



## Batch optimization of biogas yield from pasteurized slaughterhouse by-products incorporating residues from corn sieving

María José Galván<sup>a</sup>, Salvador Degano<sup>a</sup>, Mara Cagnolo<sup>a</sup>, Analia Becker<sup>a,c</sup>, Jorge Hilbert<sup>d</sup>, Mauren Fuentes<sup>e</sup>, Diego Acevedo<sup>b,c,\*</sup>

<sup>a</sup> Centro de Investigaciones y Transferencia de Villa María (CIT-VM), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de Villa María, Villa María, Argentina

<sup>b</sup> Instituto de Investigaciones en Tecnologías Energéticas y Materiales Avanzados (IITEMA), Universidad Nacional de Río Cuarto, Facultad de Ingeniería-UNRC, Dpto. de Tecnología Química, Río Cuarto, Córdoba, Argentina

<sup>c</sup> Facultad de Ciencias Exactas Físico Químicas y Naturales, Universidad Nacional de Río Cuarto, Córdoba, Argentina

<sup>d</sup> INTA Castelar, Castelar, Buenos Aires, Argentina

<sup>e</sup> Instituto de Desarrollo y Diseño (INGAR), Universidad Tecnológica Nacional – Facultad Regional Santa Fe, Santa Fe, Argentina

### ARTICLE INFO

#### Keywords:

Anaerobic digestion  
Slaughterhouse waste  
Corn sieving waste  
C/N ratio

### ABSTRACT

Batch anaerobic digestion of pasteurized pig slaughterhouse waste (SW) and corn sieving waste (CSW), and anaerobic co-digestion of CSW/SW were studied at lab scale employing several carbon to nitrogen ratio (C/N) and total solids (TS) content. The mixtures with highest biogas yield and suitable process inhibition parameter values were used to scale up to pilot scale. The results showed that SW and CSW co-digestion improved biogas yield in comparison with that obtained from mono-digestion of both substrates. Thus, it is possible to ensure that the CSW is a proper substrate to balance C/N and improve biogas yield. Moreover, this study reveals that the best biogas yield for each C/N mixture is achieved using the lowest TS content. Also, the SW/CSW mixture with C/N 15 and 5% TS achieved the highest biogas yield and the best inhibition parameters values. The pilot scale assay demonstrates that biogas yield, methane yield and Organic Matter Removal for C/N 15 mixture were 41%, 25%, and 24% higher than those using C/N 20, respectively, and the H<sub>2</sub>, CO<sub>2</sub> gasses composition presented variations. However, the methane content was similar for both C/N 15 and C/N 20 at pilot scale.

### 1. Introduction

Biogas energy is a renewable energy source obtained when organic matter decomposes producing a mixture of carbon dioxide and methane gases (biogas) [1]. The biomass energy will play a key role in meeting the world's energy demand. The International Energy Agency (IEA) has predicted that bioenergy will be the renewable energy source with the greatest growth prospects, over the 2018–2023 period [2]. Furthermore, as a result of new renewable energy legislations, the biofuels production and utilization has grown in the last years. In this context, Argentina has promoted renewable energy production to diversify its national energy matrix by passing laws such as the Act 26093/2006 (Regime of regulation and promotion for biofuels production and sustainable use) and the Act 27191/2015 (National promotion regime for the use of renewable energy sources aimed at electric power production). For this reason,

many bioethanol plants started to operate using mainly corn as a feed-stock. Globally, bioethanol plants generate a solid waste from corn cleaning and sieving processes. This residue, here named Corn Sieving Waste (CSW), contains corn grain impurities that were set apart from the bioethanol process (soil, broken or damaged grains, small cob pieces, etc.). However, the CSW presents a high carbon content, mostly in the starch form, and a soil content that does not exceed 2% w/w, that make the CSW valuable to be used in Anaerobic Digestion (AD).

On the other hand, the pig slaughtering by-products are typical environmental liabilities of the Argentina's center region. The pig slaughtering by-products, here called Slaughterhouse Waste (SW), is generally converted into a flour and then commercialized at marginal prices, however SW can be used as a substrate for AD in order to produce biogas since it is an organic waste with high protein and lipid contents [3–7]. Nevertheless, the SW employed as raw material in biogas

\* Corresponding author. Instituto de Investigaciones en Tecnologías Energéticas y Materiales Avanzados (IITEMA), Universidad Nacional de Río Cuarto, Facultad de Ingeniería-UNRC, Dpto. de Tecnología Química, Río Cuarto, Córdoba, Argentina.

E-mail addresses: [dacevedo@ing.unrc.edu.ar](mailto:dacevedo@ing.unrc.edu.ar), [dacevedofermando@exa.com](mailto:dacevedofermando@exa.com) (D. Acevedo).

<https://doi.org/10.1016/j.biombioe.2021.106136>

Received 1 February 2021; Received in revised form 12 May 2021; Accepted 23 May 2021

Available online 10 June 2021

0961-9534/© 2021 Elsevier Ltd. All rights reserved.

**Table 1**  
Lab scale co-digestion assays.

C/N	TS (% w/w)	CSW/SW	Biogas Yield (L/g <sub>SV</sub> )	OMR (%VS)	VFA (g <sub>CH<sub>3</sub>COOH</sub> /L)	TA (g <sub>CaCO<sub>3</sub></sub> /L)	TAN (g/L)	FAN (g <sub>N-NH<sub>4</sub></sub> /L)
10	5	1.3:1	336.57 ± 5.34	62.54 ± 1.74	0.77 ± 0.15	4.44 ± 0.45	4.20 ± 0.85	2.30 ± 0.41
	10	1.3:1	121.83 ± 3.53	55.23 ± 2.74	1.11 ± 0.25	6.83 ± 0.85	4.70 ± 0.70	3.65 ± 0.44
	15	1.3:1	86.02 ± 4.02	46.08 ± 0.97	3.79 ± 0.28	4.53 ± 0.44	5.35 ± 0.66	3.95 ± 0.32
15	5	3.35:1	579.01 ± 6.85	73.91 ± 1.43	1.35 ± 0.18	8.27 ± 0.68	1.32 ± 0.82	1.08 ± 0.57
	10	3.35:1	232.77 ± 1.45	56.60 ± 0.77	3.03 ± 0.33	4.24 ± 0.22	4.10 ± 0.60	3.24 ± 0.61
	15	3.35:1	74.40 ± 1.68	29.66 ± 1.33	5.57 ± 0.09	4.89 ± 0.16	5.25 ± 0.88	3.45 ± 0.39
20	5	6.65:1	404.85 ± 3.33	57.63 ± 2.27	1.05 ± 0.05	7.98 ± 0.57	1.36 ± 0.50	1.19 ± 0.60
	10	6.65:1	109.87 ± 1.50	41.80 ± 1.58	4.52 ± 0.18	3.94 ± 0.07	4.71 ± 0.80	3.48 ± 0.46
	15	6.65:1	54.06 ± 2.47	19.15 ± 1.04	5.88 ± 0.14	4.52 ± 0.11	5.10 ± 0.73	4.47 ± 0.62
30	5	30.06:1	101.98 ± 3.33	24.18 ± 1.52	2.75 ± 0.11	4.54 ± 0.06	3.45 ± 0.91	2.28 ± 0.59
	10	30.06:1	78.96 ± 1.50	19.72 ± 0.36	4.70 ± 0.15	2.63 ± 0.08	4.60 ± 0.78	3.38 ± 0.50
	15	30.06:1	40.64 ± 0.59	19.03 ± 0.62	5.52 ± 0.09	4.89 ± 0.16	5.85 ± 0.73	4.45 ± 0.36

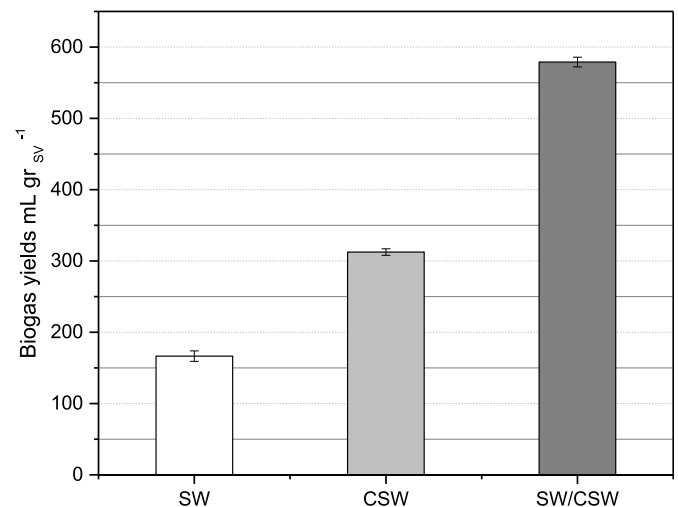
**Table 2**  
Substrate characteristics used for co-digestion and inoculum.

	Inoculum	SW	CSW
Total Solids, TS (% w/w)	2.25 ± 0.17	15.41 ± 0.75	86.16 ± 5.70
Volatile Solids, VS (% w/w) <sup>b</sup>	28.06 ± 0.38	90.54 ± 0.52	96.78 ± 0.91
Chemical Oxygen Demand, COD (g/L)	22.10 ± 1.65	200.78 ± 15.65	nm <sup>a</sup>
Biochemical Oxygen Demand, BOD <sub>5</sub> (g/L)	12.65 ± 3.58	71.40 ± 7.81	nm
BOD <sub>5</sub> /COD	0.54	0.35	nm
Nitrogen content, N (% w/w)	1.54 ± 0.31	9.76 ± 0.92	1.34 ± 0.18
Carbon content, C (% w/w)	16.31 ± 0.22	52.64 ± 0.30	48.22 ± 0.53
C/N	10.59	5.39	36.85
Proteins (% w/w) <sup>b</sup>	nm	61.00 ± 3.52	8.32 ± 1.15
Carbohydrates (% w/w) <sup>b</sup>	nm	12.54 ± 0.54	85.94 ± 3.12
Lipids (% w/w) <sup>b</sup>	nm	17.00 ± 0.06	2.52 ± 0.42
Volatile Fatty Acids, VFA (g <sub>CH<sub>3</sub>COOH</sub> /L)	0.51 ± 0.26	nm	nm
Total Alkalinity, TA (mg <sub>CaCO<sub>3</sub></sub> /L)	2.87 ± 0.52	nm	nm
pH	7.68 ± 0.52	7.35 ± 0.42	nm

<sup>a</sup> Not measured.<sup>b</sup> Dry basis.**Table 3**  
Lab scale monodigestion assays.

Substrate	Biogas yield (L/g <sub>SV</sub> )	OMR (% VS)
SW	169.20 ± 5.23	30.09% ± 3.11
CSW	315.68 ± 2.97	49.62% ± 1.34

production presents several drawbacks, such as: slow hydrolysis rates, foam generation, process inhibition on account of the high ammonia and long chain fatty acid (LCFA) concentrations [8–10]. An interesting strategy to counteract the inhibition caused by ammonia is to optimize the substrate composition, tuning the carbon/nitrogen ratio (C/N) [11]. Significant ammonia inhibition can be avoided anaerobically digesting simultaneously diverse substrates (with different C/N), in a process known as anaerobic co-digestion (AcoD). The AcoD could be considered a potential process innovation in biogas production in order to increase biogas yield [12–14]. Several authors demonstrated that one of the most important factors in AcoD is to maintain the C/N ratio between 20 and 30 [15–17]; however, other studies state that the optimum C/N value is 15 [18]. Moreover, S. Riya et al. [19] shown that a disruption in the C/N balance produces a negative effect on the microbial activity, resulting in a process depletion. A well-organized microbial community that generates high quality biogas is related to a proper C/N balance [20].

**Fig. 1.** SW (5% TS), CSW (5% TS) and SW/CSW (C/N 15 and 5% TS) biogas yields.**Table 4**  
Pilot scale co-digestion assays.

C/N	15	20
TS (% w/w)	5	5
CSW/SW	3.35:1	6.65:1
Biogas yield (mL/g <sub>SV</sub> )	602.84 ± 6.78	429.87 ± 4.60
Methane yield (mL/g <sub>SV</sub> )	316.94 ± 3.56	253.32 ± 2.71
OMR (%VS)	71.34 ± 1.29	57.33 ± 1.16
Final VFA (g <sub>CH<sub>3</sub>COOH</sub> /L)	0.92 ± 0.09	1.72 ± 0.11
Final TA (g <sub>CaCO<sub>3</sub></sub> /L)	7.01 ± 0.12	5.62 ± 0.09
VFA/TA	0.13	0.30
Final FAN (g/L)	0.87 ± 0.08	1.23 ± 0.10
Final TAN (g/L)	0.71 ± 0.11	1.00 ± 0.11

Rodriguez-Abalde et al. [21] used a mixture of pig slurry, pasteurized meat by-products and glycerin to carried out AcoD, obtaining superior methane yield and process efficiency in comparison with the only pig slurry anaerobic digestion.

Taking into account that in the best of our knowledge, the use of CSW as a substrate or co-substrate for AD is not reported in the literature, the aim of this paper is to evaluate the AcoD of CSW-SW in order to balance C/N and, thus, to optimize biogas yield by finding an appropriate substrate mixture.

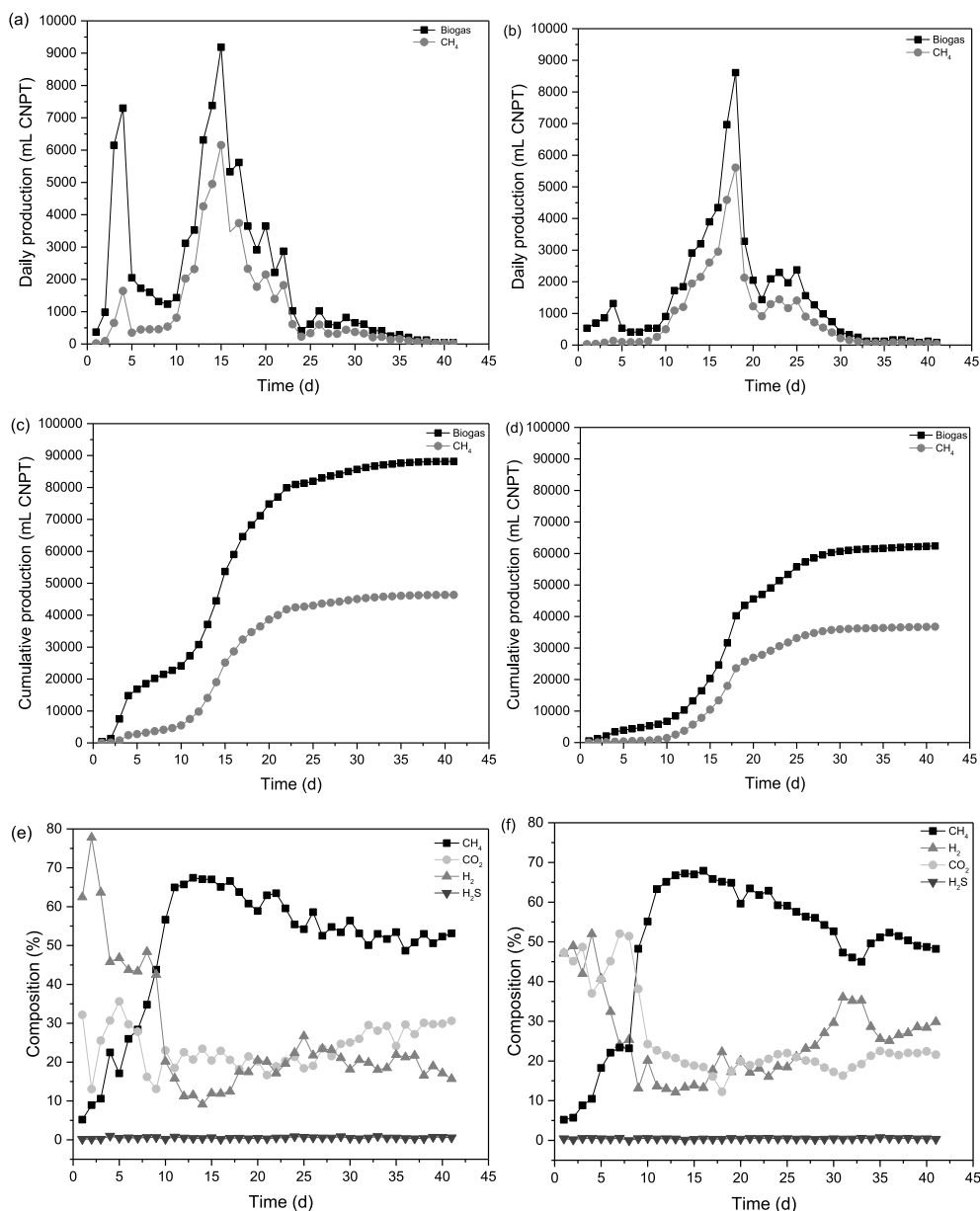


Fig. 2. Daily biogas and methane yield at pilot scale of 15 C/N (a) and 20 C/N (b); Cumulative biogas and methane yield at pilot scale of 15 C/N (c) and 20 C/N (d); Biogas composition at pilot scale of 15 C/N (e) and 20 C/N (f).

## 2. Materials and methods

### 2.1. Organic substrates and inoculum

Sludge from an active pilot scale bioreactor ( $37 \text{ }^{\circ}\text{C} \pm 1$  and 100 rpm) treating pig slurry was used as inoculum. The sampling and preparation was carried out according to the Verein Deutscher Ingenieure (VDI) 4630 method, section 5 [22]. The inoculum was conserved, degasified, and characterized according to the methodology proposed by Angelidaki et al. [23] and Holliger et al. [24].

The SW was composed of a solid fraction (30% w/w) of previously minced pig stomach, viscera, kidneys, lungs and livers and a liquid fraction (70% w/w) of pig blood. Both fractions were collected from a pig meat process industry located in Justiniano Posse, Córdoba, Argentina (Lat: S  $-32^{\circ}53'54''$  Long: W  $-62^{\circ}40'37''$ W). The SW was then pasteurized at  $70 \text{ }^{\circ}\text{C}$  for 1 h. The CSW was collected from a bioethanol production plant located in Villa María, Córdoba, Argentina (Lat: S  $-32^{\circ}41'54''$  Long: W  $63^{\circ}16'11''$ ). Both SW and CSW were dried at  $105 \text{ }^{\circ}\text{C}$

until the TS exceeded 95%, then were ground in a mill in order to obtain a particle size less than 10 mm and stored separately in zipper storage bags at room temperature. These pre-processing was done to avoid limitations due to biomass availability and to easy handling of samples.

### 2.2. Characterization of organic substrates and inoculum

The SW moisture content of solid samples, the SW total solids contain (TS) and the SW volatile solids contain (VS) were analyzed according to regulations 950.46, 950.46 and 923.153, respectively, issued by Association of Official Analytical Chemists (AOAC) [25]. The moisture content, TS and VS of the CSW solid sample were analyzed according to AOAC 950.10, 950.10 and 923.03 respectively. The TS, VS and Total Alkalinity (TA) of liquid samples were measured according to American Public Health and Association (APHA) Standard Methods 2540 B, 2540 E and 2320 B, respectively [28]. Also, the Volatile Fatty Acids (VFA) were measured according to Nordmann titration method. Furthermore, the pH was measured by HANNA HI 8424 electronic pH meter, the

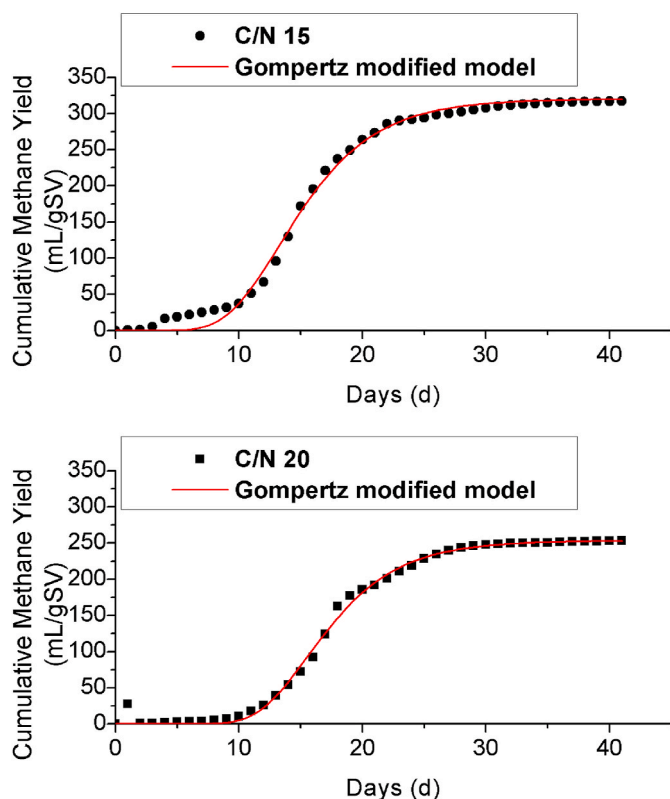


Fig. 3. Gompertz modified model fitting for 15 C/N and 20 C/N mixtures.

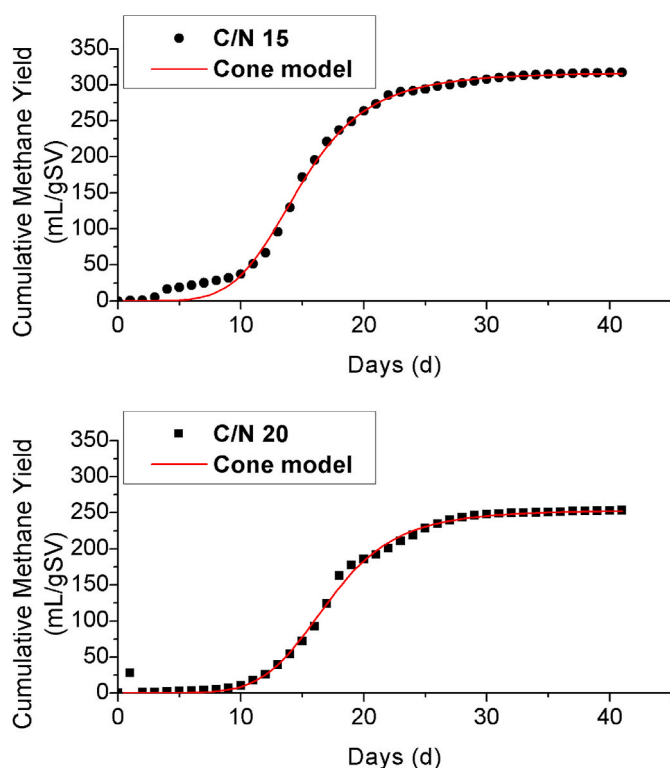


Fig. 4. Cone model fitting for 15 C/N and 20 C/N mixtures.

Chemical Oxygen Demand (COD), Total Ammonia Nitrogen (TAN) and Free Ammonia Nitrogen (FAN) were measured using HANNA spectrophotometer HI 83099 (Adaptation of USEPA 410.4 method for COD and Nessler method for TAN and FAN). Also, the Biological Oxygen Demand

Table 5

Modelling parameters of pilot scale batch tests.

	Parameter	15C/N	20C/N
Cone model	k	0.06764	0.05842
	n	5.26097	6.1646
Gompertz modified model	$\lambda$	8.99948	11.53679
	Rm	27.51159	23.11082

(BOD<sub>5</sub>) was analyzed using VELP BOD EVO Sensor System 6. Additionally, the lipids concentration was measured according to AOAC 960.39, the proteins concentration was determined by multiplying the Total Kjeldahl Nitrogen (TKN) (APHA 4500 B) by a conversion factor of 6.25 [6]; and the carbohydrates concentration was calculated as the difference between organic matter (as VS), lipids and the estimated protein content [7]. Besides, the organic carbon concentration was determined by considering an organic matter content to organic carbon ratio of 1.7241 [26].

### 2.3. Lab and pilot scale batch tests

The experimental set-up for batch assays was done according to Deutsches Institut für Normung (DIN) International Organization for Standardization (ISO) 11734 method [27], VDI 4630 method [28], Angelidaki et al. [23] and Holliger et al. [24] in order to determine biogas and methane yields of co-digestions. Several CSW/SW mixtures were made to carry out batch lab scale assays in order to obtain the desired C/N ratios. Each mixture was diluted at three different TS concentrations: 5%, 10% and 15%, see (Table 1).

In order to carry out lab AcoD experiments, each mixture was placed in 500 mL bottles with 100 mL of active inoculum, and were incubated at 37 °C into an orbital shaker at 100 rpm during 26 days. Each test was carried out in triplicate with blanks (inoculum + deionized water) and reference samples (microcrystalline cellulose). Also, the biogas yield, OMR, and inhibition parameters (VFA, TA, TAN and FAN) were measured. The biogas volume was measured daily by water displacement, and then it was converted at Standard Temperature and Pressure (STP) considering the guidelines provided by Walker et al. [29] and Strömberg et al. [30]. The mixtures that produce the higher biogas yield mixtures with a suitable inhibition parameter values were selected to be used as raw materials in the pilot scale assays due to they are consider as the best ones to produce biogas among all mixtures tested. Also, the SW and CSW with 5% TS were digested separately (mono-digested) using the same conditions to be used as references.

The scaled up assays were also run in batch and carried out in 5 L bioreactors including water-displacement gasometers to obtain larger and more representative biogas volumes than in lab scale assays in order to analyze their composition. Each bioreactor has temperature control device, rotating mixer with velocity control, and sensors that measure temperature and gas volume. The bioreactors were set-up at 37 °C±1 and 100 rpm. The biogas volume was measured daily and analyzed every 2 days to measure methane content. Methane, carbon dioxide, hydrogen and hydrogen sulfur content in the biogas were analyzed using Gas Chromatographer (Fuli Instrument) equipped with Thermal Conductivity Detector (TCD) and GDX-502 column (4 m × 3 mm). Also, the biogas yield, OMR and inhibition parameters (VFA, TA, TAN, FAN) were determined.

### 2.4. Kinetic analysis

The Gompertz modified model (Eq. (1)) and Cone model (Eq. (2)) were used to fit cumulative methane production observed for mixtures tested at pilot scale due to both model are widely used to model the batch methane production [31].

$$\text{Gompertz modified model } M(t) = M_m * e^{-e^{-\frac{Rm * t}{M_m}(\lambda - t) + 1}} \quad (1)$$

$$\text{Cone model } M(t) = \frac{M_m}{1 + (k^*t)^{-n}} \quad (2)$$

where  $M(t)$  is cumulative methane yield at a given time  $t$ ,  $M_m$  is the substrate maximum methane production,  $t$  is time,  $R_m$  is maximum specific methane production rate,  $e$  is Euler's number,  $\lambda$  is lag phase time,  $k$  is the hydrolysis rate constant, and  $n$  is the shape factor.

A nonlinear least-squares regression was used in order to determine parameters for all models [32]. The model kinetic parameters were determined and analyzed statistically using Microsoft Excel™ 2016 with a Solver add-in program (Microsoft, USA) by minimizing the RSS value and Origin V8.0 (OriginLab Corporation, USA) with a non-linear curve fitting of experimental data.

### 3. Results and discussion

#### 3.1. Inoculum and substrate characterization

Table 2 shows the inoculum and substrates characteristics and chemical composition used to carry out co-digestions. The substrates and inoculum composition showed in Table 2 is the average from measurements of different samples tooked over 2 years, also each measure was done in triplicate. It is possible to observe that inoculum presented proper characteristics in terms of VFA (<1 g CH<sub>3</sub>COOH/L), TA (>3 g CaCO<sub>3</sub>/L), and pH (7–8.5) [24]. Moreover, SW composition, VS and COD were similar to those presented by Palatsi et al. [7] and Hejnfelt and Angelidaki [6] but with different TS content. The COD and BOD<sub>5</sub> values obtained were high, and the BOD<sub>5</sub>/COD ratio showed a low OMR. Finally, the substrate characteristics used for co-digestion and inoculum presented a high protein and lipid content compared to other studies [33] and a C/N value matched those of substrates used by Mouskasis et al. [34]. Moreover, the CSW presented a high carbon content, which is appropriate to balance C/N ratio. Also, the CSW had high TS and VS content, which makes it suitable to combine with high moisture content wastes for AcoD. These values can not be compared to other studies due to the CSW characterization data and the CSW behavior in anaerobic digestion are not reported in the literature.

#### 3.2. Analysis of lab scale assays

Table 1 shows co-digestions composition and biogas yields obtained at lab scale, the OMR values and the inhibition parameters of all mixtures. It is possible to observe that TS of 5% achieved the highest biogas yields for each tested C/N mixtures. Also, the results obtained using C/N 10 showed that the biogas yields using 10% and 15% TS mixtures decreased 63.80% and 74.44%, respectively, compared with 5% TS mixture. In the case of C/N 15, the biogas yields obtained employing 10% and 15% TS mixtures dropped 59.80% and 87.15%, respectively, compared to 5% TS mixture. The data obtained for the experiences employing C/N 20 mixtures with 10% and 15% TS presented a decrease in biogas yields of 72.86% and 86.65%, respectively, when it is compared to 5% TS mixture. Finally, the biogas yields produced using the C/N 30 mixtures with 10% and 15% TS decreased 22.57% and 60.15%, respectively, in comparison to 5% TS for the same C/N. The results are in line with that obtained by other authors [6], and it is possible to observe that the same results are obtaining by adjusting the SW/CSW relationship.

When the best biogas yields among all tested C/N (TS 5%) is compared, is possible to observe that the 15C/N mixture reached the highest biogas yield. The best biogas yields for 10, 20 and 30C/N presented a reduction of 41.87%, 30.01% and 82.39%, respectively, in comparison with the C/N 15 best biogas yield. Similar studies differ in the optimum C/N ratio to produce biogas by means of AD or AcoD processes. In that sense, Rodríguez-Abalde et al. [21] determined that 10.3 was the optimum C/N ratio, while Riya et al. [19] fixed this value in

30. Also, Zheng et al. [35] proposed a C/N between 26.41 and 27.5 as ideal values, but Sievers and Brune [18] stated an optimum C/N value around 15. Based on the literature report it is possible to conclude that the optimum C/N ratio is not clear.

The VFA values for all mixtures with 5% TS were found to be around 1 g CH<sub>3</sub>COOH/L which was reported as a normal value for good quality digesters [24], except for C/N 30 which showed a concentration of 2.75 g CH<sub>3</sub>COOH/L, that present the lower biogas yield. Moreover, it possible to observe as TS increased, the VFA also increased, and both biogas yield and OMR decreased. The VFA increase and accumulation may indicate that organic matter fermented, and the methanogenic process was not occurring, probably due to bioreactor overloads (high organic matter content in the feed) that lead a pH drop, consequently the methanogens process inhibition.

TA value was higher for mixtures with C/N 15 and 20 and similar to those reported in the work by Rodríguez-Abalde et al. [21]. Moreover, FAN and TAN values for C/N 15 and 20 were within the stability range (below 2.5 g/L), while those for the other C/N mixtures were not. However, some authors established FAN and TAN limits are higher than those obtained in this work [36,37]. It is known that, high concentrations of ammonia (mostly in its free form) inhibits anaerobic digestion due to a proton imbalance in methanogens when it diffuses into the membrane cell [8].

The biogas yields and OMR of monodigestion lab scale assays are presented in Table 3. The C/N 15 and 5% TS mixture achieved the highest biogas yield, which was 3.47 and 1.85 times higher than biogas yield of SW monodigestion (5% TS) and CSW monodigestion (5% TS), respectively, as it can be seen in Fig. 1. Also, the OMR of 15C/N and 5% TS mixture was 3.42 and 2.84 times higher than SW monodigestion (5% TS) and CSW (5% TS), respectively. Thus, it is possible to conclude that co-digestion improved both biogas yield and OMR.

Based on the lab results (yields, inhibition values and OMR) the mixtures C/N 15 and 20 with 5% TS were chosen to scale up to 5 L, because they presented the best process performances among all mixtures tested.

#### 3.3. Analysis of pilot scale assays

Table 4 resumes the data obtained in the pilot scale co-digestion assays. Fig. 2a and b, show daily biogas and methane production for C/N 15 and 20 mixtures at pilot scale, respectively. The C/N 15 presented two biogas production spikes on day 5 and 15, while C/N 20 showed one biogas spike on day 18. This behavior shows that methanogens took more time to reach its maximum production for C/N 20 than for C/N 15 probable due to the more balanced relationship C/N 15 mixture (less VFA and FAN content), diminish the methanogens stress; therefore, the methane maximum production is obtained in shorter times.

Fig. 2c and d, show cumulative biogas and methane production for C/N 15 and 20 mixtures at pilot scale, respectively. The C/N 20 showed a longer lag phase than C/N 15 for both biogas and methane production. This result indicates a better bacteria adaptation when C/N 15 mixture is used, probably due to a proper C/N ratio without carbon excess that may cause environment acidification and/or nitrogen deficiency that could produce delay in bacteria growth. On the other hand, lag phase value was not higher than that reported by Palatsi et al. [7].

In Table 4 it is possible to observe the final values of inhibition parameters, biogas yields and OMR for both pilot scale assays. The biogas and methane yields and OMR for C/N 15 were 41%, 21% and 24% higher than C/N 20, respectively. The final FAN and TAN values of C/N 20 were increased to 29% when is compared to those for C/N 15. Also, the final VFA of C/N 20 was 1.87 times higher than that of C/N 15, while final TA of C/N 15 was 1.25 higher than that of C/N 20. This behavior could indicate that high carbon to nitrogen ratios may lead to an over-production of VFA, which could cause AD inhibition due to a pH decrease if the inoculum does not present high TA values. Also, as SW is

a protein-rich substrate, the SW overloads may lead to ammonia inhibition observed by high levels of FAN.

The daily biogas composition for each batch is shown in Fig. 2e and f. As it can be seen both batches presented similar methane and carbon dioxide production kinetics, maintaining methane concentration in a range between 50 and 65% until the assay was finished. However, the assay using C/N 20 the carbon dioxide composition, at the beginning of the assay, was higher than for C/N 15. This behavior could be probably due to a feeding overload, which could result from initial VFA accumulation. Also, at the beginning of the experiment, H<sub>2</sub> composition was higher for C/N 15 than for C/N 20, while the opposite situation was presented at the end of the experiment. Ward et al. [38] establish that an increased H<sub>2</sub> concentration may indicate digester overload, this observation is in agreement with the conclusion obtained from the CO<sub>2</sub> concentrations analysis.

In addition, between the days 20–40, the H<sub>2</sub> concentration did not present variations and the CO<sub>2</sub> concentration increased with time for C/N 15, while the opposite trend was observed for C/N 20. The H<sub>2</sub> increase indicates an organic disturbance caused by pulse overload [39] probably due to a reactivation of acidogenic and/or acetogenic processes after a short inhibition period, and the CO<sub>2</sub> increase may be due to a slightly acidogenic activity that remains in the reactor.

The Gompertz modified model and Cone model fitting of cumulative methane yield for pilot scale batch tests are plotted in Figs. 3 and 4, respectively. The R<sup>2</sup> obtained for both models were 0.99, which indicates good model fitness. Also, Table 5 shows both model parameters, for pilot scale batch test of C/N 15 and C/N 20 mixtures. Regarding Gompertz modified model, it can be seen that lag phase time ( $\lambda$ ) and maximum specific methane production rate (R<sub>m</sub>) for C/N 20 mixture were 28% higher and 16% lower, respectively, compared to C/N 15. Concerning the Cone model, the hydrolysis parameter (k) indicates the hydrolysis rate of organic matter [40,41], and taking into account the transformation rate of particulate organic matter into soluble organic matter, which could be considered as the rate-limiting step in anaerobic digestion process of particulate substrate [42]. For C/N 20, the k value decreased around 13% compared to C/N 15, showing that the degradation rate of organic matter is higher for C/N 15, probably due to the best C/N balance. These results show that C/N 15 mixture presented better kinetics parameter values than C/N 20 in relation to cumulative methane yield.

#### 4. Conclusion

Anaerobic digestion of pig meat byproducts presents several drawbacks when they are used as a mono-substrate. Different AD and AcoD assays carried out in this work showed that CSW could be a proper substrate to co-digest with SW in order to balance C/N and improve biogas yield. The Lab scale assays showed higher biogas yields when SW and CSW are digested together at low TS concentration due to a gradual C/N adjusting. Furthermore, the pilot scale assays of the best mixtures tested at lab scale, revealed that C/N 15 mixture presented the highest biogas and methane yields. The results allow to conclude that the AcoD synergy needs to be further studied at continuous pilot scale to provide new data, and thus, to improve biogas quality and AD stability.

#### Acknowledgements

D.F. Acevedo, and M. Fuentes are permanent research fellows of CONICET. Jorge Hilbert is permanent staff of INTA. M.J. Galván, S. Degano, M. Cagnolo, thank CONICET for a graduate fellowship. The funding of FONCYT (PICT 4627/2018, FONARSEC- FITR ENERGIA 0004/2013, ANPCyT), CONICET(PIP 2014, No. 11220130100663CO), and SECYT-UNRC, SECYT- UNVM is gratefully acknowledged. The help of R. Manno is gratefully acknowledged.

#### References

- [1] T. Abbasi, S.M. Tauseef, S.A. Abbasi, *Biogas and Biogas Energy: an Introduction*, Springer, New York, 2012.
- [2] Z. Wang, Q. Bui, B. Zhang, T.L.H. Pham, Biomass energy production and its impacts on the ecological footprint: an investigation of the G7 countries, *Sci. Total Environ.* 743 (2020) 140741.
- [3] M.A. Pereira, O.C. Pires, M. Mota, M.M. Alves, Anaerobic degradation of oleic acid by suspended and granular sludge: identification of palmitic acid as a key intermediate, *Water Sci. Technol.* 45 (10) (2002) 139–144.
- [4] Y. Suzuki, Y. Tsujimoto, H. Matsui, K. Watanabe, Decomposition of extremely hard-to-degrade animal protein by thermophilic bacteria, *Biosci. and Bioeng.* 102 (2) (2006) 77–81.
- [5] V. Vavilin, B. Fernández, J. Palatsi, X. Flotats, Hydrolysis kinetics in anaerobic degradation of particulate organic material, *Waste Manag.* 28 (6) (2008) 939–951.
- [6] A. Hejnfelt, I. Angelidaki, Anaerobic digestion of slaughterhouse by-products, *Biomass Bioenergy* 33 (8) (2009) 1046–1054.
- [7] J. Palatsi, M. Vinas, B. Fernández, X. Flotats, Anaerobic digestion of slaughterhouse waste: main process limitations and microbial community interactions, *Bioresour. Technol.* 102 (3) (2011) 2219–2227.
- [8] Y. Chen, J.J. Cheng, K.S. Creamer, Inhibition of anaerobic digestion process: a review, *Bioresour. Technol.* 99 (10) (2008) 4044–4064.
- [9] R. Rajagopal, D.I. Massé, G. Singh, A critical review on inhibition of anaerobic digestion process by excess ammonia, *Bioresour. Technol.* 143 (2013) 632–641.
- [10] N. Rasit, A. Idris, R. Harun, W. Ghani, Effect of lipid inhibition on biogas production of anaerobic digestion from oily effluents and sludges: an overview, *Renew. Sustain. Energy Rev.* 45 (2015) 351–358.
- [11] X. Wang, X. Lu, F. Li, G. Yang, Effects of temperature and carbon-nitrogen (C/N) ratio on the performance of anaerobic co-digestion of dairy manure, chicken manure and rice straw: focusing on ammonia inhibition, *PLoS One* 9 (5) (2014), e97265, <https://doi.org/10.1371/journal.pone.0097265>.
- [12] W.M. Budzianowski, A review of potential innovations for production, conditioning and utilization of biogas with multiple-criteria assessment, *Renew. Sustain. Energy Rev.* 54 (2016) 1148–1171.
- [13] M.J. Cuetos, C. Fernández, X. Gómez, A. Morán, Anaerobic co-digestion of swine manure with energy crop residues, *Biotechnol. Bioproc. Eng.* 16 (1044) (2011) 1044–1052.
- [14] R.A. Labatut, L.T. Angenent, N.R. Scott, Biochemical methane potential and biodegradability of complex organic substrates, *Bioresour. Technol.* 102 (3) (2011) 2255–2264.
- [15] C. Zhang, G. Xiao, L. Peng, H. Su, T. Tan, The anaerobic co-digestion of food waste and cattle manure, *Bioresour. Technol.* 129 (2013) 170–176.
- [16] X. Wang, G. Yang, Y. Feng, G. Ren, X. Han, Optimizing feeding composition and carbon–nitrogen ratios for improved methane yield during anaerobic co-digestion of dairy, chicken manure and wheat straw, *Bioresour. Technol.* 120 (2013) 78–83.
- [17] J. Zhu, Y. Zheng, F. Xu, Y. Li, Solid-state anaerobic co-digestion of hay and soybean processing waste for biogas production, *Bioresour. Technol.* 154 (2014) 240–247.
- [18] D.M. Sievers, D.E. Brune, Carbon/nitrogen ratio and anaerobic digestion of swine waste, *Transactions of the ASAE* 21 (1978) 537–541.
- [19] S. Riya, K. Suzuki, A. Terada, M. Hosomi, S. Zhou, Influence of C/N ratio on performance and microbial community structure of dry-thermophilic anaerobic codigestion of swine manure and rice straw, *Med. Bioengin.* 5 (1) (2016) 11–14.
- [20] R. Xu, K. Zhang, P. Liu, A. Khan, J. Xiong, F. Tian, X. Li, A critical review on the interaction of substrate nutrient balance and function in anaerobic co-digestion, *Bioresour. Technol.* 247 (2018) 1119–1127.
- [21] A. Rodríguez-Abalde, X. Flotats, B. Fernández, Optimization of the anaerobic co-digestion of pasteurized slaughterhouse waste, pig slurry and glycerine, *Waste Manag.* 61 (2017) 521–528.
- [22] Vdi 4630, *Fermentation of Organic Materials – Characterization of the Substrate, Sampling, Collection of Material Data, Fermentation Tests, VDI-Handbuch Energietechnik*, 2016.
- [23] I. Angelidaki, M. Alves, D. Bolzonella, L. Bprzacconi, J.L. Campos, A.J. Guwy, S. Kalyuzhnyi, P. Jenicek, J.B. Van Lier, Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays, *Water Sci. Technol.* 59 (5) (2009) 927–934.
- [24] C. Holliger, M. Alves, D. Andrade, I. Angelidaki, S. Astals, U. Baier, C. Bougrier, P. Buffiere, M. Carballa, V. Wilde, F. Ebertseder, B. Fernández, E. Ficara, I. Fotidis, J. Frigon, H. Fruteau de Lacos, D.S.M. Ghasimi, G. Hack, M. Hartel, J. Heerenklage, I.S. Horvarth, P. Jenicek, K. Koch, J. Krautwald, J. Lizasoain, J. Liu, L. Mosberger, M. Nistor, H. Oechsner, J.V. Oliveira, M. Peterson, A. Paus, S. Pommier, I. Porqueddu, F. Raposo, T. Ribeiro, F.R. Pfund, S. Stromberg, M. Torrijos, M. van Eekert, J. van Lier, H. Wedwitschka, I. Wierinck, Towards a standardization of biomethane potential tests, *Water Sci. Technol.* 74 (11) (2016) 2515–2522.
- [25] Association of Official Analytical Chemists, in: *Official Methods of Analysis of AOAC INTERNATIONAL, Three-Volume Set, twenty-first ed.*, 2019.
- [26] M.J. Cuetos, X. Gómez, M. Otero, A. Morán, Anaerobic digestion of solid slaughterhouse waste (SHW) at laboratory scale: influence of co-digestion with the organic fraction of municipal solid waste (OFMSW), *Bioch. Eng.* 40 (1) (2008) 99–106.
- [27] DIN EN ISO 11734, *Water Quality – Evaluation of the ‘ultimate’ Anaerobic Biodegradability of Organic Compounds in Digested Sludge – Method by Measurement of the Biogas Production*, Deutsches Institut für Normung, 1998.
- [28] American Public Health Association, *Standard Methods for the Examination of Water and Wastewater*, twentieth ed., APHA, 1998.

- [29] M. Walker, Y. Zhang, S. Heaven, C. Banks, Potential errors in the quantitative evaluation of biogas production in anaerobic digestion processes, *Bioresour. Technol.* 100 (24) (2009) 6339–6346.
- [30] S. Strömberg, M. Nistor, J. Liu, Towards eliminating systematic errors caused by the experimental conditions in Biochemical Methane Potential (BMP) tests, *Waste Manag.* 34 (11) (2014) 1939–1948.
- [31] D.D. Nguyen, B. Jeon, J.H. Jeung, E.R. Rene, J.R. Banu, B. Ravindran, C.M. Vu, H. H. Ngo, W. Guo, S.W. Chang, Thermophilic anaerobic digestion of model organic wastes: evaluation of biomethane production and multiple kinetic model analysis, *Bioresour. Technol.* 280 (2019) 269–276.
- [32] J. Pagés-Díaz, I. Pereda-Reyes, J.L. Sanz, M. Lundin, M.J. Taherzadeh, Sárvári-I. Horváth, A comparison of process performance during the anaerobic mono- and co-digestion of slaughterhouse waste through different operational modes, *J. Environ. Sci.* 64 (2018) 149–156.
- [33] R. Del Pozo, D. Okutman Tas, H. Dulkadirog lu, D. Orchon, V. Diez, Biodegradability of slaughterhouse wastewater with high blood content under anaerobic and aerobic conditions, *Chem. Technol. Biotechnol.* 78 (2003) 384–391.
- [34] F. Moukasis, E. Pellera Gidaracos, Slaughterhouse by products treatment using anaerobic digestion, *Waste Manag.* 71 (2018) 652–662.
- [35] Z. Zheng, J. Liu, X. Yuan, X. Wang, W. Zhu, F. Yang, Z. Cui, Effect of dairy manure to switchgrass co-digestion ratio on methane production and the bacterial community in batch anaerobic digestion, *Appl. Energy* 151 (2015) 249–257.
- [36] H. Siegrist, W. Hunziker, H. Hofer, Anaerobic digestion of slaughterhouse waste with UF-membrane separation and recycling of permeate after free ammonia stripping, *Water Sci. Technol.* 52 (1–2) (2005) 531–536.
- [37] M. Ortner, K. Leitzinger, S. Skupien, G. Bochmann, W. Fuchs, Efficient anaerobic mono-digestion of N-rich slaughterhouse waste: influence of ammonia, temperature and trace elements, *Bioresour. Technol.* 174 (2014) 222–232.
- [38] A. Ward, P. Hobbs, P. Holliman, D. Jones, Optimization of the anaerobic digestion of agricultural resources, *Bioresour. Technol.* 99 (2008) 7928–7940.
- [39] F. Molina, M. Castellano, C. García, E. Roca, J.M. Lema, Selection of variables for on-line control of anaerobic digestion processes, *Water Sci. Technol.* 60 (3) (2009) 615–622.
- [40] S. Sarto, R. Hildayati, I. Syaichurrozi, Effect of chemical pretreatment using sulfuric acid on biogas production from water hyacinth and kinetics, *Renew. Energy* 132 (2019) 335–350.
- [41] A.A. Ajayi-Banji, S. Sunoj, C. Igathinathane, S. Rahman, Kinetic studies of alkaline-pretreated corn stover co-digested with upset dairy manure under solid-state, *Renew. Energy* 163 (2021) 2198–2207.
- [42] Y. Zhang, Z. Yang, R. Xu, Y. Xiang, M. Jia, J. Hu, Y. Zheng, W. Xiong, J. Cao, Enhanced mesophilic anaerobic digestion of waste sludge with iron nanoparticles addition and kinetic analysis, *Sci. Total Environ.* 683 (2019) 124–133.