Thermochemical pre- and biological co-treatments to improve hydrolysis and methane production from poultry wastes

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Abstract
Poultry industry wastes, namely feathers and poultry litter, are an interesting source of substrate for biogas production. The aim of this work was to assess the biomethane potential of raw poultry wastes, as well as the possibility of enhancing this potential by favouring the hydrolysis of cellulolytic and proteinaceous material in the wastes by using bioaugmentation and thermochemical pre-treatments. Biomethane production from poultry litter and chicken feathers was assessed in batch assays. Pre-treatment with lime and sodium hydroxide was performed at different temperatures and pressures. *Clostridium cellulolyticum*, *C. saccharolyticum* and *C. thermocellum* were used as bioaugmentation strains in the anaerobic digestion of poultry litter. *Fervidobacterium pennivorans* was used to aid the hydrolysis of poultry feather. Anaerobic digestion of the raw wastes allowed a methanisation percentage (MP) of 17±2 and 33±5%, respectively from poultry litter and chicken feathers, with 2.5% total solids. The major increase in biomethanisation of poultry litter was reached after the thermochemical pre-treatment with $Ca(OH)_2$ (90 °C, 1.27 bar, 120 minutes), with an increase of 15% in the MP comparatively with the raw wastes MP. For the poultry feathers waste, none of the implemented strategies contributed to the optimization of methane production. However, it was observed that all treatments have contributed to a significant increase in the wastes solubilisation. Therefore, the conversion of soluble organic matter to methane was the limiting step of the anaerobic digestion process of poultry wastes.

Keywords
Anaerobic digestion; Biomethane potential; Feathers; Hydrolysis; Lignocellulosic biomass; Waste treatment

INTRODUCTION

In the last few years, the spread of the economical crises led to a sharp demand for cheaper products, such as poultry meat. As a result, poultry slaughterhouses are producing increasing amounts of organic wastes, such as manure and bedding material mixture (or litter), waste feed, dead birds, blood, broken eggs, and feathers (Kelleger *et al.*, 2002). Present pollution concerns, and also more restrict environmental legislation, compel poultry processing industry to adopt effective waste treatment options. Poultry litter and chicken feathers are two waste streams of concern because of their pollutant load and high volume production. Composting and direct application on land are the most widely used alternatives in handling poultry litter thus far. Chicken feathers can be incorporated in animal feed or used to produce polymers. However, both substrates have a high organic content that could be recovered as methane in an anaerobic digestion process. This alternative could have an added interest considering the present, petrol-derived energy limitation and CO_2 mitigation policies (Molinuevo-Salces *et al.*, 2010).

Poultry litter is a complex substrate, mainly composed by lignocellulosic biomass. Hydrolysis of lignocelluloses has been considered the rate limiting step during anaerobic digestion of these type of wastes, therefore constraining methane production (Pavlostathis and Giraldo-Gomez, 1991). Poultry feathers consist mainly of keratin and small amounts of lipids (Salminen *et al.*, 2003). Beta-keratin,

the insoluble structural protein of feathers, is known for its high stability and accounts for more than 90% of this waste. Pre-treatment of both lignocellulosic biomass and feathers could accelerate the hydrolysis process and improve the final biogas production from these wastes (Fernandes *et al.*, 2009; Kashani, 2009).

Waste hydrolysis can be stimulated using different methods, *e.g.* chemical, thermal, or enzymatic methods. Lime ($Ca(OH)_2$) is a cheap and safe to use compound that can be used for alkaline hydrolysis of wastes (alone or in combination with heat and/or with pressure). A practical disadvantage is that lime is a weak base and usually it is necessary a higher concentration to achieve the same performance as when using strong bases, *e.g.* sodium hydroxide (NaOH). Chemical (or themochemical) pre-treatment needs to be performed prior to anaerobic digestion and in a separate process. Addition of anaerobic hydrolytic microorganisms can be an alternative for a one-step enhanced hydrolysis-fermentation process. There are several described microorganisms with cellulolytic activity that could be used to biodegrade the lignocellulosic portion of poultry litter, namely *Clostridium cellulolyticum* (Petitdemange *et al.*, 1984) and *Caldicellulosiruptor saccharolyticus* (VanFossen *et al.*, 2009). *Fervidobacterium pennivorans* is a proteolytic bacterium that has the ability to degrade native chicken feathers (Friedrich and Antranikian, 1996).

The aim of this work was to determine the Biochemical Methane Potential (BMP) of raw poultry litter and chicken feathers. Afterwards, co- and pre-treatments were applied with the objective of enhancing the wastes solubilisation and consequently increase their conversion to methane. Thermochemical pre-treatments, using lime and sodium hydroxide, at high temperature and high pressure, and biological co-treatment with cellulolytic and proteolytic microorganisms were tested.

MATERIAL AND METHODS

Waste characterization

The two wastes used in these experiments, poultry litter and chicken feathers, were collected in a poultry industry in the north of Portugal and characterized (Table 1).

Table 1. Characterization of poultry litter and chicken feathers wastes.

	Litter	Feathers
TS (%)	77 ± 1.3	100 ± 0.5
VS (%)	70 ± 1.5	99 ± 1.4
COD (g_{COD}/kg_{waste})	915 ± 67	1408 ± 59
N-Kjeldahl (g_N/kg_{waste})	21 ± 1	137 ± 9
COD:N	40-47:1	10-11:1

Note: mean ± standard deviations of 10 observations for TS and VS, 6 observations for COD, and 4 observations for N-kjeldahl determination.

Biological Co-Treatment (Bioaugmentation)

Different microorganisms were used to bioaugment the anaerobic biodegradability tests. For the conversion of poultry litter three cellulolytic microorganisms were used: *Clostridium cellulolyticum*, *Clostridium thermocellum*, and *Caldicellulosiruptor saccharolyticus*. The chicken feathers were digested in the presence of *Fervidobacterium pennivorans.*

C. cellulolyticum, C. Saccharolyticus and *C. Thermocellum* were pre-grown with cellobiose as carbon source as described by Weimer *et al.* (1976, 1977)). *F. pennivorans* was pre-growth as described by Friedrich and Antranikian (1996). Cells were harvested during the exponential time and concentrated by centrifugation; 2.5 mL of concentrated cells of each microorganism were added

to the anaerobic biodegradability test with the respective residue. The vials were incubated at the optimal temperature of each microorganism, *i.e.*, 37ºC, 55ºC, and 65ºC for *C. cellulolyticum*, *C. thermocellum*, and *C. saccharolyticus* and *F. pennivorans*, respectively. All the assays were done with 2.5% TS_{waste} using anaerobic granular sludge as inoculum (approximately 1.30 g VS_{waste}/g $VS_{inoculum}$). Aiming at confirm the microorganisms activity over the substrates used, its incubation during 120h was performed in the same conditions as described for the anaerobic biodegradability tests but without add anaerobic inoculums, *i.e.*, only microorganism and residue.

Thermochemical pre-treatments

The wastes were pre-treated with two alkali, $Ca(OH)_2$ and NaOH at different temperatures (20 and 90 ºC), contact times (30, 60 and 120 minutes), pressure (1.01, 1.27 and 4 bar) and base concentration (0.05, 0.1 e 0.2 g_{alkali}/gTS_{waste}). Assays were made using 40 gTS_{waste}/L in 550 mL vials. Assays at 90 °C and high pressure, *i.e.* 1.27 and 4 bar, were performed in an autoclave (Hiclave HV-25L, Dublin, Ireland) and a pressure column equipped with a pressure transducer and a regulatory valve, respectively. Column pressurization was made through the injection of N_2. After pre-treatment the COD_s, TS and VS, ammonium concentration, reducing sugars, and pH were determined. Afterwards, the samples with high PS were selected in order to determine their BMP. Previously to the BMP assays pH was neutralized with HCl (8M). The anaerobic biodegradability assays were performed with 31.25 mL of pre-treated sample corresponding to 2.5% TS_{waste}, in 600 mL vials, with a ratio of 1.35 gVS_{waste}/gVS_{sludge}.

Anaerobic Biodegradability Assays

Anaerobic biodegradability batch tests were used to determine the BMP and the biomethane production rate from the poultry wastes according to the directives defined in Angelidaki *et al.* (2009). Bottles were prepared by adding the residues, inoculum, and basal medium containing $NaHCO_3$ (5 g/L) to a final volume of 50 mL. The pH was corrected to 7.0–7.2 using NaOH or HCl 2 M. The vials were sealed and the headspace flushed with N_2/CO_2 (80:20 v/v). Before incubation, the medium was reduced with $Na_2S{\cdot}9H_2O$ added to a final concentration of 1 mM. All batch tests were performed in triplicate and incubated at 37 ºC, except for the bioaugmentation tests that were incubated at optimum growth temperature of each microorganism. Parallel, blank assays in the absence of waste were also performed. Two inocula were used, anaerobic suspended sludge (VS = 13 ± 1 g/L, Specific acetoclastic activity (SAA) < 10 mL $CH_{4\ @STP}$/(gVS day), and Specific hydrogenotrophic methanogenic activity (SHMA) = 695 ± 39 mL $CH_{4\ @STP}$/(gVS day)) from a municipal wastewater treatment plant, and anaerobic granular sludge (VS = 44 ± 3 g/L; SAA = 55 ± 4 $CH_{4\ @STP}$/(gVS day); SHMA = 655 ± 39 $CH_{4\ @STP}$/(gVS day)) from a brewery industry.

The methane accumulated in the vessels headspace was measured by gas chromatography by collecting 500 µL of sample volume with a gas-tight syringe. Methane production was corrected for standard temperature and pressure (STP) conditions. For comparison with the literature and uniformity of results regardless of using different amounts of waste the BMP was determined by unit of waste of VS added to each vial:

$$\text{BMP} = \text{CH}_4 / \text{VS}_{\text{added}} = \frac{\text{COD}_{\text{removed}} - \text{CH}_4 \times 350\ (\text{CH}_4 / \text{COD}_{\text{removed}})}{\text{VS}_{\text{waste, added}}\ (\text{g})} \qquad \text{(eq. 1)}$$

The anaerobic digestion yield in terms of methane production (MP) was defined as the proportion of methane produced during the assays in relation to the biochemical methane potential (350 L CH_4/kg COD):

$$\text{MP} = \frac{\text{COD}_{\text{removed}} - \text{CH}_4 / \text{COD}_{\text{removed}}}{\text{COD}_{\text{removed}}} \times 100 \qquad \text{(eq. 2)}$$

Hydrolysis was evaluated considering the percentage of solubilisation (PS), which is the percentage of the initial COD added to the vials that is solubilised during the anaerobic biodegradability assay. The soluble COD (COD_s) detected at the end of each assay and the COD-CH_4 produced during the assay were used for calculating this parameter, as follows:

$$PS = \frac{COD_{s_{fin}} + COD\text{-}CH_4 / 0.35 - COD_{s_{ini}}}{COD_{substrate}} \times 100 \quad \text{(eq. 3)}$$

When thermochemical pre-treatments were applied, two PS were calculated, the first (PS1) corresponds to the solubilisation that occurred during the pre-treatment, and the second (PS2) to the solubilisation that occurred during the anaerobic biodegradability assay:

$$PS1 = \frac{COD_{s_{fin}}(\text{pre-treatment}) - COD_{s_{ini}}(\text{pre-treatment})}{COD_{substrate}} \times 100 \quad \text{(eq. 4)}$$

$$PS2 = \frac{COD_{s_{fin}} + COD\text{-}CH_4 / 0.35 - COD_{s_{ini}}}{COD_{substrate}} \times 100 \quad \text{(eq. 5)}$$

Analytical Methods

Total kjeldahl nitrogen (TKN), total solids (TS), and volatile solids (VS) were measured according to standard methods (APHA, 1998). Total and soluble chemical oxygen demand (COD) was determined using standard kits (Hach Lange, Düsseldorf, Germany). Ammonium was determined by the Nessler method and reducing sugars were measured using the DNS method. Volatile fatty acids (VFA) were determined by HPLC (Jasco, Japan) with a Chrompack column (6.5x30 mm^2) at 60 ºC. Sulfuric acid (0.01 N) was used as mobile phase at a flow rate of 0.6 mL/min. Detection of soluble products was made with a UV detector at 210 nm. Methane (CH_4) measurement was analyzed in a gas chromatograph (Chrompack 9000) equipped with a FID detector and a 2 m x 1/8'' Chromosorb 101 (80-120 mesh) column. Nitrogen was used as carrier gas (30 mL/min). The column, injector, and detector temperatures were 35, 110, and 220 ºC, respectively.

Statistical Analysis

The results obtained in the different anaerobic biodegradability assays were compared after a significance statistical analysis by using a single factor analysis of variance (ANOVA). Statistical significance was established at the $P<0.05$ level.

RESULTS AND DISCUSSION

Biomethane potential of the raw wastes

The first part of this work consisted in assessing the BMP of raw wastes, *i.e.* without co- or pre-treatment. Results of the biodegradability assays are shown in Table 2. Regarding the poultry litter waste, the highest cumulative methane production (292 ± 45 mg COD-CH_4) was achieved with 2.5% TS and using anaerobic granular sludge as inoculum. Of the three concentrations of waste tested with suspended biomass, the best scenario in terms of methane production was observed for the concentration of 1%; in this case a maximum specific methane production of 145±14 L CH_4/kg VS could be obtained, which corresponds to a MP of 41±3%. Results in the range of 140-220 L CH_4/kg VS have been reported by other authors (Webb and Hawkes (1985) *in* Salminen and Rintala (2002)), but in continuous reactors. All the assays showed a similar PS (approximately 39%), with a high VFA and COD_s accumulation inside the vials with high TS. The best result in terms of solubilisation was also obtained with the granular biomass (56±2 %). Relatively to the raw chicken

feathers waste, the best results were also obtained with granular sludge and 2.5% TS. The BMP was 136±22 L CH_4/kg VS and the PS was 68±5 %. The BMP obtained with suspended biomass, at different concentrations of TS, were not significantly different. Values of methane production found in literature for this type of waste range from approximately 45 to 165 L CH_4/kg VS (Kashani (2009), Salminen and Rintala (2002)).

Table 2: Experimental results obtained at the end of raw poultry wastes biodegradability tests.

		Litter				**Feathers**		
		Suspended			***Granular***	***Suspended***		***Granular***
		1% TS (0.60gCOD)	**2.5% TS** (1.49gCOD)	**5% TS** (2.98gCOD)	**2.5%TS** (1.49gCOD)	**0.5% TS** (0.35gCOD)	**1% TS** (0.70gCOD)	**2.5% TS** (1.76gCOD)
gVS_{waste}/gVS_{sludge}		1.39	3.47	6.93	1.29	0.76	1.51	1.41
CH_4 production	mgCOD-CH_4	188 ± 20	284 ± 31	126 ± 21	292 ± 45	63 ±12	134 ± 14	482 ± 71
BMP	LCH_4/kgVS	145 ± 14	87 ± 10	19 ± 3	90 ± 13	89 ± 12	88 ± 11	136 ± 22
MP	%	41 ± 3	19 ± 2	4 ± 1	17 ± 2	11 ± 1	9 ± 1	33 ± 5
PS	%	37 ± 3	39 ± 2	39 ± 1	56 ± 2	11 ± 1	10 ± 1	68 ± 5
pH		7.6	7.5	7.0	7.3	7.6	7.5	7.9
COD_s	g/L	0.66 ± 0.26	6.00 ± 1.31	20.94 ± 0.72	13.98 ± 1.52	0.04 ± 0	0.18 ± 0.01	10.40 ± 1.38
VFA	g COD/L	*0*	3.50 ± 1.18	15.53 ± 4.49	3.27 ± 0.47	0.02 ± 0	0.01 ± 0	0.73 ± 0.52
Sugars	g/L	*nd*	*Nd*	*nd*	1.68 ± 0.12	*nd*	*nd*	2.24 ± 0.33
NH_4^+-N	g/L	0.25 ± 0.03	0.70 ± 0.01	1.28 ± 0.54	0.64 ± 0.07	0.15 ± 0.04	0.40 ± 0.01	1.74 ± 0.10

Note: The values obtained in the blanks were subtracted in the data presented in the table.
*The values between brackets represent the COD added in the beginning at each assay.
nd: not determined.

Biological co-treatment (bioaugmentation)

Cellulolytic microorganisms. Results from the bioaugmented tests with mesophilic (*C. cellulollyticum*) and thermophilic (*C. thermocellum* and *C. saccharolyticus*) microorganisms are displayed in Table 3. The best results were achieved using *C. cellulolyticum*, with a specific methane production of 102±5 L CH_4/kg VS and a MP of 22±1 %. These results are significantly higher ($p<0.01$) than the obtained with the raw waste. Moreover, there was an increase in VFA concentration from 3.27±0.47 to 4.14 ± 0.69 g/L and a reduction in reducing sugar concentration from 1.68±0.12 to 0.20±0.07 g/L. In the trials with *C. thermocellum* and *C. saccharolyticus* no significant differences were observed, considering methane production. However, the bioaugmentation with these microorganisms, caused a significant increase in the PS between tests with and without co-biological treatment, *i.e.* from 56±2 to 66±1% ($p<0.01$) and 79±1% ($p<0.01$), respectively. The assays with the thermophilic microorganisms were performed at the optimum growth temperature of each microorganism, *i.e.* 55 and 65 ºC. However, the anaerobic granular sludge inoculum was mesophilic, which may be the reason for the low methane yields. In fact, in a test with 2.5% TS of waste and 2.5 mL of inoculated medium, 31 and 23% of COD removals after 120 h of inoculation with C. *thermocellum* and *C. saccharolyticus*, respectively, were observed. Thus, the failure in methane production may be explained by the inefficiency of the inoculum to use the soluble organic matter.

Table 3: Experimental results obtained at the end of the anaerobic biodegradability tests of poultry litter bioaugmented with cellulolytic microorganisms, and chicken feathers bioaugmented with a proteolytic microorganism.

		Litter (2.5% TS)			**Feathers (2.5% TS)**
		C. cellulolyticum (1.49 g COD)	*C. thermocellum* (1.49 g COD)	*C. saccharolyticus* (1.49 g COD)	*F. pennivorans* (1.76 g COD)
CH_4 production	**mg COD-CH_4**	333 ± 22	229 ± 25	310 ± 23	159 ± 32
BMP	**L CH_4/kg VS**	102 ± 5	71 ± 7	95 ± 4	45 ± 4
MP	**%**	22 ± 1	15 ± 1	21 ± 1	9 ± 1
PS	**%**	62 ± 2	66 ± 1	79 ± 1	67 ± 1
pH		7.2	7.5	7.6	7.5
COD_s	**g/L**	11.81 ± 0.94	15.16 ± 1.29	17.25 ± 0.92	20.39 ± 0.89

VFA	**g COD/L**	4.14 ± 0.69	8.66 ± 0.37	6.43 ± 0.86	18.04 ± 0.37
Sugars	**g/L**	0.20 ± 0.07	1.19 ± 0.18	1.15 ± 0.22	0.65 ± 0.10
NH_4^+-N	**g/L**	1.16 ± 0.46	0.24 ± 0.06	0.70 ± 0.04	2.81 ± 0.14

Note: The values obtained in the blanks were subtracted in the data presented in the table.
The values between brackets represent the COD, from the waste, added in the beginning at each assay.

Proteolytic microorganism. Biological co-treatment of chicken feathers with *F. pennivorans* allowed the conversion of 159±32 mg COD-CH_4 corresponding to a PM of 9 % and a BMP of 45±4 LCH_4/kgVS (Table 3). Therefore, the co-treatment did not improve the residue biodegradability. However, from the assay with only residue and *F. pennivorans* was possible to verify that the bacteria acted on the waste and, after 120 h of incubation, 31% of the initial COD was removed. The high COD_s detected after co-treatment was mainly caused by the VFA accumulation, namely acetate (10.1±0.5 g COD/L), propionate (5.1±1.1 g COD/L), and iso-butyrate (2.9±0.3 g COD/L), demonstrating the activity of the microorganism on the substrate. The high ammonium concentration, 2.8±0.1 g NH_4^+-N/L, together with a pH of 7.5, may have inhibited the methanogenesis. The efficacy of this treatment may be increased by separating the treatment in two stages.

Thermochemical pre-treatments

Results of waste pre-treatment with lime and sodium hydroxide are summarized in table 4. In the assays with lime it was possible to verify that the solubilisation significantly increased ($p<0.01$) with temperature. COD_s also increased consistently with the contact time increase from 30 to 120 min, and with the pressure increase from 1.01 to 1.27 bar. However, the pressure increase to 4 bar caused a slight decrease in the solubilisation. The highest solubilisation, *i.e.* COD_s of 13 g/L, occurred in the assay with 0.2 g $Ca(OH)_2/g_{waste}$, at 1.27 bar and 90 ºC, during 120 minutes. The effects of the several parameters tested in the assays with NaOH were similar to those obtained with lime but with higher solubilisation percentages. The assay that permitted to attain higher COD_s, around 32 g/L, was performed with 0.2 g $NaOH/g_{waste}$, at 1.27 bar and 90 ºC, during 120 minutes. As in the results with poultry litter, also in the chicken feathers waste was observed a significant ($p<0.01$) increase of the organic matter solubilised with the increase in temperature, contact time, and alkali concentration with the exception of the test at room temperature and pressure. In the assays with NaOH, similar trends were observed with the highest result obtained with 0.2 g $NaOH/g_{waste}$, at 1.27 bar and 90 ºC, during 120 minutes, *i.e.*, around 56 g COD/L.

Table 4: Soluble COD (g/L) obtained after thermochemical pre-treatment of poultry litter and chicken feathers.

	$Ca(OH)_2$					**NaOH**		
t (min)	[g/g_{waste}]	1.01 bar 20 ºC	1.01 bar 90 °C	1.27 bar 90 °C	4 bar 90 °C	[g/g_{waste}]	1.01 bar 20 ºC	1.27 bar 90 °C
Poultry Litter								
	0.05	3.77	5.30	9.08	4.34	0.05	9.62	
30	0.1	3.27	5.18	10.37	8.74	0.1	10.82	
	0.2	3.20	7.28	9.31	9.54	0.2	13.78	
	0.05	4.40	9.43	11.14	10.34	0.05	10.25	14.82
60	0.1	4.27	9.17	10.90	9.70	0.1	11.85	18.80
	0.2	3.83	9.13	11.62	9.64	0.2	14.58	21.32
	0.05	5.08	*10.90*	9.36	9.92	0.05	12.20	16.80
120	*0.1*	*5.35*	*10.90*	9.52	10.80	0.1	14.52	28.68
	0.2	4.31	10.51	***12.88***	*11.52*	***0.2***	*17.60*	***32.02***
Chicken Feathers								
	0.05	*2.40*	3.79	9.73	6.76	*0.05*	*4.60*	
30	0.1	2.40	5.50	17.72	8.32	0.1	3.56	
	0.2	1.89	5.22	16.09	7.86	0.2	3.70	
	0.05	1.82	5.82	15.36	6.74	0.05	2.80	14.70
60	0.1	1.70	8.54	17.09	22.76	0.1	3.44	33.05
	0.2	1.78	7.94	17.91	13.92	0.2	3.60	41.00
120	0.05	2.00	10.52	13.18	12.76	0.05	3.04	33.95

	0.1	2.26	16.36	26.76	20.30	0.1	3.74	50.90
	0.2	1.75	*17.76*	***33.62***	*26.62*	***0.2***	3.82	***55.60***

Based on the pre-treatment results (table 4) the wastes obtained after three conditions, namely, $Ca(OH)_2$ (90 °C/1 bar and 90 °C/1.27 bar) and NaOH (90 °C/1.27 bar), were subsequently used as substrate in the anaerobic biodegradability assays (table 5).

In the case of the residues of poultry litter, despite the fact that the pre-treatment with NaOH generates more COD_s the subsequent step to obtain methane was less effective than in the tests with $Ca(OH)_2$. Still, the hydrolysis obtained during the biodegradability assays (PS2) was higher for the test with NaOH resulting in even higher COD_s and possibly inhibiting the methanogenesis. Comparing the two tests with lime an increase in terms of absolute methane production, MP, and PS was observed with the higher pressure tested. In addition, the specific methane production was significantly ($p<0.01$) higher in the test at 1.27 bar (137±4 L CH_4/kg VS). According to Chang *et al.* (1997), the optimal conditions for the alkaline pretreatment of wheat straw are 2 h with a concentration of 0.1 g $Ca(OH)_2/g_{waste}$ at 100-120 ºC. They obtained reducing sugars concentration, after enzymatic hydrolysis, 5 times higher than with untreated biomass. In the study by Rafique *et al.* (2010), an increase of 70% in the methane yield was obtained after pretreatment with 0.05 g $Ca(OH)_2/g_{waste}$ at 70 °C.

With regards to residue of chicken feathers the test performed with high pressure (1.27 bar) presented the best results, in terms of BMP (105±3 L CH_4/kg VS), PM (22±1%), and PS2 (74±1%). Chicken feathers pre-treatment with NaOH promoted the solubilisation but inhibited methane production. In this test, high concentration of ammonium (2.7±0.2 g NH_4^+-N/L) was detected at the end of the assay, which could induce methanogenesis inhibition. Although methane production did not improve after thermochemical pretreatment, there was a significant solubilization as observed by the high PS2 values ($p<0.01$). According to Kashani (2009), the thermochemical treatments when applied to proteinaceous waste allow a significant improvement in the methane yield. From a sample with 40 g TS/L, pretreated with 0.1 g $Ca(OH)_2$/g TS, at 100 °C, during 30 minutes, this author obtained 480 L CH_4/kg VS, corresponding to 97% of the theoretical potential for methane production.

Table 5: Experimental results obtained at the end of the anaerobic biodegradability tests of poultry litter after thermochemical pre-treatment.

		$Ca(OH)_2$, 90°C & 1bar (1.55 g COD)	**$Ca(OH)_2$ 90°C & 1.27bar** (1.55 g COD)	**NaOH, 90°C & 1.27bar** (1.55 g COD)
Poultry Litter				
CH_4 production	mg COD-CH_4	460 ± 28	490 ± 15	87 ± 20
BMP	L CH_4/kg VS	129 ± 8	137 ± 4	24 ± 6
MP	%	30 ± 1	32 ± 1	6 ± 6
PS1	%	20	25	61
PS2	%	40 ± 1	42 ± 1	64 ± 1
pH		6.8	6.9	7.0
CODs	g/L	3.18 ± 0.48	3.20 ± 0.42	18.07 ± 2.12
Sugars	g/L	1.06 ± 0.30	0.74 ± 0.13	1.06 ± 0.16
NH_4^+-N	g/L	0.58 ± 0.08	0.58 ± 0.18	0.69 ± 0.08
Chicken Feathers				
CH_4 production	mg COD-CH_4	322 ± 7	374 ± 11	90 ± 9
BMP	L CH_4/kg VS	90 ± 2	105 ± 3	25 ± 3
MP	%	19 ± 1	22 ± 1	5 ± 0
PS1	%	31	59	98
PS2	%	72 ± 2	74 ± 1	*n.d.*
pH		6.8	6.9	7.0
CODs	g/L	18.17 ± 0.70	17.70 ± 0.72	36.90 ± 0.76

Sugars	g/L	0.60 ± 0.12	0.84 ± 0.07	0.64 ± 0.07
NH_4^+-N	g/L	1.02 ± 0.11	0.97 ± 0.10	2.72 ± 0.16

Note: The values obtained in the blanks were subtracted in the data presented in the table.
The values between brackets represent the COD, from the waste, added in the beginning at each assay.

CONCLUSIONS

Biomethane potential of raw poultry litter (1% TS, 1.39 gVS_{waste}/gVS_{sludge}) and chicken feathers (2.5% TS, 1.41 gVS_{waste}/gVS_{sludge}) was 145±14 L CH_4/kg VS and 136±22 L CH_4/kg VS, respectively. The best solubilisation percentages were 56±2 % and 68±5 % for poultry litter and chicken feathers at 2.5% TS of waste, respectively.

Hydrolysis of poultry litter could be enhanced by bioaugmentation with *C. saccharolyticus* (79±1 % improvement). Hydrolysis of feathers waste was improved in 74 ± 1%, with 0.2 g $Ca(OH)_2$/g waste at 90 ºC and 1.27 bar.

The biological co-treatments and the thermochemical pre-treatments with $Ca(OH)_2$ and NaOH, had a significant impact in the hydrolysis of poultry litter and chicken feathers. However, this caused an accumulation of metabolites, such as VFA and ammonia, which seemed to inhibit methanogenesis, impairing the methane production. Therefore, separating hydrolysis from the subsequent steps in anaerobic digestion of poultry residues is necessary to maximize its efficiency.

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