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Biogas from Nitrogen-Rich Biomass as an Alternative to Animal Manure Co-Substrate in Anaerobic Co-Digestion Processes

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Abstract: Nitrogen-rich biomass can be suitable for utilization as a substrate in anaerobic co-digestion (AC-D) instead of animal manure. This biomass combined with other substrates could replace animal waste in certain cases in which animal waste cannot be used to obtain methane gas. Methane is the majority component of the biogas produced in AC-D used as an energy source. In this research, a comparative study has been developed between leguminous plant biomass and pig manure in AC-D in a semicontinuous regime at different Organic Load Rate (OLR) values (1.2–1.8 g VS L_D^{-1} d^{-1}). The most elevated methane yield (494 NL CH_4 kg VS^{-1}) belongs to assays developed with nitrogen-rich biomass at 1.4 g VS L_D^{-1} d^{-1}. Methane-yield results of nitrogen-rich biomass are higher than pig manure results for all OLR studied values. The digestate obtained in the AC-D is a fertilizer of interest due to its nitrogen content and ability to save energy by replacing mineral fertilizers.

Keywords: anaerobic co-digestion; biogas; biomethane; digestate; nitrogen-rich biomass

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1. Introduction

There are large amounts of agricultural waste mainly produced by the economic sectors of agriculture and livestock farming. However, there are no records of how much waste is generated in Europe, with the exception of a few countries [1]. If agricultural waste is referred to animal or vegetal waste from agriculture, farm, forestry and fishery, the total amount generated in Spain exceeded five million tons according to the National Statistics Institute of Spain [2]. This problem and the large extensions of agricultural land at risk of desertification (more than two-thirds of the total Spanish land [3]) contribute to measurement accelerations when using waste in a sustainable manner and enriching agricultural land with organic fertilizers. Biogas production in the European Union reached 18 billion cubic metres methane (bcm) in 2015 [4] and a 4.4% biogas share in natural gas use. The RePowerEU Plan of the European Commission [5] promotes accelerating the rollout of renewables, which includes a biomethane action plan to increase production to 35 bcm by 2030, including the Common Agricultural Policy. In addition, Spain has created a Biogas Road Map to promote a progressive development of biogas in the country. This development has several positive synergies: circular economy, agro-industrial, environmental and energetic policy. Regarding the energetic use of biogas, it assists in greenhouse gas elimination and other pollutant emissions, renewable energy consumption and helps in decreasing national and European energy dependence [6].

The most common animal waste is slurry from cow, pig, sheep or chicken. This type of waste has excellent properties for carrying out a specific process to generate biogas as a source of energy. The process is called anaerobic digestion (AD), which consists of degrading organic matter in waste by using specific microorganisms to produce biogas. Another product that is generated in this process, digestate, can be used as an organic fertilizer. Biogas is mainly composed by methane, carbon dioxide, hydrogen, hydrogen sulfide and ammonia. Methane and hydrogen are components with energetic capacities. In this process, methane is the predominant component in biogas produced with a calorific capacity of 9.96 KWh m^{-3} [7]. Thus, biogas can be employed to produce heat or electrical energy. A combined heat and power system is sometimes chosen in order to use both forms of energy. Digestate is an odorless fertilizer; however, to obtain a fully deodorized fertilizer, small amounts of mineral acids have to be added, according to Samoraj et al. [8]. Moreover, the stabilization of the organic matter from digestate was mentioned by researchers such as Zilio et al. [9], and periodic measurements of greenhouse gas and methane potential determination were carried out. In digestate, NPK nutrients changed to mineral forms that are available for plants [10]. This process helps solve environmental problems related to waste management and the desertification of lands through the conversion of waste into energy and the application of digestate to lands at risk of desertification. Furthermore, digestates can be contributed as energy savings because they replace mineral fertilizers that are not being produced. In general, the combined application of digestate and mineral fertilizer is highly recommended to meet short- and long-term N demands relative to crops [11]. Gissén et al. studied energy crops and when the majority of mineral fertilizers was replaced by digestate, the decrease in primary energy input was significant at an average of 34% [12]. However, the AD process needs nutrients variability to increase biogas production, which is reached by employing several substrates from diverse origins while maintaining the C/N ratio in values close to 20–30 [8,13]. This means that substrates with elevated nitrogen content and substrates with elevated carbon content are necessary. The use of more than one substrate increases the variability of nutrients, and this is the reason why a mixture of substrates is normally employed in the anaerobic process, known as Anaerobic Co-Digestion (AC-D). There are large amounts of studies about AC-D substrates. Zhang et al. [13] investigated links between C/N ratio, synergy and microbial characteristics of AC-D of food waste, cattle manure and corn straw by employing different proportions to obtain methane yield values ranging up to 467–508 L CH_4 kg VS^{-1}. Dareioti and Kornaros [14] achieved a value of 326 L CH_4 kg VS^{-1} when digestion was developed with pretreated silage sorghum, liquid cow sludge and cheese way; pig manure was co-digested with food waste using 1:1 ratio on volatile solids (VS) basis at different total solids (TSs) content, achieving 292 L CH_4 kg VS^{-1} at 10% in TS (Wang et al. [15]). AC-D processes improve the pH buffer's capacity and decrease concentrations of volatile fatty acids (VFA) as confirmed by studies of Zarkadas et al. [16], Prabhu et al., 2020 [17], or Mosquera et al. [18]. In the same study, Zardakas et al. validated a co-digestion process for the valorization of olive-mill waste water without the requirement of manures as co-substrates. The parameter essential for controlling the pH buffer is alkalinity. High alkalinity measured by the equilibrium carbon dioxide-bicarbonate provides an excellent buffer capacity to avoid VFA accumulation and drops in pH values according Somridhivej and Boyd [19]. Nguyen et al. [20] studied a mixture of swine manure and pineapple waste by obtaining values of methane yield ranging at 825–992 mL CH_4 d^{-1} L^{-1}. Others authors [15] carried out the AC-D of pig manure and food waste by analyzing the impact of total solids on this process and the results were 279–292 CH_4 kg VS^{-1}, increasing the total solids content from 5% to 15%. When the quantity of substrates added to the digester (Organic Load Rate (OLR)) is increasing in the AC-D process, all the mentioned parameters must be controlled to avoid the inhibition of the process.

There are some cases in which it is not possible to use manure as a co-substrate in AC-D with other forms of waste. For instance, the large distance at which animal waste is generated contributes to an increase in the emissions of greenhouse gases and the transport costs render biogas plants not economically and environmentally viable. Furthermore, there are food factories where there are restrictions on access to animal waste and it cannot be used for AC-D. In these cases, it is necessary to replace it with other forms of waste, such as vegetable waste. Substrates with relatively high nitrogen contents should be employed instead of achieving an adequate C/N ratio. Biomass from legume plants could be an option as co-substrate in AC-D because of its nitrogen fixation and its high nitrogen content [21,22]. The legume plant Crotalaria (*Crotalaria latifolia* L.) is employed to increase nitrogen contents in soils as a cover crop. Its biomass could replace animal manure by providing the necessary nutrients for microorganisms in the biogas production process.

The purpose of the present study is to assess the performance and stability of digesters in increasing the OLR and establishing a comparative study to use animal manure or biomass from legume plants. Results of this study are based on a complete characterization of wastes employed (pig manure (PM), crotalaria legume (CL) biomass, prickly pear biomass (PP) and rapeseed oil (RO)). PM and RO have been investigated in previous studies and they were used as co-substrates in feed mixtures in a doctoral thesis [23]; this particular research study was carried out in the Agricultural Research Center La Orden Valdesequera from CICYTEX. In this study, PP has been introduced in the feed mixture because it has the property of fast degradability, and CL is used to study the replacement of PM (both have been examined in previous studies in CICYTEX). In addition, the four substrates studied are available in high amounts in the Extremadura region. Four assays were developed with different OLR values. The findings of this study render it possible to know the possibility of using rich nitrogen biomass instead of manure and the optimal conditions required in order to increase the methane's yield value.

2. Materials and Methods

2.1. Evaluated Raw Materials

A semi-continuous regime was applied to assays developed in this research by employing pig manure (PM), crotalaria legume biomass (CL), prickly pear biomass (PP) and rapeseed oil (RO) as co-substrates. In this study, four raw materials are employed in assays, and PP and RO are always included, but assays with PM are differentiated from assays with CL. All raw materials evaluated were collected in the Agricultural Research Center La Orden Valdesequera from CICYTEX located in Guadajira (Badajoz, Spain) (+38°51′9.6768″, −6°40′15.5418″). PM was collected in a single farm located close to the research center from a pond with a tanker. Vegetable raw materials (CL and PP) were used after a mechanical pre-treatment, but this pre-treatment was not the same for both raw materials. CL was crushed after the harvest for silage. CL silage was made from materials crushed to a size of about 3 or 4 cm, and they are introduced in vacuum bags protected from external light. This fermentation process was maintained for 3 to 4 months. After that, CL was dried at 105 °C and finally ground. For assays with CL, water was used to equalize the quantities fed into the digester because the CL substrate was solid. However, the extracted digestate was used for this purpose from the third period onwards. For PP, the PP leaves (or Nopal) were chopped into pieces to be converted by a blender into a homogeneous paste. PM and RO did not receive any pre-treatments. All raw materials were stored at room temperature with the exception of PP, which was frozen. An inoculum was employed to assist the development of specific microorganisms. The inoculum used in assays consisted of a mixture of completely degraded organic material, with a high content of methanogenic microorganisms from the CICYTEX biogas lab. The first inoculum comprised a mixture of prickly pear and pig manure in a 70:30 proportion, respectively, in anaerobic digesters. This mixture was fed for several years at approximately once a month when biogas production decreased.

2.2. Digester Used and Experimental Design

The anaerobic digesters used in the laboratory to carry out the experiments of this study are made of stainless steel and have a total volume of 6 L; 4.5 L was the volume used for these experiments. The digesters have a coated outer jacket through which hot water circulates to maintain a constant temperature and this is controlled by a thermostat. In this study, mesophilic temperature ranges (38 ± 1 °C) were employed. A mixture of substrates was completely homogenized throughout the experiment by means of a central agitator that is electrically operated and is adjustable by a potentiometer. At the beginning, biomethanisation studies from crotalaria plant (CL) were carried out to compare the biomethanisation potential from PM. The ratio inoculum to crotalaria plant was 1:2 on a volatile solid (VS) basis, and this ratio is employed by different authors and in research developments with similar substrates in previous studies carried out in CICYTEX. Then, the working procedure from semi-continuous regime assays comprised a daily feeding of substrate mixtures. The digester's total volume of substrates must remain constant; thus, the same amount introduced daily had to be extracted before feeding a new substrate into the digester. A hopper at the top of the digesters with a ball valve was employed to introduce the substrate mixture, and another valve located on the side of the digester was used to extract digestate (Figure 1). Four different OLRs (1.2 g VS L_D^{-1} d^{-1}, 1.4 g VS L_D^{-1} d^{-1}, 1.6 g VS L_D^{-1} d^{-1} and 1.8 g VS L_D^{-1} d^{-1}) were studied for each assay, employing PM or CL in the mixture. The OLR employed was selected to start the assays with a low amount of substrates, but the aim is to increase it in future studies. Each OLR evaluated was considered a study period. OLRs were calculated as a sum of the daily amount of each substrate expressed in the volatile solid and determined according to 2540 E method (specified in the Section 2.3). The effect of the progressive increase in the OLR in methane yields is the main objective of this study, and this is maintained until the Hydraulic Retention Time (HRT) is completely obtained. In Table 1, the experimental design is shown. In the periods I and II, water is employed to dilute dried CL, but in periods III and IV, a part of the daily extracted digested material was used for this dilution. The digestate (recirculated) was employed in the final two periods. The relative substrate ratios were selected according to the potential availability in the region and to obtain a correct C/N balance in the mixture.

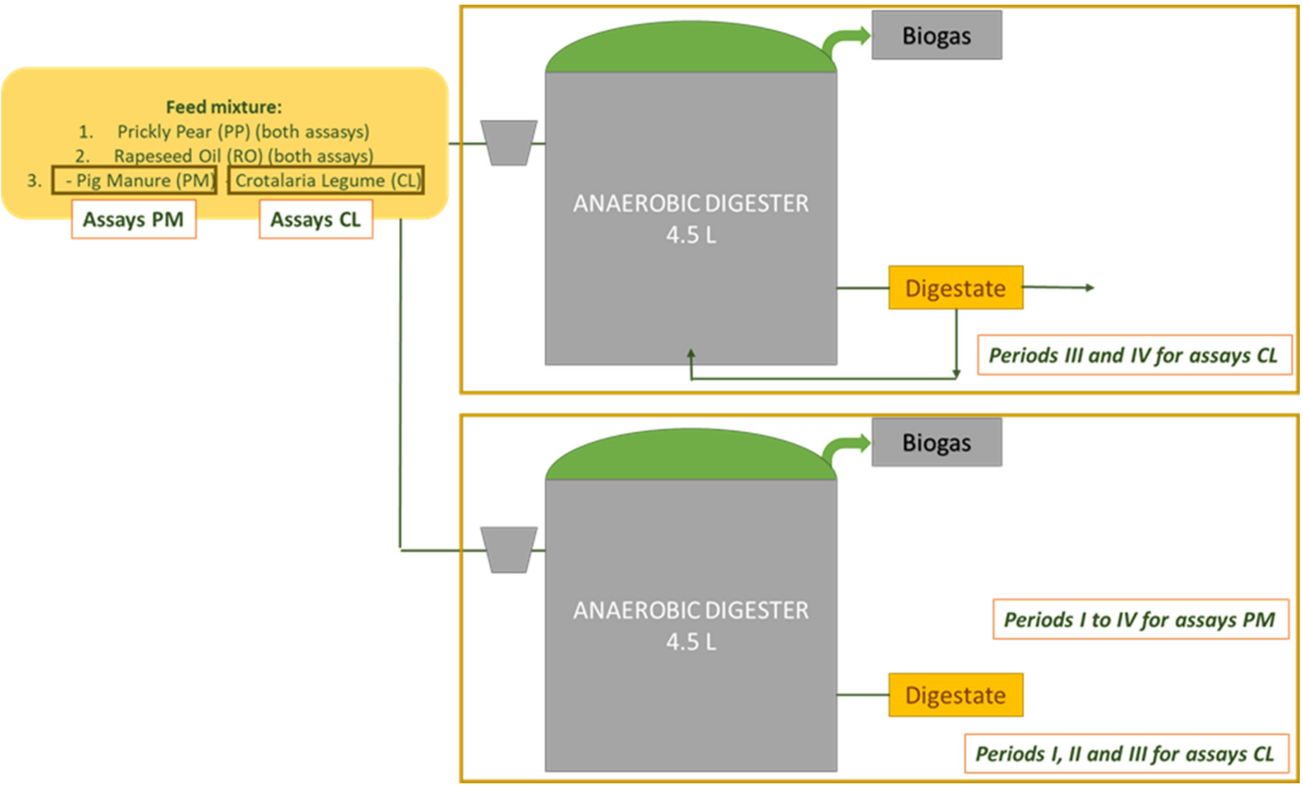

Figure 1. Experimental feeding setup.

Table 1. Experimental design in evaluated assays sets.

ASSAY	OLR, g VS l_D^{-1} d^{-1}	Mixture Composition (35% PP + 15% RO + 50% PM (or CL))				Hydraulic Retention Time (HRT), d
		PP, g	RO, g	PM, g	CL, g	
Period I	1.2 ± 0.1	27.0 ± 0.1	0.8 ± 0.1	64.0 ± 0.1	-	70
		27.0 ± 0.1	0.8 ± 0.1	-	3.2 ± 0.1 (+ 61.0 ± 0.1 water)	70
Period II	1.4 ± 0.1	31.0 ± 0.1	0.9 ± 0.1	133.0 ± 0.1	-	46
Period III	1.6 ± 0.1	31.0 ± 0.1	0.9 ± 0.1	-	3.7± 0.1 (+ 130.0 ± 0.1 water)	46
		36.0 ± 0.1	1.0 ± 0.1	152.0 ± 0.1	-	23
		36.0 ± 0.1	1.0 ± 0.1	-	4.2 ± 0.1 (+ 148.0 ± 0.1 digestate)	110
Period IV	1.8 ± 0.1	40.0 ± 0.1	1.2 ± 0.1	188.0 ± 0.1	-	19
		40.0 ± 0.1	1.2 ± 0.1	-	4.8 ± 0.1 (+ 183.0 ± 0.1 digestate)	92

2.3. Analytical Methods

The characterization of the substrates was carried out according to the APHA standard methods [24]. The total solids content was determined by drying the sample to constant weight in an oven (JP Selecta Digitheat, USA) at 105 °C for 48 h (2540 B method). The content of VS was obtained by combusting at 550 °C for 2 h in a muffle oven (Hobersal 12PR300CCH, Barcelona, Spain) (2540 E method). The pH and redox potential values were measured by using their corresponding electrodes with a pH meter (Crison Basic 20, Barcelona Spain); the alkalinity of the medium was measured according to method 2320; Chemical Oxygen Demand (COD) was measured according to method 410.4 [25]; ammonia nitrogen ($N\text{-}NH_4$) was measured by volumetric titration according to the E4500-NH_3 B method and total volatile fatty acids (VFA) was measured according to Buchauer's volumetric method [26]. The C/N ratio in the substrates was determined using a True-Spec CHN Leco 4084 elementary analyzer (USA) according to the UNE-EN 16,948 standard for biomass analysis for C, N and H [27]. The composition of the biogas and the volume generated were automatically monitored on-site throughout the experiments with an Awite System of Analysis Process series 9 analyzer (Awite Bioenergie GmbH, Langenbach, Germany). The measured system is programmed to analyze the biogas when approximately 4 L was produced in the digester. This analyzer is composed of two IR sensors that measure the methane and carbon dioxide contained in the generated biogas, and three electrochemical sensors that are responsible for measuring the content of hydrogen, hydrogen sulfide and oxygen. A gas meter (Ritter model MGC-1 V3.2 PMMA, Bochum, Germany) was used to measure the biogas produced individually, which was stored in Tedlar bags. The volume of dry gas was corrected at standard conditions (0 °C, 101,325 kPa). The elements analyzed in the digestate obtained were detected by spectroscopy using an ICP-OES Varian 715 ES (Australia). The samples for this analysis were previously digested in a Millestone Start D microwave (Italy).

2.4. Statistical Treatment of Results

A statistical treatment of ammonia nitrogen, alkalinity and VFA performance variables was carried out. These variables were subjected to a factorial ANOVA analysis (Analysis of Variance) to study the significance (p value of 0.05) in each experiment (assays with p or L substrate in the feed mixture at different OLR). The statistical program used was SPSS 15.0.

3. Results and Discussion

3.1. Chemical Characterization of Raw Materials

Raw materials employed were characterized before being introduced inside the digester in order to ascertain its mains chemical characteristics and an adequate proportion of them could be established as feed. Table 2 reflects the characterization of results obtained. As observed in the Table 2, the alkalinity from the PM and inoculum presented higher values than CL, which is necessary to buffer low pH values from PP and RO. AC-D works with alkalinity values higher than 2000 mg $CaCO_3$ L^{-1} according to Flotats et al. [28]. A weakness of CL is its instability during the process. Low values of N from PP and RO have to be supplied by PM or CL. In this case, the CL substrate has higher values of this nutrient if they are compared with the PM substrate (1.97% for N from CL versus 1.77% from PM). Therefore, in this research study, the variability of substrates (PP, RO, CL and PM) obtained a C/N ratio of 41 for mixtures studied with PM (plus PP and RO) and 18 for mixtures employing CL (with PP and RO), which are calculated from the results presented in the Tables 1 and 2, because there are substrates that provide CL to the mixture (CL, PP and RO) and substrates with high contents of N (PM and CL).

Table 2. Chemical parameters of raw materials determined.

Parameter	Inoculum	PM	CL	PP	RO
pH	7.06 ± 0.02	6.99 ± 0.05	5.18 ± 0.03	3.92 ± 0.04	6.45 ± 0.02
Redox potential, mV	−388 ± 3	−392 ± 4	144 ± 1	167 ± 1	129 ± 1
Alkalinity, mg $CaCO_3$ L^{-1}	7190 ± 7	9028 ± 4	426 ± 85	-	-
N-NH_4, mg L^{-1}	1150 ± 99	2360 ± 1	34 ± 1	<30	264 ± 2
C, %	0.46 ± 0.00	1.77 ± 0.08	44.80 ± 0.28	3.21 ± 0.44	77.61 ± 1.18
N, %	0.16 ± 0.03	0.32 ± 0.00	1.97 ± 0.06	0.01 ± 0.00	0.00 ± 0.00
C/N	2.93 ± 0.60	5.54 ± 0.32	22.38 ± 0.49	356.67 ± 48.71	74.28 ± 0.00
TS, %	1.44 ± 0.16	5.99 ± 0.07	87.14 ± 0.08	7.70 ± 0.21	100 ± 0
VS *, %	0.55 ± 0.02	3.99 ± 0.04	80.64 ± 0.12	6.65 ± 0.18	100 ± 0
Ca, ppm	1093 ± 14	1598 ± 13	6167 ± 110	2315 ± 66	211 ± 5
Fe, ppm	62 ± 3	151 ± 2	389 ± 5	57 ± 1	27 ± 1
K, ppm	3206 ± 67	1888 ± 1	13,706 ± 49	2105 ± 21	29 ± 8
Mg, ppm	858 ± 66	939 ± 21	4328 ± 3	698 ± 6	97 ± 10
Na, ppm	927 ± 12	537 ± 10	822 ± 14	221 ± 8	150 ± 5
p, ppm	152 ± 15	1486 ± 48	807 ± 17	41 ± 1	-
Al, ppm	40 ± 1	121 ± 4	3536 ± 1	76 ± 2	15 ± 1
BMP, NL CH_4 kg VS^{-1}	-	-	316	374 **	740 **

* Total volatile solid; ** [23].

3.2. Methane Potential from Crotalaria Legume Biomass (CL)

Discontinuous assays were developed to evaluate the methane potential from CL. In order to analyze the evolution of CL's methane production, two batch assays were carried out by observing that the average methane yield was 316 NL CH_4 kg VS^{-1}, and this is a higher value than methane yields from PM (188 L CH_4 kg VS^{-1} was obtained by Mao et al. [29]; Chamy and Ramos [30] obtained 195 L CH_4 kg VS^{-1} for pig manure; in the review carried out by Yerassyl et al. [31], a range between 180 amd 280 L CH_4 kg VS^{-1} was established). These results evidence that methane yields from CL should be higher than methane yields from PM in semi-continuous assays. RO and PP methane yields were determined in previous studies: values of 740 NL CH_4 kg VS^{-1} by Parralejo [23] and 374 NL CH_4 kg VS^{-1} in different assays developed, respectively.

3.3. Statistical Results from PM and CL Assays

Each experiment carried out with PM or CL substrate was monitored, and samples were taken weekly. All results obtained during this period of time were analyzed by using an analysis of variance, ANOVA. Statistical treatment results are reflected in Table 3. In this table, the different periods are represented (or OLR studied, specified in Table 1) for assays in which PM and CL were introduced into the fed mixture.

Table 3. ANOVA analysis for *p* and L assays.

Assay	Parameter			
	N-NH$_4$, mg L^{-1}	**VFA, mg L^{-1}**	**Alkalinity, mg CaCO$_3$ L^{-1}**	**VFA/Alkalinity Ratio**
Period I (Assay PM)	1270 ± 305 c	1363 ± 257 b	6989 ± 422 cb	0.20
Period I (Assay CL)	995 ± 179 b	1277 ± 93 ab	6203 ± 408 b	0.21
Period II (Assay PM)	1968 ± 270 d	410 ± 60 a	7833 ± 373 c	0.05
Period II (Assay CL)	552 ± 96 a	290 ± 27 a	4399 ± 396 a	0.07
Period III (Assay PM)	1813 ± 136 d	579 ± 82 a	9044 ± 502 d	0.06
Period III (Assay CL)	593 ± 158 a	1167 ± 128 ab	4927 ± 765 a	0.24
Period IV (Assay PM)	2690 ± 318 d	689 ± 75 a	10,150 ± 385 e	0.07
Period IV (Assay CL)	1157 ± 395 bc	1287 ± 198 ab	10,400 ± 738 e	0.12

As observed in Table 3, ammonia nitrogen, VFA and alkalinity have been measured with weekly assays. In addition, the VFA/alkalinity ratio was calculated, and the values obtained are very low (below 0.25) and increased stress was observed when values were higher than 0.5 [32]. The results collected in Table 3 show significant differences between developed assays depending on the analyzed parameter. In the ammonia nitrogen parameter, four groups were established by separating the assays developed with PM and CL substrates. Alkalinity parameter statistic treatments show significant differences in almost all assays due to the variability of values found. Despite this, a notable difference is presented in Table 3 between elevated values of PM substrates assays and low values of CL substrate assays. However, at the end of the assays, i.e., in period IV, the stability of the processes was enhanced because alkalinity values were high and they were located in the same group. Kovalev et al. [33] used recirculating digestates to maintain raised values of alkalinity. The VFA parameters studied have been maintained with low values for all assays developed.

3.4. Analysis of the PM and CL Assays Methane Yields

Four periods of semi-continuous assays were carried out for PM and CL. Each period was evaluated at different OLRs. Methane yield values for all assays are represented in the bar chart (Figure 2). A notable difference is detected between both substrates evaluated, obtaining superior results for CL. For 1.8 kg VS L_D^{-1} d^{-1}, there is no represented CL assay without recirculation because the methane yield for 1.6 kg VS L_D^{-1} d^{-1} was already showing low values; thus, the CL assay without recirculation for 1.8 kg VS L_D^{-1} d^{-1} was not carried out.

Figure 3 shows methane evolution for each period by comparing PM and CL substrates. The highest values obtained are located in period II and period III (for assay CL with recirculation) according to Figure 2. Recirculation proceses for CL assays were carried out due to the solid state of the CL substrate. This problem was solved in the two first periods by adding water; however, with a higher OLR (Periods III and IV), water was replaced with the digestate, which means recirculating a part of the digestate. Recirculation is very important in order to optimize the operating process of a biogas plant, but it is not the main aim of this study. For future studies, recirculation will be included in assays with PM. In this research study, the idea for experimentation is to ascertain the possibility of using legume plants biomass instead of animal waste and the optimal conditions required to increase methane yield values, because some biogas plants in the future will have to operate without animal waste. In line with this, the comparison between PM and CL substrates represented in Figure 3 shows that CL substrates have elevated methane yield results compared to the PM substrate. For PM assays, the theoretical methane yield (calculated with the BMP from each individual substrate and the proportion used in the feed mixture) exceeded in periods I and II (PM assays theoretical methane yield: 339 NL kg VS^{-1}). However, CL assays developed in the semi-continuous regime obtained values of methane yield higher than the theoretical methane yield (400 NL kg VS^{-1}). As previously mentioned, the methane component has a sufficient energetic capacity for applications as a biofuel. In biogas plants, it can heat and self-supply 68% of the total biogas generation and 5% for electrical supplies. In using biogas, the boiler and electric engine efficiency are established approximately at 40% and 80% for electrical and heat energy, respectively [34].

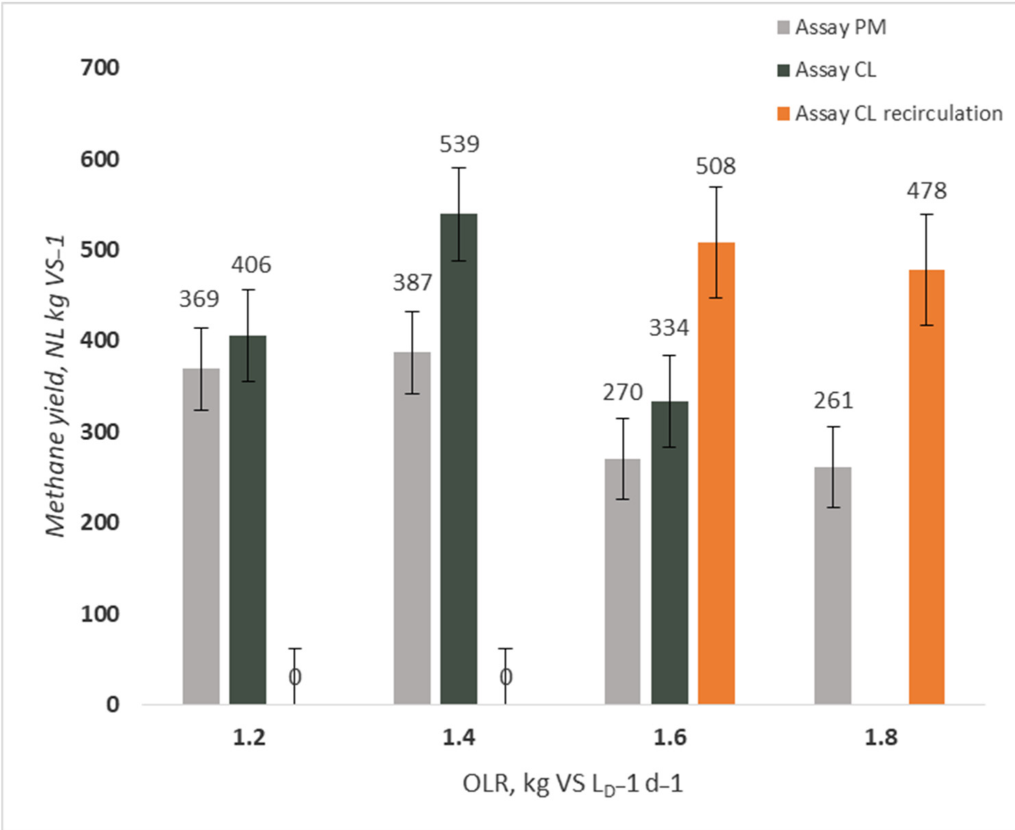

Figure 2. Methane yield values for each studied period at different OLRs.

The chemical characterization reflected in Table 2 shows higher C and VS content results in CL than in the results of PM. This could be the reason for why CL substrate methane yields are greater than the PM substrate. As observed in Figure 3, a process of stability can be confirmed, except for the CL assay in period III. In this case, a sharp drop in methane production occurred 40 days before the assay; thus, water in the feed was replaced by the recirculated digestate. A decrease in the alkalinity parameter was observed in the CL assay during period II, as observed in Figure 4, and an increase in the VFA parameter can be observed in Figure 5 for the same assay.

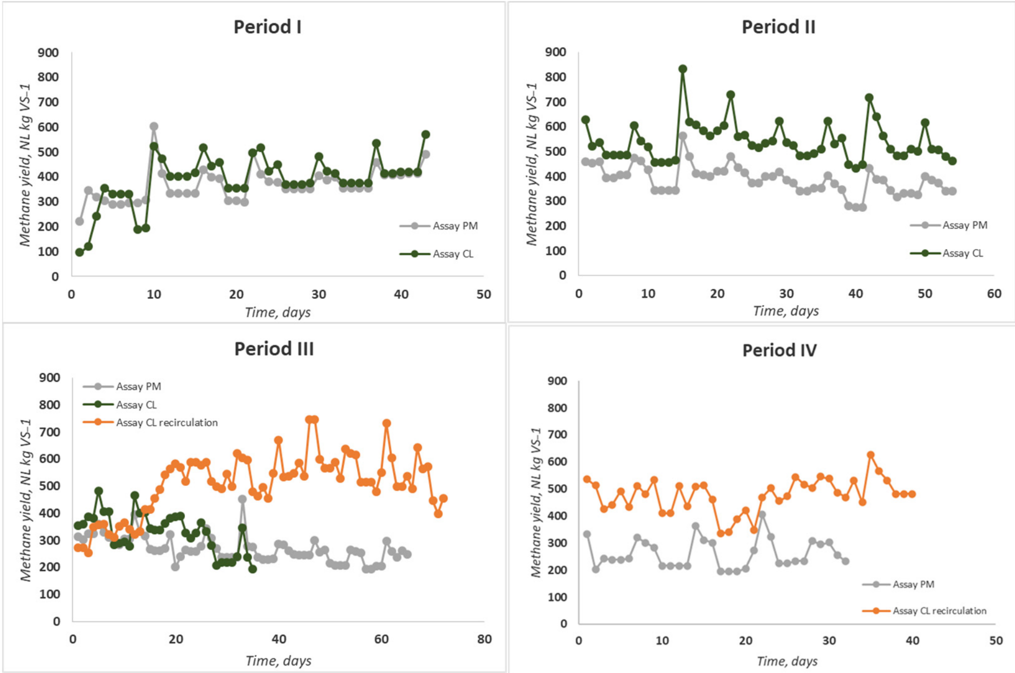

Figure 3. Methane yield evolution for each studied period at different OLRs.

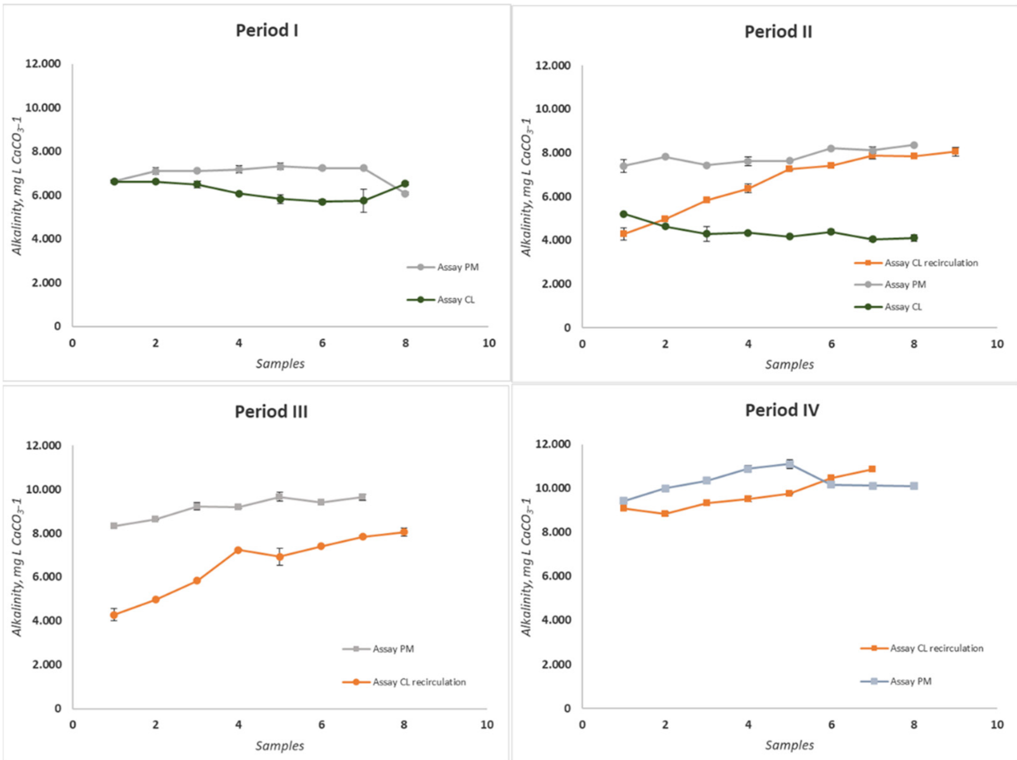

Figure 4. Alkalinity evolution for each studied period at different OLRs.

The decrease in alkalinity and the increase in VFA parameters are indications that the stability of the process is right. Many authors allude to this problem in their research: Khadka et al. [35] evaluated the effects of varying substrates relative to inoculum ratios on the kinetics of biogas production during batch mesophilic AD of simulated food waste, and the rate of biogas production was affected by the accumulation of the VFA; i.e., VFAs increase at a ratio (substrate: inoculum) of 6; Wei el at. [36] carried out their work about

AC-D with corn stover and cattle manure by employing a liquid fraction of digestate to pretreat corn stover and claimed that the buffer capacity of the system can disappear when alkalinity concentrations are below 2000 mg L^{-1}.

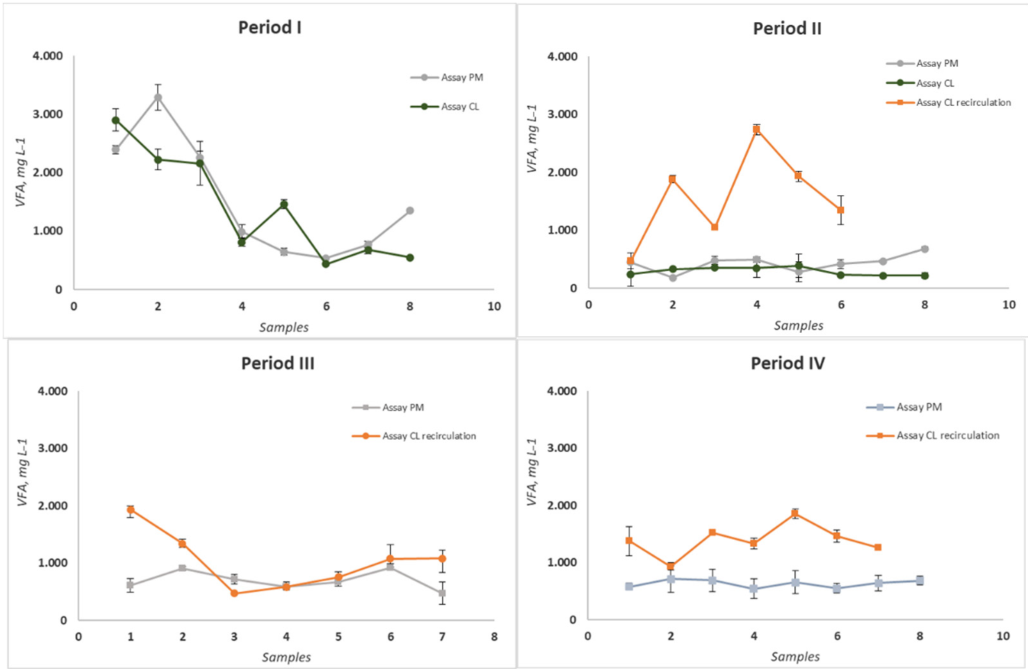

Figure 5. VFA evolution for each studied period at different OLRs.

As the OLR increased, alkalinities increased for both PM and CL substrates (Figure 4). This behavior was not observed in the VFA parameter, as shown in Figure 5. VFA values were maintained below 2000 mg L^{-1}, except at the end of period I; perhaps this is due to the predominant preliminary steps (acidogenic step) relative to the steps responsible for methane production (methanogenic step). Prior to the methanogenic step, there are other steps in which the digestion medium is more acidic and VFAs accumulate [33,37]. Higher values of the alkalinity parameter in PM assays are found than values represented by CL assays. Despite maintaining a fairly good buffering capacity in all periods studied, the PM substrate, due to its intrinsic characteristics, has higher values than the CL substrate. In the VFA parameter, the CL substrate has higher values than the PM substrate during period II to IV when the digestate recirculated. This can be due to the recirculation of the digestate, which can increase VFA accumulation [34].

3.5. Effect of OLR on Different Parameters

Interaction studies of the parameters determined in the AC-D process and different values of OLR are represented in Figure 6. An expected direct interaction is presented in alkalinity when the OLR studied increased. This means that the process's stability increased until 1.8 g VS $L_D{}^{-1}$ d^{-1} for both substrates employed. A stability of the process is one of the main findings of this study using legume plants biomass instead of animal waste. If VFA values obtained at 1.2 g VS $L_D{}^{-1}$ d^{-1} are not considered (the presence of acidogenic step is possibly the reason [33,35,36,38]), an increase in VFA and pH parameters occurred when an increase in OLR was produced. Zahan et al. [37] found the same interaction in the study of different mixtures of chicken litter, food waste, wheat straw and hay grass in anaerobic co-digestion processes in semi-continuous regimes at 2, 2.5 and 3 g TS $L_D{}^{-1}$ d^{-1}. Moreover, the VFA parameter was observed to be higher in periods III and IV of the CL assays than the PM assays. This can be due to the absence of PM, which is responsible for the high buffer capacity of the digestion medium. This indicates an increased presence of volatile fatty acids in experiments without animal manure. As Figure 6 shows, the buffer capacity values (alkalinity) of CL assays always are located below values of PM assays. However, pH values are very similar for PM and CL assays. On the other hand, pH behavior is related to the trend observed in alkalinity; an increase in alkalinity leads to an increase in pH or a stable pH. A direct relationship between ammonia nitrogen and OLR is reflected; nonetheless, a value of OLR of 1.2 g vs. L_D^{-1} d^{-1} presents a high value that is trend-breaking for the CL substrate.

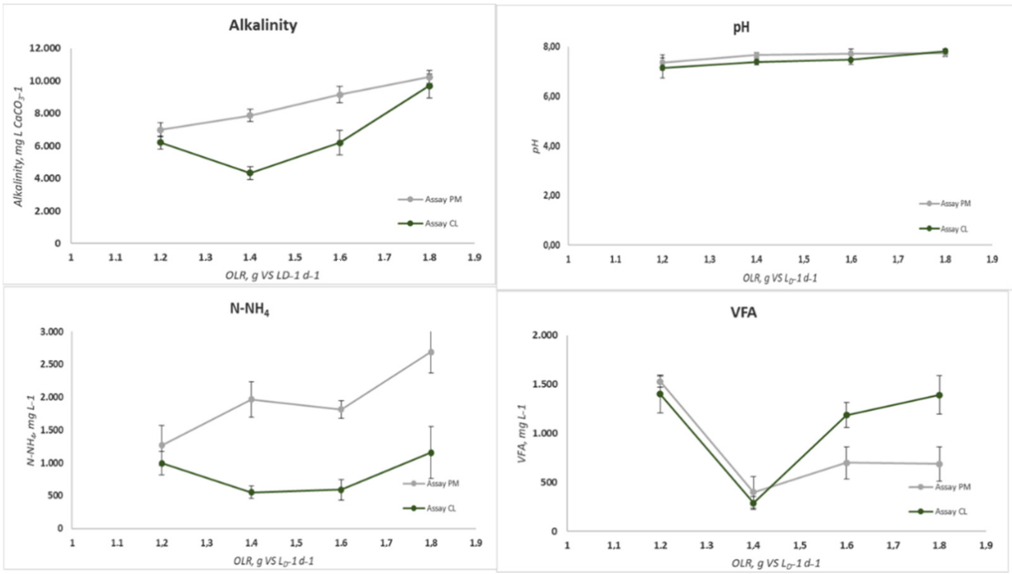

Figure 6. Alkalinity (**upper right**), pH (**upper left**), ammonia nitrogen (**bottom right**) and VFA (**bottom left**) effect on OLRs developed at PM and CL assays.

According to the results obtained, future perspectives of the research study include a more comprehensive study of the assays employed for the recirculation of PM. An operation to optimize the degradation of substrates is needed. Moreover, the digestate obtained should be completely characterized in order to be applied in crops to evaluate their production yields. Increasing the OLR in assays to obtain higher methane yields will be interesting, and with this process, developing a techno-economic analysis of a biogas plant can finally be carried out.

4. Conclusions

Legume plant biomass can be an alternative substrate that can be employed in AC-D processes at OLRs from 1.2 to 1.8 g VS L_D^{-1} d^{-1}; in fact, methane yields obtained from discontinuous assays in this study are higher than methane yields from PM obtained by other authors. For this reason, comparative semi-continuous assays have been developed among CL and PM substrates. As it has been demonstrated, it is possible to use legume plant biomass instead of animal waste because methane yields have presented higher values for CL than PM for every OLR evaluated. All experiments developed have showed that the process is stable. The biomethane and digestate obtained can reduce our dependence on energy from fossil fuels and the use of mineral fertilisers.

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