6.2×10^3
SM	ND **	2.5×10^2	8.6×10^3	ND **	<9.1	<9.1
CM	D *	<9.1	1.0×10^3	ND **	<9.1	<9.1
FW	ND **	<9.1	1.2×10^4	ND **	<9.1	1.0×10^3
PM (p)	ND **	<9.1	2.0×10^2	ND **	<9.1	<9.1
SM (p)	ND **	<9.1	<9.1	ND **	<9.1	<9.1
CM (p)	ND **	<9.1	<9.1	ND **	<9.1	64 est
FW (p)	ND **	<9.1	<9.1	ND **	<9.1	1.1×10^2
Raw material	<i>Salmonella</i> in 25 g	3rd batch		3rd batch after AD		
		<i>E. coli</i> cfu/g	<i>Enterococcus faecalis</i> cfu/g	<i>Salmonella</i> in 25 g	<i>E. coli</i> cfu/g	<i>Enterococcus faecalis</i> cfu/g
PM	ND **	45 est	2.2×10^3	ND **	<9.1	<9.1
SM	ND **	1.1×10^3	7.3×10^3	ND **	<9.1	<9.1
CM	ND **	<40	5.6×10^3	ND **	<9.1	<9.1
FW	ND **	<9.1	7.2×10^3	ND **	<9.1	<9.1
PM (p)	ND **	<9.1	1.2×10^3	ND **	<9.1	<9.1
SM (p)	ND **	<9.1	<9.1	ND **	<9.1	<9.1
CM (p)	ND **	<9.1	<9.1	ND **	<9.1	<9.1
FW (p)	ND **	<9.1	<9.1	ND **	<9.1	<40
Raw material	<i>Salmonella</i> in 25 g	4th batch		4th batch after AD		
		<i>E. coli</i> cfu/g	<i>Enterococcus faecalis</i> cfu/g	<i>Salmonella</i> in 25 g	<i>E. coli</i> cfu/g	<i>Enterococcus faecalis</i> cfu/g
PM	ND **	1.1×10^3	7.3×10^3	ND **	<9.1	<9.1
SM	ND **	<40	5.6×10^3	ND **	<9.1	<9.1
CM	ND **	<9.1	7.2×10^3	ND **	<9.1	<9.1
FW	ND **	<9.1	1.2×10^3	ND **	<9.1	<9.1
PM (p)	ND **	<9.1	<9.1	ND **	<9.1	<9.1
SM (p)	ND **	<9.1	<9.1	ND **	<9.1	<9.1
CM (p)	ND **	<9.1	<9.1	ND **	<9.1	<9.1
FW (p)	ND **	<9.1	<9.1	ND **	<9.1	<40

* D: Detected; ** ND: Not Detected.

In the unpasteurized samples, before anaerobic digestion, an absence of *Salmonella* was noted in all but one bovine sample. After anaerobic digestion, all samples were *Salmonella*-free, demonstrating the efficacy of anaerobic digestion in eliminating *Salmonella*, even in the absence of initial pasteurization. *E. coli* was marginally absent in all samples except for the swine samples before anaerobic digestion, where populations were present. This suggests that while *E. coli* is generally low in these substrates, specific types of manure, like swine manure, can harbor higher levels of this pathogen before treatment.

Concerning *Enterococcus faecalis*, its presence was elevated in all samples before pasteurization. This pathogen is known for its resilience and prevalence in animal waste.

However, after anaerobic digestion, all samples showed an absence of *E. coli* and *Salmonella* and a significant decrease in *Enterococcus faecalis*, especially in pasteurized samples. The greater reduction of *Enterococcus faecalis* in pasteurized samples compared to unpasteurized ones underscores the added benefit of combining pasteurization with anaerobic digestion for enhanced microbial safety.

Additionally, an increased microbial load was observed in food waste compared to other substrates. This highlights the variable nature of food waste and the potential for higher initial contamination levels. Nonetheless, the combined pasteurization and anaerobic digestion processes effectively reduced the microbial load, making the treated food waste safer for handling and use.

These results demonstrate the critical role of pasteurization in ensuring the microbial safety of substrates used in biogas production. By meeting the requirements of EU Regulation (EU) 142/2011 [3], pasteurization ensures that ABPs are sufficiently sanitized, reducing the risk of pathogen transmission. Moreover, the findings highlight the importance of anaerobic digestion as a complementary process that not only aids in biogas production but also further mitigates microbial risks, particularly when initial pasteurization is applied.

4. Discussion

Poultry manure: The less studied substrate in the literature. It is enriched in dry matter and has the highest concentration of it among other animal manures and this has been confirmed in the literature [22,23]. According to Hamilton [24] dry matter of poultry manure ranges from 22 to 65%.

According to Li et al. [25,26] the experimental methane yield of poultry manure is 295 mL CH₄/g VS, while in another study of poultry manure the BMP was 617 mL/gr VS_{added} and the specific methane yield was 291 mL/g VS_{added} [26]. In a study of Oklahoma University the BMP test result was 245 mL CH₄/g VS and Sakar et al. [27] found from 245–372 to 627 mL biogas/g VS. In our study the yield ranged from 362.91 to 400.02 mL biogas/g VS. Contradictorily, according to Wijaya et al. [28] poultry manure yields 114.55 NmL CH₄/g VS, that is a very ambiguous result.

So far, it has not been studied how pasteurization of poultry manure affects the biogas production, but there are a few studies about the effect of thermal pretreatment on poultry manure. According to Orlando et al. [29], when applying a thermal pretreatment on poultry manure at 70 °C the methane yield has an enhancement of about 54.6% and the yield was 518 mL CH₄/g VS, while in our research the yield ranged from 382.55 to 481.09 mL biogas/g VS. It is observed that yield was high enough.

Considering the concentration of dry matter (the highest compared to the other substrates) in poultry manure, one would expect a higher biogas yield. However, in practice, this was not observed. This phenomenon may be attributed to the high protein content in poultry manure, which limits the yield due to its high nitrogen (N) content. It is known that nitrogen compounds (NH₃, NH₄⁺) inhibit the process of methanogenesis [28].

There is some research about pasteurization of poultry manure for biogas production. Assefa et al. [30] proved that cumulative biogas yield of substrates pretreated at 60 and 80 °C was significantly higher than that of control samples and there was no significant difference between 60 and 80 °C pretreated substrates in cumulative biogas yield. The total amount in 20 days was 1190 and 1240 mL biogas, respectively, while the untreated sample accumulated about 830 mL biogas in the same period. It is obvious that temperature has a positive effect on biogas enhancement.

In general, it is concluded that poultry manure can be a fundamental substrate in a mixture for biogas production, but the proportions of the mixture and its combination with the other substrates [31] should be considered, because its high concentration of nitrogenous compounds provokes the formation of NH₃ and NH₄⁺, which inhibit methanogenesis.

Swine manure: Swine manure had the lowest biogas yield compared to the other two animal wastes. This has been confirmed in other studies conducted on laboratory-scale

biochemical methane potential (BMP) assays, as it has the lowest concentration of dry matter as indicated in the literature [32–34] that it about 2.6% TS.

Sommer et al. [34] in BMP tests on untreated swine slurry found 410 L CH₄/kg VS and Sondergraad et al. [35] found 323 ± 27 NmL of CH₄/g VS. Furthermore, according to Li et al. [25] the experimental methane yield is 322 mL CH₄/g VS. In our findings the BMP ranges from 56.21 to 479.95 mL biogas/g VS that coincides with the results from Rodriguez et al. [36] that were 437.33 mL CH₄/g Vs. Moreover, in another study from Oklahoma University [24] the BMP of untreated swine manure was very low, 130 mL CH₄/g VS, and this value was by far the lowest among other substrates of the study.

Olafsdottir et al. [37] concluded that pasteurized samples produced more CH₄ after storage compared to the control mL/g VS, while the samples treated at 90 °C had produced 495 and 505 in 30 and 90 min, respectively. The untreated sample had 435 mL/g VS. As a consequence, the methane potential of liquid phase was enhanced by 70 and 89% after a treatment at 70 and 90 °C, respectively. Moreover, Rafique et al. [4], who studied thermal pretreatment on swine manure, showed that maximum enhancement is observed at 70 °C with an increase of 78% biogas and 60% methane production. The cumulative biogas production at 70 °C was 400 mL biogas/g VS. These results were confirmed in our studies as the biogas production from pasteurized samples ranged from 115.22 to 446.85 mL biogas/g VS.

Neshat et al. [38] formulated that working in thermophilic conditions (>45 °C) can enhance the performance of anaerobic digestion because of higher solubility of organic compounds, higher chemical and biochemical reaction rates, lower solubility of gas in the liquid, lower liquid viscosity, higher pathogen deactivation, and less odor emission. On the other hand, Vergote et al. [39] claimed that the effect of low temperature (70 °C) for 1 h for liquid swine manure made no improvement in the digestion.

Thus, it is inferred that pasteurization has ambiguous results. However, according to our data it boosts both the performance and the production of biogas [3]. Possibly, heating the feedstock for one hour at 70 °C increases the solubility of organic molecules inside the digester, enhancing the hydrolysis of complex molecules into substances of lower molecular weight [40]. Additionally, pasteurization sanitizes the feedstocks, eliminating them from pathogenic agents.

However, as it contains a high water concentration and low dry matter content [41] it cannot be used as the basic substrate in the anaerobic digester. Hence, it is important to use it together with other materials rich in dry matter concentration to limit the negative effect of water. Thus, it can be an excellent solvent in the digester, acting as a buffer, particularly for feedstocks that are in solid or semi-solid form. In fact, if combined with pasteurization, it is possible to enhance the positive effect, leading to increased performance.

Bovine manure: It is a preferable substrate in anaerobic digestion. Its concentration in dry matter ranges from 7 to 9%, while the moisture from 91 to 93%, as indicated in the literature [41–43].

The biogas production from cattle manure ranges in a wide spectrum. In a BMP test in [44] the yield was 240 mL CH₄/g VS. Moreover, in another study by Sondergraad et al. [35] in a BMP test 255 ± 17 NmL of CH₄/g VS was found and Orlando et al. [29] noted performance of 37.5–270 mL biogas/g VS. The performance in our data ranged from 252.97 to 435.56 mL biogas/g VS and the mean was 325.42 mL biogas/g VS, in the scope of literature findings. Meanwhile, Wijaya et al. [28] found specific methane yield of 175.79 NmL CH₄/g VS, that is a low value compared to the above data.

According to Orlando et al. [29], in livestock waste treatments (bovine, swine, and poultry manure), thermal pretreatments improve methane production by 41%. Onsa et al. [45] ascertained that the use of system pasteurization of cow dung increases the methane concentration (15–17%) and increases the daily production rate of methane by 212%. Furthermore, Luste et al. [46] found that treating cattle slurry at 70 °C for 1 h enhanced BMP by 20% and Luste and Luostarinen [47] that the BMP of the untreated slurry was 210 ± 10 NmL CH₄/g VS_{added} and after pasteurization 280 ± 20 NmL CH₄/g VS_{added}.

an increase by 33%. Onsa et al. [45] showed the highest biogas production of 2.9 mL/g dry matter/day with methane concentration in biogas reaching 87.4% was achieved using pasteurization of bovine manure.

Furthermore Liu et al. [5] noticed that pasteurization enhances the biogas production only in some substrates, such as ABPs and manure (swine, bovine), however, it has no positive effect on grease trap sludge, various sewage sludge, slaughterhouse ABPs, etc.

In another study by Xiaojun Liu et al. [8] there was no significant difference in BMP between unpasteurized and pasteurized samples ($0.179 \pm 0.009 \text{ Nm}^3/\text{kg COD}$). Our data from pasteurized samples range from 215.8 to 785.49 mL biogas/g VS and the mean is 518.56. These results are by far higher than ones in the literature and the results from our unpasteurized samples. Obviously, pasteurization enhances hydrolytic processes resulting in methanogenesis, thus increasing biogas production [6,40]. Additionally, pasteurization sanitizes the feedstock, eliminating dangerous micro-organisms.

The result from the four experiments indicates that cattle manure is a reasonably good feedstock for biogas production. However, it should be combined with other feedstocks with higher dry matter content since its high moisture content allows it to be used as a buffer [38]. Moreover, the pasteurized cattle manure has a slightly better yield than the corresponding swine manure in terms of biogas production.

Food waste: Food waste stand out by far regarding the significantly higher yield in biogas production compared to other substrates; a fact documented by the literature too [48]. Its biogas production ranges from 100 to 1100 mL $\text{CH}_4/\text{g VS}$, which depends on the composition of the raw materials contained in these wastes [49,50]. It is worth mentioning that food wastes are rich in proteins, fats, and carbohydrates. No other substrate is as nutrient rich as food waste. Xue et al. [51] proved that a ratio of 40:40:20 for carbohydrates, lipids, and proteins in food waste has the maximum yield in biogas production. In our study we found 632.75–814.15 mL biogas/g VS, which was the substrate with the highest performance, and the mean was 752.69 mL biogas/g VS, that was an impressive value. The most plausible explanation for this phenomenon is that food wastes have an increased dry matter content and therefore much higher energy load, as they contain a surplus of organic matter and a high C/N ratio [52].

Li et al. [53] investigated the effect of thermal pretreatment on the degradation of organic compounds in FW and showed that organics could be efficiently degraded when thermal pretreatment was applied prior to anaerobic digestion. Heat pretreatment had no significant effect on the final content of protein, but it decreased the fat, oil, and grease (FOG) potential by 7–36% and increased the stagnation period of protein (35–65%) and FOG (11–82%) degradation.

According to Ariunbaatar et al. [54] all the thermally pretreated FW substrates had a higher SBP than the untreated FW ($426.0 \pm 8.5 \text{ mL CH}_4/\text{g VS}$). The highest SBP of $539.8 \pm 8.7 \text{ mL CH}_4/\text{g VS}$ was achieved with a pretreatment at 80 °C, followed by 516.1 ± 7.1 at 100 °C, 492.1 ± 16.3 at 120 °C, and 479.3 ± 7.9 at 70 °C for 1 h.

However, Zhang et al. [9] proved that methane production from unpasteurized and pasteurized substrate was very similar throughout the digestion experiment, which resulted in BMP values that were nearly equal at the end of the testing, namely $0.475 \pm 0.031 \text{ STP m}^3/\text{kg VS}$ for unpasteurized and $0.473 \pm 0.026 \text{ STP m}^3/\text{kg VS}$ for pasteurized food waste. This difference in the methane yield from unpasteurized and pasteurized food waste is statistically not significant, therefore, the hypothesis that prepasteurization increases the methane yield from food waste is to be rejected.

Furthermore, Grim et al. [11], applying pasteurization in mixtures of municipal solids waste and food waste, showed that pasteurization has no effect on methane yield, as the biogas production per day for unpasteurized samples was $13.2 \pm 0.5 \text{ L}$ and for pasteurized samples it was $13.0 \pm 0.5 \text{ L}$. Other studies revealed that substrates which do not contain high lignin or lignocellulose, such as food waste and vegetable/fruit waste, did not show a positive impact on methane yield with thermal pretreatment (Atelge et al.) [55].

In our study the performance of the pasteurized samples was also excellent, however, it fell slightly short of the corresponding unpasteurized samples. The results ranged from 530.01 to 774.9 mL biogas/g VS and the mean was 644.02 mL biogas/g VS. The probable reason why pasteurization may have yielded less than expected is thought to be because pasteurization enhances hydrolysis and rapidly increases the concentration of volatile fatty acids (due to increased lipid concentration in the substrate), which, in turn, reduces the surface tension of the solution, resulting in foam formation [9]. A consequence of this is the inhibition of the microbe growth that results in methanogenesis. It is worth mentioning here that food wastes contain very high concentrations of lipids [56], the highest concentration among all substrates.

5. Conclusions

This study assessed the performance of four substrates—poultry manure, swine slurry, cattle manure, and food waste (from both animal and plant origins)—in an anaerobic digester under mesophilic conditions at a laboratory scale, with and without pasteurization. The primary goals were to identify which substrate yielded the highest biogas production and to evaluate the impact of pasteurization on biogas yield and microbial sanitization. Key findings with practical implications for biogas production and sustainability include:

- **Biogas yield enhancement:** Pasteurization generally improved biogas yields from poultry manure, swine slurry, and cattle manure. This suggests that pasteurization breaks down complex organic compounds, making them more accessible to anaerobic bacteria and enhancing the digestion process. This can lead to more efficient biogas production from these substrates, optimizing energy recovery from organic waste.
- **Food waste considerations:** Although food waste showed a slight decrease in biogas yield postpasteurization, this highlights the complexity of its composition. The diverse mix of animal and plant materials in food waste may require tailored pasteurization processes to avoid inhibiting microbial communities crucial for biodegradation. Further research is needed to refine pasteurization techniques for food waste to maximize its biogas potential.
- **Preservation of substrate quality:** Pasteurization did not alter the physicochemical parameters or metal content of the substrates. This ensures that the structural integrity and elemental composition of the substrates are maintained, preserving their quality for effective biogas production.
- **Pathogen reduction:** Pasteurization significantly reduced pathogenic loads in the substrates, enhancing the safety of the digestate for agricultural use. This addresses public health concerns by minimizing the risk of pathogen spread through the application of treated organic waste, promoting safer and more sustainable agricultural practices.
- **Sustainability and waste management:** The study supports the use of pasteurization in anaerobic digestion processes to improve biogas yield and safety. By optimizing the digestion process, it contributes to more efficient waste management and supports renewable energy production, aligning with sustainability goals.

In conclusion, pasteurization enhances biogas production for most substrates and ensures the safety and quality of the digestate. The findings suggest the need for further investigation into food waste pasteurization to fully harness its biogas potential. These insights are valuable for optimizing anaerobic digestion processes, promoting efficient waste management, and supporting renewable energy production.

Author Contributions: Conceptualization, I.M. and A.L.; Investigation, I.M.; Resources, T.S.; Writing—original draft, I.M.; Writing—review & editing, I.G. and T.S.; Visualization, A.L.; Supervision, I.G., T.S., C.V., G.A. and A.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Conflicts of Interest: Authors Dr. Grigoriadou, Mr. Sfetsas were employed by the company QLAB Private Company. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

- Papagrigoriou, A.; Zavali, M. *Circular Economy and Entrepreneurship in the Agri-Food Sector*, 1st ed.; Kallipos, Open Academic Editions: Athens, Greece, 2023.
- Regulation, (EU). No 1774/2002 of The European Parliament and of The Council of 3 October 2002 Laying down Health Rules Concerning Animal by-Products Not Intended for Human Consumption. *Off. J. Eur. Union* **2002**, 1–95.
- Regulation, (EU). No 142/2011 of 25 February 2011 Implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down Health Rules as Regards Animal by-Products and Derived Products Not Intended for Human consumption and Implementing Council Directive 97/78/EC as Regards Certain Samples and Items from Veterinary Checks at the Border under That Directive. *Off. J. Eur. Union* **2011**, 1–254.
- Rafique, R.; Poulsen, T.G.; Nizami, A.S.; Murphy, J.D.; Kiely, G. Effect of Thermal, Chemical and Thermo-Chemical Pre-Treatments to Enhance Methane Production. *Energy* **2010**, *35*, 4556–4561. [\[CrossRef\]](#)
- Liu, X.; Lendormi, T.; Lanoisellé, J.L. A Review of Hygienization Technology of Biowastes for Anaerobic Digestion: Effect on Pathogen Inactivation and Methane Production. *Chem. Eng. Trans.* **2018**, *70*, 529–534.
- Gavala, H.N.; Yenal, U.; Skiadas, I.V.; Westermann, P.; Ahring, B.K. Mesophilic and Thermophilic Anaerobic Digestion of Primary and Secondary Sludge. Effect of Pre-Treatment at Elevated Temperature. *Water Res.* **2003**, *37*, 4561–4572. [\[CrossRef\]](#) [\[PubMed\]](#)
- Edström, M.; Nordberg, A.; Thyselius, L. Anaerobic Treatment of Animal Byproducts from Slaughterhouses at Laboratory and Pilot Scale. *Appl. Biochem. Biotech.* **2003**, *109*, 127–138. [\[CrossRef\]](#)
- Liu, X.; Souli, I.; Chamaa, M.-A.; Lendormi, T.; Sabourin, C.; Lemée, Y.; Boy, V.; Chaira, N.; Ferchichi, A.; Morancais, P.; et al. Effect of Thermal Pretreatment at 70 °C for One Hour (EU Hygienization Conditions) of Various Organic Wastes on Methane Production under Mesophilic Anaerobic Digestion. *AIMS Environ. Sci.* **2018**, *5*, 117–129. [\[CrossRef\]](#)
- Zhang, Y.; Kusch-Brandt, S.; Heaven, S.; Banks, C.J. Effect of Pasteurisation on Methane Yield from Food Waste and Other Substrates in Anaerobic Digestion. *Processes* **2020**, *8*, 1351. [\[CrossRef\]](#)
- Rodríguez-Abalde, A.; Fernández, B.; Silvestre, G.; Flotats, X. Effects of Thermal Pre-Treatments on Solid Slaughterhouse Waste Methane Potential. *Waste Manag.* **2011**, *31*, 1488–1493. [\[CrossRef\]](#)
- Grim, J.; Malmros, P.; Schnürer, A.; Nordberg, Å. Comparison of Pasteurization and Integrated Thermophilic Sanitation at a Full-Scale Biogas Plant—Heat Demand and Biogas Production. *Energy* **2015**, *79*, 419–427. [\[CrossRef\]](#)
- Hejnfelt, A.; Angelidaki, I. Anaerobic Digestion of Slaughterhouse By-Products. *Biomass Bioenergy* **2009**, *33*, 1046–1054. [\[CrossRef\]](#)
- Verein Deutscher Ingenieure VDI 4630 Fermentation of Organic Materials Characterisation of the Substrate, Sampling, Collection of Material Data, Fermentation Tests; VDI-Gesellschaft Energie und Umwelt (GEU): Düsseldorf, Germany, 2016.
- Karvonen, N. Operating Instructions. *J. Wildl. Rehabil.* **2002**, *25*, 27.
- Nathan, A.J.; Scobell, A. APHA AWWA 23rd EDITION. *Foreign Aff.* **2017**, *91*, 1689–1699.
- ISO 17294-2:2016; Water Quality—Application of Inductively Coupled Plasma Mass Spectrometry (ICP-MS)—Part 2: Determination of Selected Elements including Uranium Isotopes. International Standard: Geneva, Switzerland, 2016.
- CEN/TR 16193:2013; Sludge, Treated Biowaste and Soil—Detection and Enumeration of *Escherichia coli*. International Standard: Geneva, Switzerland, 2013.
- ISO 7899-2; Water Quality—Detection and Enumeration of Intestinal Enterococci—Part 2: Membrane Filtration Method. International Standard: Geneva, Switzerland, 2000.
- ISO 6579-1:2017; Microbiology of the Food Chain, Horizontal Method for the Detection, Enumeration and Serotyping of Salmonella—Part 1: Detection of *Salmonella* spp. International Standard: Geneva, Switzerland, 2017.
- ISO 21528-2:2017; Microbiology of the Food Chain—Horizontal Method for the Detection and Enumeration of Enterobacteriaceae—Part 2: Colony-Count Technique. International Standard: Geneva, Switzerland, 2017.
- Weik, M.H. Maintenance Manual. *Comput. Sci. Commun. Dict.* **2000**, *46*, 969.
- Markou, G. Poultry Waste (Manure): A Review of the Main Technologies for Its Management and Utilization. *e-J. Sci. Technol. (E-JST)* **2016**, 1–21.
- Sager, M. Trace and Nutrient Elements in Manure, Dung and Compost Samples in Austria. *Soil. Biol. Biochem.* **2007**, *39*, 1383–1390. [\[CrossRef\]](#)
- Hamilton, D.W. Anaerobic Digestion of Animal Manures: Methane Production Potential of Waste Materials. *Bae* **2012**, *1762*, 4–7.
- Li, Y.; Zhang, R.; Liu, G.; Chen, C.; He, Y.; Liu, X. Comparison of Methane Production Potential, Biodegradability, and Kinetics of Different Organic Substrates. *Bioresour. Technol.* **2013**, *149*, 565–569. [\[CrossRef\]](#) [\[PubMed\]](#)
- Li, Y.; Zhang, R.; Liu, X.; Chen, C.; Xiao, X.; Feng, L.; He, Y.; Liu, G. Evaluating Methane Production from Anaerobic Mono- and Co-Digestion of Kitchen Waste, Corn Stover, and Chicken Manure. *Energy Fuels* **2013**, *27*, 2085–2091. [\[CrossRef\]](#)

27. Sakar, S.; Yetilmezsoy, K.; Kocak, E. Anaerobic Digestion Technology in Poultry and Livestock Waste Treatment—A Literature Review. *Waste Manag. Res.* **2009**, *27*, 3–18. [CrossRef]
28. Wijaya, A.S.; Jariyaboon, R.; Reungsang, A.; Kongjan, P. Biochemical Methane Potential (BMP) of Cattle Manure, Chicken Manure, Rice Straw, and Hornwort in Mesophilic Mono-Digestion. *Int. J. Integr. Eng.* **2020**, *12*, 1–8.
29. Orlando, M.-Q.; Borja, V.-M. Pretreatment of Animal Manure Biomass to Improve Biogas Production: A Review. *Energies* **2020**, *13*, 3573. [CrossRef]
30. Assefa, A.; Egigu, M.C.; Kebede, A. Thermal And Chemical Pre-Treatments Of Cow Dung And Poultry Litter Enhance Biogas Production In Batch Fermentation. *Int. J. Sci. Tech. Res.* **2014**, *3*, 165–170.
31. Ogunwande, G.A.; Adeagbo, J.A.; Fakuyi, A.O. Effects of Co-Digesting Swine Manure with Chicken Manure on Biogas Production. *Ife J. Sci.* **2013**, *15*, 1–8.
32. Moral, R.; Perez-Murcia, M.D.; Perez-Espinosa, A.; Moreno-Caselles, J.; Paredes, C. Estimation of Nutrient Values of Pig Slurries in Southeast Spain Using Easily Determined Properties. *Waste Manag.* **2005**, *25*, 719–725. [CrossRef]
33. YANG, Z.; HAN, L.; LI, Q.; PIAO, X. Estimating Nutrient Contents of Pig Slurries Rapidly by Measurement of Physical and Chemical Properties. *J. Agric. Sci.* **2006**, *144*, 261–267. [CrossRef]
34. Sommer, S.G.; Hjorth, M.; Leahy, J.J.; Zhu, K.; Christel, W.; Sørensen, C.G.; Sutaryo, S. Pig Slurry Characteristics, Nutrient Balance and Biogas Production as Affected by Separation and Acidification. *J. Agric. Sci.* **2015**, *153*, 177–191. [CrossRef]
35. Søndergaard, M.M.; Fotidis, I.A.; Kovalovszki, A.; Angelidaki, I. Anaerobic Co-Digestion of Agricultural Byproducts with Manure for Enhanced Biogas Production. *Energy Fuels* **2015**, *29*, 8088–8094. [CrossRef]
36. Rodriguez-Perez, A.I.; Sucunza, D.; Pedrosa, M.A.; Garrido-Gil, P.; Kulisevsky, J.; Lanciego, J.L.; Labandeira-Garcia, J.L. Angiotensin Type 1 Receptor Antagonists Protect against Alpha-Synuclein-Induced Neuroinflammation and Dopaminergic Neuron Death. *Neurotherapeutics* **2018**, *15*, 1063–1081. [CrossRef]
37. Ólafsdóttir, S.S.; Jensen, C.D.; Lymperatou, A.; Henriksen, U.B.; Gavala, H.N. Effects of Different Treatments of Manure on Mitigating Methane Emissions during Storage and Preserving the Methane Potential for Anaerobic Digestion. *J. Environ. Manag.* **2023**, *325*, 116456. [CrossRef]
38. Neshat, S.A.; Mohammadi, M.; Najafpour, G.D.; Lahijani, P. Anaerobic Co-Digestion of Animal Manures and Lignocellulosic Residues as a Potent Approach for Sustainable Biogas Production. *Renew. Sustain. Energy Rev.* **2017**, *79*, 308–322. [CrossRef]
39. Vergote, T.L.I.; De Dobbelaere, A.E.J.; Willems, B.; Leenknegt, J.; Buysse, J.; Volcke, E.I.P.; Meers, E. Stability of Thermophilic Pig Manure Mono-Digestion: Effect of Thermal Pre-Treatment and Separation. *Front. Energy Res.* **2020**, *8*, 40. [CrossRef]
40. Yan, Y.; Chen, H.; Xu, W.; He, Q.; Zhou, Q. Enhancement of Biochemical Methane Potential from Excess Sludge with Low Organic Content by Mild Thermal Pretreatment. *Biochem. Eng. J.* **2013**, *70*, 127–134. [CrossRef]
41. Triolo, J.M.; Ward, A.J.; Pedersen, L.; Sommer, S.G. Characteristics of Animal Slurry as a Key Biomass for Biogas Production in Denmark. *Biomass Now Sustain. Growth Use* **2013**, 307–326.
42. Chastain, J.P.; Camberato, J.J. Dairy Manure Production and Nutrient Content. *Confin. Anim. Manure Manag. Certif. Program Man. Dairy Version* **2004**, 1–16.
43. Shepherd, M.; Bhogal, A.; Philipps, L.; Jackson, L. The Nutrient Content of Cattle Manures from Organic Holdings in England. *Biol. Agric. Hortic.* **2002**, *20*, 229–242. [CrossRef]
44. Abreu-Junior, C.H.; de Lima Brossi, M.J.; Monteiro, R.T.; Cardoso, P.H.S.; da Silva Mandu, T.; Nogueira, T.A.R.; Ganga, A.; Filzmoser, P.; de Oliveira, F.C.; Firme, L.P.; et al. Effects of Sewage Sludge Application on Unfertilized Tropical Soils Evaluated by Multiple Approaches: A Field Experiment in a Commercial Eucalyptus Plantation. *Sci. Total Environ.* **2019**, *655*, 1457–1467. [CrossRef]
45. Hassan Onsa -Khalid Elbadawi Elshafie, M. Increase Methane Concentration in Biogas Productions by Pasteurization Waste. Available online: https://www.academia.edu/111434513/Increase_methane_concentration_in_biogas_productions_by_pasteurization_waste?uc-sb-sw=23703042 (accessed on 7 July 2024).
46. Luste, S.; Luostarinen, S.; Sillanpää, M. Effect of Pre-Treatments on Hydrolysis and Methane Production Potentials of by-Products from Meat-Processing Industry. *J. Hazard Mater.* **2009**, *164*, 247–255. [CrossRef]
47. Luste, S.; Luostarinen, S. Enhanced Methane Production from Ultrasound Pre-Treated and Hygienized Dairy Cattle Slurry. *Waste Manag.* **2011**, *31*, 2174–2179. [CrossRef] [PubMed]
48. Izhar, T.N.T.; Zakarya, I.A.; Zaaba, S.K.; Yusof, A.H.M.; Shahril, N.M. A Review of Food Waste Characterization and Treatment in Anaerobic Digestion. *IOP Conf. Ser. Earth Environ. Sci.* **2021**, *646*, 012004. [CrossRef]
49. Pramanik, S.K.; Suja, F.B.; Zain, S.M.; Pramanik, B.K. The Anaerobic Digestion Process of Biogas Production from Food Waste: Prospects and Constraints. *Bioresour. Tech. Rep.* **2019**, *8*, 100310. [CrossRef]
50. Ferdeş, M.; Zăbavă, B.S.; Paraschiv, G.; Ionescu, M.; Dincă, M.N.; Moiceanu, G. Food Waste Management for Biogas Production in the Context of Sustainable Development. *Energies* **2022**, *15*, 6268. [CrossRef]
51. Xue, S.; Zhao, N.; Song, J.; Wang, X. Interactive Effects of Chemical Composition of Food Waste during Anaerobic Co-Digestion under Thermophilic Temperature. *Sustainability* **2019**, *11*, 2933. [CrossRef]
52. Pilarska, A.A.; Kulupa, T.; Kubiak, A.; Wolna-Maruwka, A.; Pilarski, K.; Niewiadomska, A. Anaerobic Digestion of Food Waste—A Short Review. *Energies* **2023**, *16*, 5742. [CrossRef]
53. Li, Y.; Jin, Y.; Borrión, A.; Li, H.; Li, J. Effects of Organic Composition on Mesophilic Anaerobic Digestion of Food Waste. *Bioresour. Technol.* **2017**, *244*, 213–224. [CrossRef]

54. 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. [[CrossRef](#)]
55. Atelge, M.R.; Atabani, A.E.; Banu, J.R.; Krisa, D.; Kaya, M.; Eskicioglu, C.; Kumar, G.; Lee, C.; Yildiz, Y.; Unalan, S.; et al. A Critical Review of Pretreatment Technologies to Enhance Anaerobic Digestion and Energy Recovery. *Fuel* **2020**, *270*, 117494. [[CrossRef](#)]
56. Cuetos, M.J.; Gómez, X.; Otero, M.; Morán, A. Anaerobic Digestion and Co-Digestion of Slaughterhouse Waste (SHW): Influence of Heat and Pressure Pre-Treatment in Biogas Yield. *Waste Manag.* **2010**, *30*, 1780–1789. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.