Bioresource Technology 129 (2013) 519–525

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

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

A.J. Cavaleiro [a,*], T. Ferreira [a,b], F. Pereira [a], G. Tommaso [c], M.M. Alves [a]

[a] Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[b] Spinpark – Centro de Incubação de Base Tecnológica, Avepark – Parque de Ciência e Tecnologia SA., Zona Industrial da Gandra, S. Cláudio do Barco, 4805-017 Guimarães, Portugal
[c] Laboratory of Environmental Biotechnology, Department of Food Engineering, School of Animal Science and Food Engineering, University of São Paulo, Pirassununga, Brazil

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.

ARTICLE INFO

Article history:
Received 25 June 2012
Received in revised form 17 November 2012
Accepted 19 November 2012
Available online 2 December 2012

Keywords:
Pre-treatments
Hydrolysis
Biochemical methane potential
Meat-processing wastes

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;

* Corresponding author. Tel.: +351 253 604 400; fax: +351 253 678 986.
E-mail addresses: acavaleiro@deb.uminho.pt (A.J. Cavaleiro), tommaso@usp.br (G. Tommaso), madalena.alves@deb.uminho.pt (M.M. Alves).

0960-8524/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2012.11.083

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.