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Techno-economic evaluation of anaerobic co-digestion of pepper waste and swine manure

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Abstract
Agro-industrial waste represents a major challenge for the environment. Among these, the processing of peppers generates a large amount of waste, so the search for new ways to valorise this waste is crucial. This work aims to assess the use of pepper waste for methane production with swine manure. During this study, anaerobic co-digestion was evaluated in both batch and semi-continuous operation. Specifically, batch assays were performed following a factorial design to study the effect of the initial substrate concentration and the percentage of pepper waste on methane yield and on volatile solid removal. In these assays, the highest specific methane yield was 309 NmL g VS^{-1} obtained at 2.5 g VS L^{-1} and 50% of pepper waste in the feed. Under semi-continuous operation, anaerobic co-digestion improved the methane yield up to 86% in comparison to the mono-digestion of swine manure. In addition, the economic assessment showed the viability of the process with positive indices of the anaerobic co-digestion of pepper waste and swine manure. These results proved that the anaerobic co-digestion of these substrates can be a technically and economically viable alternative for its valorisation.

Keywords Pepper waste · Swine manure · Anaerobic co-digestion · Methane · Economic viability

1 Introduction

Agro-industrial waste produced during vegetable and fruit processing is an increasing global concern. Only in Spain, around 60,000 t of peppers are processed every year and the solid waste produced accounts for 50–60% of the total processed biomass [1]. The large amounts of waste generated are usually employed as animal feed or discharged into landfills, leading to environmental problems in the areas in which they are disposed of [2]. For that reason, looking for new ways to valorise agro-industrial waste represents both the solution to an environmental issue and an economic chance to exploit zero-cost organic waste. Alternative uses for pepper waste, such as the extraction of phenolics and carotenoids or the production of solid biofuels or biogas by anaerobic digestion (AD), have been proposed in the last few years [2–4].

AD can make a significant contribution in the agro-industrial sector with regard to renewable energy generation and environmental protection, due to the reduction of greenhouse gas emissions and odours [5]. However, several limitations have been reported when anaerobically digesting pure agricultural substrates. Thus, the anaerobic digestion of agricultural substrates, such as fruit or vegetable waste, is linked to low pH values, low buffer properties and the risk of volatile fatty acids (VFA) accumulating during the process [6, 7]. Co-digestion with manure overcomes these problems due to its intrinsically high buffer capacity that allows a stable pH to be kept within the optimal interval for methanogenic microorganisms [8]. Moreover, most carbon-rich substrates are seasonally produced, so manure must be the basis for developing the co-digestion process. Swine manure also has high ammonia concentrations and other essential nutrients required by the methanogenic microorganisms during AD digestion [9, 10]. Nevertheless, the high moisture, fibre and nitrogen content of manure often leads to low methane yields [11, 12]. A high ammonium concentration, together with a high total solids (TS) concentration, and an unsuitable substrate to biomass ratio are other factors that may

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hinder the AD process [8]. The co-digestion of manures with other carbon-rich substrates, such as agro-industrial waste, solves the ammonia inhibition associated with the digestion of manure alone and allows a balance of the carbon to nitrogen (C/N) ratio in the mixed waste to be reached [13]. Sharing common installations, a better handling of the co-substrate mixture and the influence of the economy scale are other reported advantages of anaerobic co-digestion [14].

The use of pepper waste to improve methane production in co-digestion with manure has scarcely been studied. Ferrer et al. [3] used pepper waste in co-digestion with pig slurry at different feed ratios in batch experiments from 0% to 50% on a volatile solid (VS) basis and the replacement of 50% of the VS from pig slurry with pepper waste enhanced the methane yield by 44% as compared to the use of pig slurry alone. Akman et al. [5] studied the co-digestion of cow manure and pepper waste produced in greenhouses, comprised of roots, stalks, leaves and fruits, in different mixing ratios in batch experiments. These authors found that the optimal mixing ratio to obtain the highest biogas production from the anaerobic co-digestion of cow manure and pepper waste was 25:75. As far as the authors are aware, the co-digestion of swine manure with pepper waste has not been studied under a semi-continuous or continuous feeding regime. To promote the energetic potential of agricultural by-products generated in the vegetable and fruit industry and manure, specific knowledge is required, not only of the percentage in the mixture but also the substrate concentration. In addition, this information could be useful for farmers in order to choose the best mixtures of agricultural waste and pig manure in anaerobic co-digestion to get extra revenue from and diversity in the agricultural sector [3]. Likewise, providing information regarding the economic costs to potential users is necessary to successfully introduce waste valorisation technologies.

In this context, the aim of the present study was to evaluate the effect of pepper waste addition for anaerobic co-digestion with swine manure. Batch assays were performed based on a second-order factorial design to evaluate the influence of the initial substrate concentration and the percentage of the pepper waste in the substrate on methane production and VS removal. After that, the performance of the anaerobic co-digestion under semi-continuous operation was evaluated. Finally, an economic assessment of the co-digestion of swine manure with pepper waste for small- to medium-size farms was performed.

2 Materials and methods

2.1 Origin of pepper wastes, manure and inocula

The pepper waste (PW) was collected from a frozen vegetable factory located in Badajoz (Spain). It consisted of seeds, peduncle, stalk and leftover peppers. The PW was grinded with a crusher (Tecnochufa R-60) and turned into paste. Swine manure (SM) was obtained from a finishing pig farm situated in Salamanca (Spain) for the batch experiments and from a close cycle pig farm situated in Guadajira (Badajoz, Spain) for the semi-continuous experiment. SM was collected from open ponds where manure was stored for several months. The anaerobically digested sludge used as inoculum was obtained from the municipal wastewater treatment plant in Valladolid (Spain) in the case of the batch assays and from an anaerobic reactor treating swine manure and prickly pear turned into paste for 5 years in the case of the semi-continuous experiment. The pepper waste was maintained frozen at − 4 °C from its collection until its use in the co-digestion experiments. Manures and inocula were separately homogenised and thereafter stored at 4 °C for further use. The chemical characteristics of both substrates and of the inocula used are presented in Table 1.

AQ2 **Table 1** Chemical characterization of inocula, swine manures (SM) and pepper waste (PW) used in batch and semi-continuous experiments

	Inocula		SM		PW
	Batch	Semi-continuous	Batch	Semi-continuous	
TS (g kg^{-1})	18.64 ± 0.10	18.68 ± 0.17	76.63 ± 1.37	47.58 ± 1.97	58.06 ± 4.74
VS (g kg^{-1})	11.71 ± 0.10	10.79 ± 1.04	52.32 ± 1.18	29.64 ± 1.29	54.22 ± 4.48
pH	–	7.75 ± 0.01	–	7.04 ± 0.01	4.75 ± 0.05
Alkalinity (mg$CaCO_3$ L^{-1})	–	10,694 ± 93	–	9165 ± 588	183 ± 303
COD (mg O_2 L^{-1})	–	29,500 ± 707	–	44,500 ± 707	79,000 ± 1414
TAN (mg L^{-1})	–	3660 ± 85	–	2550 ± 156	n.d
VFA (mg COD L^{-1})	–	26.49 ± 3.36	–	45.84 ± 5.07	n.d
C/N ratio	–	11.57 ± 1.01	–	14.56 ± 0.99	26.66 ± 0.45

n.d. not detected

2.2 Batch assays

Batch assays were conducted at 37 ± 1 °C for 98 days based on a second-order factorial design. This factorial design is commonly comprised of a 2^k factorial nucleus, six replications of the central point and 2**k* axial points, where *k* is the number of variables studied. Two independent variables were evaluated in this study: the initial substrate concentration (SC) expressed in terms of VS concentration and the percentage of pepper waste in the mixture (%PW) expressed in terms of VS of PW in relation to VS of the feed. The evaluated range for variable one (SC) was 2.5–49.5 g VS L^{-1} and for variable two (%PW), it was 0%–100%. The axial distance to the central point (α) was calculated according to Eq. (1):

$$\alpha = 2^{k/4} \quad (1)$$

where *k* is the number of variables evaluated, two in this case.

The variables, *Xi*, were coded as *xi* according to Eq. (2), such that X_0 corresponded to the central value:

$$xi = (Xi - Xi*)/\Delta Xi, \text{ where } i = 1, 2, 3, \ldots k \quad (2)$$

where xi is the dimensionless coded value of an independent variable, *Xi* is the actual value of an independent variable for the *i*th test, *Xi** is the actual value of an independent variable at the central point and Δ*Xi* is the step change [15]. All factorial design levels were combined in 9 different treatments (T1–T9). Each treatment was conducted in duplicate, except the central point (T9), which was replicated six times in order to determine the experimental error. Table 2 summarises the codified and real values of the evaluated variables.

The evaluated responses were the methane yield, expressed as the volume of methane produced per unit of VS added, and the removal of VS.

The response surface was used for the optimization of the studied variables. This methodology allows a second-order polynomial function to be obtained to describe the process [16], Eq. (3):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad (3)$$

where *Y* represents the predicted response, whilst β_0, β_1, β_2, β_{11}, β_{22} and β_{12} are the regression coefficients. X_1 (SC) and X_2 (%PW) are the evaluated variables. The coefficient of determination (R^2) was determined to estimate the degree of data variability that can be explained by the model, and thus the quality of fit to the model. The *p*-values were used to corroborate the model with a significance of $p < 0.05$. The multiple regression analysis was carried out using Statistica software (version Release 7, Statsoft Inc., Tulsa, OK, USA). Three-dimensional plots illustrated the interactive effects of the evaluated variables on the selected responses.

Anaerobic assays were carried out using the method described by Molinuevo-Salces et al. [8]. The anaerobic experiments were performed in 0.57-L bottles filled with 0.1 L of inoculum and 0.1 L of the corresponding substrate mixture. The inoculum/substrate ratio in the bottles ranged between 0.27 and 4.68 g VS g VS^{-1} and TS concentration varied between 10.9 and 40.7 g L^{-1}. Alkalinity was not supplemented. Two blanks containing only anaerobic inoculum were also prepared to determine the endogenous methane production. The bottles were hermetically closed with a septum and the headspace was flushed with nitrogen to displace the oxygen. Mixing was performed manually once a day

Table 2 Real and codified values and responses for batch experiments of anaerobic co-digestion of swine manure and pepper waste

	Codified values		Real values		Responses	
	SC (g VS L^{-1})	% PW	SC (g VS L^{-1})	% PW	Specific methane yield (NmL CH_4 g^{-1} VS added)	VS removal (%)*
Assays						
T1	−1	1	9.38	85.36	242 ± 14	63.5
T2	−1	−1	9.38	14.64	174 ± 6	38.2
T3	1	1	42.62	85.36	165 ± 20	57.7
T4	1	−1	42.62	14.64	133 ± 13	38.2
T5	0	1.4142	26.00	100.00	145 ± 2	67.6
T6	0	−1.4142	26.00	0.00	115 ± 6	41.1
T7	−1.4142	0	2.50	50.00	309 ± 26	7.3
T8	1.4142	0	49.50	50.00	192 ± 16	51.1
T9	0	0	26.00	50.00	215 ± 21	55.2

Data are means of two replicates, except for T9 that are means of six replicates

*VS removal = 100 × (VS_o -VS_f – VS_{fblank})/VS_o, where VS_o is the VS concentration in the bottles at the beginning of the assay, VS_f is the VS concentration in the bottles at the end of each assay and VS_{fblank} is the VS concentration in the blanks at the end of the assay

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by turning the bottles upside down. The volume of biogas produced was measured by the overpressure in the headspace with time frequency [17]. They were then converted to standard temperature and pressure (0 °C and 101.325 kPa) according to Eq. (4).

$$V_{biogas,\ STP} = V_{headspace} * \left(P_{headspace}/P_{STP}\right) * \left(T_{STP}/T\right) \quad (4)$$

where $V_{biogas,\ STP}$ is the volume of biogas adjusted to standard pressure and temperature, $V_{headspace}$ is the bottle headspace volume, $P_{headspace}$ is the manometric pressure measured in the headspace and P_{STP} and T_{STP} are the standard pressure and temperature, respectively. The biogas composition was analysed once per week. The methane volumes were adjusted by subtracting the average methane volume of the blanks (endogenous production). Batch assays were finished when the daily methane production for all the treatments was less than 1% of the accumulated volume of methane [18]. The methane yield, measured as CH_4 per gram of VS added, was determined. pH, TS, VS, total nitrogen Kjeldahl (TKN), total ammonia nitrogen (TAN) and VFA were analysed at the beginning and end of each assay to determine the stability of the digestion.

2.3 Semi-continuous experiment

Anaerobic digestion was performed in two continuously stirred tank reactors (CSTRs) made of stainless steel with a total volume of 6 L and a working volume of 4 L. Each reactor had blades in a central shaft allowing optimal contact between substrates; in this way, the mechanical agitation was controlled by an independent regulator. The digesters were covered by an outer jacket through which hot water circulates to maintain a constant temperature of 38 °C (mesophilic range) controlled by a thermostat. One digester was employed for the anaerobic mono- digestion of SM and the other one was used for the anaerobic co-digestion of the SM and PW, using the optimal percentage of PW in the mixture obtained from the batch experiments described in section 2.2.

Semi-continuous operation was performed for 101 days. Three experimental periods (I to III) were run based on the organic loading rate (OLR), also including an initial acclimatisation phase (period 0) (Table 3). The same OLRs were applied to both digesters, except for this initial acclimatisation period. The OLR values were selected according to previous works, which reported that OLRs between 1 and 2 g VS L^{-1} day^{-1} were optimum for a successful AD [19, 20]. The acclimatisation period (period 0) lasted for 16 days and the applied OLR was 0.63 g VS L^{-1} day^{-1} in AD mono-digestion and 0.73 g VS L^{-1} day^{-1} for the AD of SM and PW. From there, the OLR was increased whilst methane production was also increasing, but at an OLR of 1.88 g VS L^{-1} day^{-1} in period II, the methane yield began to decrease and the OLR was consequently decreased to 1.47 g VS L^{-1} day^{-1}. Due to the different organic content of the feed used for the two digesters, and in order to maintain the same OLR for both digesters (i.e. AD mono-digestion of SM and AD of SM and PW), the hydraulic retention time (HRT) varied between the digesters. The feeding operation was performed once a day. The same feeding volume was withdrawn prior to each feeding event in order to keep a constant volume in the digesters.

Samples were taken once per week for VS, chemical oxygen demand (COD), TAN, VFA and alkalinity analyses. For each experimental period, the data obtained after one HRT were used for the mean determinations of AD parameters. A gas meter (Ritter model MGC-1 V3.2 PMMA, Germany) was used to individually measure the biogas produced, which was then stored in Tedlar bags. The volume of dry gas was corrected at standard conditions (0 °C and 101.325 kPa). The biogas composition was automatically monitored on-site throughout the experiments with an Awite System of Analysis Process series 9 analyser (Bioenergie GmbH, Germany). This analyser consists 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 hydrogen, hydrogen sulphide and oxygen content.

2.4 Analysis

The concentrations of TS, VS, TKN, TAN, alkalinity and COD were analysed in accordance with the APHA methods

Table 3 Operational parameters during the periods I, II and III in the semi-continuous AD experiment (0–101 d)

	Period I (17–34 day)		Period II (35–66 day)		Period III (67–101 day)	
	Mono-digestion	Co-digestion	Mono-digestion	Co-digestion	Mono-digestion	Co-digestion
Total $substrate_{added}$ (g L^{-1} d^{-1})	40.00	30.25	60.00	45.25	46.75	35.50
OLR (g VS L^{-1} d^{-1})	1.26 ± 0.01	1.26 ± 0.01	1.88 ± 0.02	1.88 ± 0.02	1.47 ± 0.02	1.47 ± 0.02
HRT(d)	25	33	17	22	21	28

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[21]. The pH was measured using a pH-meter Crison Basic 20 (Crison Instruments S.A., Barcelona). The initial C/N ratio in the manure and pepper waste was determined using a True-Spec CHN Leco 4084 elementary analyser (USA), in accordance with the UNE-EN 16,948 standard for biomass analysis C, N, H [22].

For batch experiments, a gas chromatograph (Agilent 7890A, USA) with a thermal conductivity detector was used to analyse biogas composition. This chromatograph was provided by an HP-Plot column (30 m, 0.53 mm, 40 µm) followed by an HP-Molesieve column (30 m, 0.53 mm, 50 µm), using helium (7 mL min^{-1}) as the carrier gas. The injection port and detector temperatures were 250 °C and 200 °C, respectively. The temperature of the oven was initially set at 40 °C for 4 min and subsequently increased to 115 °C. A gas chromatograph (Agilent 7890A, USA) equipped with a Teknokroma TRB-FFAP column of 30-m length and 0.25 mm i.d, followed by a flame ionization detector (FID), was used to monitor VFA concentration (acetate, propionate, butyrate, iso-butyrate, valerate, iso-valerate and caproate). The carrier gas was helium (1 mL min^{-1}). The temperature of the detector and the injector was 280 °C. The temperature of the oven was initially set at 100 °C for 4 min, thereafter increased to 155 °C for 2 min and subsequently increased to 210 °C. The total VFA (TVFA) concentration was calculated as the sum of the concentration of those acids after applying the COD conversion factors. For the semi-continuous experiment, the TVFA were analysed according to Buchauer [23] by the titration method.

2.5 Statistical analysis

The statistical analysis of the experimental data (TAN, alkalinity, VFA, COD and VS removal) for each period in the semi-continuous experiment was determined by applying an analysis of variance (ANOVA). The 95% confidence interval of differences ($p<0.05$) was selected to estimate the statistical significance. Samples taken for each period showed a constant evolution throughout the experiments, so it is considered that their number is sufficient to carry out a statistical analysis, as previously carried out by Zahan et al. [24] and Alqaralleh et al. [25]. Three repetitions per sample were studied. A correlation analysis was performed at the different OLR applied in each experimental period. Data analyses were performed using the SPSS Statistics 20 software.

2.6 Techno-economic study

2.6.1 Description of the farm and the biogas plant

An economic assessment of the annual cost of a biogas plant for the co-digestion of SM and PW in a small to medium sow farm of 2800 animals was performed. Considering a production of 6.12 m^3 of manure per year and per animal, the estimated generation of manure in the farm per year accounted for 17,136 m^3. The co-digestion mixture was selected to maximize methane production, according to the results obtained in the semi-continuous experiment. Pepper waste (PW) is a seasonal by-product, being produced between July and October. However, it was stated that the biogas plant is fed during the whole year with SM and a co-substrate (PW or other co-substrate with similar characteristics). In this vein, a percentage of co-substrate of 50% (on a VS basis) has been selected, which results in an amount of 6854 t of co-substrate per year. To treat this amount of co-substrate together with the SM, a reactor of 3037 m^3 is needed. The AD is performed at 37 °C, with an HRT of 33 days and wet conditions (5% VS). The 5% VS percentage in the feed corresponds to VS content of the evaluated substrates, namely swine manure and pepper waste (Table 1). Moreover, to calculate the volume of the reactor, a security factor of 1.4 has been applied [26]. The biogas plant works for 7500 h (i.e. 313 days) per year.

The daily methane production and the potential energy in the methane were calculated according to Eq. 5 and Eq. 6, respectively.

$$CH_4production\ (m^3 day^{-1}) = (M_{Feed}/\text{Plant working time}) * (Y_{CH4} * V_{Feed}/100) \quad (5)$$

where M_{Feed} is the annual mass of fresh substrate treated in the plant (in t per year); the plant working time is the number of days that the biogas plant works in a year (i.e. 313 days); Y_{CH4} is the methane yield expressed as a volume of CH_4 produced per VS and day (m^3 per t VS and day), and VS_{Feed} is the VS content in the feed expressed as a percentage.

$$PE_{CH4} = CH_4production * CV_{CH4} \quad (6)$$

where PE_{CH4} is the potential energy from methane in kWh day^{-1}; CH_4 production is the daily methane production (in m^3 per day), and CV_{CH4} is the methane calorific value (i.e. 9.96 kWh m^{-3}).

2.6.2 Cost and revenue analysis

Capital costs (CAPEX) were estimated considering the digester and the required equipment (pumps, pipelines, valves…), the engineering services, the civil and electrical works and a solid–liquid separator for digestate treatment. The cost of the digester, including a system for heat and power recovery, was calculated according to Eq. 7, proposed by Imeni et al. [27]:

$$\text{Reactor Price (€)} = 329.05 * \text{Reactor size} + 181{,}815 \quad (7)$$

A combined heat and power (CHP) system of 100 kW was chosen for the simultaneous production of electricity and heat.

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Efficiencies of 30% for electricity recovery and 50% for heat recovery were estimated for the CHP [28].

The net electricity production and the net heat production were calculated according to Eq. 8 and Eq. 9, respectively.

$$\text{Net electricity production}\ (\text{kWh day}^{-1}) = (\text{PE}_{\text{CH4}} * \text{CHP efficiency})/100 \quad (8)$$

$$\text{Net heat production}\ (\text{kWh day}^{-1}) = (\text{PE}_{\text{CH4}} * \text{CHP efficiency})/100 \quad (9)$$

The operational costs (OPEX) were calculated considering the internal energy consumption (heat and electricity) and the maintenance costs. The plant's internal consumption of electricity was estimated by multiplying the plant's daily consumption (estimated as 5% of the electricity generated [29]) by the average electricity selling price in Spain (0.1295 €/kWh). Moreover, the electricity consumption of the solid–liquid separator was added (i.e. 0.19 kW per t of digestate [30]). The maintenance costs were estimated at 7200 € per year, resulting from 720 working hours per year, with a cost of 10 € per hour [27]. The cost for the management of the liquid fraction was not taken into account, since it was considered that the farm would assume the cost of the transport of a similar volume of livestock wastewater for its use as fertiliser in the case that anaerobic digestion was not implemented [31]. Biogas cleaning was not included in the economic evaluation, since it was assumed the plant included microaeration for sulphur control [32].

Revenues from the sales of the power energy and the solid fraction of the digestate have been considered. Regarding the heat energy, it has been considered that 68% is used to heat the digester [29] and 32% for heating in the farm buildings. The savings that the farm is achieving by using this heat energy have been estimated using a diesel-powered boiler to heat the farm buildings. An estimated price, provided by a local biogas plant, of 5 € per ton has been used for the calculation of the revenues coming from the sale of the solid fraction of the digestate.

2.6.3 Economic indices

Three economic indices were calculated, namely the net present value (NPV), the internal rate of return (IRR) and the discounted payback period (DPP). A discount rate of 7% and a project life of 25 years have been chosen to calculate the said indices [27].

3 Results and discussion

3.1 Chemical characterisation of inocula, swine manure and pepper waste

Table 1 shows the characterisation of the inocula and waste used in the anaerobic experiments. Swine manure and inocula supplied alkalinity to the anaerobic reactor content to compensate for the low pH values of PW (4.75). The stability of the anaerobic co-digestion process is ensured when the alkalinity values are around 2000–3000 mg $CaCO_3$ L^{-1} [33]. On the other hand, the PW provided elevate high C/N ratio (26.7) to the feed mixture, whereas the SM had a C/N of 14.6. Values of 20–30 for the C/N ratio are favourable for the anaerobic process [34]. Inhibitory concentrations for methanogenic microorganisms range between 1500 and 7000 mg L^{-1} for TAN [35, 36]. Considering this fact, the TAN concentration could negatively affect the anaerobic digestion of the SM in the present study.

3.2 Batch experiments

The specific methane yields and VS removal obtained from each assay are shown in Table 2. The results of the regression analysis are summarised in Table S1 in the Supplementary Materials. The following second-order polynomial functions were obtained for both responses (Eq. 10 and Eq. 11):

$$Y_{CH4} = 215.44 - 35.45(SC) + 17.61(\%PW) + 14.75(SC)^2 - 45.68(\%PW)^2 - 9.14(SC)(\%PW) \quad (10)$$

$$Y_{VS} = 55.25 + 7.01(SC) + 10.29(\%PW) - 11.12(SC)^2 + 1.45(\%PW)^2 - 1.46(SC)(\%PW) \quad (11)$$

From the specific methane yield response, the regression results show that the model was significant, since the value of the *F* statistics (32.27) is higher than the calculated one (8.2×10^{-8}). Moreover, the correlation coefficient showed that the combination of the two evaluated variables (SC and %PW) had a great relevance in the specific methane yield. The *p*-values lower than 0.05 indicated that both the substrate concentration (SC) and the quadratic factor of the percentage of pepper waste (%PW) (see Table S1 in the Supplementary Materials) significantly influenced the specific methane yield.

The percentage of methane in the biogas achieved values between 47 and 70% for all the assays. As can be seen from Fig. 1, the highest specific methane yield was 309 NmL CH_4 g^{-1} VS for T7, with a substrate concentration of 2.50 g VS L^{-1} and a percentage of pepper waste in the mixture of 50%. This value was similar (344 NmL CH_4 g^{-1} VS) to that obtained by Ferrer et al. [3], when co-digesting pig slurry and pepper at a ratio of 50:50 on the VS basis. These authors demonstrated that the replacement of 50% of pig slurry VS by pepper increased the methane potential by 44%. They observed lower increments in the biochemical methane potential with a lower content of pepper in the

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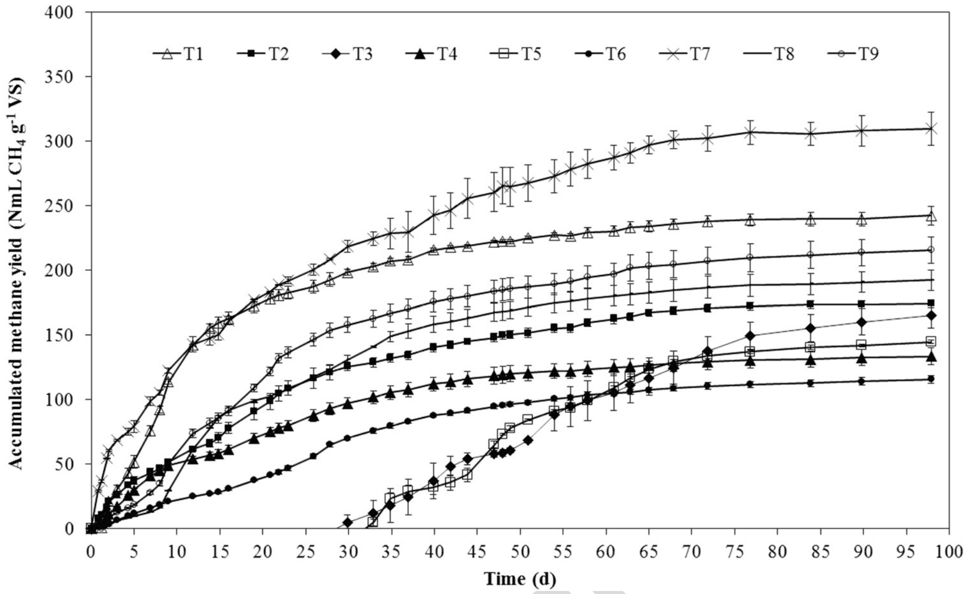


Fig. 1 Accumulated methane yield for the assays T1-T9

mixture. On the other hand, Akman et al. [5] found that the optimal mixing ratio for co-digestion of the cow manure with pepper waste was 25:75 (on a total volatile suspended solid basis) with a methane production of 223 Nml CH_4 g^{-1}VS, which was around 175% higher than the methane production achieved during the digestion of cow manure.

With regard to the assays with the same percentage of PW in the mixture (T1 and T3 or T2 and T4), the lower the substrate concentration, the higher the methane yield. Regarding the assays with the same value for SC (T5, T6 and T9), the highest specific methane yield was obtained with a percentage of PW of 50%. The same trend was observed in the response surface figure (Fig. 2a). For those treatments with the highest percentage of PW in the mixture (i.e. T3 with 85.36% and T5 with 100%), methane production started after the first 30 days (Fig. 1). In these cases, the high concentration of easily biodegradable material can lead to an accumulation of VFA. Methane production begins once the VFA are consumed by acid-degrading bacteria. The same result was reported by Molinuevo-Salces et al. [8]. Final TVFA concentrations were 254 mg COD L^{-1} and 189 mg COD L^{-1} for T3 and T5, respectively. The low alkalinity of the PW (Table 1) could negatively influence the AD process. Indeed, Chen et al. [37] found that alkalinity supplements during the AD of food waste favoured the use of VFAs by anaerobic microorganisms, increasing the methane yield in comparison with a control without the addition of alkalinity.

In the case of VS removal, the determined coefficient R^2 is 0.7386, meaning that 73.9% of the variability data can be explained by the model. The regression results show that the model is significant because the value of statistic F is 1.70, higher than the calculated one (0.35). The percentage of pepper waste is a factor that influences the VS removal response. In the response surface graph (Fig. 2b), it is clear that the VS removal efficiency increases when the percentage of pepper waste increases. In addition, the substrate concentration positively influences the VS removal in the anaerobic digestion within the studied range. These results were in agreement with those reported by Molinuevo-Salces et al. [8], who obtained an increase in the solid removal efficiency when the percentage of vegetable waste in the anaerobic co-digestion with swine manure also increased. This can be due to the higher biodegradability of the pepper waste in comparison to that of the swine manure [3]. Unexpectedly, it was not found a positive correlation between VS removal and the specific methane yield in the present study (Table 2). It could be partially attributed to the different biodegradability of the substrates, which depends on their respective chemical composition. Thus, methane production depends on the relative amount of the main organic compounds (i.e. lipids, proteins, carbohydrates and lignin) in the waste that are quantified by its VS content and that have different methane potentials [38]. Ferrer et al. [3] found that pig manure presented a lower content of carbohydrates than pepper waste, the majority (98%) being due to the presence of hemicellulose and cellulose; whereas, in pepper waste, the sum of hemicellulose and cellulose explained less than 20% of the total carbohydrates. In addition, those authors reported that pig manure also contains more lipids, proteins and lignin than pepper waste. On the other hand, operational conditions, including substrate concentration, as well as synergistic or antagonistic effects of co-digestion could affect methanogenic microorganisms, thus varying methane production [39]. Indeed, the objective of

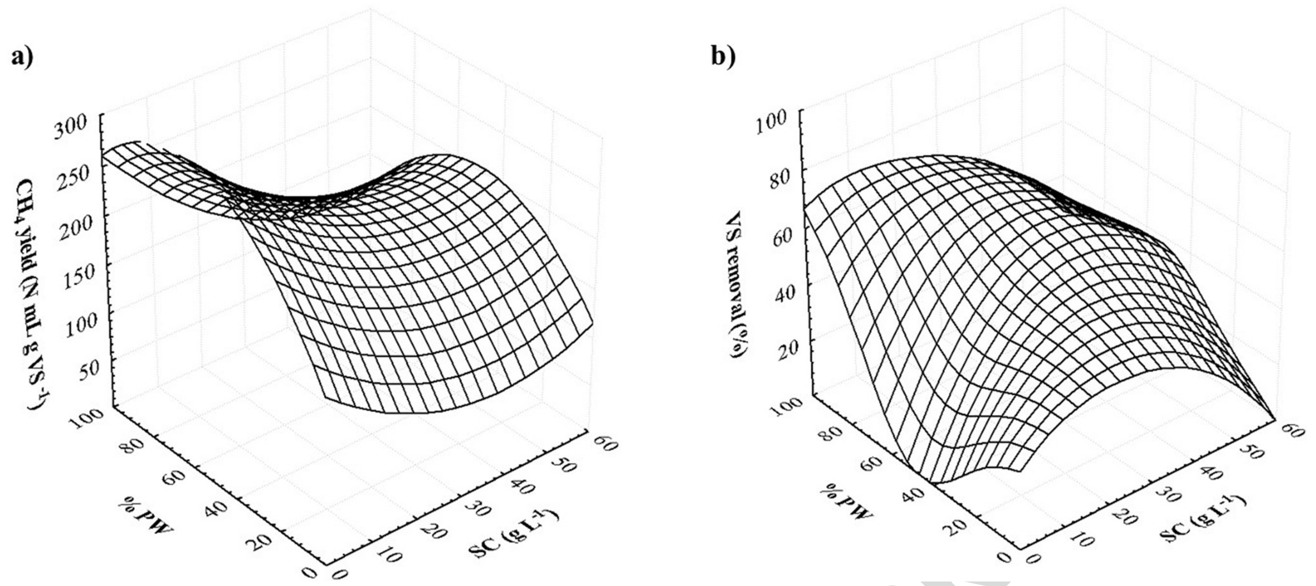


Fig. 2 Response surface graphs for the specific methane yield (**a**) and for VS removal (**b**)

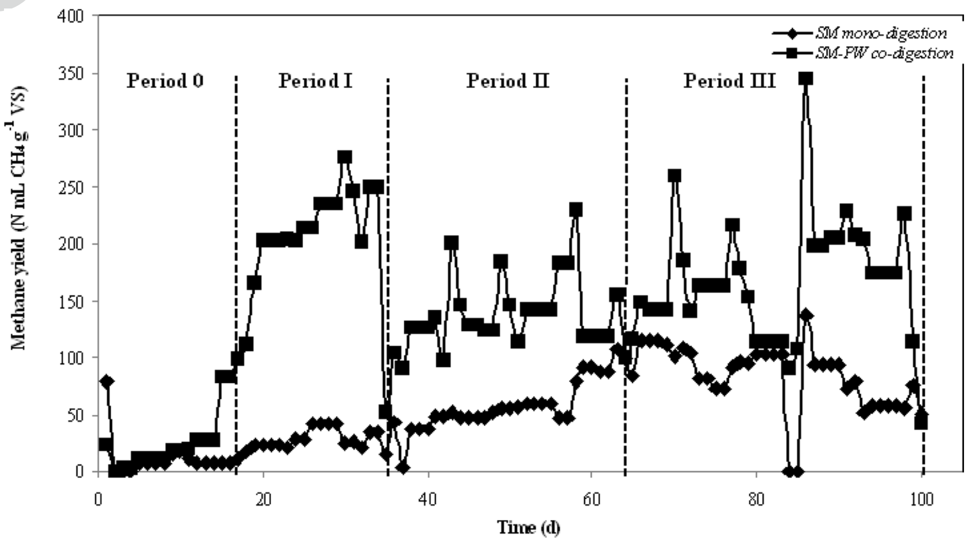
this study was to determine the conditions that allow the optimization of both responses.

3.3 Semi-continuous experiment

The best treatment in terms of specific methane yield obtained in the batch experiments (T7) for the anaerobic co-digestion of SM and PW was evaluated under semi-continuous operation. Thus, the percentage of PW used in the mixture was 50% (on the VS basis). The conditions studied in the semi-continuous experiments are shown in Table 3. Figure 3 presents the methane yields for both digesters during this experiment.

Although no significant differences were found between the alkalinity, TAN, VFA concentrations, COD removal and VS removal, significant differences were detected in methane yields for the two digesters in the different periods (Table 4). For the anaerobic co-digestion of SM with PW, the highest average methane yield (208 NmL CH_4 g^{-1} VS) was obtained when operating at the lowest OLR (i.e. an OLR of 1.26 ± 0.01 g VS L^{-1} day^{-1} during the period I (Table 4)). This result was in agreement with those obtained in the batch experiments described in section 3.2.1, in which the methane yield decreased when the substrate concentration increased. However, the highest methane yield obtained under semi-continuous operation was lower than the maximum obtained in the batch experiment (T7). Similar findings

Fig. 3 Methane yield evolution for mono-digestion and co-digestion assays



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were observed by other researchers. Parralejo et al. [40] co-digested milled grape pomace and pig manure, obtaining methane yields of 173 NmL CH_4 g^{-1} VS with an OLR of 1.34 g VS L^{-1} day^{-1} under semi-continuous operation, and 385 NmL CH_4 g^{-1} VS in batch conditions. Zarkadas et al. [41] also found differences between the results obtained in the co-digestion of olive mill wastewater with fish meal in batch vs. semi-continuous operation at 3.60 g VS L^{-1} day^{-1} (482 NmL CH_4 g^{-1} VS vs. 416 NmL CH_4 g^{-1} VS). On the other hand, a significant positive correlation ($R^2 = 0.955$) was evidenced between VS and COD removal for the AD of SM and PW mixture in period II at the highest OLR studied (see Table S2 in the Supplementary Materials). In addition, a significant positive correlation was found between the alkalinity and TAN concentration for the period I (i.e. OLR of 1.26 ± 0.01 g VS L^{-1} day^{-1}), which became negative at the highest OLR evaluated corresponding to the period II. This could be due to the short HRT applied for the highest OLR studied, which could affect the buffer capacity. This result was in agreement with that observed by Parralejo [42], who investigated the co-digestion of several agro-industrial raw materials and observed a decrease in the digestate alkalinity when the OLR exceeded 3 g VS L^{-1} day^{-1}.

For the anaerobic digestion of SM, the highest methane yield (88 NmL CH_4 g^{-1} VS) was found when operating at an OLR of 1.47 ± 0.02 g VS L^{-1} day^{-1} during period III (Table 4). The operation at a higher OLR of 1.88 ± 0.02 g VS L^{-1} day^{-1} and an HRT of 17 days in period II resulted in a lower methane production. This could be attributed to the low HRT, which would cause a quick replacement of the anaerobic reactor content and microorganisms did not then have enough time to degrade the feed, as described by Dareioti and Kornaros [43]. Low methane yields for the SM were obtained compared to previous works [44]. Such factors as the manure storage period have been reported to affect the manure methane yield [44]. A significant positive correlation was present between the VS and COD removal ($R^2 = 0.951$) in the anaerobic digestion of SM at 1.26 ± 0.01 g VS L^{-1} day^{-1} in period I. However, a significant negative correlation ($R^2 = -0.972$) was exhibited for TAN and VS removal in period III at 1.47 ± 0.02 g VS L^{-1} day^{-1} when digesting SM alone (see Table S2 in the Supplementary Materials). The different behaviour compared to the digestion of a mixture of SM and PW could be due to the composition of the digester feed (SM presented a high TAN content, whereas it was not detected in the PW, Table 1).

Overall, higher methane yields were obtained during the co-digestion of SM with PW compared to the mono-digestion of SM, regardless of the OLR applied (Fig. 3; Table 4). Specifically, the methane yield increased 86%, 57% and 48% in comparison to that achieved during the digestion of SM for periods I, II and III, respectively. The methane yield values obtained during co-digestion showed a lower stability than those obtained during the digestion of SM, especially when working at the highest OLR. Nevertheless, several peaks in the methane yields observed in Fig. 3 were due to the power outages entailing downs in the methane production obtained. The co-digestion experiment results are consistent with those obtained by other researchers. Thus, Bres et al. [28] obtained an increase in the methane yield of 31% by co-digesting fruit and vegetable waste with pig manure compared to the mono-digestion of pig manure alone, with an increase in the OLR from 0.5 to 2 g VS L^{-1} day^{-1}.

3.4 Economic analysis

The calculations for the economic approach are based on the experimental results obtained in the semi-continuous experiments (section 3.3). Specifically, a methane yield of 208 NmL CH_4 g^{-1} VS, corresponding to the highest methane yield for the co-digestion of SM and PW, was used for estimations. The daily methane production obtained after digesting the SM generated in a pig farm, together with the co-substrate (details are reported in section 2.4.1), would be

Table 4 Parameters evaluated in co-digestion and mono-digestion experiments

	Period I		Period II		Period III	
	Mono-digestion	Co-digestion	Mono-digestion	Co-digestion	Mono-digestion	Co-digestion
Methane yield (NmL CH_4 g^{-1} VS)	28 ± 8[a]	208 ± 65[b]	58 ± 10[b]	134 ± 13[a]	88 ± 16[c]	168 ± 10[ab]
VS removal (%)	53 ± 3	57 ± 10	53 ± 10	47 ± 8w	39 ± 6	42 ± 7
COD removal (%)	16 ± 4	41 ± 9	38 ± 7	32 ± 10	26 ± 3	21 ± 6
TAN (mg L^{-1})	2268 ± 815	2490 ± 202	2340 ± 286	2585 ± 319	2628 ± 432	2488 ± 324
VFA (mg L^{-1})	1376 ± 410	2399 ± 960	2962 ± 765	3118 ± 781	1794 ± 644	2158 ± 489
Alkalinity (mg$CaCO_3$ L^{-1})	10,068 ± 1186	10,308 ± 1021	10,215 ± 759	9996 ± 483	10,506 ± 896	9984 ± 717

Significant differences were determined independently for each digester. Different letters indicated significant differences between values of the same parameter for the two digesters. No significant differences were found in those parameters without any letter

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797 Nm^3 CH_4 per day. The potential energy in the produced methane would account for 7939 kWh per day, with a methane calorific value of 9.96 kWh Nm^{-3}. This would account for a net electricity production of 2382 kWh day^{-1} and a net heat production of 3970 kWh day^{-1} (Table 5).

The net present value (NPV), with a discount rate of 7%, would result in a positive value, which indicates that the money returned in the future is higher than the initial investment. The internal rate of return (IRR) of 14% indicates that this discount rate would turn the NPV to zero. The investment payback period (IPP) would indicate that 10 years are needed to recover the investment for the biogas plant (Table 5). The IPP of 10 years is in the range of previous studies evaluating the techno-economic feasibility of anaerobic digestion of different organic by-products. For instance, Imeni et al. [27] obtained an IPP of 9 years for anaerobic co-digestion of manure (70%) and cheese whey (30%) using an HRT of 28 days. Even though this approach shows a good economic performance, an economic evaluation with a lower HRT (i.e. 15 days), a smaller reactor (i.e. 1380 m^3) and a methane yield of 208 NmL CH_4 g^{-1} VS was carried out. In this manner, the initial investment for this biogas plant would account for 707,993 €, being reduced by 43% with respect to the former, whilst the investment for the biogas plant would be recovered in 5 years.

Table 5 Biogas and energy production, costs, revenues and economic indexes

Parameter	Unit	
Biogas and energy production		
Daily methane production	m^3/day	797
Potential energy in the methane	kWh/day	7939
Net electricity production	kWh/day	2382
Net heat production	kWh/day	3970
Capital costs (CAPEX)		
Digesterand required equipment	€	1,181,006
Valves and tubes	€	15,000
Electrical works	€	20,000
Engineering works	€	15,000
Solid–liquid separator	€	15,000
Civil works	€	7000
Total	€	1,253,006
Operation costs (OPEX)		
Internal AD electricity consumption	€/year	4827
Electricity consumption separator	€/year	590
Maintenance cost	€/year	7200
Total	€	12,617
Revenues		
Electricity revenues	€/year	83,926
Sales of solid fraction of digestate	€/year	27,589
Savings in heat expenses	€/year	44,845
Total	€	156,359
Economic indexes		
NPV	€	569,139
IRR	%	14
IPP	Years	10

The anaerobic co-digestion of agro-industrial by-products, such as swine manure and pepper waste, not only shows a good economic performance, but also enhances the environmental and social profile of the process in comparison with the conventional treatment. Thus, the use of this type of waste as a renewable energy source could help to mitigate greenhouse gas emissions whilst impacting on rural communities due to the creation of local jobs and the promotion of economic growth [45, 46].

4 Conclusions

The anaerobic co-digestion of SM and PW is favoured by operating with low substrate concentrations. With these low substrate concentrations, the methane production increases in line with the increase in the percentage of PW in the mixture, due to its high biodegradability. The highest specific methane yield obtained under batch conditions was 309 NmL CH_4 g^{-1} VS, obtained at values of 2.50 g VS L^{-1} and a percentage of PW in the mixture of 50% (on the VS basis). These results obtained from the batch experiments were used to evaluate anaerobic co-digestion under semi-continuous operation at different OLR values. The specific methane yield increased by up to 86% compared to that obtained from the mono-digestion of SM. The co-digestion experiment at 1.26 g VS L^{-1} day^{-1} obtained the highest value of methane yield, 208 NmL CH_4 g^{-1} VS. Under these conditions, co-digestion is economically viable, showing positive indices (net present value > 0, internal rate of return of 14% and a return of the investment in 10 years).

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