Anaerobic biodegradability of meat-processing wastes: effect of physical, chemical and enzymatic pre-treatments

A. J. Cavaleiro*, T. Ferreira*, F. Pereira*, G. Tommaso** and M. M. Alves*

* Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar, Braga, Portugal
(E-mail: *acavaleiro@deb.uminho.pt; tferreira@deb.uminho.pt; filipa.pereira@deb.uminho.pt; madalena.alves@deb.uminho.pt)*
** Laboratory of Environmental Biotechnology, Department of Food Engineering, School of Animal Science and Food Engineering, University of São Paulo, Pirassununga, Brazil
(E-mail: *tommaso@usp.br)*

Abstract
Methane is produced during the anaerobic degradation of greaves and rinds, two by-products of pig slaughter and meat-processing industry. However, although values of 914±37 and 664±35 m^3 CH_4 ton^{-1} waste (wet weight) respectively were obtained, the degradation rate in batch assays was slow and lasted between 25 and 30 days. Therefore, in order to assess the potential increase in the biodegradation rate and biodegradability of these substrates, physical (temperature), chemical (alkali) and enzymatic (lipase) pre-treatments were tested. Partial ydrolysis was achieved for all the conditions applied but the efficiency was higher in alkaline and enzymatic treatments. These conditions produced hydrolysates with higher soluble+colloidal COD, long-chain fatty acids and ammonia concentrations, comparatively with the other treatments applied. Enzymatic hydrolysis increased 14% the methane production potential of the rinds, and this increase was even higher (80%) when rinds were thermally treated before addition of the enzyme. With all other pre-treatments, anaerobic biodegradability of both wastes was not improved comparatively to the raw materials. Alkaline conditions induced a severe decreased (89%) of rinds' biodegradability. Enzymatic pre-treatment appears to be a promising strategy for increasing methane production from meat-processing wastes.

Keywords
Anaerobic biodegradability; meat-processing wastes; lipase; methane; pre-treatments.

INTRODUCTION

Slaughterhouse and meat-processing wastes contain high amounts of fats and proteins (Salminen et al., 2002), thus holding a high potential for biogas production. However, conversion of the particulate materials to methane is frequently limited by the hydrolysis step, resulting in low biodegradability (Vavilin et al., 1996; Masse et al., 2003). Therefore, anaerobic digestion of slaughterhouse and meat-processing wastes, such as greaves and rinds, may require the application of pre-treatment methods in order to accelerate the hydrolysis step.

Most of the reported works on pre-treatment of wastes for improving anaerobic biodegradability are focused on waste activated sludge (Zhang et al., 2001; Kim et al., 2003; Bougrier et al., 2008). Some studies also describe positive results from pre-treating fat-rich wastewater (Masse et al., 2003; Cammarota and Freire, 2006; Valladão et al., 2007), but only few use this approach to increase the methane production from slaughterhouse or meat-processing solid residues (Luste et al., 2009; Battimelli et al., 2009).

The anaerobic biodegradability of fatty slaughterhouse wastes was slightly increased after

thermochemical pre-treatment by saponification, as shown by the improvement in the initial reaction kinetics and total methane production, relatively to the degradation of the untreated waste (Battimmeli et al., 2009). Thermal treatment (90–210 ºC) caused efficient solubilisation of waste activated sludge (Bougrier et al., 2008), but temperatures bellow 100 ºC were shown to be more effective than higher temperatures in increasing biogas production from waste activated sludge, food industry wastewater and sewage sludge (Luste et al., 2009).

Combined thermal (120 ºC, 5 min) and enzymatic (alkaline endopeptidase) pre-treatments increased in 37-51% the methane yield of poultry feathers, whereas thermal, chemical and enzymatic treatments where less effective in yielding methane, contributing only to a 5-32% increase comparatively to the non-treated feathers (Salminen et al., 2003). Microwave and ultrasonic pre-treatments have also improved methane recovery from palm oil mill effluent (Saifuddin and Fazlili, 2009).

Despite these promising results, pre-treating the wastes does not always increase the methane potential. In a study from Luste et al. (2009), four by-products of a meat-processing industry were efficiently solubilised by the different treatments applied, but in most cases the methane yield did not increase. Long-chain fatty acids (LCFA) produced during lipids hydrolysis, and ammonia generated through protein hydrolysis, may cause problems in the anaerobic digestion process, due to potential inhibitory effects of these compounds. Therefore, enhancing biogas production from pre-treated wastes also requires that inhibition by hydrolysis products is avoided.

In this work, the effect of six different pre-treatments (physical, chemical and enzymatic) on the solubilisation and biodegradability of greaves and rinds, two wastes from meat-processing industry, was evaluated. Hydrolysis and biodegradability assays were performed separately in two phase processes.

METHODS

Wastes characterisation

Greaves and rinds were collected from Irmãos Monteiro, a meat processing industry in Portugal. After freeze-drying, wastes were characterised in terms of total and volatile solids (TS and VS), chemical oxygen demand (COD), total Kjeldahl nitrogen, total phosphorus and fats, according to the procedures described in the Standard Methods (APHA, 1989). Protein content was calculated from the Kjeldahl-N content using a conversion factor of 6.25 (Salminen et al., 2000). Free long-chain fatty acids (LCFA) were also determined (Neves et al., 2009). The characteristics of the studied materials are presented in table 1.

Pre-treatments

The physical, chemical and enzymatic pre-treatments applied in this work are described in table 2. Pre-treatments were performed in closed bottles, to avoid the loss of ammonia to the atmosphere, and before opening the bottles pH was adjusted to neutral values with an HCl 8N solution.

Thermal treatment was done at 70 ºC, in an oven. Sodium hydroxide was used for the alkaline conditions, at an alkali/waste ratio of 20-26% (w/w). The combined effect of base and temperature was also studied, either at 55 ºC (in a water bath,) or at 121 ºC (in an autoclave).

Lipase from *Candida rugosa* (Sigma) with an activity of 20 U mg^{-1} was used in the enzymatic assays (E and AE). One unit of enzyme activity was defined as the amount of enzyme that liberates 1 μmol of free fatty acid per minute at pH 7.2 and 37 ºC. The lipase was dissolved in a phosphate

buffer solution (0.1 M pH 7.3) and mixed with the wastes at a final activity of 10 U g^{-1} fat (based on the work of Jeganathan et al., 2007), corresponding to an enzyme/waste ratio of 0.2% (w/w). The hydrolysis was performed at room temperature, with gentle agitation, during 24 hours, after which the enzyme was denatured in a water batch at 60 ºC for 15 min. In the thermal+enzymatic pre-treatment (AE), wastes were heat-treated at 121 ºC for 20 min before addition of the enzyme to the bottles.

Table 1. Characterisation of greaves and rinds wastes

	Greaves	**Rinds**
Total solids (%)	88±0.4	65±2.4
Volatile solids (%)	86±0.5	65±2.4
Total COD (g kg^{-1} wet waste)	1045±254	611±75
Kjeldahl-N (g kg^{-1} wet waste)	70±2	73±15
Total phosphorus (g kg^{-1} wet waste)	0.56	0.33
Sugars (g kg^{-1} wet waste) (*)	154	163
Proteins (g kg^{-1} wet waste)	440±15	458±95
Fats (g kg^{-1} wet waste)	406±4	379±80
Free LCFA (g kg^{-1} wet waste)	23±1	32±1

(*) Calculated according to Álvarez et al., 2010.

Table 2. Pre-treatment conditions applied

Pre-treatment	**Code**	**Conditions**			
		NaOH	**Temperature**	**Enzyme**	**Time**
Base	B	0.3 g g^{-1} TS	25 ºC	-	24 hours
Base+Thermal	BT	0.3 g g^{-1} TS	55 ºC	-	24 hours
Base+Thermal2	BA	0.3 g g^{-1} TS	121 ºC	-	20 min
Thermal	T	-	70 ºC	-	24 hours
Enzymatic	E	-	25 ºC	10 U g^{-1} fat	24 hours
Thermal+Enzymatic	AE	-	121ºC	10 U g^{-1} fat	24 hours

At the end of the treatments, hydrolysis efficiency was evaluated by analysing the liquid fractions produced. Soluble+colloidal COD was determined after centrifugation of the hydrolysates at 15000 rpm for 15 min (APHA, 1989). LCFA were quantified by GC after extraction with dichloromethane (Neves et al., 2009). Volatile fatty acids (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_2SO_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. Ammonium concentration was quantified by a titrimetric method (APHA, 1989) in all the hydrolysates except the ones resulting from the treatment with the lipase (E and AE).

Biodegradability assays

Anaerobic biodegradability of the hydrolysates was studied in batch assays and compared with the anaerobic digestion of the raw substrates. Granular sludge from an industrial wastewater treatment plant treating brewery wastewater was incubated in 160 mL vials (80 mL working volume, VS content around 10 g $L^{-}1$), at 37 ºC, 150 rpm, under strict anaerobic conditions. The procedure was according to the method described by Angelidaki et al. (2009). Blank assays (without substrate) were also prepared, and all the tests were performed in triplicate.

To avoid immediate inhibition of the anaerobic microbial communities due to VFA, LCFA or ammonium, the amount of substrate added to each bottle was calculated based on the physico-chemical characterisation of the raw wastes and hydrolysates. Each bottle received 0.25 g soluble COD g^{-1} VS, corresponding to 0.3-0.8 g total COD g^{-1} VS and less than 0.5 g COD-free LCFA g^{-1} VS. This threshold value for LCFA was defined considering the kinetics proposed by Pereira et al. (2004), where specific LCFA contents above 1 g COD-LCFA g^{-1} VS decrease the capacity of the anaerobic microorganisms to produce methane from the LCFA. A ratio $VS_{inoculum}/VS_{substrate}$ between 2 and 10 was established in all the bottles.

The methane content of the accumulated biogas was periodically measured in a Micro-GC CP-4900 (Varian Inc.). A 10 m PPU column was heated at 80 °C and helium (at 150 kPa) was used as carrier gas. Temperature of the thermal conductivity detector and injection port was 55 ºC and 110 °C, respectively. Methane production values were corrected for standard temperature and pressure conditions (STP).

At the end of the biodegradability assays, samples from the liquid medium were analysed for soluble+colloidal COD, VFA, LCFA and ammonium, according to the methods described in the pre-treatments section. The maximum theoretical amount of sodium chloride that could be present in the bottles prepared with the alkaline hydrolysates was calculated, and was always lower than 3.6 g L^{-1}.

RESULTS AND DISCUSSION

Greaves and rinds are by-products of meat processing industry that present a very low or even null market value. Anaerobic digestion of these wastes may contribute for simultaneous waste treatment and energy generation in the form of biogas. However, direct methane production of the raw wastes is usually not easy. Hydrolysis is considered to be the limiting step in this process and, therefore, pre-treating the organic materials prior to the anaerobic digestion may increase their methane potential.

In this work, the different pre-treatments applied allowed the solubilisation of the organic materials in all the assays (table 3), but the most effective were the alkaline and enzymatic. The hydrolysates produced under these conditions presented higher concentrations of soluble+colloidal COD and free LCFA compared with the other conditions applied. Oleate (C18:1) and palmitate (C16:0) were the most abundant LCFA released during the hydrolysis of the lipids, accounting respectively for 30-50% and 15-35% of the free LCFA. Higher LCFA formation was obtained by the addition of base to the greaves, and through the combined action of base and temperature (BT treatment) on the rinds (table 3). The higher ammonium concentrations were quantified when NaOH was combined with temperature at 55 ºC (BT treatment).

Table 3. Characterisation of the hydrolysates produced by the different pre-treatments

Pre-treatment	$COD_{sol+coll}$ (g kg^{-1})*		LCFA (g kg^{-1})*		NH_4^+ (g N kg^{-1})*	
	Greaves	Rinds	Greaves	Rinds	Greaves	Rinds
Base (B)	672	896	42±0	183±0	4.8±0	1.1±0.1
Base+Thermal (BT)	1220	504	188±126	127±0	7.6±0.1	2.7±0.5
Base+Thermal2 (BA)	532±13	550±2	31±5	41±7	n.d.	n.d.
Thermal (T)	520	578	30±10	62±0	2.9±0.1	0.9±0.0
Enzymatic (E)	1115±13	634±8	103±4	91±4	n.d.	n.d.
Thermal+Enzymatic (AE)	981±1	984±4	95±3	111±11	n.d.	n.d.

* Values expressed in g per kg of wet initial waste. n.d. – not determined.

A comparison between the LCFA and NH_4^+ values measured in the hydrolysates, and the maximum theoretical values that could be expected considering the physico-chemical characterisation of the raw wastes, show that the hydrolysis was not complete. Hydrolysis efficiency for proteins and fats was lower than 8% and 54%, respectively. Enzymatic hydrolysis released 27-33% of the maximum expected LCFA.

In order to evaluate the effect of the different pre-treatments in the biodegradability of the greaves and rinds, biodegradability assays were performed with the untreated and the pre-treated wastes. In the BMP assays, methane production achieved values of 914±37 and 664±35 m^3 CH_4 ton^{-1} wet for the raw greaves and rinds, respectively (table 4). These values are slightly higher than the ones reviewed by Salminen et al. (2002) for several slaughterhouse wastes (50-300 m^3 ton^{-1} wet weight).

Any of the pre-treatments tested had a positive effect in the biomethane potential of greaves (Table 4). However, conversion of the rinds to methane was improved by the thermal (T) and enzymatic (E and AE) pre-treatments (table 4). The maximum increase in the methane production (80%) was achieved when the rinds were heat-treated before addition of the lipase (AE treatment) (table 4 and figure 1). In this case, the high temperature (121 ºC) applied may have caused a first partial hydrolysis of the waste, making it more accessible to the enzyme and, therefore, facilitating the enzymatic hydrolysis. With all other pre-treatments, anaerobic biodegradability of the rinds was not improved, and alkaline conditions even induced a severe decreased (89%) of rinds' biodegradability.

At the end of the biodegradability assays, liquid samples where collected and characterised in terms of VFA, LCFA and NH_4^+ (table 5). Interestingly, although the wastes had a similar composition, a significant different pattern of biodegradation occurred. No VFA or LCFA were detected in the graves' bottles whereas in the assays amended with the rinds' alkaline and thermal hydrolysates, high VFA and LCFA concentrations were also detected in the liquid, besides ammonium, which indicates that methanogenesis was not complete, likely due to an inhibition effect of ammonia and LCFA. Unionised ammonia inhibits methanogenesis at initial concentrations of ca. 0.1-1.1 g N L^{-1}, and small amounts of sulphide may contribute to increase this inhibition (Salminen et al., 2002).Acetic and n-butyric acids were the major VFA quantified, accounting for approximately 50% and 30% of total VFA, respectively; palmitate represented 40-68% of the total free LCFA measured. These results suggest that the incomplete hydrolysis induced by the pre-treatments continued during the anaerobic biodegradability assays, producing intermediary compounds that inhibited the methanogenesis. Reinforcing this suggestion, in the bottles where enzymatic

hydrolysates from the rinds (rinds' E and AE) were added, LCFA and VFA did not accumulate, and higher amount of methane was produced, in relation to the raw waste.

Table 4. Maximum cumulative methane production (m^3 CH_4 ton^{-1} wet treated waste) obtained in the biodegradability assays of the raw wastes and hydrolysates

	Greaves	**Rinds**
Raw waste	914±37	664±35
Base (B)	770±98	72±1
Base+Thermal (BT)	614±59	73±4
Base+Thermal2 (BA)	358±18	406
Thermal (T)	487±70	851±47
Enzymatic (E)	676±25	758±60
Thermal+Enzymatic (AE)	686±97	1194±234

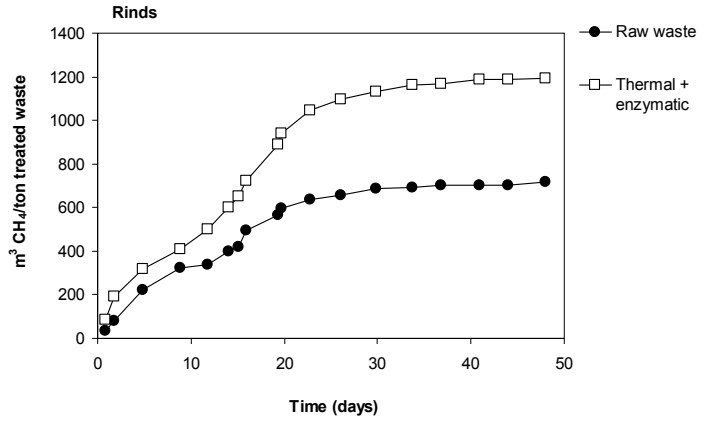

Figure 1. Cumulative methane production obtained from the rinds during the anaerobic biodegradability assay of the raw waste and thermal+enzymatic hydrolysate.

Table 5. Characterisation of the liquid samples collected at the end of the biodegradability assays

	VFA (mg L^{-1})		**LCFA (mg L^{-1})**		**NH_4^+ (mg N L^{-1})**	
	Greaves	**Rinds**	**Greaves**	**Rinds**	**Greaves**	**Rinds**
Base (B)	0	3645	0	1152	925±0	915±58
Base+Thermal (BT)	0	3411	0	1192	897±112	1023±99
Base+Thermal2 (BA)	n.d.	n.d.	0	1487	392±49	364±49.
Thermal (T)	0	69	0	129±100	1289±28	1196±16
Enzymatic (E)	0	0	0	0	402±58	430±16
Thermal+Enzymatic (AE)	0	0	0	0	411±16	346±16

Based on these results, enzymatic hydrolysis with a lipase appears to be the best strategy to increase the methane production from the rinds. However, the physical and chemical pre-treatments of both

wastes may also prove to be adequate if a more extensive hydrolysis can be promoted before the biodegradability assay, in order to avoid inhibition of the anaerobic microbial consortium by the intermediary products, provided a two-step process is applied. For that, different concentration of the hydrolysing agents or higher contact times could be tested. The combined action of lipase with a protease could also contribute to enhance even more the biomethane potential of both wastes. Methane production from solid slaughterhouse or meat-processing wastes may require a two-phase approach, increasing the process stability and efficiency due to the physical separation of the hydrolytic pre-treatment from the methanogenesis steps.

CONCLUSIONS

A partial hydrolysis was achieved for meat processing wastes (greaves and rinds) with pre-treatments based on temperature, alkaline conditions, combinations of alkaline and thermal conditions, enzymatic and combination of enzymatic and thermal conditions. Alkaline and enzymatic conditions lead to a wider solubilisation and thermal+enzymatic treatment applied to the rinds, improved the methane potential in 80%. Anaerobic biodegradability was not improved by the other pre-treatments, probably due to inhibition of the microbial communities by ammonia, VFA or/and LCFA.

Aknowledgements

The authors thank Cristiana Gonçalves for her help with the preparation of the enzymatic solution. The financial support from Portuguese Inovation Agency (ADI), through the project FatValue (QREN n.º 3491), is gratefully acknowledged. Professor Giovana Tommaso thanks the EMUNDUS15 - Erasmus Mundus External Cooperation Window (Lot15) that provided financial support for her stay in the University of Minho, in Portugal.

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