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Biochemical methane potential of raw and pre-treated meat-processing wastes

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HIGHLIGHTS

▶ Biochemical methane potential (BMP) of pre-treated greaves and rinds were studied.
▶ BMP of rinds was improved 25% by exposure to 70 °C for 24 h.
▶ Incomplete hydrolysis of rinds caused inhibition of methanogens in the BMP assays.
▶ For greaves, hydrolysis was the limiting step even after the applied pre-treatments.

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ABSTRACT

Raw and pre-treated greaves and rinds, two meat-processing wastes, were assessed for biochemical methane potential (BMP). Combinations of temperature (25, 55, 70 and 120 °C), NaOH (0.3 g g^{-1} waste volatile solids) and lipase from Candida rugosa (10 U g^{-1} fat) were applied to promote wastes hydrolysis, and the effect on BMP was evaluated. COD solubilisation was higher (66% for greaves; 55% for rinds) when greaves were pre-treated with NaOH at 55 °C and lipase was added to rinds after autoclaving. Maximum fat hydrolysis (52–54%) resulted from NaOH addition, at 55 °C for greaves and 25 °C for rinds. BMP of raw greaves and rinds was 707 ± 46 and 756 ± 56 L CH$_4$ (at standard temperature and pressure) kg^{-1} VS, respectively. BMP of rinds improved 25% by exposure to 70 °C; all other strategies tested had no positive effect on BMP of both wastes, and anaerobic biodegradability was even reduced by the combined action of base and temperature.

1. Introduction

Slaughterhouses and meat processing industries generate large amounts of wastes and by-products, e.g. carcasses, feet, offal, hides, bones and blood, corresponding to 40–50% of the total animal weight slaughtered (Cuadros et al., 2011; FAOSTAT, 2012). Anaerobic digestion of organic wastes is a highly sustainable process, since it combines waste treatment with energy production in the form of biogas and nutrients recycling. Animal wastes are typically rich in fats and proteins (Salminen and Rintala, 2002), therefore representing a good substrate for biogas production. However, the conversion of these complex particulate materials to methane in anaerobic digesters is frequently limited by the hydrolysis step (Masse et al., 2003; Vavilin et al., 1996). Efficient hydrolysis is crucial to make complex substrates accessible to anaerobic bacteria and ultimately optimize methane production.

Several pre-treatment techniques have been applied to enhance hydrolysis and anaerobic biodegradability of organic wastes (Cammarota and Freire, 2006; Carrère et al., 2010; Costa et al., 2012; Hejnfelt and Angelidaki, 2009; Luste et al., 2009). Physical treatments such as high temperature, microwaves, ultrasounds, grinding and maceration destroy aggregated particles, decrease particles size and disrupt cell structure. The molecular structure of the material can be changed through the addition of acids or bases, or through the action of enzyme-producing microorganisms (bioaugmentation) or enzyme preparations. The majority of the studies have been focused on pre-treating waste activated sludge or wastewaters (Bougrier et al., 2008; Cammarota and Freire, 2006; Carrère et al., 2010; Masse et al., 2003; Valladão et al., 2007; Zhang and Jahng, 2010), and only few authors tested this approach on animal wastes (Table 1).

In most cases the hydrolysis of the wastes is not complete and only partial solubilisation of the materials or particle size reduction is achieved. Additionally, methane production from the hydrolysates is not always higher than that obtained from untreated wastes, and in some cases it even decreases (Costa et al., 2012;

Table 1
Examples of reported studies on pre-treatment and anaerobic digestion of slaughterhouse and meat-processing wastes.

Pre-treatment conditions applied	Wastes	Anaerobic digestion conditions	Reference
Thermal			
70 °C, 60 min	DTC, DSW, DAF, GTS	Batch, 35 °C, substrate:inoculum 1:1	Luste et al. (2009)
70 °C, 60 min	MPW	Batch, 37 °C and 55 °C	Hejnfelt and Angelidaki (2009)
133 °C, P > 3 bar, 20 min	MPW	Batch, 37 °C and 55 °C	Hejnfelt and Angelidaki (2009)
133 °C, P > 3 bar, 20 min	SHW	Semi-continuously fed CSTR	Cuetos et al. (2010)
Ultrasound			
5600 ± 300 kJ kg^{-1} TS, 25 °C	DTC, DSW, DAF, GTS	Batch, 35 °C, substrate:inoculum 1:1	Luste et al. (2009)
Alkaline			
NaOH 2 M (6–14%), 4 h	DTC, DSW, DAF, GTS	Batch, 35 °C, substrate:inoculum 1:1	Luste et al. (2009)
NaOH 50–100 g kg^{-1} VS	MPW	Batch, 37 °C and 55 °C	Hejnfelt and Angelidaki (2009)
Acid			
HCl 6 M (2–8%), 4 h	DTC, DSW, DAF, GTS	Batch, 35 °C, substrate:inoculum 1:1	Luste et al. (2009)
Termo-chemical			
NaOH, 0.04 mol g^{-1} COD, 60 °C, 30 min	DAF, FF	Fed-batch reactors, 0.1–0.2 g COD g-1 VS, 35 °C and 55 °C	Battimelli et al. (2009)
NaOH, 0.156 g g^{-1} VS, 60–150 °C, 3 h	DAF, FF	Fed-batch reactors, 35 °C, 1–5 g COD L-1	Battimelli et al. (2010)
Bacterial product			
Liquid certizyme 5 TM, 60 mg L^{-1}, 24 h, 25 °C	DTC, DSW, DAF	Batch, 35 °C, substrate:inoculum 1:1	Luste et al. (2009)
Enzymatic			
Pancreatic lipase, 250 mg enzyme L^{-1}, 5.5 h, 25 °C	PFPSW	Anaerobic sequencing batch reactor, 25 °C	Masse et al. (2003)

DTC – digestive tract content; SDW – slaughterhouse drumsieve waste; DAF – sludge from dissolved air flotation; GTS – grease trap sludge from meat-processing industry; MPW – mixed pork waste (all non-commercial parts of one slaughtered pig); SHW – slaughterhouse waste (mixture of entrails, contents of the stomach and intestines); FF – flesh fats from animal carcasses; PFPSW – pork fat particles in slaughterhouse wastewater. CSTR – continuously stirred tank reactor.

Cuetos et al., 2010; Hejnfelt and Angelidaki, 2009; Luste et al., 2009). For example, none of the five pre-treatments tested by Luste et al. (2009) was able to improve the biochemical methane potential (BMP) of slaughterhouse drumsieve waste, despite a 26–76% increase in the soluble chemical oxygen demand (COD) measured after pre-treatments (Table 1), and the BMP values obtained for the pre-treated materials were actually 20–39% lower than those of the untreated waste (400 m^3 CH$_4$ t^{-1} VS). Cuetos et al. (2010) also reported a lower biogas production (2.9 L day^{-1}) during the digestion of slaughterhouse waste (SHW) previously submitted to the combined action of temperature and pressure (133 °C, >3 bar, 20 min), than during digestion of the untreated SHW (3.2 L day^{-1}) in continuously stirred tank reactors (CSTR).

This reduced methane production is probably caused by inhibitory compounds, such as long-chain fatty acids (LCFA) and ammonia, produced during the hydrolysis of lipids and proteins (Alves et al., 2009; Salminen and Rintala, 2002). LCFA tend to adsorb onto the surface of the microbial cell wall, possibly hindering the transfer of substrates and products (Pereira et al., 2005). Although these effects are not irreversible, long periods of time are frequently needed for complete conversion of these compounds to methane (Alves et al., 2009; Cavaleiro et al., 2008). The toxicity of ammonia is related to changes in the intracellular pH caused by the non-ionized form NH$_3$ that easily penetrates the microbial cell membrane. Moreover, ammonia inhibition of enzymes involved in methane formation has also been suggested (Kadam and Boone, 1996).

Optimal methane production from meat-processing wastes is still challenging. Improvement of the hydrolysis step while preventing inhibition of the microbial communities by the hydrolysis products is required. Physical separation of the hydrolysis step from the anaerobic conversion of the hydrolysates to methane, in a two-phase process, can possibly circumvent this inhibition and contribute to enhanced methane production from these materials. In the present study, the effects of six different pre-treatments on the hydrolysis and BMP of greaves and rinds, two wastes from pig meat-processing, were evaluated. In a first stage, pre-treatments were performed in closed bottles and the hydrolysates were characterized in terms of COD, LCFA and NH$_4^+$. Thereafter, anaerobic biodegradability of the hydrolysates was studied in batch assays

and compared with the BMP of the untreated wastes. The amount of substrate added to the anaerobic bottle was based on LCFA and NH$_4^+$ concentrations in the hydrolysates.

2. Methods

2.1. Meat-processing wastes

Two wastes from a meat-processing plant located in Portugal were used: (i) greaves, the residue that remains after the rendering of pig tallow, and (ii) rinds, the tough outer covering of bacon or pig meat. The materials were reduced to small particles less than 3–5 mm in size in the factory and stored at −20 °C. Total and volatile solids (TS and VS), total Kjeldahl nitrogen (TKN), total phosphorus, fats and free long-chain fatty acids (LCFA) were determined after freeze-drying. Protein content was calculated from the TKN value using a conversion factor of 6.25 (Salminen et al., 2000). Total theoretical COD was calculated by applying stoichiometric conversion factors, i.e. 1.50 and 2.87 g COD g^{-1} for proteins and lipids, respectively (Palatsi et al., 2011). The characteristics of the studied materials are presented in Table 2.

Table 2
Characterisation of greaves and rinds (average values and their standard deviation are presented).

Parameter	Greaves	Rinds
Color	Brownish	Whitish
Total solids (%)	88 ± 0	65 ± 2
Volatile solids (%)	86 ± 0	65 ± 2
Total theoretical COD (g kg^{-1})[a]	1846	1774
TKN (g kg^{-1})	70 ± 2	73 ± 15
Total phosphorus (g kg^{-1})	0.6	0.3
Proteins (g kg^{-1})[a]	440 ± 15	458 ± 95
Fats (g kg^{-1})	406 ± 4	379 ± 80
Free LCFA (g kg^{-1})	23 ± 1	32 ± 1
Oleate (C18:1) (%)	41 ± 2	38 ± 2
Stearate (C18:0) (%)	30 ± 4	12 ± 0
Palmitate (C16:0) (%)	29 ± 2	42 ± 3

[a] Calculated as described in Section 2.1.

2.2. Pre-treatments

Six pre-treatments were tested and the conditions are summarized in Table 3. A 24-h contact period was applied in all the pre-treatments, except the ones performed in the autoclave. All the pre-treatments were performed in closed bottles to avoid the loss of ammonia to the atmosphere. Before opening the bottles, the pH was adjusted to neutral values with 8 N HCl.

The lipase from *Candida rugosa* (Sigma, 20 U mg^{-1}) was dissolved in 0.1 M sodium phosphate buffer solution (pH 7.3) and mixed with the wastes at a final activity of 10 U g^{-1} fat (Jeganathan et al., 2007), corresponding to an enzyme/waste ratio of 0.02% (w/w). One enzyme unit (U) was defined as the amount of enzyme that liberates 1 µmol of free fatty acid per min at pH 7.2 and 37 °C. Hydrolysis was performed at 37 °C, with gentle agitation, for 24 h, after which the enzyme was denatured in a water batch at 60 °C for 15 min. In the autoclaving + enzyme pre-treatment (AE), wastes were heat-treated at 121 °C for 20 min prior to addition of the enzyme.

After the pre-treatments, soluble + colloidal COD and free LCFA were analyzed in the liquid fractions. Ammonium concentrations were quantified for all the hydrolysates, except the ones resulting from the treatment with lipase (E and AE).

2.3. Anaerobic biodegradability assays

Anaerobic biodegradability of the untreated and treated wastes (hydrolysates) was studied in batch assays. Granular sludge from a brewery wastewater treatment plant was used as inoculum, with a specific methanogenic activity of 53 ± 3 and 508 ± 105 mL CH$_{4@STP}$ g^{-1} VS day^{-1} in the presence of acetate and H$_2$/CO$_2$, respectively. Anaerobic basal medium was prepared as described by Angelidaki et al. (2009) and transferred to 160-mL bottles (80 mL working volume) already containing substrate and inoculum at a final concentration of 8 g VS L^{-1}. For untreated wastes, an inoculum:substrate ratio of 4 g VS$_{inoculum}$ g^{-1} COD$_{total}$ was used. For the hydrolysates, different amounts were added to each bottle, considering the free LCFA concentrations determined after pre-treatments, and a maximum threshold concentration of 0.5 g COD-free LCFA g^{-1} VS$_{inoculum}$. This value was based on the kinetics proposed by Pereira et al. (2004), who found that specific LCFA contents above 1 g COD-free LCFA g^{-1} VS$_{inoculum}$ decreased the capacity of the anaerobic microorganisms to produce methane from LCFA. Therefore, 4 g VS$_{inoculum}$ g^{-1} COD$_{soluble+colloidal}$ and 1.3–3.3 g VS$_{inoculum}$ g^{-1} COD$_{total}$ were applied in the bottles amended with the hydrolysates. N-NH$_4^+$ concentrations were lower than 25 mg L^{-1}. Bottles were sealed with butyl rubber septa and aluminum screw caps, and the headspace was flushed with a mixture of N$_2$/CO$_2$ (80:20 v/v). Sodium sulphide was added as reducing agent, to a final maximum concentration of 1 mM. Blank assays (without substrate) were prepared in a similar way. All the tests were performed in triplicate and incubated at 37 °C, 150 rpm, under strict anaerobic conditions.

Methane content of the accumulated biogas was periodically measured by gas chromatography, and the methane production values were corrected for standard temperature and pressure (STP) conditions. BMP values were expressed per VS of the original waste, i.e. the cumulative methane production obtained at the end of the biodegradability tests (after subtraction of the value obtained in the blank assays) was divided by the equivalent VS content of the amount of waste that was pre-treated and transferred to the biodegradability tests. To compare the BMP values obtained with the theoretical potentials of the raw wastes, methane recovery (%) was calculated for the different conditions studied (Eq. (1)):

$$CH_4\ recovery\ (\%) = \frac{BMP \times 100}{Theoretical\ CH_4\ potential\ (raw\ waste)} \tag{1}$$

The theoretical methane potential of the wastes (greaves or rinds) was calculated considering its physico-chemical characterization (Table 2) and the following theoretical values of methane production: 0.63 L CH$_4$ g^{-1} protein and 0.99 L CH$_4$ g^{-1} lipid at STP conditions (Li et al., 2002). In the assays prepared with the enzymatic hydrolysates (E and AE), the potential methane production from the degradation of the inactivated enzyme was less than 0.1 mL methane at STP conditions. Therefore, it was considered irrelevant.

At the end of the biodegradability assays, samples from the liquid medium were analyzed for soluble + colloidal COD, volatile fatty acids (VFA), free LCFA and ammonium. The maximum theoretical amount of sodium chloride that could be present in the bottles prepared with the alkaline hydrolysates was always lower than 3.6 g L^{-1}.

2.4. Analytical methods

Solids (TS and VS), COD, TKN, ammonium and fats were measured according to standard methods (APHA et al., 1989). Soluble + colloidal COD was determined after centrifugation of the sample at 22,000g for 15 min. Total phosphorus was quantified using standard kits (Hach Lange, Düsseldorf, Germany). LCFA quantification was performed as described by Neves et al. (2009). Briefly, esterification and extraction of free fatty acids was performed with a mixture of propanol, HCl and dichloromethane, for 3.5 h at 100 °C. The extracts were analyzed by gas chromatography (Varian 3800, flame ionization detector, Teknokroma TR-WAX eq. CP-Sil 52 CB 30 m × 0.32 mm × 0.25 µm capillary column). Helium was used as carrier gas at a flow rate of 1.0 mL min^{-1}. Initial oven temperature was set at 50 °C for 2 min and the final temperature of 225 °C was attained with a ramp rate of 10 °C min^{-1}. Injector and detector temperatures were 220 °C and 250 °C, respectively. VFA were determined by HPLC (Jasco, Japan), using a Chrompack organic acids analysis column (30 × 6.5 mm) and a mobile phase of 5 mM H$_2$SO$_4$ at a flow rate of 0.6 mL min^{-1}. The column was set at 60 °C, and the detection was made spectrophotometrically at 210 nm. The methane content of the accumulated biogas was measured in a Micro-GC CP-4900 (Varian Inc.). A 10-m PPU column was

Table 3
Pre-treatment conditions applied.

Pre-treatment	Code	NaOH	Temperature	Enzyme	Time	Equipment
Base	B	0.3 g g^{-1} TS[a]	25 °C	–	24 h	–
Base + temperature	BT	0.3 g g^{-1} TS[a]	55 °C	–	24 h	Water bath
Base + autoclaving	BA	0.3 g g^{-1} TS[a]	121 °C	–	20 min	Autoclave
Temperature	T	–	70 °C	–	24 h	Oven
Enzyme	E	–	37 °C	10 U g^{-1} fat[b]	24 h	–
Autoclaving + enzyme	AE	–	121 °C/37 °C	10 U g^{-1} fat[b]	20 min/24 h	Autoclave

[a] Based on the work of Neves et al. (2006).
[b] U = enzyme unit. This value is based on the work of Jeganathan et al. (2007).

heated at 80 °C and helium (at 150 kPa) was used as carrier gas. The temperature of the thermal conductivity detector and injection port was 55 and 110 °C, respectively.

2.5. Statistical analysis

The statistical significance of the differences detected in the BMP values was evaluated using single factor analysis of variances (ANOVA). Statistical significance was established at $P < 0.05$.

3. Results

3.1. Effects of the different pre-treatments

The use of physical, chemical and enzymatic methods to pre-treat greaves and rinds produced hydrolysates with different characteristics, as shown by the soluble + colloidal COD, free LCFA and NH_4^+ concentrations (Table 4).

A maximum value of 1220 g $COD_{sol+coll}$ kg^{-1} raw waste was obtained for greaves with the combined action of base and temperature (BT), although high concentrations around 1000 g $COD_{sol+coll}$ kg^{-1} raw waste were also accomplished with enzymatic pre-treatments (E and AE). For rinds, maximum values of 900–1000 g $COD_{sol+coll}$ kg^{-1} were obtained with base and autoclaving + enzyme addition (Table 4). The highest percentage of fat hydrolysis (52–54%) occurred for the pre-treatment of greaves with base + temperature (BT) and for the pre-treatment of rinds with base alone (B). The free LCFA concentrations were around 188 g free LCFA kg^{-1} raw waste. The C. rugosa lipase only achieved 26–33% of fat hydrolysis in both wastes (Table 4). In all cases, oleate (C18:1) and palmitate (C16:0) were the most abundant LCFAs released during the hydrolysis of lipids, accounting for 30–50% and 15–35% of the free LCFAs, respectively.

3.2. Anaerobic biodegradability assays

A potential methane production of 794 and 1028 L $CH_{4@STP}$ kg^{-1} VS was calculated for raw greaves and rinds, respectively, based on the lipid and protein contents of the wastes (Table 2), and on theoretical values of methane production from these macromolecules, as described in Section 2.3. The BMP values calculated at the end of the biodegradability assays for the raw and treated wastes are compiled in Table 5. By comparing these BMP values with the theoretical potential of the untreated wastes, methane recovery was calculated for the different conditions studied (Eq. (1), Table 5).

3.2.1. Anaerobic biodegradability of greaves

In bottles amended with untreated greaves, methane production accounted for 89% of the maximum potential value

(BMP = 707 ± 46 L $CH_{4@STP}$ kg^{-1} VS), showing good biodegradability of the raw waste (Table 5). Pre-treating the greaves did not improve BMP values and imposing temperatures between 55–121 °C (in treatments BT, BA and T) even decreased the methane recovered by 36–57% (Table 5). Moreover, none of the strategies tested was able to improve the methane production rate, as shown by the slope of the cumulative methane production curves in Fig. 1.

Results of the analysis performed in the mixed liquor content of the vials at the end of the biodegradability tests are presented in Table 5. In all the assays amended with untreated and treated greaves, VFA and LCFA did not accumulate, whereas concentrations higher than 390 mg N-NH_4^+ L^{-1} where measured. Digestion of untreated greaves generated 934 ± 43 mg N-NH_4^+ L^{-1}, and this value was only exceeded in the assays amended with hydrolysates produced by heat-treatment (T).

3.2.2. Anaerobic biodegradability of rinds

In the assay prepared with untreated rinds, a maximum cumulative methane production of 756 ± 56 L $CH_{4@STP}$ kg^{-1} VS was achieved after approximately 30 days of incubation, corresponding to 74 ± 5% of the maximum potential value for this waste (Table 5, Fig. 2).

A 25% higher BPM value was obtained in assays amended with rinds pre-treated with heat (Table 5, Fig. 2). In the other situations, BMP was not significantly different (AE treatment) or was lower (B, BT, BA and E) than that of untreated waste. Anaerobic biodegradability of B- and BT-treated rinds was severely reduced, since methane production in these assays was lower than that of the blanks (where approximately 60 mL $CH_{4@STP}$ was measured). Methane production rate was not improved by any of the strategies applied (Fig. 2).

VFA and free LCFA concentrations around 3500 mg L^{-1} and 1000–1500 mg L^{-1}, respectively, were observed at the end of the experiment in bottles amended with physical–chemical hydrolysates, although these intermediary compounds were not detected after anaerobic degradation of enzymatic hydrolysates (E and AE) or of untreated waste. Acetic and n-butyric acids were the major VFAs, accounting for approximately 50% and 30% of total VFA, respectively; palmitate represented 40–68% of the total free LCFAs measured. Ammonium was present in all the vials at concentrations higher than 345 mg N-NH_4^+ L^{-1}, reaching maximum values of around 1000 mg N-NH_4^+ L^{-1} in the B, BT and T treatments (Table 5).

4. Discussion

Greaves and rinds are produced during pig meat-processing operations and have a very low or even no market value. Mainly composed of fats and proteins, these materials present a high potential for methane production, i.e. 794 and 1028 L $CH_{4@STP}$ kg^{-1} VS

Table 4
Soluble + colloidal COD, free LCFA and ammonium nitrogen concentrations in the hydrolysates obtained after the pre-treatments, and calculated efficiencies of COD solubilisation (E_{COD}) and fat hydrolysis (E_{fats}). Data are average values and their standard deviations, calculated from triplicate measurements.

Waste	Pre-treatment	$COD_{sol+coll}$ (g kg^{-1} RW)	E_{COD} (%)	Free LCFA (g kg^{-1} RW)	E_{fats} (%)	NH_4^+ (g N kg^{-1} RW)
Greaves	Base (B)	672	36	42 ± 0	12 ± 0	5 ± 0
	Base + temperature (BT)	1220	66	188 ± 126	52 ± 3	8 ± 0
	Base + autoclaving (BA)	532 ± 13	29 ± 1	31 ± 5	8 ± 1	n.d.
	Temperature (T)	520	28	30 ± 10	8 ± 3	3 ± 0
	Enzyme (E)	1115 ± 13	60 ± 1	103 ± 4	28 ± 1	n.d.
	Autoclaving + enzyme (AE)	981 ± 1	53 ± 0	95 ± 3	26 ± 1	n.d.
Rinds	Base (B)	896	50	183 ± 0	54 ± 16	1 ± 0
	Base + temperature (BT)	504	28	127 ± 0	37 ± 1	3 ± 0
	Base + autoclaving (BA)	550 ± 2	31 ± 0	41 ± 7	12 ± 2	n.d.
	Temperature (T)	578	33	62 ± 0	18 ± 7	1 ± 0
	Enzyme (E)	634 ± 8	36 ± 1	91 ± 4	27 ± 1	n.d.
	Autoclaving + enzyme (AE)	984 ± 4	55 ± 0	111 ± 11	33 ± 3	n.d.

RW = raw waste. n.d. – not determined.

Table 5
BMP values, methane recovery and concentrations of intermediary compounds quantified at the end of the biodegradability assays (data are averages values and their standard deviations, calculated from triplicate measurements).

Waste	Pre-treatment	BMP (LCH$_{4@STP}$ kg^{-1} VS)[a]	CH$_4$ recovery (%)[b]	LCFA (mg L^{-1})	VFA (mg L^{-1})	NH$_4^+$ (mg N L^{-1})
Greaves	Raw waste (RW)	707 ± 46	89 ± 6	233	0	934 ± 43
	Base (B)	630 ± 108	79 ± 14	0	0	925 ± 0
	Base + temperature (BT)	453 ± 77	57 ± 10	0	0	897 ± 112
	Base + autoclaving (BA)	305 ± 21	38 ± 3	0	n.d.	392 ± 49
	Temperature (T)	414 ± 84	52 ± 11	0	0	1289 ± 28
	Enzyme (E)	641 ± 36	81 ± 5	0	0	402 ± 58
	Autoclaving + enzyme (AE)	662 ± 53	83 ± 7	0	0	411 ± 16
Rinds	Raw waste (RW)	756 ± 56	74 ± 5	0	0	878 ± 144
	Base (B)	[c]	[c]	1152	3645	915 ± 58
	Base + temperature (BT)	[c]	[c]	1192	3411	1023 ± 99
	Base + autoclaving (BA)	533	52	1487	n.d.	364 ± 49
	Temperature (T)	941 ± 57	92 ± 6	129 ± 100	69	1196 ± 16
	Enzyme (E)	609 ± 34	59 ± 3	0	0	430 ± 16
	Autoclaving + enzyme (AE)	919 ± 223	89 ± 22	0	0	346 ± 16

[a] L methane per kg VS of raw waste (as described in Section 2.3).
[b] Calculated relatively to the theoretical methane production of the wastes (as described in Section 2.3).
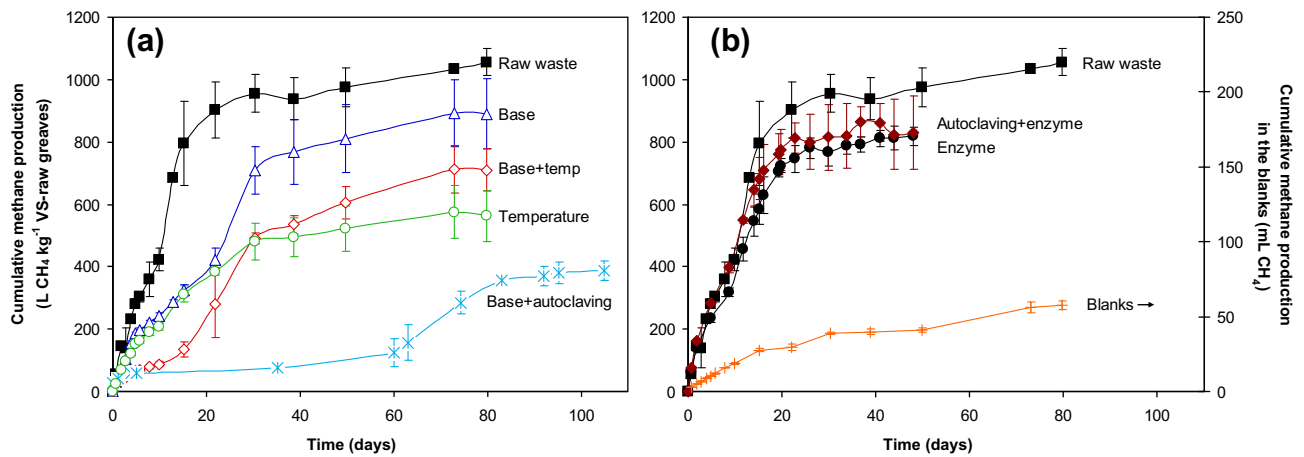[c] Methane production in these assays was lower than the value obtained in blank assays.

Fig. 1. Cumulative methane production from the raw and pre-treated greaves, after physical, chemical and physico-chemical pre-treatments (a), and enzymatic pre-treatments (b). Raw waste (■), base (△), base + temperature (◇), base + autoclaving (✳), temperature (○), enzyme (●) and autoclaving + enzyme (◆). The contribution of the blank was not subtracted in these curves, and is presented (+) in (b). Error bars represent standard deviations of triplicate measurements.
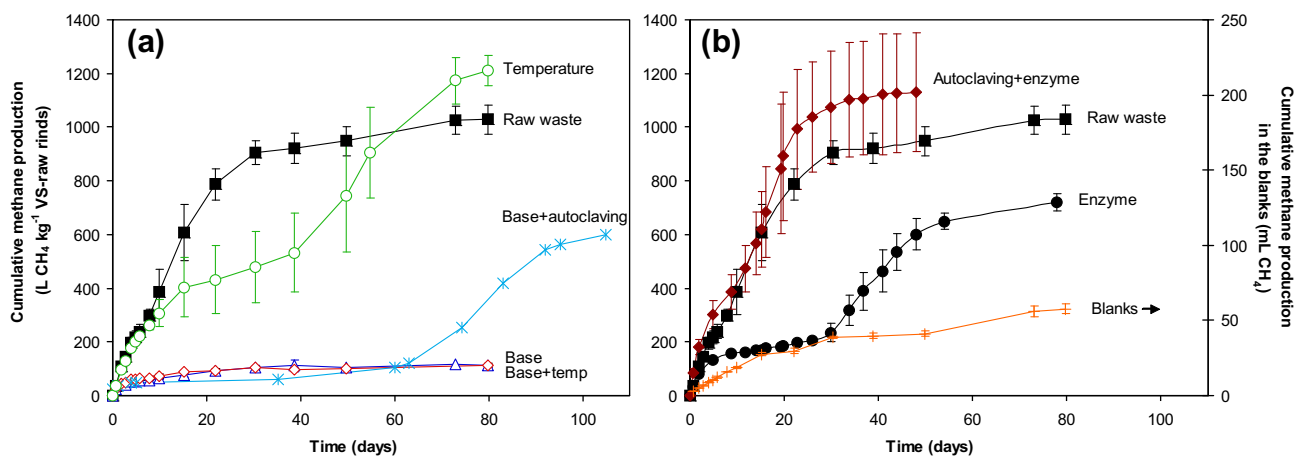

Fig. 2. Cumulative methane production from the raw and pre-treated rinds, after physical, chemical and physico–chemical pre-treatments (a), and enzymatic pre-treatments (b). Raw waste (■), base (△), base + temperature (◇), base + autoclaving (✳), temperature (○), enzyme (●) and autoclaving + enzyme (◆). The contribution of the blank was not subtracted in these curves, and is presented (+) in (b). Error bars represent standard deviations of triplicate measurements.

can be expected from the anaerobic biodegradation of greaves and rinds, respectively. In practice, BMP values of 707 ± 46 L $CH_{4@STP}$ kg^{-1} VS-greaves and 756 ± 56 L $CH_{4@STP}$ kg^{-1} VS-rinds were obtained in the biodegradability tests, corresponding to $89 \pm 6\%$ and $74 \pm 5\%$ of the calculated theoretical methane potentials, respectively. These values, and the absence of a lag phase in the methane production curves (Figs. 1 and 2), show good biodegradability of the raw wastes, with greaves being slightly more biodegradable at anaerobic conditions than rinds.

However, despite the good biodegradability of the wastes, methane production rates were low, since maximum cumulative methane production was only attained after approx. 30 days of incubation. Therefore, pre-treatment of the organic materials prior to the anaerobic digestion was used as a possible strategy to accelerate methane production rates and eventually increase wastes biodegradability even further.

Of the different strategies tested, alkaline treatments appear to be the best for improving hydrolysis of the organic materials. Treatments performed with NaOH (at 25 °C or 55 °C) yielded high soluble + colloidal COD concentrations and simultaneously stimulated the hydrolysis of fats (Table 4). Nevertheless, the positive effects of NaOH were not intensified by the 20-min application of 121 °C and 138 kPa imposed in the autoclave.

Enzymatic treatments only released 26–33% of the LCFA present in the wastes. Difficult access of the enzymes to the substrates or inadequate hydrolysis conditions may have limited the efficacy of this approach.

Although non-inhibitory concentrations of free LCFA and NH_4^+ were assured at the start of the biodegradability assays, and NaCl and Na$^+$ concentrations in the assays amended with alkaline-treated substrates were non-inhibitory (3.6 and 1.4 g L^{-1}, respectively) (de Baere et al., 1984; Rinzema et al., 1988; Zhang and Jahng, 2010), BMP was only significantly increased (25%, $p < 0.0002$) when rinds where exposed to a temperature of 70 °C (Table 5). Reduced BMP were especially observed in the assays amended with rinds pre-treated with base or base + temperature (Table 5, Figs. 1 and 2). Although hydrolysis was incomplete, the pre-treatments may have promoted a faster release of the remaining hydrolysis products in the biodegradability batch assays in comparison with the raw waste BMP assays. Ammonia accumulation was not likely inhibitory since a maximum concentration of 20 mg N-NH$_3$ L^{-1} (pH approx. 7.2) was calculated from the NH_4^+ values in the mixed liquor at the end of these assays (Table 5). This value is much lower than the inhibitory concentrations reported for non-acclimated sludge, i.e. 100–1100 mg N-NH$_3$ L^{-1} (Salminen and Rintala, 2002). LCFA and VFA accumulation were probably the reason for the lower BMP values (Table 5). Palmitate was the major LCFA measured, accounting for 40–68% of the total free LCFA. Palmitate accumulation in lipid/LCFA-inhibited anaerobic digestion processes has been previously reported (Pereira et al., 2002) and shows that β-oxidation reactions are impaired. This fact is indicative of unbalanced syntrophic relations between LCFA-degrading bacteria and methanogenic archaea. Acetic and butyric acids were the main VFA detected, and total VFA concentrations exceeded 3000 mg L^{-1}, which has been suggested as the threshold value for methanogenic toxicity (Ahring et al., 1995). Pre-treatment could have promoted the fast build-up of LCFA and VFA, causing inhibition of methanogenesis. In this case, the use of an inoculum adapted to lipids could have decreased or avoided inhibition, and accelerated wastes conversion to methane. In the thermal pre-treatment of rinds, the higher BMP was likely due to a slower release of LCFA and VFA in the anaerobic batch test, allowing a slow but steady conversion to methane. The lower hydrolysis efficiency in this case, could be one of the reasons for this behaviour.

In the anaerobic assays amended with hydrolysates of pre-treated greaves, LCFA and VFA were not detected at the end of

the experiments, despite the fact that methane yields and methane production rates were lower than those obtained with untreated greaves. These observations suggest that methanogenesis was not the rate-limiting step; instead, hydrolysis appears to be the limiting step, even after all the pre-treatments applied. None of the pre-treatments improved the methane production rate from both wastes, and more than 30 days were needed to achieve the plateau in the cumulative methane production (Figs. 1 and 2).

Optimization of the pre-treatment conditions (e.g. different concentration of hydrolysing agents or contact times), or alternative strategies, as the addition of enzyme mixtures or bioaugmentation with enzyme-producing microorganisms, can be tested as future strategies for improving BMP and methane production rates of this type of waste. Considering the high cost of enzymes and the better tolerance of microorganisms to changes in environmental conditions, bioaugmentation is a possible option. In that case lipolytic and proteolytic microorganisms may also be combined with bioaugmentation of LCFA-degrading bacteria to improve the overall process.

5. Conclusions

Pre-treating greaves and rinds with physical, chemical and enzymatic methods did not improve wastes conversion to methane and, in some cases, BMP were lower than those of untreated wastes. For rinds, this was possibly due to an improved accessibility of the treated materials to anaerobic microorganisms. LCFA and VFA accumulated in the batch vials and inhibited methanogenesis. For greaves, hydrolysis was the limiting step even after pre-treatment. Further studies are necessary to increase hydrolysis efficiency and to optimize the ultimate methane production from this type of meat-processing wastes.

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