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Study of the Influence of Pre-Treatment in the Grape Waste Biogas Generation

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Abstract: In this study, the influence of the treatment of grape pomace residues on the methane potential obtained in batch and semi-continuous regime during the anaerobic digestion process is evaluated. The results of methane potentials corresponding to experiments carried out in this work with residues subjected to milling treatments are better than those that have not been treated in this way (295 LN kg VS^{-1} and 285 LN kg VS^{-1} versus 390 LN kg VS^{-1} and 380 LN kg VS^{-1}). As a consequence, semi-continuous experiments have been carried out studying mixtures of the grape pomace residue together with other substrates in the digester using milled and unmilled residue. Additionally, the anaerobic digestion process has been evaluated at the pilot plant scale in order to verify the results obtained in the laboratory and establish the way to proceed in a real installation.

Key words: grape pomace, biogas, methane yield, milling

1. Introduction

In compliance with the 2020 energy and climate objectives, the European Union has created new industries and jobs in Europe with the aim of enhancing technological innovation, thus reducing technology costs. The renewable energy revolution is the best example of this. The share of renewable energy in final energy consumption has increased from 9% in 2005 to 17% in 2018.

The transformation towards sustainable, healthy, nutrition-sensitive, resource-efficient, resilient, circular and inclusive food and farming systems needs to accelerate [1]. A significant amount of emissions to the atmosphere is generated in the livestock sector. One of the most efficient solutions to reduce the mentioned emissions is the treatment of manure in anaerobic digesters. Treatment of manure in anaerobic digesters would reduce non-CO_2 emissions and produce biogas [2].

Various residues from the food or fruit processing industries can be used as biogas raw material in co-digestion with manure.

The area of EU vineyards was 3.362 million hectares in 2016 [3]. In Spain, according to the Survey on Surfaces and Yields of Crops ESYRCE of MAGRAMA, with data for 2015 analyzed by the Spanish Wine Market Observatory, the area of Vineyard in Spain was 954,659 hectares. Regarding the Autonomous Communities, Castilla-La Mancha has the largest area with 473,268 hectares, that is, 49.6% of the total area in Spain. It follows, Extremadura with 80,391 hectares [4]. Generally, the grape waste is used for distillation, and it has been estimated that only 3% of the national production is destined for animal feed due mainly to its low nutritional value because of the

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presence of highly lignified fiber and the content of secondary compounds such as tannins.

The grape pomace or grape waste is the residue of the pressing of the grape, composed of pulp (55%), seeds (20%) and rachis (25%). Seeds, from which oil is obtained, can be separated from the grape pomace without alcohol; calcium tartrate, from which tartaric acid is obtained; and biomass that is used as solid biofuel in boilers. Another option under study is the silage of grape waste in cylindrical or rectangular bales for animal feed application. The yield of the pressing process in wineries is around 30 kg of grape pomace per 100 L of wine, with the potential Spanish production being around 750,000 tons per year.

In the present study, the use of silage grape waste has been studied in anaerobic digestion processes in batch and semi-continuous regimes, mainly evaluating the influence of the milling of the seeds contained in the grape pomace on the methane yield obtained.

2. Material and Methods

2.1 Substrates Studied

The substrate or residue evaluated in this work was grape pomace. It is a very heterogeneous waste (rachis, seeds and skin) produced seasonally, which means that it must be kept for use at any time of the year. The form of storage of the grape waste has been the silage, carried out in plastic bags in which the vacuum has been made to keep the substrate in the absence of oxygen and kept in darkness. Prior to silage, the grape pomace has required a crushing process to reduce the size. Grape pomace has been studied under two different conditions, crushed and ensiled grape pomace (T1 experiments) and crushed, silage and milled grape pomace (T2 experiments). The milling process carried out has had as its main objective the breakage of the seeds contained in the grape waste.

The inoculum used in the tests consisted of mixtures of completely degraded organic material, with a high content of methanogenic microorganisms. All the inoculums used for each of the experiments performed have been characterized (Table 1). The study of the experiments in semi-continuous regime has needed to use more substrates together to the grape pomace, among them pig manure, sorghum and triticale. The pig slurry has been collected from a pig farm located in Guadajira (Badajoz, Spain) (+38°51'9.6768", -6°40'15.5418"). Sorghum and triticale have been used milled, which means that it has required previous pretreatments. These pretreatments have consisted of crushing prior to the silage process, after the harvest of both crops. Before the anaerobic digestion the material has been dried at 105°C, and finally ground. All substrates used in this study have been analyzed to obtain characteristic physical-chemical parameters of the anaerobic digestion process whose analysis methods are detailed in section 2.3.

2.2 Digester Used and Experimental Design

The anaerobic digesters that have been used in the laboratory to carry out the experiments corresponding to this work are made of stainless steel and have a total volume of 6 L, 4.5 L was the volume used for this tests. The digesters are coated of an outer jacket through which hot water circulates to maintain a constant temperature of the substrate controlled by a thermostat. In this study we have worked in the mesophilic temperature range (38°C). The substrate to be digested has been completely homogenized by means of a central agitator electrically operated and adjustable by a potentiometer.

The pilot plant digester used to carry out the semi-continuous experience has a useful volume of 1500 L, is made of stainless steel, and a shaft with propellers homogenized the sample, and is and is heated through an internal coil through which hot water circulates. The pilot plant has a feeding system that pumps the liquid substrate from a stainless steel tank, and an auger screw through which the solid sample is introduced. The pilot plant has a gas line that consists of a water trap, a biogas meter, a gasometer and a compressor that conducts the gas to the energy use equipment. In this work, two different operating regimes, batch and semi-continuous, have been studied in duplicate (A and B). In each operating regime, two types of experiments (T1 and T2) have been performed. Subsequently, an experience in a semi-continuous pilot plant has been developed. Table 2 shows a design of the experiments that have been carried out and evaluated in this work.

Table 1 Physical-chemical characterization of initial substrates.

Experiment regime	Batch			Semi-continuous			
Parameter/Substrate	**Inoculum**	**Grape pomace 1 (T1)**	**Grape pomace 2 (T2)**	**Inoculum T1A**	**Inoculum T1B**	**Inoculum T2A**	**Inoculum T2B**
pH	8.14	4.69	4.19	9.03	8.67	7.94	7.73
Potential redox, mV	-438	111	35	-128	-253	-166	-318
Alkalinity, mg $CaCO_3$ L^{-1}	9258	-	-	11154	11042	10567	10985
COD^a, mg O_2 L^{-1}	16000	410000	410000	250000	61000	19000	15000
VFA^d, mg L^{-1}	479	-	-	1958	628	516	691
Ammoniam nitrogen, mg L^{-1}	2400	156	<30	1840	2320	2240	2360
Ratio C/N	9.32	26.86	-	32.12	12.17	5.09	4.36
TS^b, %	1.75	36.36	39.63	1.53	1.86	1.53	1.69
VS^c, %	1.11	32.72	36.38	0.75	1.03	0.79	0.89

Experiment regime	Semi-continuous				Pilot plant semi-continuous		
Parámetro/Sustrato	**Pig manure T1**	**Pig manure T2**	**Sorghum T1 and T2**	**Triticale T1 and T2**	**Inoculum PP**	**Grape pomace PP**	**Pig manure PP**
pH	7.95	7.79	3.64	4.71	9.01	3.98	7.59
Potential redox, mV	-370	-409	188	155	-422	209	-400
Alkalinity, mg $CaCO_3$ L^{-1}	10374	8631	-	-	10313	-	12287
COD^a, mg O_2 L^{-1}	18175	13000	800000	1170000	14000	488000	54500
VFA^d, mg L^{-1}	436	721	-	-	1169	-	2859
Ammoniam nitrogen, mg L^{-1}	2885	440	412	230	2520	209	3040
Ratio C/N	9.06	16.01	53.03	79.47	5.08	33.8	5.62
TS^b, %	1.86	1.49	100.00	100.00	1.45	38.15	5.05
VS^c, %	0.99	0.79	94.65	93.93	0.64	34.54	3.44

Table 2 Experimental design.

Substrate		Work regime			
		Batch			
Inoculum, g		3500			
Grape Pomace, g		265			
		Semi-continuous OLR: 1.69 g VS L_D^{-1} d^{-1} (T1) and 1.34 g VS L_D^{-1} d^{-1} (T2)			
		T1A	T1B	T2A	T2B
FEEDING	*Pig manure, g*	338			
	Sorghum, g	0.90			
	Triticale, g	0.90			
	Grape Pomace T1,g	1.80		-	
	Grape Pomace T2,g	-		4.64	
		SEMI-CONTINUOUS (PILOT PLANT) OLR: 1.75 kg VS $m^3{}_D{}^{-1}$ d^{-1}			
FEEDING	*Purín, kg*	50			
	Grape Pomace T2, kg	2.20			

2.3 Analytical Methods

The characterization of the substrates was carried out according to the standard methods [5]. 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 Volatile Solids (VS) was obtained by drying at 550°C for 2 h in a muffle oven (Hobersal 12PR300CCH, Spain) in an inert atmosphere (2540 E method). The pH and redox potential values were measured through their corresponding electrodes with a pH meter (Crison Basic 20, Spain), the alkalinity of the medium according to method 2320, Chemical Oxygen Demand (COD) according to method 410.4 [6], ammonia nitrogen (N-NH4) by volumetric titration according to the E4500-NH3 B method and Total Volatile Fatty Acids (VFA) according to Buchauer's volumetric method [7]. The initial C/N ratio in the substrates was determined using a True-Spec CHN Leco 4084 elementary analyzer (USA), according to the UNE-EN 16948 standard for biomass analysis C, N, H [8]. 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 (Bioenergie GmbH, Germany). 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. The gas meter (Ritter model MGC-1 V3.2 PMMA, Germany) was used to individually measure the biogas produced, 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 and Kinetic Adjustment of Results

A statistical treatment of results of the methane, ammonia nitrogen, alkalinity and VFA performance variables has been carried out. These variables have been subjected to an ANOVA analysis (Analysis of Variance) of a factor to study the significance (p value of 0.05) in each experiment (different treatment of grape waste). The statistical program used was Minitab 18.

In addition, the results obtained from methane volume accumulated over time in experiments carried out in batch regime have been adjusted to a first order kinetic model. This model assumes that the limiting stage in the speed of the anaerobic digestion process is hydrolysis. The adjustment of the results obtained is carried out according to the linearized expression (1).

$$G = G_m \left[1 - e^{k_o t}\right] \quad (1)$$

where G (LCH_4 $kgVS^{-1}$) is the methane yield accumulated in a time t (d), Gm is the maximum methane yield and k_o (d^{-1}) is the methane production rate constant.

3. Results and Discussion

3.1 Experiments in Batch Regime

Two experiments were performed in duplicate in batch mode. It was studied the effect of the grape waste mechanical milling treatment on the methane potential obtained, and on other analyzed variables considered of interest. In Table 3 it can be seen how the treatment 2 with the grape waste milled had higher methane yields than the treatment 1. Furthermore, the values shown in the reduction of VS have been slightly higher for the T2 tests performed. However, in the values obtained in the COD reduction no differences were found in both types of treatment studied.

Table 3 Results obtained in batch assays realized with grape pomace T1 and T2.

Parameter	T1A	T1B	T2A	T2B
COD reduction, %	53.54	37.14	35.78	38.84
VS reduction, %	49.11	52.69	62.57	57.74
Methane yield, LN kg VS^{-1}	295	285	390	380
Average methane concentration, %	56	49	63	61
k, d^{-1}	0.0875	0.0955	0.0874	0.0967
r^2	0.9605	0.9681	0.9207	0.8915

r^2: Linear regression coefficient

Regarding the methane yields shown in Table 3, there were increases around 25% in the T2 experiments with respect to the T1 experiments. This increase in methane yields obtained can be related to the milling treatment carried out on the seeds contained in the grape waste. The oil inside the seeds is that, when released, can increase the levels of methane concentration in the biogas generated. This behavior can be seen illustrated in Fig. 1 in which the evolution of methane yield accumulated over time for the batch experiments performed is showed.

Some authors [9] have studied anaerobic digestion of pulp and ground grape seeds separately and have obtained methane yield values of 343 LN kg VS^{-1} and 214 LN kg VS^{-1}, respectively. The results obtained in this research work are higher probably due to the synergy of the different fractions that make up the grape waste.

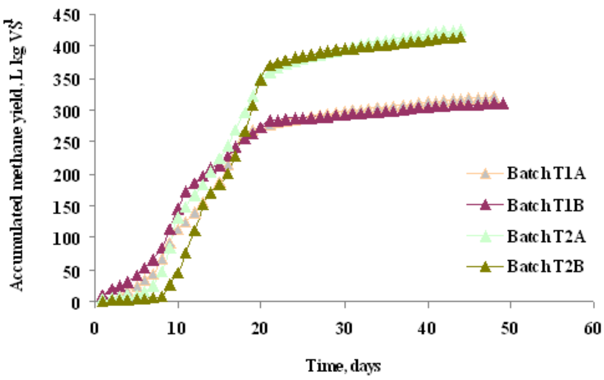

Fig. 1 Accumulated methane yield in batch duplicated assays realized with grape pomace T1 and T2.

From the results of the kinetic adjustments developed on the accumulated methane yields over time to a first order equation, the values of the kinetic constants shown in Table 3 were obtained. In T2B test the methane yield values have not been adjusted to the equation evaluated. From the values of the kinetic constants corresponding to the T1A, T1B and T2A experiments (0.0875 d^{-1}, 0.0955 d^{-1} and 0.0874 d^{-1}) an equal methane generation rate is observed in tests T1 and T2. This tendency can be observed in Fig. 1. Values of kinetic constants obtained by other authors are comparable to the values obtained in this work. Thanikal et al. [10] obtained values of kinetic constants of 0.10 d^{-1} for anaerobic digestion of the grape. According to a study by Zahan et al. [11] the kinetic constant determined for the anaerobic digestion process in wheat straw vegetable substrates was 0.06 d^{-1}. While another type of substrate used in anaerobic digestion such as microalgae have been studied by researchers Zhen et al. [12] and have presented values of kinetic constants of 0.12 d^{-1}.

3.2 Experiments in Semi-Continuous Regime

Once methane generation potentials of the grape waste were determined in treatments 1 and 2, semi-continuous experiments were developed (Table 2) in duplicate. The behaviors of the variables in the anaerobic digestion process were evaluated over time for the grape waste substrate undergoing treatment 1 and 2. First, the evolution of the daily methane yield was shown corresponding to each type of experiment (Fig. 2). In this case, the same influence of treatment 2 has been observed in methane yield as in the experiments carried out in batch regime. The mean values of methane yields obtained for the T1 experiments were 81 LN kg VS$_{added}^{-1}$ and 91 LN kg VS$_{added}^{-1}$, respectively for the A and B replicas, while the average methane yield values corresponding to the T2 experiments were 173 LN kg VS$_{added}^{-1}$ for replicas A and B. In the case of substrates of plant origin, results collected by Croce et al. [13] obtained from wheat straw a methane yield of 178 L kg VS^{-1} for a VCO of 1 g VS L_D^{-1} d^{-1} was obtained, value exceeded in the case of T2 experiments.

In the case of the experiments carried out in semi-continuous regime, the increase in methane yields achieved with treatment 2 of the grape waste with respect to treatment 1 is 50%. It is possible that the oil from the seeds of the grape waste extracted with the milling in treatment 2 requires more time for degradation (not evaluated in the trials in batch regime for treatment 2), but once the oil is present in the digestion medium begins to degrade and the methane yields obtained are higher.

The rest of studied parameters in the experiments that have been carried out in semi-continuous regime, are showed in Figs. 3 and 4. The trend shown in Fig. 3 for the VFA parameter reflects higher values for experiments T1A and T1B than for experiments T2A and T2B. However, in both cases the VFA values represented are below the threshold values established as inhibitory for the anaerobic digestion process (4000 ppm [14]).

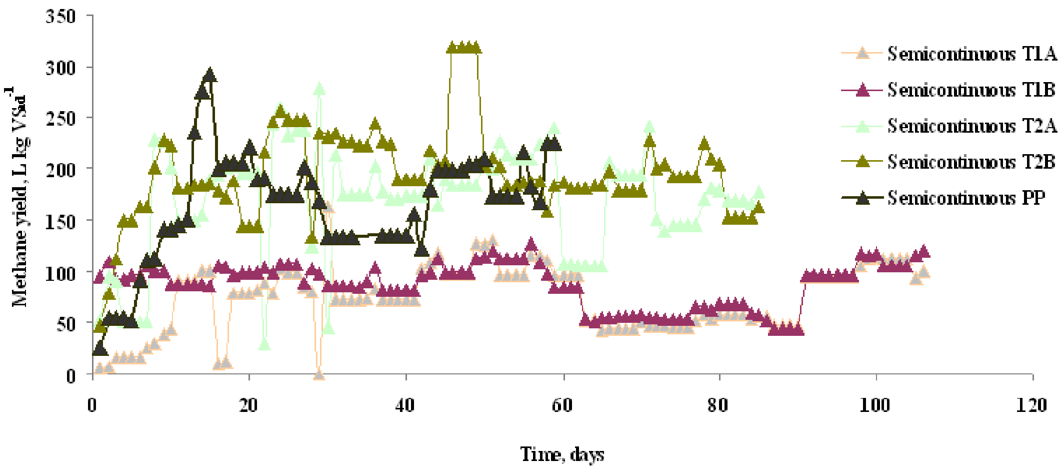

Fig. 2 Methane yield evolution belong to semicontinuous regime duplicated assays with grape pomace T1 and T2, and a pilot plant experience.

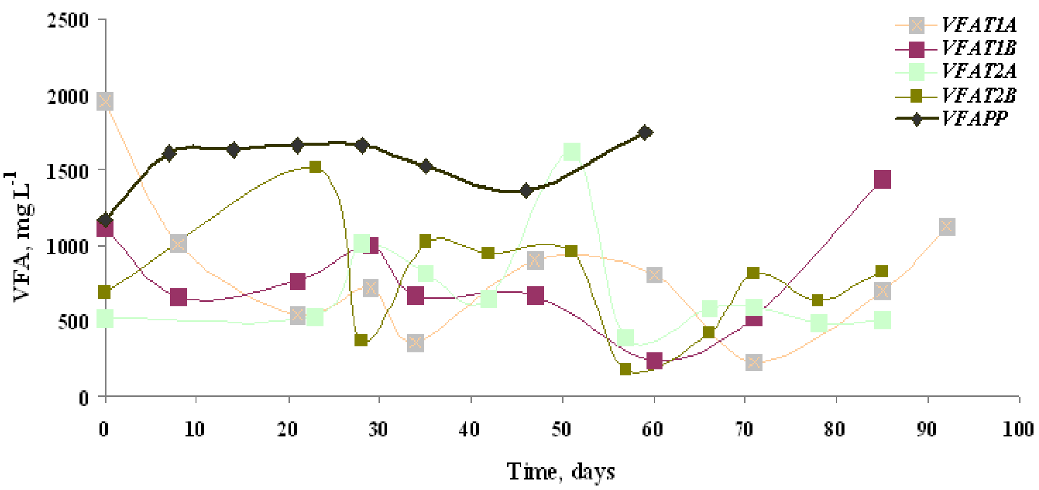

Fig. 3 VFA concentration evolution belong to semicontinuous regime duplicated assays with grape pomace T1 and T2, and a pilot plant experience.

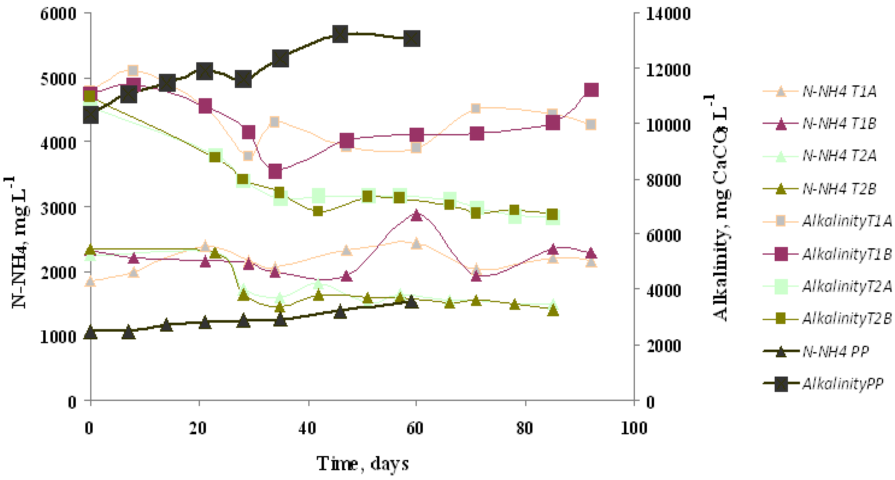

Fig. 4 Ammonia nitrogen and alkalinity concentration evolution belong to semicontinuous regime duplicated assays with grape pomace T1 and T2, and a pilot plant experience.

With respect to the trend observed in the evolution of the results obtained in the ammonia nitrogen and alkalinity parameters belonging to the experiments carried out in semi-continuous regime in the laboratory (Fig. 4), it can be said that all are located in suitable values for the anaerobic digestion process (5000 mg L^{-1} for ammonia nitrogen [14] and greater than 1500 mg L^{-1} for alkalinity [15]). The results obtained for both parameters shown in Fig. 4 are slightly lower in the semi-continuous experiments corresponding to treatment 2 than in the semi-continuous experiments belonging to treatment 1. Specifically, the behavior represented for the parameters ammonia nitrogen and alkalinity in experiments T1 and T2 reflects very similar values at the beginning of the experiments, appearing differences in these parameters between experiments T1 and T2 after approximately 30 days. These differences are probably caused by the physicochemical characteristics of the pig manure used in the feed in each experiment because the pig manure used in T1 experiments (higher alkalinity and ammonia nitrogen values, Table 1) was different to the one used in T2 experiments.

All parameters (methane yield, VFA, ammonia nitrogen and alkalinity) have been subjected to a single-factor ANOVA statistical analysis for all the experiments carried out in semi-continuous regime. The results extracted from this analysis are showed in Table 4. It can be seen in Table 4 and in Fig. 5 how significant differences are established between treatments 1 and 2 in all the parameters evaluated, except in the VFA parameter. In this last parameter, only an increase in the average value is observed for the pilot plant experiment. In relation to the average values showed in Table 4 of the parameters ammonia nitrogen and alkalinity, three clearly differentiated groups are established, the group corresponding to the experiments pertaining to the treatment 1 in the laboratory, the group corresponding to the experiments developed in the laboratory with the treatment 2, and finally, the pilot plant experiment.

Table 4 ANOVA analysis of methane yield, VFA, ammonia nitrogen and alkalinity concentration results obtained in semicontinuous regime assays.

Assay	Parameter			
	Methane yield, LN kg VS_{ad}^{-1}	VFA, mg L^{-1}	$N\text{-}NH_4$, mg L^{-1}	Alkalinity, mg $CaCO_3$ L^{-1}
T1A	81±42 b	828±203 b	2240±155 b	9971±1061 b
T1B	91±16 b	836±324 b	2274±301 b	10285±804 b
T2A	173±39 a	648±205 b	1623±108 c	7256±405 c
T2B	173±33 a	652±328 b	1543±95 c	7152± 464 c
PP	173±55 a	1601±127 a	2954±353 a	12094±824 a

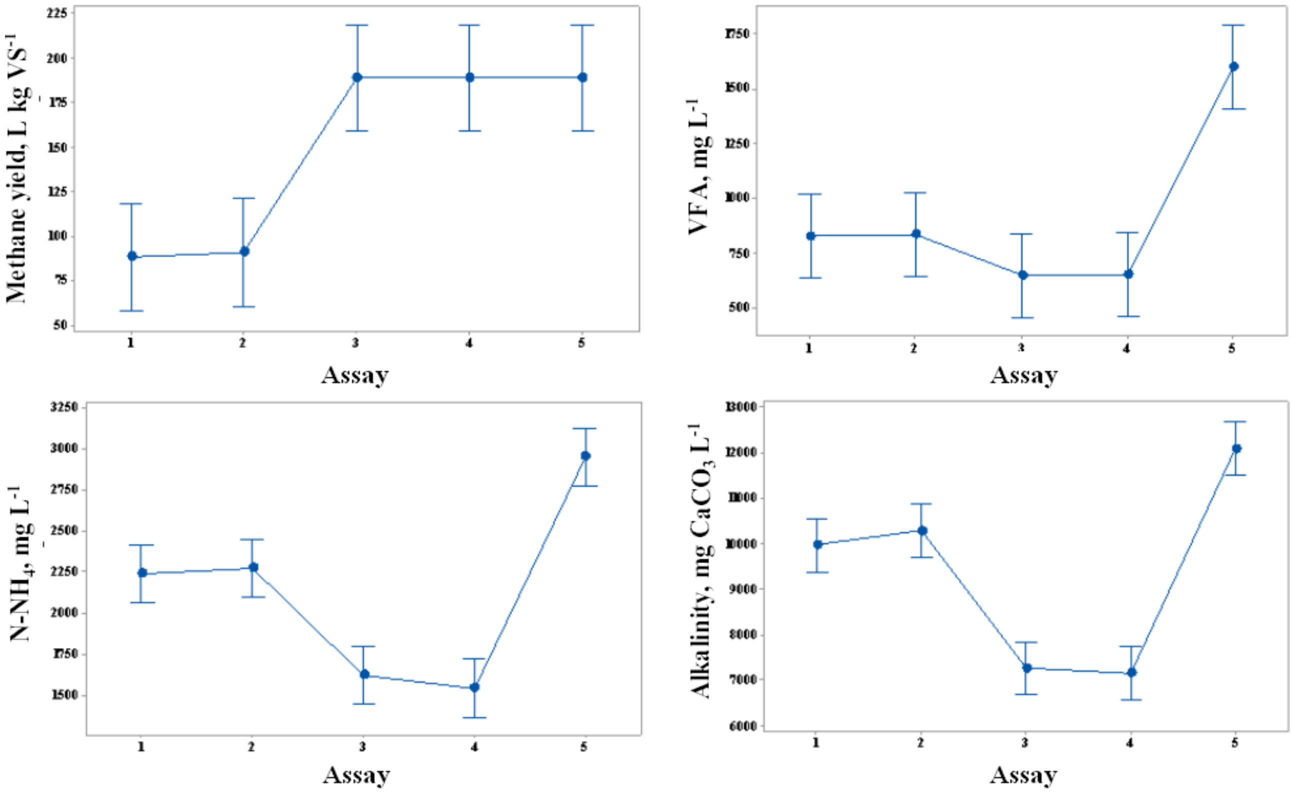

Fig. 5 Average obtained values results in VFA, ammonia nitrogen and alkalinity concentration, and methane yield for semicontinuous regime assays (1:T1A, 2:T1B, 3:T2A, 4:T2B and 5:PP).

3.3 Pilot Plant Experiment in Semi-Continuous Regime

The experiment that was developed in a semi-continuous pilot plant obtained results of methane yields similar to those obtained in the T2 experiments carried out in laboratory (Fig. 2 and Table 4). In this experiment, the mixture used as substrate was composed only of pig manure and grape waste undergoing treatment 2. The study of the variables VFA, ammonia nitrogen and alkalinity indicates a constant or slightly upward evolution (Figs. 3 and 4), causing higher average values in the ANOVA analysis that classify the experiment into an independent group. This can be explained as a consequence of the absence of co-substrates in the mixture that compensate for the high values of said parameters in the pig manure substrate. Despite this, the average values reached in the parameters studied are far from the inhibitory threshold values, therefore they do not affect the results obtained in methane yield. The digestate obtained from the pilot plant experiment was been evaluated and the results are presented in Table 5.

Table 5 Elemental composition analysis of digestate generated in pilot plant experience.

Element	Digestate PP
N, %	0.31
P, %	0.17
K, %	0.42
Ca, %	0.31
Fe, %	0.02
Mg, %	0.09
Na, %	0.42
Al, %	0.01
Cd, ppm	16
Cr, ppm	97
Cu, ppm	22
Ni, ppm	93
Mn, ppm	20
Zn, ppm	71

As for the dose of NPK that can be contributed to the soil as a biofertilizer with the digestate obtained after the experience of anaerobic digestion, it corresponds to 0.31% of N, 0.39% of P_2O_5 and 0.50% of K_2O. These values are very similar to those published by other authors, such as Reyes [16] who presents in his work values of 0.50% for K_2O and N, and 0.30% for P_2O_5, in pig slurry samples.

4. Conclusions

The influence of the milling treatment of the grape waste on the anaerobic digestion process was positive with respect to the methane yield obtained in both batch regime (295 LN kg VS^{-1} and 285 LN kg VS^{-1} for T1A and T1B versus at 390 LN kg VS^{-1} and 380 LN kg VS^{-1} for T2A and T2B) as in semi-continuous regime (81 LN kg VS_{added}^{-1} and 91 LN kg VS_{added}^{-1} for T1A and T1B versus 173 LN kg VS_{added}^{-1} for T2A and T2B). The variables studied alkalinity and ammonia nitrogen were located within the appropriate values for the optimal development of anaerobic digestion and have allowed according to an ANOVA analysis to establish three differentiated groups between the T1, T2 experiments and the pilot plant experiment. The digestate obtained in the experiment developed in the pilot plant can provide a dose to the ground of 0.31% of N, 0.39% of P_2O_5 and 0.50% of K_2O.

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