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Performance of semi-continuous anaerobic co-digestion of poultry manure with fruit and vegetable waste and analysis of digestate quality: A bench scale study

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ABSTRACT

Poultry manure (PM) can contain ammonium and ammonia nitrogen, which may inhibit the anaerobic process. The aim of this work was to evaluate the performance of anaerobic digestion of PM co-digested with fruit and vegetable waste. Two semi-continuous bench scale (19L) stirred tank reactors were used. The operating conditions were: 34.5 °C, 2 gVS/L.d (organic load rate), 28 d of hydraulic retention time and 100 revolutions per m (1 h × 3 times by day) for the agitation. The reactors were fed PM and a mixture of PM and fruit and vegetable waste (FVW) at equal proportions (based on wet weight). The performance of the anaerobic process was assessed through biogas and methane yields, reduction of organic matter, release of nitrogen compounds and the monitoring of stability indicators (pH, volatile fatty acids (VFA), total (TA) and partial (PA) alkalinity). Moreover, the digestate quality was evaluated to determine potential risk and benefits from its application as biofertilizer. Toxicity was assessed using *Daphnia magna* immobilization tests. Results showed that biogas and methane yields from PM-FVW were 31% and 32% higher than PM alone, respectively. Values of organic matter, pH, *alpha* (PA/TA) and VFA revealed that stability was approached in PM and PM-FVW. The co-digestion of PM with FVW led to the highest methane and biogas yields, lower FAN and TAN concentrations, and a better digestate quality compared to mono-digestion of this manure.

1. Introduction

Organic waste is produced by a range of industries, such as agriculture, livestock farming, beverage manufacture and food industry in appreciable quantities (Callaghan et al., 1999). The continuous development of animal production has led to an increase of livestock manures in the rural areas. Argentina only registered 42.3 million laying hens in 2016 and this represents a loading of 1.4 million tonnes of laying hen manures produced annually (Agro-industry Ministry from Argentina, 2016a). When the manure is improperly managed (e.g. landfilling), severe consequences to the environment are evidenced, such as malodours, pest prevalence, pathogens, water pollution, and emission of greenhouse gases (Nelson and Lamb, 2002; Ward et al., 2008).

On the other hand, the generation of fruit and vegetable waste (FVW) is increasing and is a rising concern when landfilled due to its high biodegradability (Khalid et al., 2011). In the city of Buenos Aires (Argentina), about 30,000 tonnes of solid waste was produced in the largest wholesale food distribution centre of the country (Central Market Corporation of Buenos Aires) in 2016. About 70% of this waste includes fruits and vegetables which is disposed of to landfills (Rosso et al., 2016). More of 75% of the total of greenhouse gas (GHG) is represented by the CH_4 emitted from landfills. Calabrò et al. (2015) reported that an integrated system of municipal solid waste (MSW) based on the optimized use of available technologies (composting, anaerobic digestion, recycling, incineration with energy recupery) has already led to high reductions of GHG emissions. Adopting high level of separation collection, efficient energy recovery in waste to energy plants and very limited landfill disposal could become a carbon sink with a potential of up to 40 $MtCO_{2eq}$/year.

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Poultry manure (PM) is a plentiful source of biomass for energy production via anaerobic digestion. Several batch studies on PM as a single substrate have reported yields of 0.14–0.37 L CH_4/kgVS added (Costa et al., 2012; Nie et al., 2015; Salminen and Rintala, 2002). This manure has not been fully utilized so far due to problems associated to inhibition caused by ammonia (Abouelenien et al., 2014). The anaerobic decomposition of proteins and urea present in manure result in the production of high amounts of ammonia and ammonium ions (Abouelenien et al., 2009; Chen et al., 2008; Yenigün and Demirel, 2013).

The co-digestion of manure with other waste, such as sewage sludge, agro-waste, agro-industrial and municipal solid waste can improve biogas yields (Álvarez et al., 2010; Alvarez and Lidén, 2008; Fantozzi and Buratti, 2009; Gelegenis et al., 2007b; Ho and Ho, 2012;Li et al., 2011; Magbanua et al., 2001).

The addition of FVW to PM can adjust the mixture's C/N ratio to optimal values and can reduce the toxicity attributed to the high ammonia concentration commonly encountered in PM alone. Seadi et al. (2008) reported that FVW produce 0.25–0.50 LCH_4/gVS added whereas Gunaseelan (2004) reported a range of 0.18–0.73 L/gVS added for 54 FVW as single substrate. These studies reveal a high methane potential compared to other organic waste. However, others studies have indicated problems and limitations in the anaerobic digestion of FVW (Jiang et al., 2012). The FVW have a high organic matter content which is easily hydrolysed to total volatile fatty acids (VFA). If VFAs build-up rapidly during digestion, this may lead to acidification of the system (Molinuevo-Salces et al., 2010; Ward et al., 2008). Thus, this unstability could be avoided with the addition of other substrates. The generation of alkaline compounds during the degradation of PM can add a buffering capacity to the system (Kafle and Kim, 2013).

The co-digestion of FVW with cattle slurry (CS) and PM has been studied by Callaghan et al. (2002) in continuously stirred tank reactor (18 L). According to them, an increase of the proportion of FVW from 20% to 50% improved the methane yield from 0.23 to 0.45 m^3CH_4/kgVS. However, the co-digestion of CS with PM from 30 to 50% PM caused a reduction of volatile solids of 50% to 30% and a decrease of methane yield from 0.10 to 0.05 m^3CH_4/kgVS. This effect could be attributed to an inhibition of the sytem by an increase of free ammonia concentration (>100 mg/L).

Molinuevo-Salces et al. (2013) studied anaerobic digestion of livestock waste (swine manure and poultry litter (PL)) and vegetable processing waste mixtures (VPW) in batch experiments. The results showed that the co-digestion of PL with 50% and 75% (based on dry weight) of VPW improved the specific methane yield from 158 to 179 and 223 mL CH_4/gVS added, respectively. The addition of PL to VPW increased the C/N ratios in the range that is optimal for anaerobic digestion (Wang et al., 2012). Thus, the methane yield was increased substantially (41%), as well, particularly in the highest C/N (25).

Although several studies reported the co-digestion of PM with others waste in batch experiment, effects of co-digestion of PM and FVW in semi-continuous bench scale reactors, their performance at steady state and the digestate quality are poorly known.

Anaerobic digestion leads to the release of macro and micronutrients, which is a valuable soil fertilizer. Compared to raw animal manure, digestate has improved fertilizer abilities, higher homogeneity and nutrient availability, a higher C/N ratio and a significantly reduced odour potential than the raw waste (Seadi et al., 2008). In addition, digestate contains heavy metals. Although, most of the heavy metals and micro-elements are essential for plants, high amounts can produce toxic effects. Therefore, the content of contaminants in digestates, should be carefully monitored and the concentrations must not exceed the legal limits set in each country (Lukehurst et al., 2010).

The aim of this study was to evaluate the performance and the digestate quality of the mono-digestion and co-digestion of PM with FVW at equal proportions (wet weight) in semi-continuous anaerobic reactors at a bench scale (19 L). The performance of the anaerobic process was evaluated through the biogas and methane yields, the reduction of organic matter, the release of nitrogen compounds and the monitoring of selected stability indicators. Digestate quality was assessed via measurements of physical, chemical parameters and toxicity tests so that to evaluate the potential of digestate application as biofertilizer.

2. Materials and methods

2.1. Collection and preparation of raw materials

Poultry manure (PM) and fruit and vegetable waste (FVW) were selected for this study. Raw PM was collected from a laying hen farm that used the cage system. Manure was removed two or three times per week. Lime was typically added to dry manure to prevent generation of larvae of flies. FVW were collected from an organic solid waste management company. The FVW composition was (% w/w): 15.6% eggplants, 6.5% green onions; 3.8% cabbage leaves, 11.4% bean sprout, 7.7% onion, 1.9% corn and husk, 11.3% carrot and butternut squash, 15.3% lettuce, 2.5% broccoli, 5.8% globe squash, 10.8% oranges, 1.4% grapes, 4.0% tomatoes and 2.0% sweet potatoes. The FVW was shredded to a small size and homogenized.

The PM was mixed with FVW at a 1:1 wet weight ratio (50% PM and 50% FVW) to increase C/N ratio and to reduce the potential inhibitory ammonia levels. Molinuevo-Salces et al. (2013) mentioned that higher FVW:PM ratios resulted in an increase of up to 40% methane yield compared with PM alone. In the present work, we evaluated a lower FVW:PM ratio, because our main goal was to treat the PM rather than the FVW. In this sense, we selected a mixture than can be co-digested in stable conditions and can provide a sufficient methane production using the highest amount of PM possible.

PM and PM-FVW were characterized, fractionated by daily feed amount and stored at −18 °C until use. Substrates were brought at ambient temperatures before the daily feeding.

2.2. Experimental set-up

Two semi-continuous stirred tank reactors that operated at the mesophilic range were used. The reactors had a working volume of 19 L, so that the scale of the experiment can be classified as bench scale. Due to the use of bench (and not laboratory) scale reactors here, we decided not to use replications in our experimental design but to rather dedicate more reactors to investigate different treatments. We consider the use of replications absolutely necessary at the laboratory scale (e.g. such as in BMP tests in vessels < 1L); however, replications could be omitted when larger scale systems (such as the ones used here) are present. The reactors were stirred at 100 revolutions per minute (1 h × 3 times by day) and were heated by water through a thermostatic bath to maintain the operating temperature (34.5 ± 1.4 °C). A detailed design of the reactors can be found in Bres et al. (2011).

Sludge from dairy effluents treatment anaerobic lagoon was used as inoculum in the three reactors. Each reactor was fed with PM and PM-FVW diluted with tap water to reach 8% of total solids (TS). The organic loading rate (OLR) calculated by the volatile solid (VS) was increased gradually (0.5, 1, 1.5 and 2 gVS/L.d) to generate an acclimated biomass for each substrate over a seven month period. An OLR of 2 gVS/L.d was maintained for a period of two times the hydraulic retention time (i.e. 56 days, since HRT was 28 d) to reach steady state conditions. Then, the experimental period started up and digestate samples were taken in triplicates weekly for a total period of 114 d (a total of 16 sampling events).

Biogas was collected in a 50 L capacity gasometer. The daily production was measured according to the water height reached in the gasometer. The biogas production was calculated at standard temperature and pressure conditions (0 °C; 1 atm). Biogas samples were taken once weekly in a gas sampling bag to determine the composition over the period of 80–114 days.

2.3. Analytical measurements

PM, FVW and the mixture of PM-FVW were characterized for TS, VS, chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), total phosphorus (TP) and total elements Ca, Mg, K, Na, Fe, Zn, Mn and Cu.

Digestate samples were analysed for pH, electrical conductivity (EC), COD, TS, VS, volatile fatty acids (VFA), total alkalinity (TA) and partial alkalinity (PA) once weekly during all experiments. In addition, TP, selected elements (Ca, Mg, K, Na, Fe, Zn, Mn and Cu), TKN, total ammonia nitrogen (TAN) and free ammonia nitrogen (FAN) were determined in digestate samples over the last six weeks of operation.

EC, pH, COD, TS, VS and TP were measured according to standard methods (APHA, 1992). The samples were digested by microwave equipment (Mars 5, CEM) for the determination of TP and total elements according to method microwave-assisted nitric acid digestion (USDA and USCC, 2001). Total elements were measured by atomic absorption spectrophotometry (Varian, 2020 A). TA, PA and VFA were measured according to Jenkins et al. (1983). These parameters were used to determine the *alpha* ($\alpha = PA/TA$) and the VFA/TA process indicators. TKN and TAN were determined using the standard method by Foss Tecator automatic analyser. Equation (1) was used to calculate FAN (free NH_3) concentrations, according to Hansen et al. (1998).

$$FAN = TANx\left(1 + \frac{10^{-pH}}{10^{-\left(0.09018 + \frac{2729.92}{T(K)}\right)}}\right)^{-1} \tag{1}$$

Methane and carbon dioxide contents in the biogas were measured by gas chromatography (Hewlett Packard 5890 GC System) according to method ASTM D 1945-14 (2014) using a molecular sieve 13X and HP PLOT Al_2O_3, a thermal conductivity detector (TCD and helium as a carrier gas at a flow rate of 2.1 mL/min. The column temperature was kept at 90 °C whereas the injector and detector temperatures were set at 130 °C and 250 °C, respectively. The biogas yield (Specific Gas Production) was calculated according to Equation (2).

$$SGP = \frac{V_{biogas}}{substrate\ mass}\left[\frac{NL_{biogas}}{KgSV_{added}}\right] \tag{2}$$

where SGP is the Specific (Bio)Gas Production, V_{biogas} is the accumulated volume of biogas in standard conditions, and substrate mass is the weight of volatile solids in the substrate added to reactor (VS of manure in PM and VS of mixture in PM-FVW). In addition, the methane yield was expressed as Specific Methane Production (SMP) replacing V_{biogas} for $V_{methane}$ in Eq. (2).

2.4. Toxicity test

Acute toxicity tests were carried out using *Daphnia magna* immobilization test (USEPA, 1996) in digestate samples from the two reactors at the end of the experiment. Neonates of *Daphnia magna* used for experiments were obtained from a broodstock maintained at the aquarium facilities of the *Instituto Nacional de Tecnología Agropecuaria* (INTA, Argentina). Briefly, ten neonates (<24 h after hatching) were exposed for 48 h in a static-flow system, containing 30 mL of sample dilution or control water. Eight concentrations from each digestate sample (0, 0.1, 1, 3, 5, 9, 15 and 25% v/v) and one positive control were tested in triplicate. The culture medium (dechlorinated and aerated water; pH = 8.1 ± 0.3; electrical conductivity = 642 ± 24 µS/cm) was used as control water to prepare sample dilutions. Experiments were conducted under controlled laboratory conditions in a controlled room (23 ± 2 °C and 16:8-h light: dark photoperiod). The quality controls used were immobilization under 10% in negative control (concentration of 0%) and Cr^{+6} ($K_2Cr_2O_7$) as a reference toxic compound in positive control (Díaz Baez et al., 2004). Toxicity endpoints assessed were effective concentration 50 at 48 h (EC_{50}), LOEC (Lowest Observed Effect Concentration), and NOEC (No Observed Effect Concentration).

2.5. Statistical analysis

A paired *t*-test was performed to compare the physical and chemical parameters between treatments, when data passed the D'Agostino-Pearson normality test ($p < 0.05$). NOEC and LOEC were determined by one-way analysis of variance (ANOVA) and the Dunnet's *post-hoc* test ($p < 0.05$). A Pearson correlation analysis among physicochemical parameters was performed. Data analyses were performed using InfoStat® and Prism® software.

3. Results

3.1. Raw materials

Physical and chemical properties of raw materials are included in Table 1. The C/N ratio was 6 and 9 for PM and for the mixture of PM-FVW, respectively.

3.2. Biogas and methane yields

The cumulative biogas production at the end of the experiment were 996 NL and 764 NL for PM-FVW and PM respectively. Although the same temperature, agitation and OLR conditions were applied to all two reactors, the highest biogas production was observed for the PM-FVW substrate. The co-digestion of PM-FVW increased biogas production by 30% compared to mono-digested PM.

The steady state percentages of methane was 62.6% ± 2.4 and 62.8% ± 4.0 in the biogas of PM-FVW and PM respectively. The methane content in the total gas volume remained approximately constant from day 78 until the end of the experiment in both reac-

Table 1
Physical and chemical properties of the raw materials.

Parameter	Unit	PM	FVW	PM-FVW
Dry mass	% wb	30.2 ± 1.6	11.7 ± 0.6	17.9 ± 0.7
VS	% db	79.8 ± 1.7	85.4 ± 0.9	74.2 ± 2.1
TKN	mg/g	56.9 ± 1.0	22.1 ± 0.2	42.2 ± 1.8
TP	mg/g	17.1 ± 2.4	2.9 ± 0.4	4.7 ± 0.4
COD	g/g	1.0 ± 0.2	1.4 ± 0.2	0.8 ± 0.0
Ca	mg/g	23.9 ± 3.6	7.4 ± 0.5	19.4 ± 4.4
Mg	mg/g	6.9 ± 0.8	1.8 ± 0.3	1.7 ± 0.2
K	mg/g	29.0 ± 2.7	24.3 ± 2.5	10.5 ± 0.4
Na	mg/g	4.3 ± 0.4	3.8 ± 0.5	2.0 ± 0.3
Fe	mg/g	1.1 ± 0.1	2.6 ± 0.3	0.4 ± 0.1
Zn	µg/g	255 ± 33	62 ± 2	82 ± 7
Mn	µg/g	359 ± 33	96 ± 9	133 ± 16
Cu	µg/g	21.2 ± 4.8	2.2 ± 1.0	11.2 ± 0.9

Average (±SD) based on $n = 3$; PM: poultry manure; FVW: fruit and vegetable wastes; wb: wet weight basis; db: dry weight basis; VS: volatile solids; TKN: total Kjeldahl nitrogen; TP: total phosphorous; COD: chemical oxygen demand; except dry mass, all others expressed on a dry weight basis.

tors (six sampling events). No significant differences ($p < 0.05$) in the content of CH_4 (%) in the two reactors were observed.

The specific biogas (SGP) and methane (SMP) yields are shown in Fig. 1a and b, respectively. According to Fig. 1a, the steady state condition in PM-FVW was achieved after 30 d and remained stable till the rest of the experiment with an average biogas yield of 0.33 ± 0.01 NL/gVS_{added}. A different behaviour was observed in SGP for PM, which it slowly declined from 30 d. Although the maximum value in PM was 0.39 NL_{biogas}/gVS_{added} at day 30, stable conditions were achieved between 94 d and 114 d with an average value of 0.25 ± 0.01 NL_{biogas}/gVS_{added}. The SGP in PM-FVW was 32.0% higher than PM.

The SMP values (Fig. 1b) were approximately constant for both reactors. However, a drop in the last point of the curve in PM was observed. The average values of SMP were 0.21 ± 0.01 and 0.16 ± 0.03 $NLCH_4/gVS$ in PM-FVW and PM respectively. The co-digestion of PM with FVW led to an increase of SMP by 31.2% compared to the mono-digestion of PM. Biogas and methane yields had a positive correlation ($R^2 = 0.89$; $p < 0.001$).

3.3. Effect of nitrogen compounds

The TKN and TAN concentrations over time in both reactors are shown in Fig. 2a. Average concentrations of TKN and TAN were 3.45 ± 0.45 g/L and 2.68 ± 0.27 g/L for PM-FVW and 4.44 ± 0.20 g/L and 3.54 ± 0.14 g/L for PM, respectively. PM-FVW had the maximum value at 63 d and then, the concentration was decreasing over time. PM had relatively constant concentrations with a range of 4.1–4.5 g/L over time. In addition, a peak at 114 d was observed in both reactors. TKN and TAN showed significant differences between PM and PM-FVW ($p < 0.01$ and $p < 0.001$, respectively). TAN had a positive correlation with TKN ($R^2 = 0.98$; $p \leq 0.001$), and high TAN/TKN ratios were observed (78.5% and 79.2% in PM-FVW and PM, respectively). As expected, PM and PM-FVW showed high contents of nitrogen compounds with a high transformation of nitrogen to ammonia in solution.

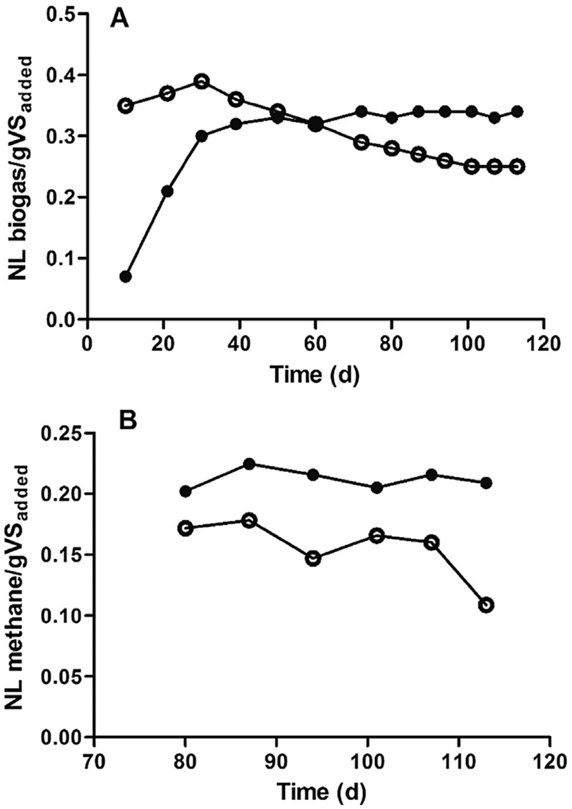

Fig. 1. Biogas and methane yields in PM-FVW (●) and PM (○). (a) Specific Biogas Production; (b) Specific Methane Production during the stable period (80–114 days).

FAN showed a positive correlation with pH during all experiments (Fig. 2b; $R^2 = 0.90$; $p < 0.001$). FAN concentration was significantly the highest in PM ($p < 0.01$). In PM and PM-FVW, FAN concentrations increased from 42.0 to 161.6 mg/L and from 22 to 98.2 mg/L during the 70–105 d period, respectively. On the other hand, pH values showed no significant differences between treatments ($p > 0.05$). The highest FAN concentrations were reached at 114 d when pH values were higher than 8 in both reactors.

Fig. 2c reveals a non-linear correlation between the FAN/TAN ratio (y-axis) and pH (x-axis), which follows the exponential equation $y = 2.34^{-7} * exp^{(2.16*x)}$ ($R^2 = 0.993$). Values of FAN/TAN ratio were lower than 2% for pH < 7.4, whereas this ratio increased exponentially when pH was > 7.4. At pH > 8, the FAN concentrations increased by 12–15% in both PM and PM-FVW.

3.4. System stability

The selected stability indicators (pH, *alpha*, VFA/TA) and the organic matter removal in PM-FVW and PM are included in Table 2.

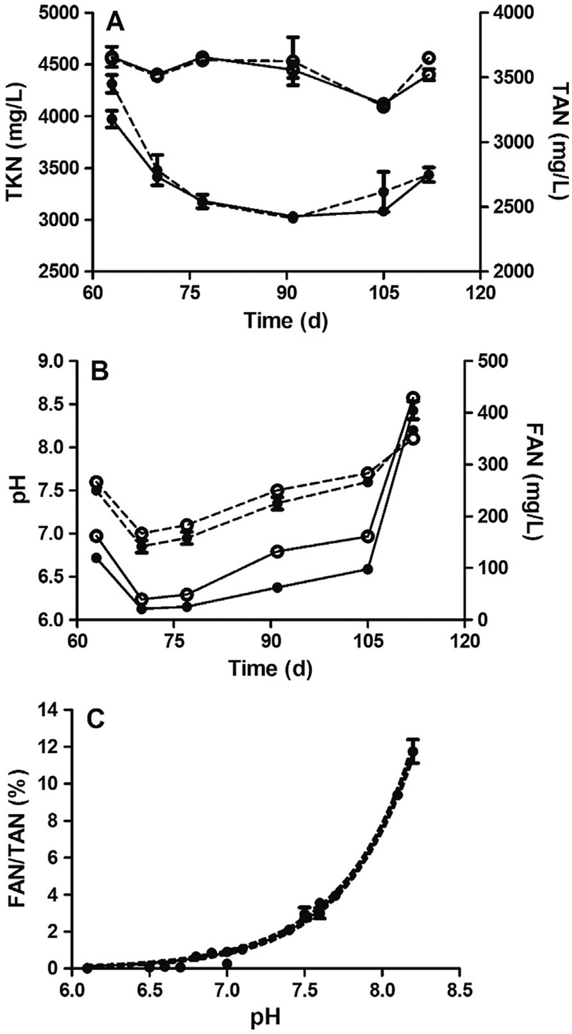

Fig. 2. Variation of average (± SD) nitrogen compounds in PM-FVW (●) and PM (○). (a) TAN (—) and TKN (– – –) concentrations over time; (b) FAN concentrations (—) and pH values (– – –) over time; (c) Relation between pH and percentage of FAN/TAN ratio (dashed lines indicate the 95% confidence intervals of the regression line).

Table 2
Stability indicators and organic matter removals.

Variable	Unit	PM-FVW	PM
pH		7.46 ± 0.31^{a}	7.56 ± 0.24^{a}
alpha		0.84 ± 0.06^{a}	0.85 ± 0.06^{a}
VFA/TA		0.10 ± 0.04^{a}	0.11 ± 0.06^{a}
VS_r	%	64.9 ± 8.9^{b}	58.5 ± 7.4^{a}
COD_r	%	72.0 ± 10.3^{a}	65.1 ± 3.2^{a}

Average (±SD) based on $n = 16$; *alpha*: ratio of partial alkalinity/total alkalinity; VFA: volatile fatty acids; TA: total alkalinity; VS_r: volatile solids removal; COD_r: chemical oxygen demand removal. Different letters indicate significant differences between treatments ($p < 0.05$).

Table 3
Physical and chemical properties and toxicity endpoints of the two reactor digestates in the last sampling event.

Parameter	Unit	PM-FVW	PM
EC	mS/cm	16.8 ± 2.6^{a}	19.6 ± 0.6^{b}
COD	g/L	12.5 ± 3.8^{a}	33.8 ± 3.7^{b}
Ca	mg/L	1630.0 ± 272.4^{a}	1771.0 ± 454.6^{b}
Mg	mg/L	163.5 ± 53.8^{a}	277.3 ± 75.8^{a}
K	mg/L	1960.4 ± 498.4^{a}	2117.5 ± 270.4^{a}
Na	mg/L	701.8 ± 36.2^{a}	702.3 ± 60.9^{a}
Zn	mg/L	10.2 ± 2.5^{a}	20.7 ± 3.7^{b}
Mn	mg/L	6.3 ± 2.5^{a}	19.1 ± 2.7^{b}
Cu	mg/L	1.6 ± 0.5^{a}	1.7 ± 0.5^{a}
Fe	mg/L	57.0 ± 17.3^{a}	76.5 ± 12.5^{a}
EC_{50}	%	2.6	1.8
NOEC	%	1.0	0.5
LOEC	%	3.0	1.0

Average (± SD) based on $n = 6$ for physical and chemical properties and final sampling for *Daphnia magna* toxicity test. EC: electrical conductivity; COD: chemical oxygen demand; EC_{50}: effective concentration 50%; NOEC: no observed effect concentration; LOEC: lowest observed effect concentration. Different letters indicate significant differences between treatments ($p < 0.05$).

A high efficiency of substrate removal was observed, reaching 60% (as COD and VS) in PM and PM-FVW. PM-FVW showed values of VS reduction significantly higher than PM ($p \leq 0.05$). Although no significant differences between treatments were observed for CODr, a higher tendency for COD removal was recorded in PM-FVW. COD_r and VS_r showed a negative correlation with TKN ($R^2 = -0.70$; $p \leq 0.05$).

3.5. Digestate quality

EC, COD, Mn, Ca and Zn values were statistically higher in PM than in PM-FVW (Table 3). Particularly, concentrations of Zn (based on dry weight)) were 391 ± 89 mg/kg (10.2 mg/L) and 596 ± 100 mg/kg (20.7 mg/L) in PM-FVW and PM respectively.

Toxicity tests were in accordance with the criteria established by the quality controls. The average value of immobilized neonates of *D. magna* in the negative controls was 2.20%, lower than the recommended limit (10%) by USEPA (1996). The EC_{50} average value in the positive controls was 0.35 ± 0.06 ($n_{\text{bioassays}} = 10$) mg/L of Cr^{+6}. The values of EC_{50}, NOEC and LOEC were: 1.8%, 0.5% and 1.0% in PM and 2.6%, 1.0% and 3.0% in PM-FVW, respectively. Toxicity endpoints had a positive asociation with pH, whereas had a negative asociation with EC, TAN, and TKN.

3.6. Correlations

CE correlated positively with TAN ($R^2 = 0.79$; $p \leq 0.01$) and TKN ($R^2 = 0.73$; $p \leq 0.01$) (Table 4). Zn had positive correlations with Mn ($R^2 = 0.82$; $p \leq 0.01$), Mg ($R^2 = 0.85$; $p \leq 0.001$), TAN ($R^2 = 0.85$; $p \leq 0.05$) and TKN ($R^2 = 0.88$; $p \leq 0.05$), whereas it had negative correlations with biogas ($R^2 = -0.82$; $p \leq 0.05$) and methane yields ($R^2 = -0.93$; $p \leq 0.01$). Mg and Mn showed a positive correlation with FAN ($R^2 = 0.96$; $p \leq 0.05$) and Fe ($R^2 = 0.72$; $p \leq 0.05$) respectively, whereas those metals showed a negative correlation with biogas ($R^2 = -0.72$ and -0.78, respectively; $p \leq 0.05$).

COD had a positive correlation with TAN ($R^2 = 0.86$; $p \leq 0.001$) and TKN ($R^2 = 0.87$; $p \leq 0.001$), whereas it had a negative correlation with the methane yield ($R^2 = -0.83$; $p \leq 0.01$). Finally, TAN and TKN had a negative correlation with the biogas ($R^2 = -0.74$ and -0.73, respectively; $p \leq 0.01$) and the methane yields ($R^2 = -0.86$ and -0.89; $p \leq 0.01$).

4. Discussion

4.1. Performance of the anaerobic process

The high nutrient and organic matter contents (as VS and COD) in the raw materials indicated a favourable conditions for a biologic process. The characteristics of PM and FVW in this experiment were similar to previous work (Alvarez and Lidén, 2008; Borowski and Weatherley, 2013; Gangagni Rao et al., 2011; Nicholson et al., 1999; Quiroga et al., 2010; Salminen and Rintala, 2002; Wang et al., 2012; Zhao et al., 2014). Particularly, relatively high concentrations of N, P, Ca, Mg, K, Na, Mn, Cu and Zn were found in PM. Poultry excrete around 80% of the nitrogen and phosphorus consumed in the diet (Bujoczek et al., 2000; Burton and Turner, 2003). The high nitrogen content in PM could affect the anaerobic degradation because the high concentration of free NH_3 can inhibit the process (Abouelenien et al., 2009; Borowski et al., 2014; Fricke et al., 2007). Also, the C/N ratio in PM was low (C/N = 6), the PM mixture with FVW resulted in an increase of C/N ratio from 6 to 9. Although, this co-substrate increased

Table 4
Pearson correlation coefficients among physical and chemical parameters in both reactors.

	Ca	Zn	Mn	Fe	COD_r	VS_r	TAN	TKN	FAN	Biogas	CH_4
pH	-0.72^{**}								0.90^{***}		
CE							0.79^{**}	0.73^{**}			
Mg		0.85^{***}							0.96^{*}	-0.72^{*}	
Zn			0.82^{**}				0.85^{*}	0.88^{*}		-0.82^{*}	-0.93^{**}
Mn				0.72^{*}						-0.78^{*}	
COD					-0.81^{***}		0.86^{***}	0.87^{***}			-0.83^{**}
COD_r								-0.70^{*}			
VS						-0.99^{***}					
VS_r								-0.70^{*}			
TAN								0.98^{***}		-0.74^{**}	-0.86^{**}
TKN										-0.73^{**}	-0.89^{**}
Biogas											0.89^{***}

Asterisks indicate significant correlations between paired parameters. * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$; VS_r: volatile solids removal; COD_r: chemical oxygen demand removal the C/N ratio by 33%, the optimal C/N ratio (25–30) for anaerobic digestion was still not reached.

The biogas analysis showed that the co-digestion process of PM improved the biogas and methane yields (>31%). This could be likely due to the synergistic effect of the nutrient composition of FVW and PM. Although C/N ratio in PM-FVW did not reach the optimal range, this mixture had lower nitrogen concentrations than the manure alone. Moreover, the higher biogas yield of the mixture could be attributed to the higher level of biodegradable matter and methane potential in fruit and vegetable waste (75% of DM is sugar and hemicellulose) compared to manure alone (Bouallagui et al., 2005; Gelegenis et al., 2007a). On the other hand, a likely slight inhibition was found in PM, which SGP declined after 30 d.

The percentage of methane remained approximately constant (>62% v/v) and indicated stable anaerobic degradation in both reactors. In contrast to SGP, the SMP in PM was approximately constant during the evaluated period. The acetoclastic species are, generally, more sensitive than hydrogenotrophic species to toxic compounds such as free ammonia. Therefore, at high FAN levels, methane can be generated by hydrogen and carbon dioxide, which can lead to lowering of biogas production with no impact on methane content in total gas volume (Borowski et al., 2014). On the other hand, a drop in SMP was observed in PM at 114 d. It could indicate that the highest FAN concentration affected the methanogenic bacteria.

The effect of nitrogen compounds was analysed in both reactors. TKN, TAN and FAN were lower in co-digested than the mono-digested PM. This effect could be due to the addition of fruit and vegetables that resulted in the dilution of the nitrogen compounds in the feed of the co-digestion anaerobic process.

Nitrogen concentrations in PM and PM-FVW did not cause complete inhibition of the anaerobic process. The lack of complete inhibition of the anaerobic process by nitrogen compounds in this experiment could be attributed to the use of pre-acclimated biomass. For an adapted process, several studies reported a tolerance of up 4 g/L for TAN (Angelidaki and Ahring, 1993; Yenigün and Demirel, 2013) and of up to 1.1 g/L for FAN (Hansen et al., 1998). Although there was no complete inhibition in both treatments, a slight inhibition was observed in PM, evidenced by a decrease of the biogas yield. Duan et al. (2012) indicated that a slight inhibition can occur when FAN levels are ranging from 250 to 400 mg/L. In these experiments, the FAN concentrations was always below 200 mg/L for both treatments, except during the last sampling event, in which FAN reached above 400 mg/L for both treatments (see Fig. 2B). Although a slight inhibition was observed in PM alone, it was not observed in PM-FVW; thus, the inhibition cannot be attributed to the high FAN concentration, since it was similar in both treatments. Future studies would be necessary to better explain this inhibition, which might be only associated to the higher TKN concentration (>4500 mg/L) for PM alone (see Fig. 2A) compared to PM-FVW (around 3500 mg/L) at the last sampling events.

A wide range of ammonia concentrations capable of inhibiting the process has been reported. The differences of the inhibitory TAN and FAN levels can be attributed to different substrate type, dilution, acclimation period, pH and work temperature (Gallert et al., 1998; Hansen et al., 1998; Hashimoto, 1986; Krylova et al., 1997). In our case, the highest FAN levels were observed at pH > 8 for PM and PM-FVW. The increased pH led to a higher percentage of free ammonia compared to the ionic form. Other studies reported that pH > 7 can negatively impact biogas generation, due to the high presence of molecular ammonia that becomes toxic to microbes (Hadj et al., 2009; Kayhanian, 1994).

The relation between FAN/TAN ratio and pH to a constant temperature and independent time allowed clarifying the dissociation of TAN as a function of pH (Fig. 2c). When pH was higher than 7.4, the FAN increased exponentially, reaching 12–15% for pH > 8. A similar result was reported by Rajagopal et al. (2013), who found that if the anaerobic digesters operated at pH 7 and 35 °C, FAN was lower than 1% of TAN; at pH 8, the FAN increased to 10% of TAN. The FAN/TAN ratio and pH could be, thus, a sufficient indicator of toxicity and inhibition during the anaerobic process. In this experiment, a drop of SMP in PM was observed at a FAN/TAN ratio equal to 12% and at a pH 8.1.

In addition to the above, the stability of the anaerobic process was evaluated via the pH, *alpha* and VFA/TA indicators (Table 2). The results showed that the stability was reached in both reactors. Anaerobic degradation is considered to be stable when the *alpha* factor is >0.7, VFA/TA is < 0.3–0.4 and the pH value is in the range of 6.5 to 7.5 (Callaghan et al., 1999; Jenkins et al., 1991; Khanal, 2008; Wilawan et al., 2014). In this study, *alpha*, VFA/TA and pH revealed stability throughout the whole experiment, which indicated a high buffering capacity in both treatments. However, these indicators could not properly reflect the system instability caused by ammonia. A similar effect was found by Duan et al. (2012), who reported a slight inhibition in the process while the indicators showed stable conditions in the same time. These authors explained that the increase in FAN concentration contributes to increase TA, which in turn leads to an increase on VFA concentration and then, the system can reach a new steady state. A similar result was reported by Nie et al. (2015), who showed that stable anaerobic conditions were reached despite the slight inhibition observed during the mono-digestion of PM (FAN < 0.6 g/L; VFA/TA between 0.20 and 0.35).

Furthermore, the organic matter removal was evaluated via the COD and VS contents, which had maximum values similar to those reported by Sakar et al. (2009). These authors reported in a review that the anaerobic digestion of PM had a range of 32%-78% of COD and VS removal. High organic matter removal indicated an efficient anaerobic degradation in both reactors. In addition, the highest VS_r was obtained in co-digested PM, which could be associated with the higher values of biogas and methane yields measured. On the other hand, the negative correlations between CODr and VSr with TKN indicated that the lower degradation of organic matter in manure alone could be associated with higher nitrogen compounds compared to the mixture of FVW and this manure.

Chen et al. reported that inhibitors commonly present in anaerobic reactors are ammonia, sulphides, metals, and organic compounds. Our results on the significant negative correlations suggest that the high organic matter (COD), the high content of nitrogen compounds (TAN and TKN), and the high content of certain metals (Zn, Mg and Mn) could be indicators of the reduced biogas and methane yields.

4.2. Evaluation of digestate quality

The regulation for the use of digestate as biofertiliser has not been established yet in Argentina. Accordingly, the values obtained in two digestates were compared to the regulatory limits set in Germany (RAL-QAS), Switzerland (ASCP) and United Kingdom (PAS 110).

Both PM and PM-FVW showed high EC values. Although EC is not included as a control parameter in international regulations for application as biofertilizer, excessive doses or continued applications of digestates could lead to an increase in soil salinity and plant growth inhibition (Alburquerque et al., 2012). Burton and Turner (2003) reported that the addition of organic waste with EC > 4 mS/cm to soils of arid and semiarid zones could cause salinization and the crop production could be affected.

The average values of Ca, Mg, K, and Na in PM were similar to those reported by Nkoa (2014) and Voća et al. (2005). Various digestates derived from PM, pig manure, sudan grass and organic household waste were valuable fertilizers suitable for agricultural production (Amon et al., 2007; Lukehurst et al., 2010; Voća et al., 2005; Weiland, 2010). It is noted that international regulations do not establish limit values for these nutrients. In addition, the management of good agricultural practices is recommended to ensure greater efficiency of digestate as fertilizer.

Zn concentration was higher in PM than PM-FVW. The co-digestion of PM had an effect of dilution of this metal in the digestate. Moreover, both digestates showed high concentration of this metal. The regulation of ASCP and PAS 110 established the limit of 400 mg/kg dry weight of Zn. The Zn concentration in PM-FVW (391 mg/kg) was below this limit established, while PM (596 mg/kg) was above. This metal can potentially cause a damage on the sustainability of agricultural soils through soil accumulation and phytotoxicity (Nkoa, 2014).

The Mn concentration was higher in mono-digestion than co-digestion of this manure. Once again, the effect of dilution of substrate was observed in co-digested digestate. Repeated long-term applications of digestate onto lands may result in Mn and organic matter accumulation, factors that favour Mn toxicity in soils with low Mn sorption capacity (Nkoa, 2014).

The use of *D. magna* in whole effluent toxicity tests is recommended by international organisms (USEPA, 2002). Acute toxicity tests allowed the determination of the toxicity level of PM and PM-FVW digestates at the end of the experiment. The analysis of the endpoints showed that the highest toxicity was associated with PM digestate, which was closely followed by PM-FVW. The complex mixture of many chemicals in the digestate causes toxicological interactions, such as synergism and antagonism (Gupta and Kelly, 1990). For example, plants exposed to digestate from livestock effluents showed growth stimulation at low concentrations (Alburquerque et al., 2012; Pivato et al. 2016), whereas showed seed germination inhibition at high concentrations. Particularly, Gupta and Kelly (1992) reported toxicity endpoints from various species exposed to poultry litter leachate, such as *D. magna* and *Vibrio* (*Photobacterium*) *phosphoreum*. Also, these authors found that poultry litter causes mutagenic effects using the Ames test. Our results are related to the differences in several physicochemical parameters among the digestates. In particular, toxicity was associated with EC, VFA, and nitrogen compounds, such as several authors have reported for aquatic animals (Olivero-Verbel et al., 2008; Pablos et al., 2011; Young et al., 2016), microorganisms (Juvonen et al., 2000), and plants (Boluda et al., 2011; Di María et al., 2014; Tigini et al., 2016; Young et al., 2012; Young et al., 2016).

5. Conclusions

Poultry manure co-digested with fruit and vegetable waste led to the highest biogas and methane yields and organic matter removal (VS_r), whereas it had the lowest nitrogen concentrations and lower digestate toxicity. Specifically:

- The presence of FVW improved the biogas and methane yields by >31%. This could be due to the intrinsic characteristics of this waste (high biodegradability and methane potential), the increase of C/N ratio and the dilution of nitrogen compounds.
- The mono-digestion of PM led to a slight inhibition in the biogas yield, but the methane yield was not affected. This could be associated to the high TAN (3.54 mg/L) and TKN (4.44 mg/L) concentrations.
- The stability indicators (pH, *alpha* and VFA/TA) showed that the system achieved stable conditions in both treatments during both experiments. Nevertheless, these indicators did not reflect the decrease in the biogas yield for the PM alone.
- Toxicity was associated to the salinity, total elements (Zn, Mn, Mg) and nitrogen compounds. Special care is needed with regard to the application of mono-digested poultry manure derived digestate as biofertilizer, due to the high Na, EC, Mn and Zn concentrations. The standardized bioassays, such as the *D. magna* immobilization test used here, can provide useful information on the safety related to the application of digestates as biofertilizers.
- Significant negative correlations suggest that organic matter (COD), nitrogen compounds (TAN and TKN), and total elements (Zn, Mg, Mn) could be indicators of reduced biogas and methane yields.

This experiment allowed to study the performance of anaerobic process of poultry manure alone and when co-digested with other organic waste in semicontinuous bench scale reactors. Future studies can include replications to quantify the inherent variance of the process.

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References

Abouelenien, F., Kitamura, Y., Nishio, N., Nakashimada, Y., 2009. Dry anaerobic ammonia-methane production from chicken manure. Appl. Microbiol. Biotechnol. 82, 757–764. https://doi.org/10.1007/s00253-009-1881-3.

Abouelenien, F., Namba, Y., Kosseva, M.R., Nishio, N., Nakashimada, Y., 2014. Enhancement of methane production from co-digestion of chicken manure with agricultural wastes. Bioresour. Technol. 159, 80–87. https://doi.org/10.1016/j.biortech.2014.02.050.

Agro-industry Ministry from Argentina. 2016a. Good management practices and use of chicken manure and chicken bedding (in Spanish). . http://www.agroindustria.gob.ar/sitio/areas/ prensa/folletos_digitales/contenido/Manual_Avicola.pdf (accessed 26 February 2018)

Alburquerque, J.A., de la Fuente, C., Ferrer-Costa, A., Carrasco, L., Cegarra, J., Abad, M., Bernal, M.P., 2012. Assessment of the fertiliser potential of digestates from farm and agroindustrial residues. Biomass Bioenergy 40, 181–189. https://doi.org/10.1016/j.biombioe.2012.02.018.

Álvarez, J.A., Otero, L., Lema, J.M., 2010. A methodology for optimising feed composition for anaerobic co-digestion of agro-industrial wastes. Bioresour. Technol. 101, 1153–1158. https://doi.org/10.1016/j.biortech.2009.09.061.

Alvarez, R., Lidén, G., 2008. Semi-continuous co-digestion of solid slaughterhouse waste, manure, and fruit and vegetable waste. Renew. Energy 33, 726–734. https://doi.org/10.1016/j.renene.2007.05.001.

Amon, T., Amon, B., Kryvoruchko, V., Machmüller, A., Hopfner-Sixt, K., Bodiroza, V., Hrbek, R., Friedel, J., Pötsch, E., Wagentristl, H., Schreiner, M., Zollitsch, W., 2007. Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations. Bioresour. Technol. 98, 3204–3212. https://doi.org/10.1016/j.biortech.2006.07.007.

APHA, 1992. Standard methods for examination of water and wastewater.18th ed., American Public Health Association (APHA), American Water Works Association (AWWA) and Water Pollution Control Federation (WPCF), Washington DC, USA

Angelidaki, I., Ahring, B.K., 1993. Thermophilic digestion of livestock waste: the effect of ammonia. Appl. Microbiol. Biotechnol. 38, 560–564. https://doi.org/10.1016/j.biortech.2007.01.057.

ASTM, 2014. Standard test methods for analisis of gas natural by gas chromatography. ASTM D 1945-14. ASTM International, West Conshohocken, PA, USA. http://doi.org/10.1520/D1945-14

Boluda, R., Roca-Pérez, L., Marimón, L., 2011. Soil plate bioassay: an effective method to determine ecotoxicological risks. Chemosphere 84, 1–8. https://doi.org/10.1016/j.chemosphere.2011.02.013.

Borowski, S., Domański, J., Weatherley, L., 2014. Anaerobic co-digestion of swine and poultry manure with municipal sewage sludge. Waste Manag. 34, 513–521. https://doi.org/10.1016/j.wasman.2013.10.022.

Borowski, S., Weatherley, L., 2013. Co-digestion of solid poultry manure with municipal sewage sludge. Bioresour. Technol. 142, 345–352. https://doi.org/10.1016/j.biortech.2013.05.047.

Bouallagui, H., Touhami, Y., Ben Cheikh, R., Hamdi, M., 2005. Bioreactor performance in anaerobic digestion of fruit and vegetable wastes. Process Biochem. 40, 989–995. https://doi.org/10.1016/j.procbio.2004.03.007.

Bres, P., Beily, M.E., Crespo, D., O'Farrel, J., 2011. Co-digestion of the organic fraction of household solid waste with sawdust in semi-continuous anaerobic reactors (in Spanish). ASADES 15, 9–16. ISSN 0329–5184.

Bujoczek, G., Oleszkiewicz, J., Sparling, R., Cenkowski, S., 2000. High solid anaerobic digestion of chicken manure. J. Agric. Eng. Res. 76, 51–60. https://doi.org/10.1006/jaer.2000.0529.

Burton, C.H., Turner, C., 2003. Manure management: treatment strategies for sustainable agriculture. Sisloe Research Institute, Sisloe, p. 451. ISBN 0953128261.

Calabrò, P.S., Gori, M., Lubello, C., 2015. European trends in greenhouse gases emissions from integrated solid waste management. Environ. Technol. 36 (16), 2125–2137. https://doi.org/10.1080/09593330.2015.1022230.

Callaghan, F.J., Wase, D.A.J., Thayan–ithy, K., Forster, C.F., 1999. Co-digestion of waste organic solids: batch studies. Bioresour. Technol. 67, 117–122. https://doi.org/10.1016/S0960-8524(98)00108-4.

Callaghan, F.J., Wase, D.A.J., Thayanithy, K., Forster, C.F., 2002. Continuous co-digestion of cattle slurry with fruit and vegetable wastes and chicken manure. Biomass Bioenergy 27, 71–77. https://doi.org/10.1016/S0961-9534(01)00057-5.

Chen, Y., Cheng, J.J., Creamer, K.S., 2008. Inhibition of anaerobic digestion process: a review. Bioresour. Technol. 99, 4044–4064. https://doi.org/10.1016/j.biortech.2007.01.057.

Costa, J.C., Barbosa, S.G., Alves, M.M., Sousa, D.Z., 2012. Thermochemical pre- and biological co-treatments to improve hydrolysis and methane production from poultry litter. Bioresour. Technol. 111, 141–147. https://doi.org/10.1016/j.biortech.2012.02.047.

Di María, F., Sordi, A., Cirulli, G., Gigliotti, G., Massaccesi, L., Cucina, M., 2014. Cotreatment of fruit and vegetable waste in sludge digesters. An analysis of the relationship among bio-methane generation, process stability and digestate phytotoxicity. Waste Manag. 34, 1603–1608. https://doi.org/10.1016/j.wasman.2014.05.017.

Díaz Baez, M.C., Pica Granados, Y., Ronco, A., 2004. Ensayo de toxicidad aguda con Daphnia magna. In: Castillo Morales, G. (Ed.), Ensayos toxicológicos y métodos de evaluación de calidad de aguas: Estandarización, intercalibración, resultados y aplicaciones, Mexico (in spanish).

Duan, N., Dong, B., Wu, B., Dai, X., 2012. High-solid anaerobic digestion of sewage sludge under mesophilic conditions: Feasibility study. Bioresour. Technol. 104, 150–156. https://doi.org/10.1016/j.biortech.2011.10.090.

Fantozzi, F., Buratti, C., 2009. Biogas production from different substrates in an experimental Continuously Stirred Tank Reactor anaerobic digester. Bioresour. Technol. 100, 5783–5789. https://doi.org/10.1016/j.biortech.2009.06.013.

Fricke, K., Santen, H., Wallmann, R., Hüttner, A., Dichtl, N., 2007. Operating problems in anaerobic digestion plants resulting from nitrogen in MSW. Waste Manag. 27, 30–43. https://doi.org/10.1016/j.wasman.2006.03.003.

Gallert, C., Bauer, S., Winter, J., 1998. Effect of ammonia on the anaerobic degradation of protein by a mesophilic and thermophilic biowaste population. Appl. Microbiol. Biotechnol. 50, 495–501. https://doi.org/10.1007/s002530051326.

Gangagni Rao, A., Surya Prakash, S., Joseph, J., Rajashekhara Reddy, A., Sarma, P.N., 2011. Multi stage high rate biomethanation of poultry litter with self mixed anaerobic digester. Bioresour. Technol. 102, 729–735. https://doi.org/10.1016/j.biortech.2010.08.069.

Gelegenis, J., Georgakakis, D., Angelidaki, I., Christopoulou, N., Goumenaki, M., 2007a. Optimization of biogas production from olive-oil mill wastewater, by codigesting with diluted poultry-manure. Appl. Energy 84, 646–663. https://doi.org/10.1016/j.apenergy.2006.12.001.

Gelegenis, J., Georgakakis, D., Angelidaki, I., Mavris, V., 2007b. Optimization of biogas production by co-digesting whey with diluted poultry manure. Renew. Energy 32, 2147–2160. https://doi.org/10.1016/j.renene.2006.11.015.

Gupta, G., Kelly, P., 1990. Toxicity (EC50) comparisons of some animal wastes. J. Water Air Soil Pollut. 53, 113–117. https://doi.org/10.1007/BF00154995.

Gupta, G., Kelly, P., 1992. Poultry litter toxicity comparison from various bioassays. J. Environ. Sci. Hlth. A27 (4), 1083–1093. https://doi.org/10.1080/10934529209375781.

Gunaseelan, V.N., 2004. Biochemical methane potential of fruits and vegetable solid waste feedstocks. Biomass Bioenergy 26, 389–399. https://doi.org/10.1016/j.biombioe.2003.08.006.

Hadj, B.E., Astals, S., Galí, A., Mace, S., Mata-Áivarez, J., 2009. Ammonia influence in anaerobic digestion of OFMSW. Water Sci. Technol. 59, 1153–1158. https://doi.org/10.2166/wst.2009.100.

Hansen, K.H., Angelidaki, I., Ahring, B.K., 1998. Anaerobic digestion of swine manure: Inhibition by ammonia. Water Res. 32, 5–12. https://doi.org/10.1016/S0043-1354(97)00201-7.

Hashimoto, A.G., 1986. Ammonia inhibition of methanogenesis from cattle wastes. Agric. Wastes 17, 241–261. https://doi.org/10.1016/0141-4607(86)90133-2.

Ho, L., Ho, G., 2012. Mitigating ammonia inhibition of thermophilic anaerobic treatment of digested piggery wastewater: use of pH reduction, zeolite, biomass and humic acid. Water Res. 46, 4339–4350. https://doi.org/10.1016/j.watres.2012.05.016.

Jenkins, S.R., Morgan, J.M., Sawyer, C.L., 1983. Measuring digestion simple and alkalimetric sludge by titration a growth. Water Pollut. Control Fed. 55, 448–453.

Jenkins, S.R., Morgan, J.M., Zhang, X., 1991. Measuring the usable carbonate alkalinity of operating anaerobic digesters. Res. J. Water Pollut. Control Fed. 63, 28–34. https://doi.org/10.2307/25043948.

Jiang, Y., Heaven, S., Banks, C.J., 2012. Strategies for stable anaerobic digestion of vegetable waste. Renew Energy. https://doi.org/10.1016/j.renene.2012.01.012.

Juvonen, R., Martikainen, E., Schultz, E., Joutti, A., Ahtiainen, J., Lehtokari, M., 2000. A battery of toxicity tests as indicators of decontamination in composting oily waste. Ecotox. Environ. Saf. 47, 156–166. https://doi.org/10.1006/eesa.2000.1943.

Kafle, G.K., Kim, S.H., 2013. Anaerobic treatment of apple waste with swine manure for biogas production: batch and continuous operation. Appl. Energy 103, 61–72. https://doi.org/10.1016/j.apenergy.2012.10.018.

Kayhanian, M., 1994. Performance of a high solids anaerobic digestion process under various ammonia concentrations. J. Chem. Technol. Biotechnol. 59, 349–352. https://doi.org/10.1002/jctb.280590406.

Khalid, A., Arshad, M., Anjum, M., Mahmood, T., Dawson, L., 2011. The anaerobic digestion of solid organic waste. Waste Manag. 31, 1737–1744. https://doi.org/10.1016/j.wasman.2011.03.021.

Khanal, S.K., 2008. Environmental factors. In: Khanal, S.K. (Ed.), Anaerobic biotechnology for bioenergy production: principles and applications. John Wiley & Sons Inc, New York, pp. 43–63.

Krylova, N.I., Khabiboulline, R.E., Naumova, R.P., Nagel, M.A., 1997. The influence of ammonium and methods for removal during the anaerobic treatment of poultry manure. J. Chem. Technol. Biotechnol. 70, 99–105. https://doi.org/10.1002/(SICI)1097-4660(199709)70:1<99::AID-JCTB684>3.0.CO;2-C.

Li, C., Champagne, P., Anderson, B.C., 2011. Evaluating and modeling biogas production from municipal fat, oil, and grease and synthetic kitchen waste in anaerobic co-digestions. Bioresour. Technol. 102, 9471–9480. https://doi.org/10.1016/j.biortech.2011.07.103.

Lukehurst, C., Frost, P., Al Seadi, T., 2010. Task 37, Utilisation of digestate from biogas plants as biofertiliser, in: IEA Bioenergy. pp 24. http://www.centri-force.co.uk/ (accessed 26 February 2018)

Magbanua, B.S., Adams, T.T., Johnston, P., 2001. Anaerobic codigestion of hog and poultry waste. Bioresour. Technol. 76, 165–168. https://doi.org/10.1016/S0960-8524(00)00087-0.

Molinuevo-Salces, B., García-González, M.C., González-Fernández, C., Cuetos, M.J., Morán, A., Gómez, X., 2010. Anaerobic co-digestion of livestock wastes with vegetable processing wastes: a statistical analysis. Bioresour. Technol. 101, 9479–9485. https://doi.org/10.1016/j.biortech.2010.07.093.

Molinuevo-salces, B., Gómez, X., Morán, A., García-gonzález, M.C., 2013. Anaerobic co-digestion of livestock and vegetable processing wastes : Fibre degradation and digestate stability. Waste Manag. 33, 1332–1338. https://doi.org/10.1016/j.wasman.2013.02.021.

Nelson C., Lamb, J., 2002. Final Report: haubenschild farms anaerobic digester. The Minnesota project, St. Paul, MN., pp 35.

Nicholson, F.A., Chambers, B.J., Williams, J.R., Unwin, R.J., 1999. Heavy metal contents of livestock feed and animal manures in England and Wales. Bioresour. Technol. 70, 23–31. https://doi.org/10.1016/S0960-8524(99)00017-6.

Nie, H., Jacobi, H.F., Strach, K., Xu, C., Zhou, H., Liebetrau, J., 2015. Mono-fermentation of chicken manure: Ammonia inhibition and recirculation of the digestate. Bioresour. Technol. 178, 238–246. https://doi.org/10.1016/j.biortech.2014.09.029.

Nkoa, R., 2014. Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: a review. Agron. Sustain. Dev. 34, 473–492. https://doi.org/10.1007/s13593-013-0196-z.

Olivero-Verbel, J., Padilla-Bottet, C., De la Rosa, O., 2008. Relationships between physicochemical parameters and the toxicity of leachates from a municipal solid waste landfill. Ecotox. Environ. Saf. 70, 294–299. https://doi.org/10.1016/j.ecoenv.2007.05.016.

Pablos, M.V., Martini, F., Fernandez, C., Babin, M., Herraez, I., Miranda, J., Martinez, J., Carbonell, G., San-Segundo, L., García-Hortigüela, P., 2011. Correlation between physicochemical and ecotoxicological approaches to estimate landfill leachates toxicity. Waste Manag. 31, 1841–1847. https://doi.org/10.1016/j.wasman.2011.03.022.

Pivato, A., Vanin, S., Raga, R., Lavagnolo, M.C., Barausse, A., Rieple, A., Laurent, A., Cossu, R., 2016. Use of digestate from a decentralized on-farm biogas plant as fertilizer in soils: an ecotoxicological study for future indicators in risk and life cycle assessment. Waste Manag. 49, 378–389. https://doi.org/10.1016/j.wasman.2015.12.009.

Quiroga, G., Castrillón, L., Fernández-Nava, Y., Marañón, E., 2010. Physico-chemical analysis and calorific values of poultry manure. Waste Manag. 30, 880–884. https://doi.org/10.1016/j.wasman.2009.12.016.

Rajagopal, R., Massé, D.I., Singh, G., 2013. A critical review on inhibition of anaerobic digestion process by excess ammonia. Bioresour. Technol. 143, 632–641. https://doi.org/10.1016/j.biortech.2013.06.030.

Rosso, A., Muzlera, A., Pettigiani, E., Mazzeo, N., Poliak, R., 2016. Characterization and management proposal for the waste generated by the Central Market Corporation of Buenos Aires. Report of the National Institute of Industrial Technology (in Spanish). www.mercadocentral.gob.ar/proyectos/informe-caracterizacion-y-gestion-INTI.pdf (accessed 27 April 2017)

Sakar, S., Yetilmezsoy, K., Kocak, E., 2009. Anaerobic digestion technology in poultry and livestock waste treatment: a literature review. Waste Manag. Res. 27, 3–18. https://doi.org/10.1177/0734242X07079060.

Salminen, E., Rintala, J., 2002. Anaerobic digestion of organic solid poultry slaughterhouse waste: a review. Bioresour. Technol. 83, 13–26. https://doi.org/10.1016/S0960-8524(01)00199-7.

Seadi, T.A., Rutz, D., Prassl, H., Köttner, M., Finsterwalder, T., Volk, S., Janssen, R., 2008. Biogas Handbook. Igarss 2014. https://doi.org/10.1533/9780857097415.1.85.

Tigini, V., Franchino, M., Bona, F., Varese, G.C., 2016. Is digestate safe? A study on its ecotoxicity and environmental risk on a pig manure. Sci. Total Environ. 551–552, 127–132. https://doi.org/10.1016/j.scitotenv.2016.02.004.

USDA., USCC, 2001. Test Methods for the Examination of Composting and Compost (TMECC). Edaphos International, Houston, USA.

USEPA, 1996. Ecological Effects Test Guidelines OPPTS 850.1010. Aquatic invertebrate acute toxicity test, freshwater Daphnids. EPA 712-C-96-114 Environmental Protection Agency, USA.

USEPA, 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Environmental Protection Agency, USA. EPA-821-R-02-012.

Voća, N., Krička, T., Ćosić, T., Rupić, V., Jukić, Ž., Kalambura, S., 2005. Digested residue as a fertilizer after the mesophilic process of anaerobic digestion. Plant, Soil Environ. 51, 262–266.

Wang, X., Yang, G., Feng, Y., Ren, G., Han, X., 2012. 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, 78–83. https://doi.org/10.1016/j.biortech.2012.06.058.

Ward, A.J., Hobbs, P.J., Holliman, P.J., Jones, D.L., 2008. Optimisation of the anaerobic digestion of agricultural resources. Bioresour. Technol. 99, 7928–7940. https://doi.org/10.1016/j.biortech.2008.02.044.

Weiland, P., 2010. Biogas production: current state and perspectives. Appl. Microbiol. Biotechnol. 85, 849–860. https://doi.org/10.1007/s00253-009-2246-7.

Wilawan, W., Pholchan, P., Aggarangsi, P., 2014. Biogas production from co-digestion of Pennisetum pururem cv. Pakchong 1 grass and layer chicken manure using completely stirred tank. Energy Procedia 52, 216–222. https://doi.org/10.1016/j.egypro.2014.07.072.

Yenigün, O., Demirel, B., 2013. Ammonia inhibition in anaerobic digestion: a review. Process Biochem. 48, 901–911. https://doi.org/10.1016/j.procbio.2013.04.012.

Young, B.J., Riera, N.I., Beily, M.E., Bres, P.A., Crespo, D.C., Ronco, A.E., 2012. Toxicity of the effluent from an anaerobic bioreactor treating cereal residues on *Lactuca sativa*. Ecotoxicol. Environ. Saf. 76, 182–186. https://doi.org/10.1016/j.ecoenv.2011.09.019.

Young, B.J., Rizzo, P.F., Riera, N.I., Della Torre, V., López, V.A., Molina, C.D., Fernández, F.E., Crespo, D.C., Barrena, R., Komilis, D., Sánchez, A., 2016. Development of phytotoxicity indexes and their correlation with ecotoxicological, stability and physicochemical parameters during passive composting of poultry manure. Waste Manag. 54, 101–109. https://doi.org/10.1016/j.wasman.2016.05.001.

Zhao, Y., Shepherd, T.A., Swanson, J.C., Mench, J.A., Karcher, D.M., Xin, H., 2014. Comparative evaluation of three egg production systems: housing characteristics and management practices. Poul. Sci. 94, 475–484. https://doi.org/10.3382/ps/peu077.