ANAEROBIC DIGESTION OF ORANGE PEEL WASTE AND SWINE MANURE: A CENTRAL COMPOSITE DESIGN

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***Abstract*- This work studies the methane potential for the anaerobic co-digestion of swine manure with orange peel waste. The influence of the initial substrate concentration and the percentage of orange peel waste were evaluated in batch experiments based on a central composite design. Both factors have a significant influence on specific methane production, observing an increase in specific methane production when the two factors were evaluated at their maximum levels. These results suggest that for a plant scale implementation maximum levels of substrate concentration and content of orange peel waste should be selected.**

Keywords—anaerobic digestion, orange peel waste, swine manure.

I. INTRODUCTION

Spain was the large producer of oranges in the European Union (EU) in 2018 according to EU statistic office [1]. Concretely, Spain generated 54.8 % of the total production of oranges in the EU. An orange production of 3,717.4 millions of tons has entailed an increase of 10.4 % over the last year production´s.

The extraction of high added-value compounds supposes an important valorisation of orange scratch and juice. The orange waste is composed by peel without scratch. The orange waste can be used as a raw material for energy generation throughout anaerobic co-digestion with swine manure. In this way, the orange waste is probably an excellent co-substrate in the anaerobic co-digestion processes because it does not contain certain inhibitory compounds that favors the right development of these processes. Firstly, the citrus essential oils extracted from the citrus waste of the fruit contents a high limonene concentration that can be inhibitory for anaerobic co-digestion process [2,3]. Several works have evaluated the effect of limonene removal on anaerobic digestion [4,5,2,6]. Moreover, polyphenol compounds from orange scratch and orange juice are considered as inhibitory compounds in the anaerobic co-digestion processes. These compounds can be extracted from scratch or juice of different fruits to obtain bioactive compounds or other high added-value products [7,8].

The main aim of this work was to evaluate anaerobic co-digestion process of orange peel waste (OPW) and swine manure (SM) in batch experiments using a central composite design (CCD). The orange peel waste employed in the present study did not contain the scratch due to the possibility of further valorization for other purposes. The influence of the initial substrate concentration and the percentage of OPW were evaluated in terms of methane yield. In addition, the obtained digestates were characterized for their classification as fertilizers.

II. MATERIALS AND METHODS

A. *Origin of substrates and inocula*

Swine manure (SM) was collected from a pig farm located in Guadajira (Badajoz, Spain) (+38° 51' 9.6768",-6° 40' 15.5418"). The orange peel waste (OPW) was simulated after the extraction of the orange juice and the scratch peel to orange. This waste was grinded to obtain a paste. Both substrates were stored at -4 ºC for further use. The anaerobic sludge used as inocula was obtained from an anaerobic reactor treating pig manure and prickly pear turned into paste for 5 years. Table 1 shows the chemical characterization of each substrate and the anaerobic sludge.

TABLE 1. CHEMICAL CHARACTERIZATION OF INOCULA, OPW AND SM.

Parameter/Substrate	Inocula	OPW	SM
pH	7.77 ± 0.16	3.51 ± 0.13	7.85 ± 0.11
Alkalinity (mg $CaCO_3$ L^{-1})	8750 ± 57	-	11619 ± 78
C/N	2.84 ± 0.13	92.89 ± 6.00	4.12 ± 1.44
$N\text{-}NH_4$ (mg L^{-1})	1760 ± 226	<30	3240 ± 339
COD_a (mg O_2 L^{-1})	16500 ± 3536	288000 ± 15556	38500 ± 6364
Redox potential (mV)	-393 ± 21	-86 ± 19	-426 ± 8
TS_b (%)	1.68 ± 0.08	18.54 ± 0.14	3.97 ± 0.22
VS_c(%)	0.84 ± 0.01	18.39 ± 0.00	2.65 ± 0.01
$TVFA_d$ (mg L^{-1})	794 ± 131	-	1449 ± 69

[a]Chemical Oxygen Demand; [b]Total Solids; [c]Total Volatile Solids; [d]Total Volatile Fatty Acid.

B. *Anaerobic digesters*

Assays were carried out using 6 L cylindrical waterproof reactors built in stainless steel with a working volume of 4.5 L. A water jacket surrounding each digester allowed temperature remaining constant at approximately 38 °C (mesophilic range), checked by a thermostat. Mechanical agitation in the biodigesters was controlled by an independent regulator allowing optimal contact between substrates. Batch experiments were carried out based on a central composite design explained in section II.C. An inocula volume of 0.75 L was introduced into each reactor at the beginning of each batch experiment.

C. *Experimental design*

A central composite design (CCD) was carried out to study the anaerobic co-digestion of OPW and SM. Two factors, namely the substrate concentration based on VS (SC) and the proportion of OPW (% OPW) in the co-digestion mixture (based on VS), were selected for the experimental design. The selected range for factor 1 (i.e. substrate concentration) was from 2.5 to 27.5 g VS L^{-1}. The selected range for factor 2 (i.e. proportion of OPW) was between 0 and 100%. All the treatments were carried out in duplicate except for the central point (T9) which was repeated 6 times in order to estimate the experimental error. Batch reactors were prepared as previously explained in II.B. The experiments lasted for 73 days.

Response surface methodology was used to fit the experimental data into a second-order polynomial equation (1). The experimental response selected was the specific methane yield. This equation describes the influence of the two selected factors over the response:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_{12} + \beta_{22} X_{22} + \beta_{12} X_1 X_2 \qquad (1)$$

where Y is the predicted response value, namely methane yield. β_0, β_1, β_2, β_{11}, β_{22} y β_{12} are the regression coefficients. X_1 and X_2 are the evaluated factors (SC and % OPW). Excel was used to obtain the regression coefficients from the data set. The determination coefficient (R^2) was calculated to assess the quality of the fit of the polynomial model equation. The impact of the regression coefficients on the predicted response was determined by p-values and significant model terms were indicated by p-values lower than 0.05.

D. . *Analytical methods*

Substrates characteristics were analyzed according to the Standard Methods [9]. Total solid (TS) 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 Volatile solid (VS) content was obtained by heating the dried TS to constant weight at 550 °C for 2 h in a muffle furnace (Hobersal 12PR300CCH, Spain) at inert atmosphere (2540 E method). pH and redox potential were measured with their corresponding electrodes with a pH meter (Crison Basic 20, Spain), alkalinity of the medium through 2320 method, Chemical Oxygen Demand (COD) according to 410.4 method [10], $N\text{-}NH_4$ through E4500-NH3 B volumetric titration method and the Total Volatile Fatty Acids (TVFA) according to Buchauer [11] by titration methods. The initial C/N ratio in substrates was determined using an elemental analyser True-Spec CHN Leco 4084 (USA), according to the standard UNE-EN 16,948 for analysis of biomass C, N, H [12]. Gas composition and total gas volume were automatically monitored *in-situ* during the experiments with a gas analyser Awite System of Analysis Process serie 9 (Bioenergie GmbH, Germany). This analyser was composed of two IR sensors to take methane and carbon dioxide measurements, and three electrochemical sensors that supply values of hydrogen, sulfhidric acid and oxygen content in the produced biogas. Gas counters (Ritter model MGC-1 V3.2 PMMA, Germany) were used to measure produced biogas stored in tedlar bags. Dry gas volume was corrected to standard conditions (0 °C, 101.325 kPa). Some elements (Cd, Cr, Cu and Ni) in the digestate were detected by spectroscopy technique using an ICP-OES Varian 715 ES (Australia). Previously, samples were digested in a laboratory microwave Millestone Start D (Italy).

III. RESULTS AND DISCUSSION

A. *Anaerobic co-digestion study*

Anaerobic co-digestion of OPW and SM was investigated by a central composite design (CCD). The coded and actual values corresponding to the studied factors (concentration of substrate (SC) and proportion of OPW (% OPW)) and response (i.e. methane yield) from the batch tests are presented in Table 2.

TABLE 2. A) CODIFIED, REAL VALUES AND RESPONSE FOR SWINE MANURE CO-DIGESTION IN BATCH EXPERIMENTS. B) REGRESSION RESULTS FOR SWINE MANURE CO-DIGESTION IN BATCH EXPERIMENTS.

A)

*Data are means of two replicates, except T9, which data are means of six replicates. Standard deviation is shown in brackets.

	Codified values		Real values		Real response *
	SC ($g\ VS\ L^{-1}$)	**% OPW**	**SC ($g\ VS\ L^{-1}$)**	**% OPW**	**Y ($mL\ CH_4\ g^{-1}$ VS added)**
Treatments					
T1	-1	1	6.16	85.36	212.4 (107.0)
T2	-1	-1	6.16	14.64	50.8 (8.0)
T3	1	1	23.84	85.36	46.8 (1.6)
T4	1	-1	23.84	14.64	81.6 (12.6)
T5	0	1.4142	15.00	100.00	334.3 (14.5)
T6	0	-1.4142	15.00	0.00	52.43 (61.3)
T7	-1.4142	0	2.50	50.00	158.4 (68.7)
T8	1.4142	0	27.50	50.00	278.6 (15.1)
T9	0	0	15.00	50.00	201.2 (55.2)

B)

	Y ($mL\ CH_4\ g^{-1}$ VS added)	
	Coefficient	**Prob**
β_0	220.9	<0.001
β_1	44.7	0.005
β_2	102.9	<0.001
β_{11}	-7.0	0.645
β_{22}	-23.9	0.130
β_{12}	31.4	0.160
R^2= 0.8437, Adj. R^2= 0.7836, r= 0.9185 F value= 14.04, Prob>F= $7.5*10^{-5}$		

R^2, correlation coefficient; Adj. R^2, adjusted correlation coefficient; r, regression coefficient; F value, value resulted from the F-test

When considering methane production obtained from SM and OPW co-digestion it was observed that all treatments raised the expected methane potential production, except T3 (Fig. 1). This treatment was characterized by high concentration of solids and orange peel waste content. Molinuevo-Salces et al. [13] observed an organic overload during the co-digestion of swine manure and vegetable wastes for T3 that resulted in TVFA accumulation. When TVFA were steady consumed, methane production started, however methane production for T3 seemed not to be completely stopped at the end of an experimental time of approximately 90 days, leading to underestimating response values. The high substrate/microorganisms ratio resulted in high TVFA formation with a delay on methane production due to partial inhibition over methanogenic bacteria. The same behavior was obtained in the experiences developed by Ruiz and Flotats [3] when digesting grinded orange peel. Based on the previous statement, T3 values were excluded when adjusting data to the model.

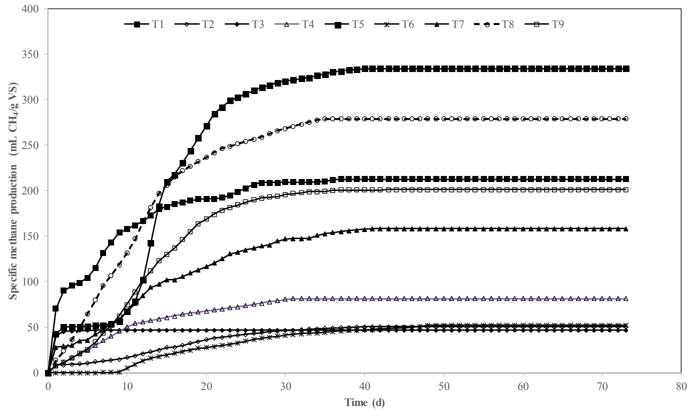

Figure 1. Accumulated methane yield for T1-T9.

The regression analysis for the co-digestion of SM and OPW resulted in Eq. (2) for the specific methane yield:

$$Y_{CH4} = 220.9+44.7\ (SC)\ +102.9\ (\%\ OPW) - 7.0\ (SC)^2 - 23.9\ (\%\ OPW)^2 + 31.4\ (SC)\ (\%\ OPW) \quad (2)$$

The response model presented a determined R^2 coefficient of 0.8437, which means that the assessed factors and their interactions are able to explain 84% of the data variability found in the response specific methane yield. As previously stated, the response did not take into consideration data obtained from treatment T3. Regression results shows a statistically significant model, since the actual F-value (14.04) is higher than the calculated one (7.5 x 10^{-5}). P-values were lower than 0.05 for both studied factors (Table 1), indicating that both of them have a significant influence on specific methane production.

Treatments with a constant value of SC (T5, T6 and T9) exhibited an increase of methane production with an increment in OPW. This influence can be observed in the response surface plot (Fig. 2). The improvement in methane yield seems to be related with the high biodegradability of OPW added as co-substrate. The large biodegradability of this substrate was proven when digesting 100% of OPW and resulted in the highest methane yield (T5). A similar behavior was observed for systems T1 and T2 presenting both the same value of factor SC with T1 system being evaluated at a higher level of factor OPW.

On the other hand, treatments with a constant value of OPW (T7, T8 and T9) evaluated at different levels of SC factor follow the same tendency (Table 2). The highest specific methane production (276 mL CH_4 g VS $_{added}$ $^{-1}$) corresponded to T8 (with a SC of 27.5 g L^{-1}). This behavior can be observed in the response surface plot (Fig. 2).

As conclusion, based on experimental results obtained from the two responses analyzed it is observed that, in general an increase in specific methane production was observed whenever the two factors are evaluated at their maximum levels. In this sense, when considering implications related to plant scale implementation maximum levels of substrate concentration and content of OPW may be selected.

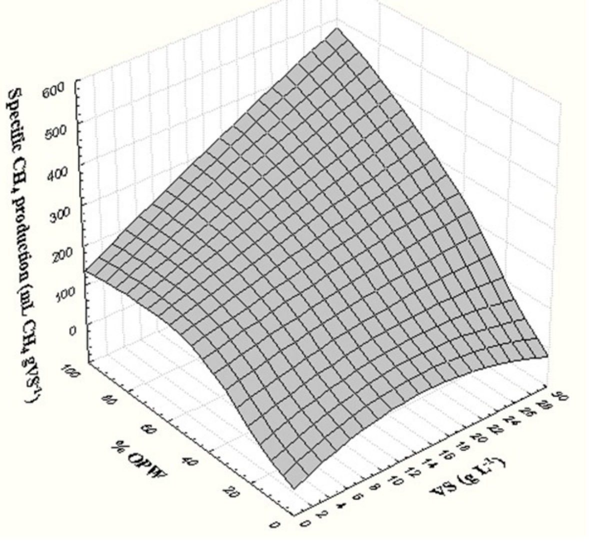

Figure 2. Surface respose plot for specific methane yield response.

TABLE 3. CHEMICAL CHARACTERIZATION FOR DIGESTATES OBTAINED IN ANAEROBIC CO-DIGESTION ASSAYS.

Parameter /Substrate	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**
pH	7.91 ± 0.02	7.91 ± 0.53	5.29 ± 0.08	7.63 ± 0.05	7.30 ± 0.01	7.30 ± 0.01	7.64 ± 0.04	7.73 ± 0.03	7.79 ± 0.24
Alkalinity, (mg $CaCO_3$ L^{-1}*)*	3009 ± 60	5594 ± 274	5722 ± 677	9459 ± 332	4746 ± 70	6148 ± 205	4714 ± 184	4015 ± 97	5166 ± 790
C/N	24.11 ± 13.24	7.65±1.81	14.08±2.71	5.22±0.27	10.57 ± 2.26	20.96±5.10	21.07 ± 6.83	6.62 ± 0.48	8.51 ± 0.74
N-NH_4*, (mg* L^{-1}*)*	900 ± 85	1540 ± 141	970 ± 99	2440 ± 0	1120 ± 113	1540 ± 28	1460 ± 311	2060 ± 255	1333 ± 136
COD[a]*, (mg* O_2 L^{-1}*)*	55000 ± 0	45000	36500 ± 7778	27500 ± 2121	69500	64000 ± 5657	59000	21500 ± 4950	15833 ± 4875
Redox potential(mV)	28 ± 6	-267 ± 146	-98 ± 30	-379 ± 16	-254 ± 11	-243 ± 168	-106 ± 12	-251 ± 42	-124 ± 44
TS(%)	0.43 ± 0.02	1.99 ± 0.31	1.34 ± 0.07	2.78 ± 0.22	1.77 ± 0.30	1.6 0± 0.08	2.35 ± 0.13	2.39 ± 0.34	1.19 ± 0.17
VS(%)	0.21 ± 0.00	1.19 ± 0.00	0.91 ± 0.00	1.69 ± 0.00	1.04 ± 0.00	0.95 ± 0.00	1.40 ± 0.00	1.38 ± 0.00	0.67 ± 0.01
TVFA, (mg L^{-1}*)*	407 ± 35	656	7826 ± 1160	12089 ± 26	928	466	928	901 ± 214	627 ± 2 89

B. Evaluation of the obtained digestate as fertilizer

The characterization of the digestates obtained after anaerobic co-digestion assay is detailed in Table 3. The high TVFA concentration obtained in digestates from T3 explained the low methane production in this treatment. pH value in this digestate was also very low which it is typical of acidogenic/acetogenic stage, that occurs before methanogenic stage.

Digestates from treatments with a constant value of SC (T5, T6 and T9) presented a decrease of pH and alkalinity with the increment of OPW in the mixture. On the other hand, digestates from treatments with a constant value of OPW (T7, T8 and T9) showed a slight increase concentration of the inhibitory parameters (ammonia nitrogen and TVFA) when SC increased in the mixture. However, the anaerobic co-digestion process is not affected by this increase; thus, among these treatments, the highest methane yield was obtained for T8, that was the treatment with the highest SC amount in the mixture digested.

A classification of fertilizers obtained from waste treated by anaerobic digestion process is contemplated in the RD 999/2017 about fertilizer products in Spain [15]. The classification based on heavy metal concentration is shown in Table 4. The results obtained for the digestates composition in some heavy metals are presented in the Table 5. Digestates obtained in this study are set in the class A for digestates from T3, T4 and T8, and in the class B for the rest of digestates.

TABLE 4. MAXIMUM LEVEL ALLOWED FOR HEAVY METALS IN FERTILIZERS FROM DIGESTATES ACCORDING TO RD 999/2017.

Fertilizer classification	**A**	**B**	**C**
Cd, ppm	0.7	2	3
Cu, ppm	70	250	300
Cr, ppm	70	300	400
Ni, ppm	25	90	100

TABLE 5. CLASSIFICATION OF FERTILIZER BASED ON THE CONCENTRATION OF HEAVY METALS.

Heavy metal (ppm) /Digestate	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**
Cd	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cu	101.62 ± 4.81	177.32 ± 19.55	13.21 ± 0.24	29.78 ± 1.33	231.41 ± 5.88	194.51 ± 4.23	159.62 ± 14.62	20.93 ± 0.78	78.59 ± 4.59
Cr	13.86 ± 4.39	n.d.	n.d.	n.d.	n.d.	14.30 ± 4.38	n.d.	n.d.	n.d.
Ni	33.91 ± 4.80	6.57 ± 2.03	<5	7.52 ± 0.43	11.09 ± 1.30	24.01 ± 5.34	13.99 ± 1.70	<5	22.76 ± 4.64
Fertilizer classification	B	B	A	A	B	B	B	A	B

IV. CONCLUSIONS

Anaerobic co-digestion of OPW and SM was investigated by a central composite design (CCD) and two factors were selected: the substrate concentration based on VS (SC) and the proportion of OPW (% OPW) in the co-digestion mixture (based on VS). The response model presented a determined R^2 coefficient of 0.8437. Experimental results obtained from 9 different designed treatments showed an increase in specific methane production whenever the two factors are evaluated at their maximum levels.

A classification of fertilizers obtained from waste treated by anaerobic digestion according to Spanish normative (RD 999/2017) based on heavy metal concentration was carried out. Digestates from T3, T4 and T8 were included in the class A and the rest of digestates in the class B.

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