# Efficient poultry manure management: anaerobic digestion with short hydraulic retention time to achieve high methane production

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ABSTRACT The efficient treatment or appropriate final disposal of poultry manure (PM) to avoid serious environmental impacts is a great challenge. In this work, the optimization of a 2-stage anaerobic digestion system (ADS) for PM was studied with the aim of reaching a maximal methane yield with a short hydraulic retention time (HRT). Three activities were performed: The first activity, ADS 1, consisted of evaluating the effect of the substrate concentration and the HRT on the process, with a constant organic loading rate (**OLR**) of  $3.66 \pm 0.21$  gVS L<sup>-1</sup> d<sup>-1</sup>. The second activity, ADS 2, consisted of decreasing the HRT from 9.09 to 2.74 d with a constant substrate concentration. In the third activity, ADS 3, the substrate concentration was increased from 10.09  $\pm$  1.41 to 35.25  $\pm$ 6.20 gVS  $L^{-1}$  with an average HRT of 4.66  $\pm$  0.11 d.

Maximal methane yields of 0.22, 0.21, and 0.22  $LCH_4 \text{ gVS}^{-1}$  were reached for ADS 1, ADS 2,

and ADS 3, respectively, at a low HRT (3.38 to 4.66 d) and high free ammonia concentration (between 323.05  $\pm$  56.48 and 460.93  $\pm$  135.40 mgN-NH<sub>3</sub> L<sup>-1</sup>). These methane yields correspond to the production of 40.36 and 42.28 cubic meters of methane per ton of PM, respectively, and a laying hen produces between 47.45 and 54.75 kg of PM per year in Chile.

Finally, this is the first study of the separate and combined effects of OLR, HRT and substrate concentration on the anaerobic digestion of PM. The results demonstrate the technical feasibility of the two-stage ADS treatment of PM with a short HRT; the system tolerates variations in the total ammonia nitrogen concentration of PM throughout the year and achieves a high methane yield when the correct operational conditions are selected.

Key words: poultry manure, waste valorization, energy, anaerobic digestion, inhibition

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### INTRODUCTION

Poultry manure (**PM**) is an organic waste generated by the poultry industry that carries several impacts on the environment if it is disposed inadequately due to air, water and soil contamination caused by released odors and gases or by its content of nitrogen and pathogens (Yetilmezsoy and Sakar, 2008; Roeckel et al., 2017; Pizarro et al., 2019). The efficient treatment or final disposal of PM is a great challenge. The Chilean poultry industry has 47.7 million birds, of which 26.7% are designated for egg production, i.e., 12.7 million laying hens (ODEPA, 2018), and a laying hen produces between 47.45 and 54.75 kg of manure per year.

The high content of organic matter makes PM an adequate substrate for anaerobic digestion (**AD**) processes, which lead to pathogen stabilization and waste valorization by the production of methane. AD is a very complex process carried out by different anaerobic microorganisms, involves several biochemical reactions and has different requirements, such as substrate affinity, inhibitors, optimum pH, and temperature. In brief, the main reactions are grouped into four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis; therefore, it is necessary to reach the correct balance between these reactions (Angelidaki et al., 1990; Pavlostathis and Giraldo-Gomez, 1991).

Volatile fatty acid (**VFA**) concentrations over 6 gVFA  $L^{-1}$  exert an inhibitory effect on biogas production (Siegert and Banks, 2005). VFA build-up is a consequence of an organic overload of the system rather than an inhibition since the VFA consumption rate is lower than the VFA production rate. This situation affects the buffering capacity of the system, decreasing methane production. This imbalance can be detected easily and quickly by alkalinity measurements (Pérez and Torres, 2008), which give information about VFA accumulation. To avoid VFA accumulation, it is useful

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to work with a 2-stage anaerobic digester since this configuration allows the system to work with optimal conditions for each bacterial group, giving time for methanogens to consume VFA produced in the previous stage. Many authors have studied the optimal configuration for a 2-stage anaerobic digestion system (**ADS**), and they determined that a thermophilic-mesophilic configuration gives higher methane production and organic matter removal (Lo et al., 1986; Dugba and Zhang, 1999; Zhang et al., 2000).

When treating organic wastes such as food wastes, poultry and swine manure, it is possible to reach high methane yields, over  $0.18 \text{ m}^3 \text{ CH}_4 \text{ kgVS}^{-1}$  (Hansen et al., 1998; Chae et al., 2008; Nagao et al., 2012), but long reaction times are required, i.e., hydraulic retention times (**HRTs**) or batch operation experiments of over 15 d.

The maximal methane yield reported for PM is  $0.27 \text{ m}^3\text{CH}_4 \text{ kgVS}^{-1}$  (Huang and Shih, 1981). Some authors achieved good performance for the AD of PM, near the maximal methane yield, but used long **HRTs**, over 20 d (Li et al., 2014; Bayrakdar et al., 2017), which involves high investment and operational costs. There is not enough information available on the AD of PM with a low HRT. Since PM is a solid waste, the main difficulty in applying a low HRT is the high solids content, including egg shells, feathers, and other residues, which causes tube clogging and solids build-up in the reactor.

Moreover, one of the bottlenecks of the AD of PM is the elevated content of total ammonia nitrogen (TAN), which inhibits biogas production (Roeckel et al., 2017). In solution, ammonia  $(NH_3)$  is in equilibrium with ammonium  $(NH_4^+)$ , which is called TAN. Of  $NH_3$  and  $NH_4^+$ , the main inhibitor of anaerobic digestion is free ammonia  $(NH_3, FA)$  since FA is a unionized molecule capable of passively diffusing through the cellular membrane of biomass and altering the process. The proposed mechanism of inhibition is a change in intracellular pH, an increase in maintenance energy requirements and the inhibition of a specific enzymatic reaction (Wittmann et al., 1995). Otherwise, among the four types of anaerobic microorganisms, methanogens are the least tolerant and the most likely to cease growth due to ammonia inhibition (Chen et al., 2008).

According to the above, to achieve sustainable and efficient PM management, it is necessary to improve methane production, and this is possible by avoiding TAN inhibition and tube clogging when operating at short HRTs. Additionally, a low HRT has great importance in the investment costs of these systems since at a lower HRT, smaller reactors will be required to treat a specific amount of PM. The aim of this work is to maximize the methane yield of the AD of PM under a high TAN concentration, from 0.67 to 3.73 gN-TAN L<sup>-1</sup>, and short HRT in a two-stage ADS, which will allow the reduction of the costs of this process by decreasing the operating unit size and by producing energy from this waste.

### MATERIALS AND METHODS

### Substrate

The raw material used was PM from laying hens, provided by Avícola Coliumo, an associated local company. Once the necessary supply was received, it was diluted and prepared to be fed to the digester. As PM has a high content of solids and impurities, a process of dilution and filtration was carried out to remove excess sand, feathers and other impurities. From this treatment, a concentrated substrate was obtained, with a TS content of approximately 7%. Then, the substrate was prepared at the desired concentration according to the different operational conditions needed for each activity.

### Anaerobic Digestion System

A continuous-feed 2-stage ADS was used to treat the PM (Figure 1). Both stages were performed in an upflow anaerobic sludge blanket agitated by gas recirculation and are provided of a jacket heat transfer system where heated water circulate to maintain the desired temperature constant. The first-stage reactor had a working volume of 1 L and was operated at 55°C (hydrolytic stage), and the second stage had a working volume of 4.32 L and was operated at 35°C (methanogenic stage). The substrate was kept in a feed tank, cooled at 4°C and mechanically agitated at 250 rpm.

The ADS was inoculated with biomass from a mesophilic one-stage AD reactor; therefore, an acclimation period was necessary.

## Experimental Design

In this work, 3 activities were carried out to determine the different optimal operating parameters of an ADS fed with PM, with the aim of maximizing the methane yield.

In the first activity (ADS 1), the effect of the substrate concentration and the HRT (corresponding to the global HRT, i.e., the sum of both stages) on the process was evaluated stepwise, with a constant OLR of  $3.66 \pm$ 0.21 gVS L<sup>-1</sup> d<sup>-1</sup>. Then, the substrate concentration and the HRT were increased in each step. Four conditions were assayed for this activity, as shown in Table 1. This activity provided the optimal substrate concentration and HRT to evaluate in the following assays.

During the second activity (**ADS 2**), the effect of HRT on ADS efficiency was evaluated. The global HRT was decreased from 9.09 to 2.74 d, and 5 conditions were evaluated (9.09, 5.97, 4.38, 3.38, and 2.74 d). An average substrate concentration in the feed flow of 18.46  $\pm$  2.56 gVS L<sup>-1</sup> during the whole activity was used.

In the third activity (**ADS 3**), the effect of the substrate concentration on the ADS efficiency was evaluated. The OLR was increased by increasing the substrate concentration from  $10.09 \pm 1.41$  to  $35.25 \pm 6.20$  gVS L<sup>-1</sup> with an average HRT of  $4.66 \pm 0.11$  d. Five conditions were evaluated (shown in Table 1).



Figure 1. Scheme of the anaerobic digestion system. The hydrolytic stage has a working volume of 1 L and was operated at 55°C. The methanogenic stage has a working volume of 4.32 L and was operated at 35°C. Agitation of both stages was performed by gas recirculation.

# Analytical Methods

Samples from each ADS were collected twice a week to measure nitrogen compounds (TAN,  $NO_2^-$  and  $NO_3^{-}$ ), total organic carbon (**TOC**), chemical oxygen demand (COD), total solids (TS), volatile solids (VS), pH and alkalinity. Nitrogen compounds were spectrophotometrically measured with a flow injection analyzer (FIAlab, 2500/2700, 1.0607, Seattle, WA, USA) using a USB400-VIS-NIR detector (Sánchez et al., 2005), TOC was measured via combustion analysis followed by a nondispersive infrared gas analyzer (Shimadzu, TOC-5000<sup>a</sup>, Japan). For both analyses, samples were filtered through 0.45- $\mu m$  cellulose. COD, TS and were measured according to standard methods. Measurements of pH were carried out with a pH meter (UB-10, Denver Instrument, Denver, CO, USA). Alkalinity was determined according to Pérez and Torres (2008). The biogas flow was measured by liquid displacement of the released biogas by overpressure, and the biogas composition was determined by a gas chromatograph (HP 5890 Series II, Hewlett Packard, Avondale, PA, USA) equipped with a Porapak Q column, 80/100 mesh.

### Calculations

The FA concentration in solution is a function of the TAN concentration, temperature, and pH and can be determined by the following equation (Anthonisen et al., 1976):

$$\mathrm{NH}_3 - \mathrm{N}\left[rac{\mathrm{mg}\,\,\mathrm{N}}{\mathrm{L}}
ight] = rac{\mathrm{TAN}\left[rac{\mathrm{mg}\,\,\mathrm{N}}{\mathrm{L}}
ight] imes 10^{\mathrm{pH}}}{\mathrm{e}^{rac{6344}{(273+\mathrm{T})}}+10^{\mathrm{pH}}}$$

For the methane yield determination, to normalize the biogas flow to standard conditions, a correction factor  $(C_N)$  was calculated according to the following equation:

$$C_N = \frac{V_N}{V} = \frac{(p - p_{H_2O}) \cdot T_N}{p_N \cdot T}$$

where:

- V<sub>N</sub>: gas volume under standard conditions
- V: gas volume at the experimental pressure and temperature
- p: experimental atmospheric pressure (mbar)
- $p_{H_2O}$ : water pressure at the experimental temperature (mbar)
- $p_N$ : standard pressure = 1013.25 mbar

 $T_N$ : standard temperature = 273.15 K

T: experimental temperature (K)

The biogas flow was measured at an average temperature of 294 K, the average pressure was 1013 mbar and the water pressure at these conditions was 24.88 mbar. Then, experimental measurements of biogas flow were normalized to  $C_{\rm N} = 0.906$ .

The methane yield was calculated as follows:

$$Y_{CH_4} = \frac{Q_{biogas} \cdot \frac{\% CH_4}{100}}{OLR \cdot V_R} \cdot C_N \left[ \frac{L_{CH_4}}{gVS \text{or}gCOD} \right]$$

where  $Q_{biogas}$  is the measured biogas flow (L d<sup>-1</sup>), %CH<sub>4</sub> is the methane percentage measured by gas chromatography, OLR is the organic loading rate (expressed as gVS L<sup>-1</sup> d<sup>-1</sup> or g COD L<sup>-1</sup> d<sup>-1</sup>) and V<sub>R</sub> is the total working volume of ADS, equal to 5.32 L.

## **RESULTS AND DISCUSSION**

# Effects of Substrate Concentration and HRT with Constant OLR

During the first activity (ADS 1), the efficiency of the anaerobic digestion of PM was studied. The substrate concentration and the HRT were increased while keeping a constant organic loading rate (OLR), between 3.42 to 3.86 gVS  $L^{-1}d^{-1}$ , with a variation below 10% (see Table 1). ADS 1 was carried out during 81 d of operation. The results revealed a maximum methane

sludge blanket (UASB). The first reactor was operated at 55°C (working volume of 1 L), and the second reactor was operated at 35°C (working volume of 4.32 L). The substrate system 1; the methane yield was evaluated with a constant OLR. ADS 2: Anaerobic digestion system 2; the effect of HRT on **Fable 1.** Summary of operational conditions and results. The study was carried out in an anaerobic digestion system that has 2 stages, both consisting of an upflow anaerobic methane yield and VS removal was evaluated. ADS 3: Anaerobic digestion system 3; the effect of substrate concentration on methane yield and VS removal was evaluated. OLR: organic loading rate; HRT: hydraulic retention time, corresponding to the global HRT of the system; TAN: total ammonia nitrogen; VS: volatile solids; COD: chemical oxygen was poultry manure. ADS 1: Anaerobic digestion demand.

Reactor	$OLR gSV/Ld (gCOD/Ld)^1$	HRT d	${ m TAN_{input}}{ m gN-TAN/L}$	${ m VS_{input}}{ m gVS/L}$	${ m COD}_{ m input}$ gCOD/L	VS removal $\%$	COD removal %	Methane Yield LCH <sub>4</sub> /gVS (LCH <sub>4</sub> /gCOD) <sup>1</sup>
ADS 1	3.55(7.46)	2.88	$1.39 \pm 0.13$	$10.23 \pm 2.29$	$21.49 \pm 2.17$	$56.74 \pm 13.33$	$69.97 \pm 4.35$	$0.16 \pm 0.01 \ (0.07 \pm 0.01)$
ADS 1	3.87 (7.14)	4.30	$2.03 \pm 0.36$	$16.61 \pm 3.59$	$30.70 \pm 0.55$	$62.91 \pm 10.63$	$64.70 \pm 1.88$	$0.22 \pm 0.01 \ (0.12 \pm 0.01)$
ADS 1	3.81(7.49)	5.75	$2.44 \pm 0.26$	$21.88 \pm 2.32$	$43.00 \pm 4.80$	$68.62 \pm 7.62$	$72.26 \pm 2.87$	$0.14 \pm 0.01 \ (0.07 \pm 0.01)$
ADS 1	3.42(7.15)	8.21	$2.08 \pm 0.64$	$28.10 \pm 4.86$	$58.74 \pm 16.48$	$66.16 \pm 8.93$	$65.97 \pm 10.66$	$0.10 \pm 0.01 \ (0.05 \pm 0.01)$
ADS 2	1.75(2.67)	9.09	$0.74 \pm 0.61$	$15.88 \pm 2.81$	$24.24 \pm 2.87$	$83.65 \pm 0.62$	$82.02 \pm 6.35$	$0.08 \pm 0.01 \ (0.05 \pm 0.01)$
ADS 2	2.96(3.87)	5.97	$0.77 \pm 0.32$	$17.70 \pm 2.88$	$23.13 \pm 2.87$	$80.40 \pm 3.38$	$78.22 \pm 5.56$	$0.10 \pm 0.01 (0.08 \pm 0.01)$
ADS 2	4.06(7.02)	4.38	$1.35 \pm 0.44$	$17.78 \pm 1.97$	$30.75 \pm 6.55$	$69.97 \pm 6.09$	$71.31 \pm 4.34$	$0.09 \pm 0.01 \ (0.05 \pm 0.01)$
ADS 2	5.38(8.03)	3.38	$2.10~\pm~0.35$	$18.17 \pm 0.04$	$27.14 \pm 3.79$	$61.12 \pm 8.51$	$69.65 \pm 5.05$	$0.21 \pm 0.01 (0.14 \pm 0.01)$
ADS 2	$8.31 \ (11.55)$	2.74	$2.02 \pm 0.46$	$22.76 \pm 3.70$	$31.63 \pm 1.54$	$54.72 \pm 10.34$	$69.41 \pm 6.38$	$0.14 \pm 0.01 \ (0.10 \pm 0.01)$
ADS 3	2.12(3.44)	4.76	$0.67 \pm 0.20$	$10.09 \pm 1.41$	$16.39 \pm 3.86$	$71.60 \pm 6.23$	$71.97 \pm 10.75$	$0.07 \pm 0.01 (0.05 \pm 0.01)$
ADS 3	3.04(4.77)	4.73	$0.86 \pm 0.11$	$14.35 \pm 3.97$	$22.53 \pm 7.49$	$80.47 \pm 3.05$	$72.43 \pm 5.48$	$0.07 \pm 0.01 (0.04 \pm 0.01)$
ADS 3	4.03(6.48)	4.68	$1.26 \pm 0.47$	$20.15 \pm 6.78$	$30.36 \pm 3.34$	$79.34 \pm 8.01$	$77.23 \pm 4.11$	$0.09 \pm 0.01 \ (0.06 \pm 0.01)$
ADS 3	5.54(8.93)	4.66	$2.07 \pm 0.42$	$25.81 \pm 3.07$	$41.58 \pm 6.38$	$61.04 \pm 11.83$	$63.85 \pm 7.08$	$0.22 \pm 0.01 \ (0.13 \pm 0.01)$
ADS 3	7.89(12.77)	4.47	$3.73 \pm 0.56$	$35.25 \pm 6.20$	$57.01 \pm 3.60$	$59.14 \pm 2.13$	$52.89 \pm 12.71$	$0.15 \pm 0.01 \ (0.09 \pm 0.01)$

yield of  $0.22 \pm 0.01 \text{ LCH}_4 \text{ kgVS}_{\text{in}}^{-1} (0.12 \pm 0.01 \text{ LCH}_4 \text{ kgCOD}_{\text{in}}^{-1})$  when operating at a substrate concentration of  $16.61 \pm 3.59 \text{ gVS L}^{-1} (30.70 \pm 0.55 \text{ gCOD L}^{-1})$ , a TAN concentration of  $2.03 \pm 0.36 \text{ g}$  N-TAN L<sup>-1</sup> and an HRT of 4.3 d. The VS removal was not affected by the substrate or the TAN concentration (Table 1). Then, the subsequent activities were carried out based on these results.

# Effect of HRT

During the second activity (ADS 2), the OLR was increased by decreasing the HRT while the substrate concentration remained constant and corresponded to the optimal substrate concentration found in ADS 1 (Table 1). This activity was evaluated during 182 d of operation. The results of ADS 2 show that the efficiency of the process decreases at higher HRTs (see Figure 2), reaching a maximum VS removal of  $83.65 \pm$ 0.62% (COD removal of  $82.02 \pm 6.35\%$ ); nevertheless, the methane yield was higher at higher OLRs, reaching a maximum of 0.21  $\pm$  0.01 LCH<sub>4</sub> gVS<sup>-1</sup> with an OLR of 5.38 gVS  $L^{-1} d^{-1}$  (8.03 gCOD  $L^{-1} d^{-1}$ ). HRT of 3.38 d and influent TAN concentration of  $2.10 \pm 0.35$ g N-TAN  $L^{-1}$ . Therefore, it is possible to achieve good performance in AD with a high methane vield when operating at a high TAN concentration and low HRT.

# Effect of Substrate Concentration

The third activity (ADS 3) consisted of increasing the OLR by increasing the substrate concentration from 10.09  $\pm$  1.41 to 35.25  $\pm$  6.20 gVS  $\rm L^{-1}$  with constant HRT (see Table 1) and was evaluated during 181 d of operation. As seen in Figure 3, the reactor reached a maximum VS removal of near 80%, and later, the efficiency decreased to 60% by simply increasing the organic matter input. These results are in accordance with the literature, which suggests that there are optimal operating conditions for the performance of an anaerobic digester (Mahmoud et al., 2003). The methane yield also reached a maximum (0.22  $\pm$  0.01  $LCH_4$  gVS<sup>-1</sup>), but not at the same OLR value as that for the maximum VS removal. This could be explained by the accumulation of biogas in the sludge bed, forming stable gas pockets that lead to the incidental lifting of parts of the bed and a pulse-like eruption of gas from this zone (Kalyuzhnyi et al., 1998; Elmitwalli et al., 1999). At high influent concentrations, the upflow velocity is not sufficient to achieve good mixing. Then, the optimal operating conditions are those that realize a trade-off between methane yield and VS removal.

Despite having found a maximum, it is interesting to analyze whether the methane yield decrease was due to the high ammonia concentration. The last conditions were operated at inhibitory TAN and FA concentrations of  $3.73 \pm 0.56$  gN-TAN L<sup>-1</sup> and  $1086.34 \pm 178.82$  mgN-NH<sub>3</sub> L<sup>-1</sup>, respectively. Nevertheless, the FA level in ★ Substrate concentration ↔ FA concentration



Figure 2. Performance of the two-stage anaerobic digestion system during the second activity (ADS 2) treating poultry manure. The effect of the global HRT was evaluated by decreasing the HRT from 9.09 to 2.74 d (i.e., increasing OLR from 1.75 to 8.31 gVS  $L^{-1} d^{-1}$ ). OLR: organic loading rate, FA: free ammonia and VS: volatile solids.

the methanogenic stage was higher than the inhibitory values (53.4 to 183 mgN-NH<sub>3</sub> L<sup>-1</sup>) (Sung and Liu, 2003; Yenigün and Demirel, 2013), and it was possible to reach a high methane yield of 0.15 LCH<sub>4</sub> gVS<sup>-1</sup>.

### Ammonia Inhibition

Although the ammonia concentration ensures a sufficient buffer capacity of the methanogenic medium in AD and then increases the stability of the digestion process (Rajagopal et al., 2013), as mentioned above, FA is also the main inhibitor of anaerobic digestion. According to the equation of Anthonisen et al. (1976), at higher temperatures, there is a higher concentration of FA; thus, thermophilic operations are more sensitive than mesophilic anaerobic digestion. For example, (Angelidaki and Ahring, 1994) studied the effect of temperature in the range of 40 to 64°C at TAN concentrations of 2.5 and 6.0 gN L<sup>-1</sup>. They determined that with a higher TAN concentration, a decrease in temperature below

 $55^{\circ}$ C resulted in an increase in biogas yield and better process stability. Additionally, they observed that at FA concentrations greater than 0.7 g L<sup>-1</sup>, the biogas yield decreased, and the VFA concentration increased.

Nevertheless, a wide range of inhibitory concentrations has been described for mesophilic and thermophilic anaerobic digestion since the inhibitory concentration depends on the biomass acclimation to different substrates. Sung and Liu (2003) evaluated ammonia inhibition in the thermophilic AD (at 55°C) of synthetic wastewater. FA concentrations of 92.0 and 53.4 mg N-NH<sub>3</sub>  $L^{-1}$  decreased the methane production by as much as 39 and 64%, respectively, with respect to the control. Despite the large number of inhibitory values reported, Rajagopal et al. (2013) summarized the effects of ammonia as beneficial, no antagonistic effect, inhibition and toxic at TAN concentration ranges of 50 to 200, 200 to 1000, 1500 to 3000, and over  $3000 \text{ mgTAN-N } \text{L}^{-1}$ , respectively. Regrettably, these values were not summarized in terms of FA. In this



Figure 3. Performance of the 2-stage anaerobic digestion system during the third activity (ADS 3) treating poultry manure. The effect of substrate concentration was evaluated by increasing the substrate concentration from  $10.09 \pm 1.41$  to  $35.25 \pm 6.20$  gVS L<sup>-1</sup> with an average HRT of  $4.66 \pm 0.11$  d. OLR: organic loading rate, FA: free ammonia and VS: volatile solids.

work, the results in Figure 2 showed that in ADS 2, with a FA concentration of  $323.05 \pm 56.48 \text{ mgN-NH}_3$  L<sup>-1</sup>, the methane yield was 0.21 LCH<sub>4</sub> gVS<sup>-1</sup> and decreased to 0.14 LCH<sub>4</sub> gVS<sup>-1</sup> with 874.08 ± 161.74 mgN-NH<sub>3</sub> L<sup>-1</sup> (i.e., a decrease of 33.33%); furthermore, in ADS 3 (Figure 3), with 460.93 ± 135.40 mgN-NH<sub>3</sub> L<sup>-1</sup>, the methane yield was 0.22 LCH<sub>4</sub> gVS<sup>-1</sup> and decreased by 31.82% to 0.15 LCH<sub>4</sub> gVS<sup>-1</sup> with 1086.34 ± 178.82 mgN-NH<sub>3</sub> L<sup>-1</sup>.

On the other hand, the TAN concentration in the raw substrate varied throughout the year of operation, so there is not always linearity between TAN and VS content. Moreover, the FA concentration presented higher variation because of pH variation in the system. Then, ADS was stable under variations in the TAN content of the raw substrate, reaching a high methane yield even when working under high FA concentrations. It is possible to avoid or reduce the inhibitory effect of high FA concentrations when other operational parameters are optimized, such as the AD configuration, substrate concentration, temperature or HRT.

#### Methane Yield

Ma et al. (2018) reached at day 75 of a batch assay, a methane yield of 0.206  $LCH_4$  gVS<sup>-1</sup> treating PM. Bayrakdar et al. (2017) codigested PM with poppy straw in a CSTR with an HRT of 23 d and OLR of  $3.56 \text{ gSV } \text{L}^{-1} \text{d}^{-1}$  and reached a maximal methane yield of 0.290 LCH<sub>4</sub> gVS<sub>in</sub><sup>-1</sup> with a VS removal of 67.0% and FA concentration of 284 mg N-NH<sub>3</sub>  $L^{-1}$ . Li et al. (2014) also carried out codigestion in a CSTR, treating PM and corn stover. The maximal methane yield and the VS removal reached were  $0.255 \text{ LCH}_4 \text{ gVS}^{-1}$  and 79.0%, respectively, operating with an OLR of 1.00 gVS  $L^{-1}d^{-1}$ , HRT of 90 d and FA concentration of approximately 18 mg N-NH<sub>3</sub>  $L^{-1}$  (calculated from the published data). Finally, the ADS of this work was able to achieve as good a methane yield as other studies with the advantage that the system allows treating higher OLRs at lower HRTs than published previously (HRTs of 4.3, 3.38, and 5.54 d for ADS 1, ADS 2 and ADS 3, respectively).

On the other hand, the methane composition of biogas remained relatively constant during the overall work, the maximal and minimal methane percents for each activity were 49.88 to 64.52%, 33.81 to 60.51% and 49.49 to 59.50% for ADS 1, ADS 2, and ADS 3, respectively.

Finally, considering a VS content in raw PM of 210.65 kgVS  $(m^3_{\rm PM})^{-1}$  and a density of 1096 kg  $(m^3)^{-1}$  (Alejo-Alvarez et al., 2016), the maximal methane yields from this research give values of 40.36 and 42.28 cubic meters of methane per ton of PM  $(m^3CH_4 \ (ton_{\rm PM})^{-1})$ . Finally, a laying hen produces between 47.45 and 54.75 kg of PM per year in Chile; i.e., with one laying hen, it is possible to produce between 1.93 and 2.31 m<sup>3</sup> of methane per year.

# CONCLUSIONS

The operation of 2-stage ADS to treat PM was successful since high methane yields were reached. The OLR was optimized using a low HRT. Moreover, the ADS presented good performance despite substrate variations during 1 yr of operation.

This is the first study of the separate and combined effects of OLR, HRT, and substrate concentration on the anaerobic digestion of PM.

We conclude that it is possible to reach high methane yields of 0.21 and 0.22  $LCH_4$  gVS<sup>-1</sup> by working with a low HRT of 4.66 to 3.38 d when operating a thermophilic-mesophilic 2-stage ADS.

The ADS shows optimal performance at FA concentrations of 323 to 460 mgN-NH<sub>3</sub> L<sup>-1</sup>. Since TAN concentration variations were only caused by changes in PM composition throughout the year, this study demonstrates the technical feasibility of this process, where the 2-stage ADS is able to tolerate changes in substrate composition and achieve a high methane yield.

The thermophilic conditions of the first stage allowed substrate solubilization, reducing the tube clogging caused by the heterogeneity of the substrate. The latter is of great interest since it is possible to treat high OLRs with lower HRTs than reported in the literature. Moreover, the thermophilic stage can be used for pathogen stabilization of the effluent, which would allow the effluent to be used as a soil improver. Then, 2stage ADS would give a second valuable product from a waste. This advantage of the system must be studied in future research in terms of toxicity and pathogen content with the aim of corroborating its innocuousness to the environment.

Finally, PM treatment with two-stage ADS provides an environmental solution and provides 2 valuable products from a waste: biogas and a soil improver.

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### REFERENCES

- Alejo-Alvarez, L., V. Guzmán-Fierro, K. Fernández, and M. Roeckel. 2016. Technical and economical optimization of a full-scale poultry manure treatment process: total ammonia nitrogen balance. Environ. Technol. 37:2865–2878.
- Angelidaki, I., and B. K. Ahring. 1994. Anaerobic thermophilic digestion of manure at different ammonia loads: Effect of temperature. Water Res. 28:727–731.
- Angelidaki, I., S. P. Petersen, and B. K. Ahring. 1990. Effects of lipids on thermophilic anaerobic digestion and reduction of lipid inhibition upon addition of bentonite. Appl. Microbiol. Biotechnol. 33:469–472.
- Anthonisen, A. C., R. C. Loehr, T. B. Prakasam, and E. G. Srinath. 1976. Inhibition of nitrification by ammonia and nitrous acid. J. Water Pollut. Control Fed. 48:835–852.
- Arriagada, C., V. Guzmán-Fierro, E. Giustinianovich, L. Alejo-Alvarez, J. Behar, L. Pereira, V. Campos, K. Fernández, and M. Roeckel. 2017. NOB suppression and adaptation strategies in the partial nitrification–Anammox process for a poultry manure anaerobic digester. Process Biochem. 58:258–265.
- Bayrakdar, A., R. Molaey, R. Ö. Sürmeli, E. Sahinkaya, and B. Çalli. 2017. Biogas production from chicken manure: Co-digestion with spent poppy straw. Int. Biodeterior. Biodegradation 119:205–210.
- Chae, K. J., A. Jang, S. K. Yim, and I. S. Kim. 2008. The effects of digestion temperature and temperature shock on the biogas yields from the mesophilic anaerobic digestion of swine manure. Bioresour. Technol. 99:1–6.
- Chen, Y., J. Cheng, and K. Creamer. 2008. Inhibition of anaerobic digestion process: a review. Bioresour. Technol. 99:4044–4064.
- Dugba, P. N., and R. Zhang. 1999. Treatment of dairy wastewater with two-stage anaerobic sequencing batch reactor systems - thermophilic versus mesophilic operations. Bioresour. Technol. 68:225–233.
- Elmitwalli, T., M. Zandvoort, G. Zeeman, H. Bruning, and G. Lettinga. 1999. Low temperature treatment of domestic sewage in upflow anaerobic sludge blanket and anaerobic hybrid reactors. Water Sci. Technol. 39:177–185.
- Hansen, K. H., I. Angelidaki, and B. K. Ahring. 1998. Anaerobic digestion of swine manure inhibition by ammonia. Water Sci. Technol. 32:5–12.
- Huang, J. J. H., and J. C. H. Shih. 1981. The potential of biological methane generation from chicken manure. Biotechnol. Bioeng. 23:2307–2314.
- Kalyuzhnyi, S., L. Estrada de los Santos, and J. Rodriguez Martinez. 1998. Anaerobic treatmen of raw and preclarified potato-maize wastewaters in a UASB reactor. Bioresour. Technol. 66:195–199.
- Li, Y., R. Zhang, Y. He, C. Zhang, X. Liu, C. Chen, and G. Liu. 2014. Anaerobic co-digestion of chicken manure and corn stover in batch and continuously stirred tank reactor (CSTR). Bioresour. Technol. 156:342–347.
- Lo, K. V., P. H. Liao, and N. R. Bulley. 1986. Two-phase mesophilic anaerobic digestion of screened dairy manure using conventional and fixed-film reactors. Agric. Wastes 17:279–291.
- Ma, J., M. Amjad Bashir, J. Pan, L. Qiu, H. Liu, L. Zhai, and A. Rehim. 2018. Enhancing performance and stability of anaerobic digestion of chicken manure using thermally modified bentonite. J. Clean. Prod. 183:11–19.
- Mahmoud, N., G. Zeeman, H. Gijzen, and G. Lettinga. 2003. Solids removal in upflow anaerobic reactors, a review. Bioresour. Technol. 90:1–9.
- Nagao, N., N. Tajima, M. Kawai, C. Niwa, N. Kurosawa, T. Matsuyama, F. M. Yusoff, and T. Toda. 2012. Maximum organic loading rate for the single-stage wet anaerobic digestion of food waste. Bioresour. Technol. 118:210–218.

- Odepa, OdeEyP. A. 2018. Huevos. Available at https://www.odepa. gob.cl/rubros/huevos?mobile=off (verified 8 February 2019).
- Pavlostathis, S. G., and E. Giraldo-Gomez. 1991. Kinetics of anaerobic treatment : a critical review. Crit. Rev. Environ. Control:411– 490.
- Pérez, A., and P. Torres. 2008. Indices de alcalinidad para el control del tratamiento anaerobio de aguas residuales fácilmente acidificables (Alkalinity indices for control of anaerobic treatment of readily acidifiable wastewaters). Ing. y Compet. 10: 41–52.
- Pizarro, M. D., G. Céccoli, F. F. Muñoz, L. S. Frizzo, L. D. Daurelio, and C. A. Bouzo. 2019. Use of raw and composted poultry litter in lettuce produced under field conditions: microbiological quality and safety assessment. Poult. Sci. 98:2608–2614.
- Rajagopal, R., D. I. Massé, and G. Singh. 2013. A critical review on inhibition of anaerobic digestion process by excess ammonia. Bioresour. Technol. 143:632–641.
- Roeckel, M., C. Arriagada, and V. Guzmán-Fierro. 2017. Innovative Nitrogen and Carbon Removal. Pages 9–29 in Nitrification and Denitrification. Ivan X. Zhu, ed. IntechOpen.

- Sánchez, O., E. Aspé, M. C. Martí, and M. Roeckel. 2005. Rate of ammonia oxidation in a synthetic saline wastewater by a nitrifying mixed-culture. J. Chem. Technol. Biotechnol. 80:1261–1267.
- Siegert, I., and C. Banks. 2005. The effect of volatile fatty acid additions on the anaerobic digestion of cellulose and glucose in batch reactors. Process Biochem. 40:3412–3418.
- Sung, S., and T. Liu. 2003. Ammonia inhibition on thermophilic anaerobic digestion. Chemosphere 53:43–52.
- Wittmann, C., A. P. Zeng, and W. D. Deckwer. 1995. Growth inhibition by ammonia and use of a pH-controlled feeding strategy for the effective cultivation of *Mycobacterium chlorophenolicum*. Appl. Microbiol. Biotechnol. 44:519–525.
- Yenigün, O., and B. Demirel. 2013. Ammonia inhibition in anaerobic digestion: a review. Process Biochem. 48:901–911.
- Yetilmezsoy, K., and S. Sakar. 2008. Improvement of COD and color removal from UASB treated poultry manure wastewater using Fenton's oxidation. 151:547–558.
- Zhang, R. H., J. Tao, and P. N. Dugba. 2000. Evaluation of two-stage anaerobic sequencing batch reactor systems for animal wastewater treatment. Trans. ASAE 43:1795–1802.