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

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ABSTRACT

The biochemical methane potential (BMP) of raw poultry litter waste was assessed in batch assays. Biological co-treatment with *Clostridium cellulolyticum*, *Caldicellulosiruptor saccharolyticum* and *Clostridium thermocellum* as bioaugmentation strains, and thermochemical pre-treatments with lime and sodium hydroxide performed at different temperatures and pressures were applied as strategies to improve the BMP by favouring the hydrolysis of the cellulolytic material in the waste. Anaerobic digestion of the raw waste allowed a specific methane production of 145 ± 14 L CH_4 kg^{-1} VS, with 1% total solids and 0.72 g $VS_{inoculum}$ g^{-1} VS_{waste}. The pre- and co-treatments contributed to a significant increase (up to 74%) in the waste solubilisation when using *C. saccharolyticum*, but methane production did not improve considerably. Therefore, the conversion of soluble organic matter to methane was the limiting step of the anaerobic digestion process of poultry litter waste.

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1. Introduction

Over the past few years, the spread of the economical crises led to a sharp demand for cheaper meat products, such as poultry meat and poultry derivates. As a result, poultry industry is producing increasing amounts of organic wastes, *e.g.* poultry litter (mixture of manure and bedding material), waste feed, dead birds, blood, broken eggs, and feathers (Kelleher et al., 2002). Current environmental policies and legislation compel poultry industry to adopt effective waste treatment options. Poultry litter is a waste stream of concern because of its high pollutant load. Thus far, composting and direct applications on land have been the most widely used alternatives in handling poultry litter. However, on land over-application of this material can lead to nutrients leach into the natural environment, with consequences on the spread of pathogens, the production of phytotoxic substances, the eutrophication of water bodies, and also the emission of greenhouse gases and other air pollutants (Kelleher et al., 2002).

Anaerobic digestion of organic wastes is an alternative to waste composting and could be applied to the treatment of poultry litter. Organic material present in poultry litter could be converted to biogas in an anaerobic digestion process. Biogas is a renewable energy carrier, mainly formed by methane (CH_4) and carbon dioxide (CO_2). Therefore, this alternative could have an added interest considering the present fossil fuel derived energy limitation and CO_2 mitigation policies (Molinuevo-Salces et al., 2010). Poultry litter can generate more biogas than piggery and cattle wastes (Rao et al., 2011). Nevertheless, poultry litter is a complex substrate containing a high fraction of bedding material, which might include straw, sawdust, wood shavings, shredded paper and peanut or rice hulls. It is generally high in lignocellulose and therefore more recalcitrant to biological degradation (Kelleher et al., 2002; Singh et al., 2010; Mosier et al., 2005). Hydrolysis of lignocellulose has been considered the rate limiting step during anaerobic digestion of this type of waste, thus constraining methane production (Pavlostathis and Giraldo-Gomez, 1991). Pre-treatment of lignocellulosic biomass could accelerate the hydrolysis process and improve the final biogas production from lignocellulosic wastes (Fernandes et al., 2009).

Hydrolysis of lignocelluloses biomass can be stimulated using chemical, thermal, or enzymatic methods. Chemical and thermochemical pre-treatments have been considered unattractive due to the high costs involved, namely regarding the chemicals and energy cost. Lime ($Ca(OH)_2$) is an inexpensive and safe to use compound that can be used for alkaline hydrolysis of wastes (alone or in combination with heat and/or pressure). A practical disadvantage is that lime is a weak base and usually a higher concentration or exposure time is necessary to achieve the same performance obtained with strong bases, *e.g.* sodium hydroxide (NaOH). In previous works it was found that the pre-treatment time, temperature, and lime concentration have greater impacts on digestibility of wastes than water loading and biomass particle size (Chang et al., 2001).

Addition of anaerobic hydrolytic microorganisms can be an alternative for a one-step enhanced hydrolysis–fermentation process. There are several described microorganisms with cellulolytic

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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). Low energy and no chemical requirements are the main advantages of biological pre-treatment. However, the rate of hydrolysis is low in most biological pre-treatment processes (Sun and Cheng, 2002).

The aim of this work was to evaluate the biochemical methane potential (BMP) of poultry litter. Due to the recalcitrance of lignocellulosic materials, bioaugmentation with cellulolytic microorganisms and pre-treatments were applied with the objective of enhancing the waste solubilisation and consequently increase their conversion to methane. Thermochemical pre-treatments, using lime and sodium hydroxide, at high temperature and pressure were tested.

2. Methods

2.1. Waste characterisation

The waste used in these experiments was collected in a poultry industry in the north of Portugal. The poultry litter had 77 ± 1.3% of total solids (TS) and 70 ± 1.5% of volatile solids (VS). Chemical oxygen demand (COD) of the waste was 915 ± 67 g COD/kg_{waste} and total kjeldahl nitrogen (TKN) was 21 ± 1 g N/kg_{waste} (COD:N ratio of 44:1). This characterisation resulted from an average of 10 measurements.

2.2. Anaerobic biodegradability of raw poultry litter

Anaerobic biodegradability of poultry litter was tested using different concentrations of waste solids, *i.e.* 1.0%, 2.5% and 5.0% of TS_{waste}. An active anaerobic inoculum (suspended sludge or granular sludge) was added to the tests to a final concentration of 6.6 g VS L^{-1}. The ratio inoculum:waste was 0.72, 0.29 and 0.14 g $VS_{inoculum}$ $g^{-1}VS_{waste}$ for the tests with 1.0%, 2.5% and 5.0% of TS_{waste}, respectively. Blank assays without the addition of poultry litter were also performed. Detailed procedure of biodegradability tests in Section 2.5.

2.3. Biological co-treatment (bioaugmentation)

2.3.1. Medium composition and microorganisms cultivation
C. cellulolyticum (DSM 5812), *Clostridium thermocellum* (DSM 1237), and *C. saccharolyticus* (DSM 8903) used in the bioaugmentation assays were purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Braunschweig, Germany). *C. cellulolyticum* and *C. thermocellum* were pre-grown in CM3 medium using 6 and 5 g L^{-1} of cellobiose as carbon source, respectively. The composition of CM3 was (per L): yeast extract, 2.0 g; $(NH_4)_2SO_4$, 1.3 g; KH_2PO_4, 1.5 g; $K_2HPO_4 \cdot 3H_2O$, 2.9 g; $MgCl_2 \cdot 6H_2O$, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.075 g; $FeSO_4 \cdot 7H_2O$, 1.25 mg; resazurin, 0.5 mg; cysteine–$HCl \cdot H_2O$, 0.5 mg; trace elements solution SL-10, 1 mL. The trace element solution contained (per L): hydrochloric acid (25%), 10 mL; $FeCl_2 \cdot 4H_2O$, 1.5 g; $ZnCl_2$, 70 mg; $MnCl_2 \cdot 4H_2O$, 100 mg; H_3BO_3, 6 mg; $CoCl_2 \cdot 6H_2O$, 190 mg; $CuCl_2 \cdot 2H_2O$, 2 mg; $NiCl_2 \cdot 6H_2O$, 24 mg; $Na_2MoO_4 \cdot 2H_2O$, 36 mg. The pH of the medium was adjusted to 6.0 with hydrochloric acid and dispensed into serum bottles. The bottles were sealed with butyl rubber septa and aluminium crimp caps and the headspace was flushed with N_2 (1.7 × 105 Pa). After autoclaving, pH of the medium was adjusted to 7.2 by adding a sterile anoxic Na_2CO_3 solution (5% w/v) prepared under a mixture of $N_2:CO_2$ (80:20%). *C. cellulolyticum* and *C. thermocellum* cultures were incubated shaking (100 rpm) in the dark at 37 or 55 °C, respectively. *C. saccharolyticus*

was pre-grown with 1 g L^{-1} cellobiose in medium containing (per L): yeast extract, 2 g; tripticase, 1.5 g; NH_4Cl, 0.9 g; NaCl, 0.9 g; KH_2PO_4, 0.75 g; K_2HPO_4, 1.5 g; $MgCl_2 \cdot 6H_2O$, 0.4 g; $FeCl_3 \cdot 6H_2O$, 2.5 mg; resazurin, 0.5 mg; cysteine–$HCl \cdot H_2O$, 0.75 g; trace elements solution SL-10, 1 mL. The pH of the medium was adjusted to 7.2, dispensed into serum bottles and the bottles' headspace was flushed with N_2 (1.7 × 10^5 Pa). *C. saccharolyticus* cultures were incubated shaking (100 rpm) at 65 °C in the dark.

2.3.2. Bioaugmented poultry litter biodegradability tests
C. cellulolyticum, *C. thermocellum*, and *C. saccharolyticus* cells were harvested during the exponential phase by centrifugation (1500g, 10 min); 2.5 mL of 25× concentrated cells of each microorganism were used in the anaerobic biodegradability tests containing poultry waste (2.5% TS_{waste}) and anaerobic granular sludge (approximately 0.77 g $VS_{inoculum}$ g^{-1} VS_{waste}). Biodegradability test vials were incubated at the optimal temperature of each microorganism, *i.e.* 37, 55, and 65 °C for *C. cellulolyticum*, *C. thermocellum*, and *C. saccharolyticus*, respectively. Controls, in which each of the microorganisms was incubated with poultry litter, but without anaerobic sludge, were also performed. Non-bioaugmented vials, containing waste and anaerobic sludge, were also set-up. Biodegradability tests procedure in Section 2.5.

2.4. Thermochemical pre-treatment

2.4.1. Thermochemical pre-treatments
Poultry litter was pre-treated with two alkali, *i.e.* lime and sodium hydroxide. Variables tested were: (i) temperature (20 and 90 °C), (ii) contact time (30, 60 and 120 min), (iii) pressure (1.01, 1.27 and 4 bar), and (iv) base concentration (0.05, 0.1 e 0.2 $g_{alkali}/$ g TS_{waste}). Thermochemical pre-treatment tests were made using 40 g TS_{waste} L^{-1}. Assays at 90 °C and with overpressure (1.27 and 4 bar) were performed in an autoclave (Hiclave HV-25L, Dublin, Ireland) and in a pressure column equipped with a pressure transducer and a regulatory valve, respectively. Column pressurisation was made through the injection of N_2. Soluble COD concentration (COD_s) was determined before and after thermochemical treatment. The percentage of solubilisation in the pre-treatment ($PS_{pre-treatment}$), was calculated as the percentage of the initial COD added to the vials that was solubilized during the thermochemical treatment (Eq. (1)).

$$PS_{pre-treatment} = \frac{\text{mg } COD_s \text{ (end pre-treatment)} - \text{mg } COD_s \text{ (initial)}}{\text{mg } COD_{added}} \times 100$$

(1)

Thermochemical treatments yielding high percentage of organic matter solubilisation were selected for subsequent biodegradability tests.

2.4.2. Anaerobic biodegradability assays of pre-treated poultry waste
Anaerobic biodegradability tests of raw and pre-treated poultry wastes were performed according to the procedure detailed in Section 2.5. Prior to the tests, pH was neutralized with hydrochloric acid (8 M). The assays were performed with 31.25 mL of pre-treated sample corresponding to 2.5% TS_{waste}, using a ratio of inoculum:waste of 0.74 g $VS_{inoculum}$ g^{-1} VS_{waste}.

2.5. Anaerobic biodegradability assays

Anaerobic biodegradability batch tests were used to determine the BMP and maximum methane production rate (Rm) from the poultry wastes.

2.5.1. Inoculum source

Poultry litter without co- or pre-treatment (Section 2.2) was inoculated with anaerobic suspended sludge from a municipal wastewater treatment plant (VS = 13 ± 1 g L^{-1}, Specific Acetoclastic Activity (SAA) < 10 mL $CH_{4 \text{ @STP}}$ g^{-1} VS d^{-1}, and Specific Hydrogenotrophic Methanogenic Activity (SHMA) = 695 ± 39 mL $CH_{4 \text{ @STP}}$ g^{-1} VS d^{-1}). The assay with 2.5% TS poultry litter was repeated using granular sludge from a brewery industry (VS = 44 ± 3 g L^{-1}; SAA = 55 ± 4 mL $CH_{4 \text{ @STP}}$ g^{-1} VS d^{-1}; SHMA = 655 ± 39 mL $CH_{4 \text{ @STP}}$ g^{-1} VS d^{-1}), in order to test a more active inoculum. The SAA and SHMA were determined according to Colleran et al. (1992) and Alves et al. (2001). Assay with granular sludge (which has a higher acetoclastic activity than suspended sludge) was done for testing the possibility of accelerating methane production rate, and therefore decrease the time required for BMP testing.

2.5.2. Experimental procedure

Biodegradability assays were performed according to the directives defined by Angelidaki et al. (2009). Bottles were prepared by adding the substrate (poultry litter), inoculum (anaerobic sludge), and basal medium containing $NaHCO_3$ (5 g L^{-1}) to a final volume of 50 mL. pH of the medium was corrected to 7.0–7.2 with 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 amended with $Na_2S \cdot 9H_2O$, to a final concentration of 1 mM. All batch tests were performed in triplicate and incubated at 37 °C, except for the bioaugmentation tests that were incubated at optimum growth temperature of each microorganism. Blank and control assays were also performed as described in previous sections.

Methane in the vessels headspace was monitored by gas chromatography using 500 µL of gas sample collected with a gas-tight syringe. Methane production was corrected for standard temperature and pressure (STP) conditions. BMP was determined by unit of VS of waste added to each vial:

$$BMP = L\ CH_4/kg\ VS = \frac{kg\ COD - CH_4 \times 350\ (L\ CH_4/kg\ COD)}{VS_{\text{waste added (kg VS)}}} \quad (2)$$

Relative methane production (MP) was defined as the amount of methane produced during the assays in relation to the theoretical biochemical methane potential (*i.e.* 350 L CH_4/kg COD):

$$MP = \frac{mg\ COD - CH_4}{mg\ COD_{\text{added}}} \times 100 \quad (3)$$

Hydrolysis extent was evaluated considering the percentage of solubilisation (PS), which is the percentage of the initial COD added to the vials that is solubilized during the anaerobic biodegradability assay (Eq. (4)).

$$PS = \frac{mg\ COD_s\ (\text{end assay}) - mg\ COD_s\ (\text{initial}) + mg\ COD - CH_4}{mg\ COD_{\text{added}}} \times 100 \quad (4)$$

2.6. Analytical methods

TKN, TS, and VS were measured according to standard methods (APHA, 1998). Total and soluble COD was determined using standard kits (Hach Lange, Düsseldorf, Germany). Ammonium was determined by the Nessler method (APHA, 1998) and reducing sugars were measured using the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). Volatile fatty acids (VFA) were determined by HPLC (Jasco, Japan) equipped with a UV detector (210 nm) and a Chrompack column (6.5 × 30 mm^2) at 60 °C and using sulphuric acid (0.01 N) as mobile phase at a flow rate of 0.6 mL/min. Methane in biogas was analyzed in a gas chromatograph (Chrompack 9000) equipped with a FID detector and a 2 m × 1/8″ Chromosorb 101
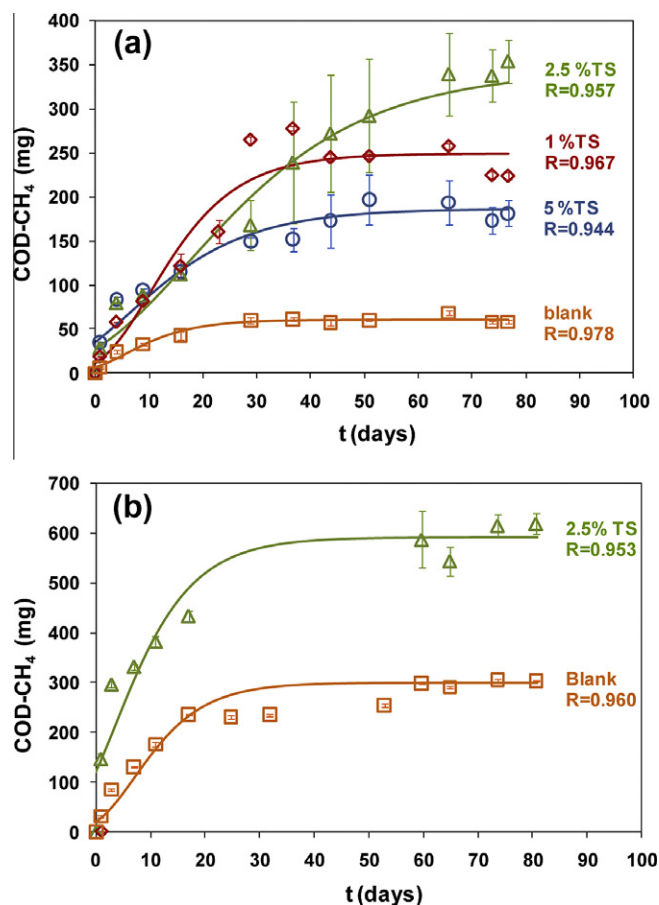

Fig. 1. Cumulative methane production (mg COD-CH_4) during anaerobic biodegradability tests at different poultry litter concentrations with anaerobic suspended sludge (a) and granular sludge (b). 0% TS (blank) (□), 1% TS (◇), 2.5% TS (△), and 5% TS (○). The symbols represent the experimental data with respective standard deviation (*n* = 3 points). The lines represent the predicted data by the modified Gompertz equation.

(80–120 mesh) column and using nitrogen as carrier gas (30 mL/min); column, injector, and detector temperatures were 35, 110, and 220 °C, respectively.

2.7. Statistical analysis

The modified Gompertz equation (Eq. (5)) was used to describe the progress of cumulative methane production obtained from the batch experiments (Zwietering et al., 1990).

$$M(t) = P\ \exp\left[-\exp\left[\frac{Rm^e}{p}(\lambda - t) + 1\right]\right] \quad (5)$$

where $M(t)$ is the methane cumulative production (mg COD-CH_4), P is the maximum methane production (mg COD-CH_4), Rm is the maximum methane production rate (mg COD-CH_4 d^{-1}), e equals to 2.7182818, and λ is the lag-phase (days).

Waste biodegradability results 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.

Table 1
Methane-production parameters predicted by the modified Gompertz equation and experimental results obtained at the end of raw poultry wastes biodegradability tests.

		Suspended			Granular
		1% TS	2.5% TS	5% TS	2.5% TS
Concentration					
g $VS_{inoculum}$ g^{-1} VS_{waste}		0.72	0.29	0.14	0.77
P	mg COD-CH_4	188 ± 20	284 ± 31	126 ± 21	292 ± 45
BMP	LCH_4 kg^{-1} VS	145 ± 14	87 ± 10	19 ± 3	90 ± 13
Rm	mg COD-CH_4 d^{-1}	10.1	6.7	5.5	27.3
MP	%	31 ± 3	19 ± 2	4 ± 1	17 ± 2
PS	%	32 ± 3	34 ± 2	34 ± 1	52 ± 2
pH		7.6	7.5	7.0	7.3
COD_s	g L^{-1}	0.66 ± 0.26	6.00 ± 1.31	20.94 ± 0.72	13.98 ± 1.52
Sugars	g L^{-1}	nd	nd	nd	1.68 ± 0.12
NH_4^+–N	g L^{-1}	0.25 ± 0.03	0.70 ± 0.01	1.28 ± 0.54	0.64 ± 0.07
Total VFA	g COD L^{-1}	0	3.50 ± 1.18	15.53 ± 4.49	3.27 ± 0.47
Acetic acid	mg COD L^{-1}	0	1412	5193	1427
Propionic acid	mg COD L^{-1}	0	1412	3928	1557
iso-Butyric acid	mg COD L^{-1}	0	676	3808	287
n-Butyric acid	mg COD L^{-1}	0	0	2602	0
Ethanol	mg COD L^{-1}	0	177	3352	3410
Butanol	mg COD L^{-1}	0	0	0	3898

Note: The values obtained in the blanks were subtracted in the data presented in the table.
nd: Not determined.

Table 2
Methane-production parameters predicted by the modified Gompertz equation and experimental results obtained at the end of the anaerobic biodegradability tests of poultry litter (2.5% TS) bioaugmented with cellulolytic microorganisms.

		C. cellulolyticum	*C. thermocellum*	*C. saccharolyticus*
P	mg COD-CH_4	335 ± 17	241 ± 21	310 ± 23
BMP	LCH_4 kg^{-1} VS	102 ± 5	71 ± 7	95 ± 4
Rm	mg COD-CH_4 d^{-1}	25.2	12.3	10.5
MP	%	23 ± 1	16 ± 1	21 ± 1
PS	%	57 ± 2	62 ± 1	74 ± 1
pH		7.2	7.5	7.6
COD_s	g L^{-1}	11.81 ± 0.94	15.16 ± 1.29	17.25 ± 0.92
Sugars	g L^{-1}	0.20 ± 0.07	1.19 ± 0.18	1.15 ± 0.22
NH_4^+–N	g L^{-1}	1.16 ± 0.46	0.24 ± 0.06	0.70 ± 0.04
Total VFA	g COD L^{-1}	4.14 ± 0.69	8.66 ± 0.37	6.43 ± 0.86
Acetic acid	mg COD L^{-1}	3277	4802	3251
Propionic acid	mg COD L^{-1}	183	3246	2480
iso-Butyric acid	mg COD L^{-1}	683	598	701
n-Butyric acid	mg COD L^{-1}	0	0	0

Note: The values obtained in the blanks were subtracted in the data presented in the table.

3. Results and discussion

3.1. Biomethane potential of the raw poultry litter

The first part of this research consisted in assessing the BMP of the raw poultry litter, *i.e.* without co- or pre-treatment. The profiles of cumulative methane production, using suspended sludge as inoculum, are shown in Fig. 1a. The best scenario in terms of specific methane production was observed for solids concentration of 1% TS; in this case a maximum specific methane production of 145 ± 14 L CH_4 kg^{-1} VS could be obtained, which corresponds to approximately MP of 41 ± 3% (Table 1). Specific methane production in the range of 140–220 L CH_4 kg^{-1} VS have been reported by other authors (Webb and Hawkes, 1985), but in continuous reactors. According to Bujoczek et al. (2000), anaerobic digestion of chicken manure is feasible up to a solid concentration of 10% TS. However, in this experiment with poultry litter (a mixture of manure, straw and feathers), an inhibitory effect occurred in the vials with high solids concentration, especially with 5% TS. This is possibly related with VFA, alcohols and COD_s accumulation inside the vials, although no drop in the pH of the medium was observed

due to high VFA concentrations (Table 1). Ammonium concentration at the end of the batch assays increased from 0.25 ± 0.54 to 1.28 ± 0.54 g NH_4–N L^{-1} in assays with 1% and 5% TS, respectively (Table 1). However, unionized ammonium concentrations detected never reached inhibitory levels, since a maximum of 27.5 mg NH_3–N L^{-1} was obtained for the assay with 2.5% TS. According to Rao et al. (2008) unionized ammonia in the range of 0.1–1.1 g N L^{-1} inhibits methanogenesis.

Since the time required for BMP testing was too long with suspended sludge, a test with granular sludge was also performed. Considering that 1% TS is a very low solids concentration for practical purposes, it was decided to test the intermediate TS concentration of 2.5%. As expected, the BMP values obtained for 2.5% with suspended and granular sludge were similar, but the maximum methane production rate (*Rm*) was 4× higher in the assay with granular sludge (27.3 mg COD-CH_4 d^{-1} vs. 6.7 mg COD-CH_4 d^{-1}). Percentage of COD solubilisation of the poultry waste was similar for all the tests in which suspended inoculum was used (PS of approximately 34%, for all tested% TS_{waste}), A higher solubilisation percentage was obtained in the assays with granular sludge and 2.5% of TS_{waste} (*i.e.* PS of 52 ± 2%).
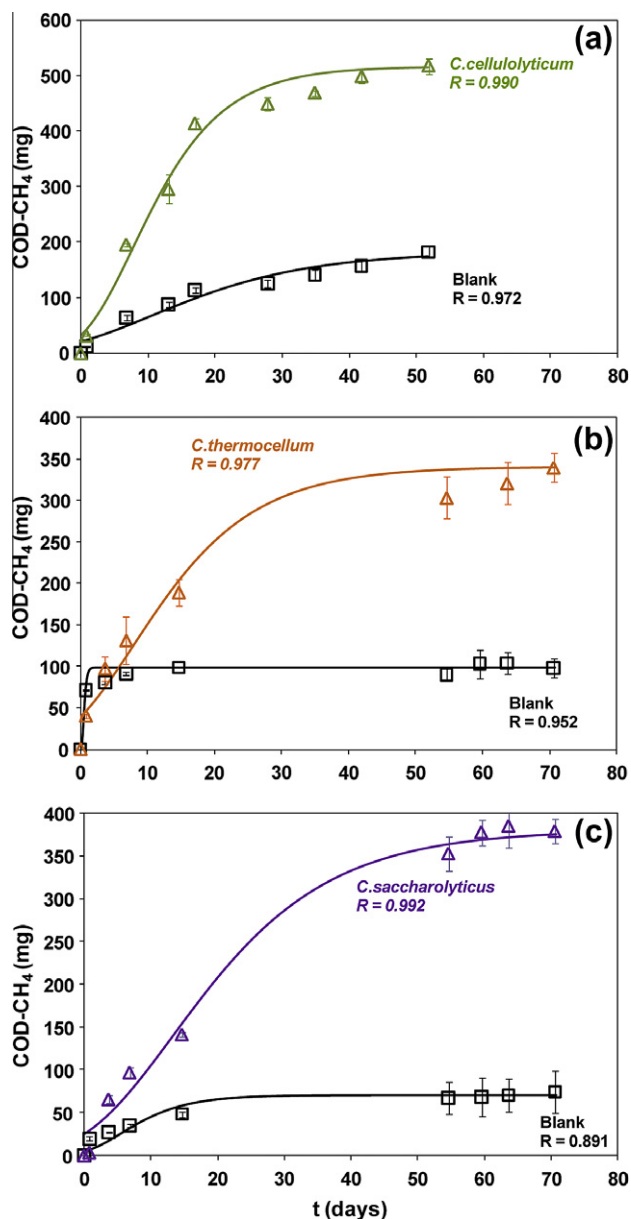
Fig. 2. Cumulative methane production (mg COD-CH$_4$) during anaerobic biodegradability tests of poultry litter bioaugmented with: (a) *C. cellulolyticum* (△); (b) *C. thermocellum* (△); and, (c) *C. saccharolyticus* (△). The respective blank assay (□) without waste and microorganism is presented in all figures. The symbols represent the experimental data with respective standard deviation (*n* = 3 points). The lines represent the predicted data by the modified Gompertz equation.

3.2. Biological co-treatment (bioaugmentation)

Results from the bioaugmented tests with mesophilic (*C. cellulollyticum*) and thermophilic (*C. thermocellum* and *C. saccharolyticus*) microorganisms are displayed in Table 2. The profiles of cumulative methane production and predicted data by the modified Gompertz equation are presented in Fig. 2.

Addition of *C. cellulolyticum* had a clear positive effect in the cumulative methane production (*P* = 335 ± 17 mg COD-CH$_4$, value approximately 15% higher than in the non-bioaugmented test). Specific methane production in *C. cellulolyticum* bioaugmented test was 102 ± 5 L CH$_4$ kg^{-1} VS. These results are significantly higher (*P* < 0.01) than the obtained in the raw waste non-bioaugmented tests. VFA concentration increased from 3.27 ± 0.47 g L^{-1}, in non-bioaugmented tests, to 4.14 ± 0.69 g L^{-1} in *C. cellulolyticum*

bioaugmented tests. This might indicate an enhanced hydrolysis and subsequent acidogenesis of the waste material. *C. cellulolyticum* was isolated from decayed grass and was proven to be capable of hydrolyzing cellulose and fermenting sugars (Petitdemange et al., 1984). Sugars concentration at the end of the assay was lower in *C. cellulolyticum* bioaugmented tests (*i.e.* 0.20 ± 0.07 g L^{-1}) than in the non-bioaugmented tests (*i.e.* 1.68 ± 0.12 g L^{-1}). Therefore, low sugar concentrations might be related to *C. cellulolyticum* activity.

In the trials with *C. thermocellum* and *C. saccharolyticus* as bioaugmented species no significant differences on methane production were observed, when compared to the non-bioaugmented assays. However, the bioaugmentation with these microorganisms caused a significant increase (*P* < 0.01) in the solubilisation of the organic matter in the waste: PS value in non-bioaugmented tests was 52 ± 2%, while in *C. thermocellum* and *C. saccharolyticus* bioaugmented tests PS values were 62 ± 1% and 74 ± 1%, respectively. This indicates an efficient hydrolysis of the cellulosic material in the poultry litter. *C. thermocellum* is a thermophilic bacterium with high cellulolytic activity that ferments lignocellulosic substrates to mainly hydrogen, acetate and ethanol (Lynd et al., 1989; Weimer and Zeikus, 1977). Nevertheless, some of the by-products generated during hydrolysis accumulated in the medium and were not converted to methane, which indicates that methanogenesis was the rate-limiting step in the conversion of cellulosic material. Apparent low methanogenic activity could be associated to the fact of using a mesophilic methanogenic inoculum for *C. thermocellum* bioaugmentation tests, which were performed at 55 °C. Results with *C. saccharolyticus* were similar, *i.e.* higher solubilisation of the organic material present in the poultry waste but no improvement in methane production, when compared with non-bioaugmented assays. Also here incubations were performed at high temperature, *i.e.* 65 °C, which might have negatively influenced methanogenic archaea present in the inoculum sludge. In the control assays with 2.5% TS$_{waste}$ and bioaugmenting strains, a COD removal of 33 ± 2% and 32 ± 1% was observed after 120 h of inoculation with *C. thermocellum* and *C. saccharolyticus*, respectively. These results confirm that the hydrolytic microorganisms effectively act on the substrate. The results obtained in the co-treatment assays with the three strains suggest that instead of a co-treatment, a two stage strategy consisting in pre-treatment/acidification followed by the methanogenic phase, should be considered in further studies.

3.3. Biomethane potential of thermochemical pre-treated poultry litter

Results of waste pre-treatment with lime and sodium hydroxide are summarized in Table 3. In the assays with lime, solubilisation significantly increased with temperature (*P* < 0.01). Soluble COD increased with contact time (up to 120 min) and pressure (up to 1.27 bar). However, an overpressure of 4 bar caused a slight decrease in the solubilisation of the organic matter in the waste. The highest solubilisation, corresponding to a COD$_s$ of 13 g L^{-1} at the end of the pre-treatment, was observed in the assay with lime concentration of 0.2 g Ca(OH)$_2$ g^{-1}$_{waste}$, at 1.27 bar and 90 °C, during 120 min. Use of sodium hydroxide as chemical agent resulted in higher solubilisation of the organic matter (Table 3). Thermochemical hydrolysis with 0.2 g NaOH g^{-1}$_{waste}$, at 1.27 bar and 90 °C for 120 min, yielded a soluble COD in the medium as high as 32 g L^{-1}. Similarly to lime-catalyzed hydrolysis, COD solubilisation in sodium hydroxide pre-treatment tests improved with contact time, pressure and temperature.

Biodegradability tests of the mixed liquor resulting from pre-treated wastes were done upon analysis of the soluble COD, and considering the highest yields obtained. Pre-treated wastes under three different conditions were selected, namely: Ca(OH)$_2$ (90 °C/ 1 bar and 90 °C/1.27 bar) and NaOH (90 °C/1.27 bar). Cumulative

Table 3
Soluble COD (g/L) obtained after thermochemical pre-treatment of poultry litter.

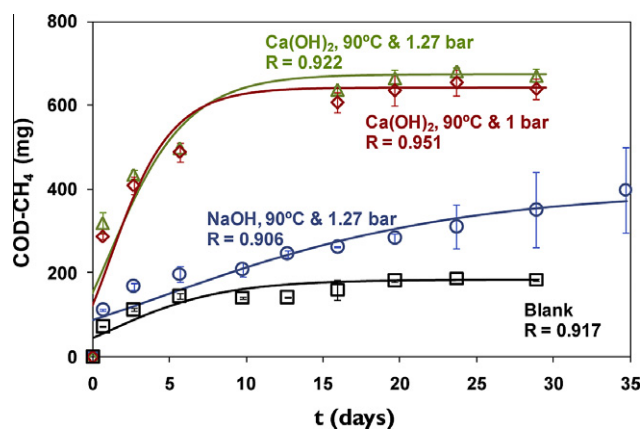|		Ca(OH)$_2$				NaOH	
t (min)	[g/g$_{waste}$]	1.01 bar 20 °C	1.01 bar 90 °C	1.27 bar 90 °C	4 bar 90 °C	1.01 bar 20 °C	1.27 bar 90 °C
30	0.05	3.77	5.30	9.08	4.34	9.62	nd
	0.1	3.27	5.18	10.37	8.74	10.82	nd
	0.2	3.20	7.28	9.31	9.54	13.78	nd
60	0.05	4.40	9.43	11.14	10.34	10.25	14.82
	0.1	4.27	9.17	10.90	9.70	11.85	18.80
	0.2	3.83	9.13	11.62	9.64	14.58	21.32
120	0.05	5.08	10.90	9.36	9.92	12.20	16.80
	0.1	5.35	10.90	9.52	10.80	14.52	28.68
	0.2	4.31	10.51	12.88	11.52	17.60	32.02

nd: Not determined.

Fig. 3. Cumulative methane production (mg COD-CH$_4$) during anaerobic biodegradability tests of pre-treated poultry litter. Blank without waste (□), pre-treated with Ca(OH)$_2$ at 90 °C and 1 bar (◇); Ca(OH)$_2$ at 90 °C and 1.27 bar (△), and NaOH at 90 °C and 1.27 bar (○). The symbols represent the experimental data with respective standard deviation (n = 3 points). The lines represent the predicted data by the modified Gompertz equation.

Table 4
Methane-production parameters predicted by the modified Gompertz equation and experimental results obtained at the end of the anaerobic biodegradability tests of poultry litter after thermochemical pre-treatment. The % TS in the BMP assays after the pre-treatment was 2.5%.

		Ca(OH)$_2$, 90 °C and 1 bar	Ca(OH)$_2$ 90 °C and 1.27 bar	NaOH, 90 °C and 1.27 bar
P	mg COD-CH$_4$	460 ± 28	490 ± 15	215 ± 102
BMP	LCH$_4$ kg^{-1} VS	129 ± 8	137 ± 4	59 ± 28
Rm	mg COD-CH$_4$ d^{-1}	102.1	85.0	12.9
MP	%	30 ± 1	32 ± 1	14 ± 7
PS$_{pre-treatment}$	%	15	20	56
PS	%	19 ± 1	16 ± 1	8 ± 1
pH		6.8	6.9	7.0
COD$_s$	g L^{-1}	3.18 ± 0.48	3.20 ± 0.42	18.07 ± 2.12
Sugars	g L^{-1}	1.06 ± 0.30	0.74 ± 0.13	1.06 ± 0.16
NH$_4^+$–N	g L^{-1}	0.58 ± 0.08	0.58 ± 0.18	0.69 ± 0.08

Note: The values obtained in the blanks were subtracted in the data presented in the table.

methane production in these assays is displayed in Fig. 3. Methane-production parameters predicted by the modified Gompertz equation, relative methane production, solubilisation percentages, and soluble products formed during the anaerobic digestion of these mixed liquors are shown in Table 4.

Although soluble COD resulting from thermochemical pre-treatment was higher when using sodium hydroxide, subsequent substrate conversion to methane was less effective in this case than in the tests subjected to pre-treatment with lime. Wu et al. (2009) observed that the addition of sodium hydroxide, at 131 °C, inhibited methane production from meat and bone meal. The high solubilisation observed during the pre-treatment with sodium hydroxide (PS$_{pre-treatment}$ = 56%) may have generated some metabolites, like VFA, ammonia, furfural, etc., which could be responsible for methanogenesis inhibition (Table 4). Zhang and Jahng (2010), concluded that lime was better effective than sodium hydroxide and potassium hydroxide in terms of volumetric methane production rates and removal of organic compounds from a piggery wastewater. The suggested reason was that the cations Na$^+$ and K$^+$ were strong methanogenic inhibitors when compared with Ca^{2+}.

According to Chang et al. (1997), the optimal conditions for the alkaline pre-treatment of wheat straw were 2 h with a concentration of 0.1 g Ca(OH)$_2$ g$^{-1}_{waste}$ at 100–120 °C. Reducing sugars concentration five times higher than with untreated material were obtained. In the study by Rafique et al. (2010), an increase of 70%

in the methane yield was obtained after pre-treatment with 0.05 g Ca(OH)$_2$ g$^{-1}_{waste}$ at 70 °C. In the present study, 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^{-1} VS) (Table 4).

4. Conclusions

Biomethane potential of raw poultry litter (1% TS, 0.72 g VS$_{inoculum}$ g^{-1} VS$_{waste}$) was 145 ± 14 L CH$_4$ kg^{-1}. The biological co-treatments and the thermochemical pre-treatments with lime and sodium hydroxide had a significant impact in the hydrolysis of poultry litter. Bioaugmentation with *C. saccharolyticus* caused 74 ± 1% of waste solubilisation. However, the improvement in the substrate hydrolysis resulted in the accumulation of metabolites, such as VFA, which seemed to inhibit methanogenesis and impaired methane production. Therefore, separating hydrolysis from the subsequent steps in anaerobic digestion of poultry residues may be necessary to maximize its efficiency.

help given with the high-pressure reactor used for waste physico-chemical pre-treatment.

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