

ANAEROBIC DIGESTION OF ANIMAL BY-PRODUCTS: EFFECT OF SUBSTRATE CONCENTRATION

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Abstract

In this work, the anaerobic valorization of Category 2 Animal By-Products (ABP) to biogas is assessed. Category 2 ABP other than manure, digestive tract content and milk can be considered as a suitable substrate for anaerobic digestion, due to their protein and grease content. Anaerobic biodegradability tests were performed by ‘multiple bottle’ reactor method: the initial conditions were the same in all bottles and it is assumed that the digestion process is similar for all the bottles. The quantitative analysis was performed over time in the content of each bottle. Three concentrations of substrate were tested: 2, 5 and 10% of total solids (TS). The concentration of 2% TS yielded higher cumulative methane production than the other concentrations. Between the 20th and 30th day of incubation soluble COD reached approximately 500 mg COD g⁻¹VS for the three tested TS concentrations. After that period, the soluble COD concentration started to decrease for 2% TS while it continued to increase for 5 and 10% TS, suggesting the occurrence of methanogenic inhibition for the higher substrate concentrations. Total volatile fatty acids were not the limiting parameter for methanogenesis, however ammonium concentrations higher than 6000 mg NH₄⁺-N L⁻¹ were observed for 5 and 10% TS.

1- INTRODUCTION

The European Parliament and the Council on the 3rd October 2002 [1] established the possible applications and processing rules of animal by-products not intended for human consumption through the Regulation (EC) 1774/2002, which has been changed by other regulations adopted between 2003 and 2009. There are 3 categories of animal by-products (ABP): Category 1 is a high risk material; Category 3 is low risk ABP that are not intended for human consumption but can be used as a raw matter for animal feeds; and Category 2 comprises all ABP that are neither included in Category 1 nor in Category 3. Category 2 ABP other than manure, digestive tract and milk are sterilized and transformed by rendering process (designed as Category 2 ABP* hereafter) and then can be eliminated by incineration, co-incineration, transformed into biogas, composted, used to produce fertilizers or disposed in landfill. The most common practice in Portugal is landfill disposal or fertilizer production. Due to the high organic matter content of Category 2 ABP*, there is a great potential for the valorisation of this residue by conversion into biogas in anaerobic digestion processes. In addition, the EU Landfill Directive (99/31/EC) sets specific targets for disposing the biodegradable wastes in landfill sites. The reduction targets are 50% and 65% of 2001 levels by 2013 and 2020, respectively [2]. Since lipid and protein degradation through anaerobic digestion process may cause inhibitory effects in biogas production through long chain fatty acids (LCFA) and short chain volatile fatty acids (VFA) concentration or ammonia accumulation, which concentration of Category 2 ABP* can be used? To answer this question, a study with different concentrations of ABP with VFA, pH and ammonia control is done.

The aim of this work was to evaluate the methane potential of Category 2 ABP* at different concentrations: 2, 5 and 10% of total solids and establish the concentration more suitable for anaerobic digestion and the key factors responsible for process inhibition.

2- MATERIALS AND METHODS

Category 2 ABP*

The substrate was provided by a processing plant of Category 2 ABP according Regulation No. 1774/2002. At the premises of the company, the ABP were crushed to particles with no more than 30 mm in diameter. Then, the material was agitated and pre-cooked at 101-104 °C in a steam-heated vessel. After pre-cooking the ABP were sterilized at a temperature of 140 °C and a pressure of 3 bars for 20-25 minutes. The finished product (Category 2 ABP*) is a fine granular material that is medium to light brown in color (Figure 1). This substrate was characterized for total solids (TS), volatile solids (VS), total Kjeldhal Nitrogen (TKN), total phosphorus (TP), chemical oxygen demand (COD) and oil/fat (Table 1) according to *Standard Methods for the Examination of Water and Wastewater* [3].



Figure 1: Image of Category 2 ABP*, also known as ‘meat and bone flour’

Inoculum

The inoculum used in this study was digested sludge from a wastewater treatment plant (DSWW) collected at the outlet of an anaerobic reactor working at 35 °C with a HRT of 20 days. TS and VS contents of the inoculum are shown in Table 1.

	Parameter ± SE					
	TS (%)	VS (% TS)	TKN (mgN g ⁻¹ TS)	TP (mgP g ⁻¹ TS)	COD (mgO ₂ g ⁻¹ TS)	Oil/fat (%)
Category 2 ABP*	85.0 ± 0.4	90 ± 3	89.4 ± 0.8	9.2 ± 0.2	1510 ± 6	37.41 ± 0.03
DSWW	1.556 ± 0.008	60.1 ± 0.3				

Table 1: Category 2 ABP* and DSWW characterization

Experimental set-up

The experiment was performed in a ‘multiple bottle’ reactor [4], where several bottles represent one large reactor. At the start of the experiment, all bottles have the same contents and it is assumed that the progress of the anaerobic process is similar in all bottles. Each time the reactor is sampled one whole bottle is used and its contents are analyzed [4]. In this experiment, 1100 mL bottles were used for methane analysis and 110 mL bottles for aqueous content analysis. The initial conditions of each bottle are described in Table 2. The headspace was flushed with N₂ and residual oxygen in the medium was reduced with 0.125 M Na₂S and incubated at 35 °C without stirring. The test was conducted during 130 days. The volume of methane (CH₄) produced was calculated by the ideal gas equation and converted to standard conditions (0°C and 1 atm). Soluble COD, VFA, pH and NH₄⁺ were measured during the experiment. Methane and VFA analyses were performed by GC-FID using a 30 m x 0.53 mm x 0.45 µm Nukol capillary column. Helium was used as carrier and auxiliary gas. For CH₄ determination the conditions were: carrier gas flow = 3 mL min⁻¹ and temperatures of 40 °C for the oven, 120 °C for the injector and 130 °C for the detector. For the

determination of VFA, approximately 2 mL of sample was centrifuged at 10000 rpm for 10 min. The centrate was filtered by 0.2 μm syringe filter. A volume of 20 μL of 85% phosphoric acid, a suitable volume of the filtered centrate, Millipore water and 100 μL of phenol (as internal standard) at 5000 mg L^{-1} were added into a vial, giving a final volume of 1 mL. The GC operational conditions were: carrier gas flow of 30 mL min^{-1} ; temperatures of 200 $^{\circ}\text{C}$ for the injector and 250 $^{\circ}\text{C}$ for the detector, and a temperature ramp (105 $^{\circ}\text{C}$ for 4 min, then raised 10 $^{\circ}\text{C}$ per min until 190 $^{\circ}\text{C}$ and maintained for 2 min) for the oven. Ammonium was analyzed by ion chromatography (Dionex DX-120), using a Dionex Ionpac column CS12A 4 \times 250 mm and a suppressor CSRS@300 4 mm. Isocratic elution was made with 20 mM methanesulfonic acid at a flow rate of 1.0 mL min^{-1} .

Modeling

To compare the effect of different concentrations tested, a modified Gompertz model was used to describe the cumulative gas production:

$$\text{CH}_4(t) = P \cdot \exp \left\{ -\exp \left[\frac{R_{\max} \cdot e}{P} (\lambda - t) + 1 \right] \right\} \quad (1)$$

where CH_4 is the cumulative methane production ($\text{mL CH}_4 \text{ g}^{-1} \text{ VS}_{\text{substrate added}}$), P is the maximum methane production ($\text{mL CH}_4 \text{ g}^{-1} \text{ VS}_{\text{substrate added}}$), R_{\max} is the maximum methane production rate ($\text{mL CH}_4 \text{ g}^{-1} \text{ VS}_{\text{substrate added}} \text{ day}^{-1}$), λ is the lag phase time (day), and t is the digestion time (day). The parameters P , R_{\max} and λ were estimated by non-linear regression using the Solver function of the Microsoft Excel software by minimizing the residual sum of squared errors between the average experimental results and the model curve.

Concentration (% TS)	V_{bottle} (mL)	V_{working} (mL)	$m_{\text{substrate}}$ (g)	COD substrate (g L^{-1})	V_{inoculum} (mL)	$V_{\text{H}_2\text{O}}$ (mL)	m_{NaHCO_3} (g)	$V_{\text{Na}_2\text{S}}$ (mL)
2%	1100	100	2.35	30.1	70	26.85	0.5	0.8
5%	1100	100	5.88	75.3	70	23.32	0.5	0.8
10%	1100	100	11.76	150.6	70	17.44	0.5	0.8
2%	110	50	1.18	30.1	35	13.42	0.25	0.4
5%	110	50	2.94	75.3	35	11.66	0.25	0.4
10%	110	50	5.88	150.6	35	8.72	0.25	0.4

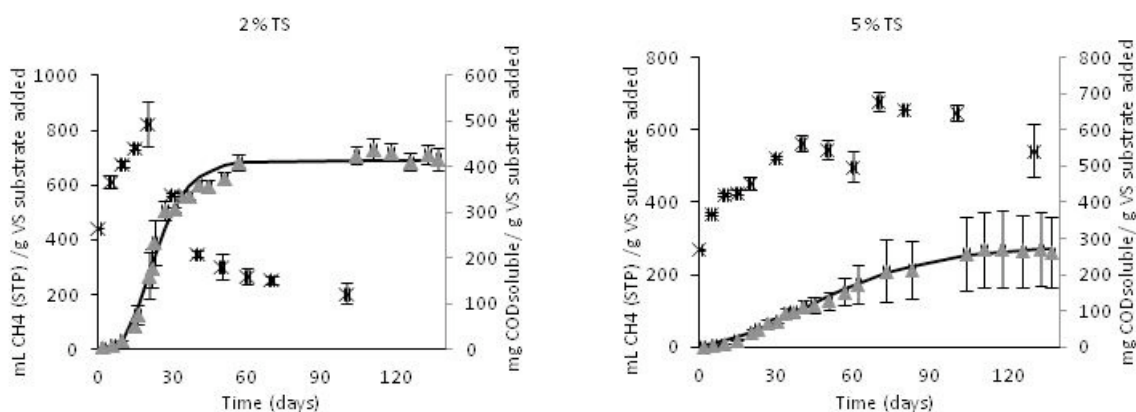
Table 2- "Multiple bottle" reactor description

3- RESULTS AND DISCUSSION

The potential of the Category 2 ABP* for anaerobic digestion was assessed by studying the conversion to methane of the substrate, in suspensions of 2, 5 and 10% TS (30.2, 75.5 and 151 g COD L^{-1} , respectively). The cumulative methane production (after subtracting the blank values), normalized by the VS content of the substrate, and the curves obtained by fitting the Gompertz model to the experimental data are presented in Figure 2. The better performance ($P = 692 \pm 12 \text{ mL CH}_4 \text{ (STP) g}^{-1} \text{ TS}_{\text{substrate}}$ and $R_{\max} = 28 \pm 2 \text{ mL CH}_4 \text{ (STP) g}^{-1} \text{ TS}_{\text{substrate}} \cdot \text{d}^{-1}$) was found for the lowest solids load (2% TS). P value was about 59 and 97% and R_{\max} 87 and 97% higher than for 5 and 10% TS, respectively. The lag period was similar for 2 and 5% TS.

There was almost no methane production for 10% TS, which is probably due to inhibition by some compounds resulting from substrate hydrolysis.

An accumulation of acetate, propionate, butyrate, iso-butyrate, valerate and iso-valerate was observed at some intervals for 5 and 10% TS. According to Grady et al. 1999 [5] the inhibition caused by VFA will be of little concern as long as the pH remains within the normal range for growth of methanogens (6.8 to 7.4); for pH values below this range, impacts associated to no ionized VFAs will be significant. The pH varied in the ranges 7.00 – 7.95 for 2% TS, 6.76 – 7.70 for 5% TS and 6.69 - 7.18 for 10% TS. The lowest values of pH were measured on the 10th day, when NH₄⁺ concentrations were 1100, 2600 and 1900 mg N L⁻¹ and total VFA presented values of 17500, 16500 and 24000 mg COD L⁻¹ for 2, 5 and 10% TS, respectively. Then, total VFA concentration for 2% TS decreased along time until 220 mg COD L⁻¹. Oppositely, for 5 and 10% TS raised slowly up to 20000 and 29500 mg COD L⁻¹. At this time (day 130) the pH was higher due to ammonia formation in the range 2500 to 12000 mg N L⁻¹, and then total VFA should not have inhibitory effects [5]. The excess ammonia in the medium can cause inhibitory effects since for pH in the range 6.5 - 8.5, the percentage of free NH₃ accounts for 0.33 - 33% of the total ammonia nitrogen. In this study, at the end of the experiment, the NH₃ concentrations were 309, 406 and 237 mg N L⁻¹ for 2, 5 and 10% TS, respectively. These values were in the range of the concentration considered to have no adverse effects on anaerobic digestion [6]. Krylova et al., 1997 [7] studied the effect of NH₄Cl on the anaerobic digestion of poultry manure and concluded that concentrations between 520 mg N L⁻¹ to 2600 mg N L⁻¹ of NH₄⁺ do not affect biogas and methane production but concentrations between 2600 and 7800 mg N L⁻¹ reduced the production in 50-60% and 80-90%, respectively for biogas and methane. They also concluded that high concentrations of NH₄⁺ probably inhibit the methanogens but there was not an apparent effect on hydrolytic and acetogenic steps. These findings are in accordance with the results obtained in this study. For 2% TS the concentration of NH₄⁺-N reached 1850 mg N L⁻¹ at the 30th day, which caused the methane production to slow down. As shown in Figure 2, there is a marked decrease in methane production after the 30th day and a reduction of soluble COD consumption after day 40. Between 30th and 100th days the reduction on methane activity for 5% TS was 59% of that obtained for 2% TS, with an average NH₄⁺-N concentration around 5000 mg N L⁻¹. A value close to 6100 mg N L⁻¹ was reached at the 130th day resulting in 100% methanogenic activity inhibition. Moreover, the soluble COD consumption was not so evident, between 40th and 60th days, as for 2% TS. For 10% TS the NH₄⁺-N concentration of 6500 mg N L⁻¹ after the 30th day ceased the methanogenic activity. As show in Figure 2, the methane production stopped and soluble COD never decreased along time.



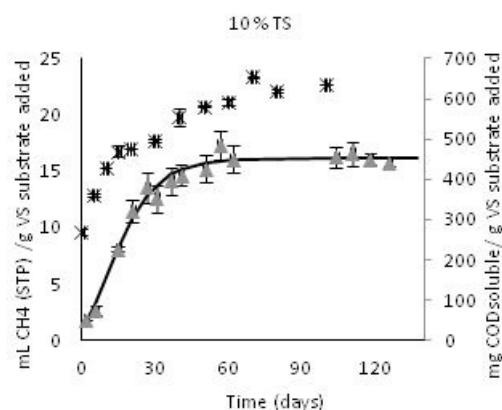


Figure 2: Accumulated methane production (\blacktriangle), soluble COD ($*$) and respective Gompertz Model adjustment (—) for the three Category 2 ABP* concentrations ($P = 691.6 \pm 12$, 283 ± 5 and $16.2 \pm 0.2 \text{ mL CH}_4 \text{ g}^{-1} \text{ VS}_{\text{substrate added}}$, $R_{\text{max}} = 28 \pm 2$, 3.5 ± 0.1 and $0.55 \pm 0.04 \text{ mL CH}_4 \text{ g}^{-1} \text{ VS}_{\text{substrate added}} \text{ day}^{-1}$, $\lambda = 11 \pm 1$, 11 ± 1 and 0.1 ± 1 for 2, 5 and for 10% TS, respectively)

4- CONCLUSIONS

Biodegradation of Category 2 ABP* in different concentrations, by anaerobic digestion at mesophilic conditions, was assessed in this work. Three concentrations were tested: 2, 5 and 10% TS. Analyses during the process revealed high concentrations of total VFA, but the pH remained in the interval where no inhibition by VFA occurs. The most probable cause of inhibition for 5 and 10% TS (41% and 3% of the maximum methane production obtained for 2% TS) was the ammonia resulting from the hydrolysis step. So, the conversion to biogas of Category 2 ABP* in concentrations higher than 2% TS may be feasible by a co-digestion process, which requires the addition of a substrate rich in carbon and poor in nitrogen.

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