Biogas production from slaughterhouse waste: effect of blood content and fat saponification

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Keywords

Anaerobic digestion, animal by-products, nitrogen-inhibition, saponification.

Abstract

The effect of fat saponification and the inclusion or exclusion of blood in slaughterhouse mixtures were assessed in terms of anaerobic digestion performance. Mixtures of animal by-products (ABP) were collected for 1 year, whereby following the daily activity and waste generation at a slaughterhouse facility, seasonal fluctuations were found. The blood content of ABP mixtures was variable, affecting both the methane yield and the production rate (287.8-320.5 NL_{CH4} kg_{COD}^{-1} and 80.3-94.7 and NL_{CH4} kg_{COD}^{-1} d^{-1}, respectively). The saponification of fatty ABP materials was studied to assess the methane production rate, singularly or combined, with and without the addition of blood. Data showed that saponification significantly reduced the lag phase, from 2.2 to 1.5 days in winter mixtures and from 1.5 to 0.9 days in summer mixtures (all with blood), and from 0.3 to 0.1 days in summer mixtures without blood. Finally, the percentage of energy demand at the slaughterhouse potentially covered by net biogas energy was estimated, finding that the facility could be 100% energy self-

sufficient in winter, whereas this would be reduced to 85 % in the summer due to different methane yields of ABP mixtures based on season.

1. Introduction

Meat consumption increased worldwide in recent years and is expected to continue increasing up to 366 million tonnes by 2029 (OECD/FAO, 2020). Taking the pork industry as an example, the number of slaughtered pigs in the European Union (EU) reached 256 million in 2019 (European Commission, 2019). This aside, the amount that remains unfit for human consumption, between 25 - 50% per animal (on a wet basis) (European Commission, 2005), is called animal by-product (ABP) and its management must meet with EU regulations on sanitary risks (European Community, 2009). ABPs are classified into 3 categories depending on the health risk for people and animals. The mandatory sanitation process depends on each category, as well as the recovery processes and subsequent uses (European Community, 2009) (European Community, 2011).

Slaughterhouse ABPs include different animal body components, mainly blood, fat, and clipping parts (internal organs, etc.) plus lesser amounts of manure and digestive tract content. The blood content, which contributes to the protein content of ABP mixtures (Ortner et al., 2014) (Palatsi et al., 2010), can differ depending on market demand due to specific blood valorisation alternatives, such as ingredients in animal feedstocks or in organic fertilizer products (Ofori and Hsieh, 2014) (Toldrá et al., 2016). ABPs, usually treated by energy consuming processes such as rendering or carcase incineration (European Commission, 2005), are an attractive substrate for biogas production because of their high content of organic matter, measured both as volatile solids (VS) of 80–100 g_{VS} kg^{-1} or as chemical oxygen demand (COD) of 180–260 g_{COD} kg^{-1} (Ortner et al., 2014). Anaerobic digestion is a well-known biological degradation process (Tsui and

Wong, 2019) likely to be applied in slaughterhouse facilities which could be of help in solving their consistently high energy consumption that ranges 110-760 kWh t pig-carcase-1 for refrigeration and water heating for scalding, singeing, splitting and cleaning activities (European Commission, 2005). Nevertheless, only ABP of categories 2 and 3 (i.e. manure, digestive tract content, blood, and carcases and internal organs of healthy slaughtered animals) can be submitted to biogas production process, always combined with a particle size reduction up to 50 mm and the corresponding specific sanitation procedure, such as pressure sterilization at 133ºC & 2 bars for 20 minutes or pasteurization at 70ºC for 60 minutes for category 2 and 3, respectively (European Community, 2009).

ABPs have been proved to be an interesting feedstock for biogas production due to their biogas yield potential of 620 - 852 $Nm^3_{CH4}\ t_{VS}^{-1}$ (Hejnfelt and Angelidaki, 2009) (Rodríguez-Abalde et al., 2011a) (Ortner et al., 2014), values in between theoretical yields of 496 $Nm^3_{CH4}\ t_{VS}^{-1}$ for proteins and 1,014 $Nm^3_{CH4}\ t_{VS}^{-1}$ lipids (Angelidaki and Sanders, 2004). In this regard, previous works showed that sanitation treatments do not significantly affect the ABP characteristics (Hejnfelt and Angelidaki, 2009) and even encourage their methane yields (Rodríguez-Abalde et al., 2011a). Some ABPs are regular feedstocks in full-scale co-digestion biogas plants (Schnurer et al., 2011) (Ortner et al., 2015), but they are usually restricted to low loadings to avoid operational imbalances linked to fat and/or protein overloads. In the case of fats, failure depends upon their concentration (Cirne et al., 2007), or even on their particulate size. found that sizes since up to 450 µm were found to slow down biogas production compared to smaller particles (Masse et al., 2002). As non-soluble compounds, the fat mass transfer during the hydrolysis stage of anaerobic digestion may be rate-limited, especially when high amounts of solids are present (Chen et al., 2008). The degradation-intermediate

compounds of fats, long chain fatty acids which are surface-active compounds that adsorb onto microbial cell membranes and tend to generate foams, contributing to biomass wash out (Masse et al., 2001). Besides, some inhibitory effects, linked to the microbial consortia and oxidation process of fats, depend upon hydrogen production – consumption imbalance due to hydrogenotrophic methanogen activity (Masse et al., 2002) . In the case proteins, highly present in ABPs depending on blood content, inhibitory effects have been widely reported by ammonia or proteins intermediate product of the acidogenic stage of anaerobic digestion (Salminen and Rintala, 2002). Nitrogen has been identified as a methanogen inhibitor with total ammonia nitrogen (TAN) levels >1.7 $g_N\ L^{-1}$ (Chen et al., 2008), reducing the methane yield, increasing the volatile fatty acids (VFA) and finally resulting in microbial instability (Angelidaki and Ahring, 1993). However stable continuous production of biogas has been reported with TAN ranged 3.5 - 7.7 $g_N\ L^{-1}$ (Ortner et al., 2014) due to a microbial population shift towards slow growers such as hydrogenotrophic methanogens and homoacetogenic bacteria or syntrophic acetogens. In addition, an excess of hydrogen could also inhibit syntrophic acetogens and hydrogenotrophic methanogens (Angelidaki et al., 2018) (Demirel and Scherer, 2008).

Several strategies could be studied to prevent and overcome inhibition caused by lipids and nitrogen of ABPs. Feedstock codigestion with agricultural wastes encompasses dilution of the inhibitory substances, resulting in stable continuous performance (Moukazis et al., 2018) (Salama et al., 2019). The addition of iron salts or trace elements have been proved to reduce process instability by ammonia, resulting in near zero VFA concentration (Ortner et al., 2014). In the case of fat overload, the enzymatic hydrolysis with as lipases addition enhanced the stability by reducing particle size up to 75 % (Masse et al., 2001).Among others, saponification of fatty materials has the

advantage of producing soaps that are soluble and available for microorganisms. Saponified aero-flotation fats and flesh fats from animal carcasses have proved to be more easily broken down than non-saponified ABP, increasing both methane yield and production rate (Battimelli et al., 2009).

Some researchers have assessed the effect of proteins or fats on methane production, but there are few studies regarding both effects in slaughter waste materials. Besides, literature does not always report the real situation of a full scale biogas plant in which there is a seasonal variation in the protein-fat profile of the incoming ABP mixture. The aim of this work is to assess the combined effect of the protein (from blood) content and the saponification pre-treatment of ABP of category 2, regarding both methane yield and production rate. For that purpose, all individual ABP fractions were collected in two different periods of 1 year. Representative slaughterhouse mixtures were defined based on real slaughterhouse conditions and activity, defining 2 representative ABP mixtures (summer and winter). Focusing on protein and fat profiles, sterilized ABP fractions, saponified fatty-ABP fractions, and representative slaughterhouse mixtures were submitted to biochemical methane potential (BMP) tests to identify limiting steps and to separately confirm the effect of blood and saponification in methane yield and production rate. Additionally, to better assess a real scale situation, an estimation of the net energy gain due to biogas production was carried out for the 2 identified periods of the slaughterhouse activity.

2. Materials and methods

2.1. Slaughterhouse waste collection and characterisation

The ABP generation was monitored for one year in a slaughterhouse (MAFRICA S.A., Barcelona, Spain) with a slaughter capacity of 500,000 pigs per year. The generation was registered and the samples collection (5 kg per fraction to obtain representative

126 samples) were performed in 2 campaigns in 2019 (winter 2019 and summer 2019).
127 Eight ABP fractions were collected per campaign: pig manure from animal storage area
128 (MA), sewage sludge (SL) from the wastewater treatment plant, blood (BL), digestive
129 tract fat (DT), internal organs (IO), fillet fat (FF), abdominal fat (AF) and clipping parts
130 (CP). Bones, hooves, or pig hair were not considered because of their low
131 biodegradability. All samples were preserved at -20ºC. No sample alterations were
132 detected after sample defrost. Shredded ABP samples were freeze dried (Model
133 Cryodos50, Telstar, Spain) and homogenised previous to their physic-chemical
134 characterization, based on the following parameters: total and volatile solids (TS, VS);
135 total Kjeldahl and ammonia nitrogen (TKN, TAN) (APHA, AWA, 2005); total
136 chemical oxygen demand (COD) (Noguerol-Arias et al., 2012); total fat (TF) (US
137 Environmental Protection Agency, n.d.) (Soxhlet™ 2050 extraction equipment, Foss,
138 Spain); organic nitrogen (org-N), as the difference between TKN and TAN contents;
139 and total protein content (TP), using the Jones factor (conversion factor of 6.25 g-TP kg
140 org-N-1 (Salminen et al., 2000a)). The total carbohydrate (CBH) content was estimated
141 with equation 1 (Ware and Power, 2016).
142 Eq. 1. CH (g kg^{-1}) = VS (g kg^{-1}) – TP (g kg^{-1}) – TF (g kg^{-1}) – Ash (g kg^{-1})

143 **2.2. Experimental design**

144 The present work was divided into 2 parts after the initial mixture characterization.
145 Firstly, characteristics of the ABP fractions were determined and typical ABP mixtures
146 were identified based on generation data registered at the slaughterhouse during the
147 sampling campaign in winter (w) and summer (s) (M1w and M5s). Once the typical
148 winter mixture was defined, sterilisation pre-treatment (for typical winter mixture M1w
149 and typical winter mixture without blood) and combined sterilisation and saponification
150 (for mixture M2w, typical winter mixture with rich fat fractions –AF and FF-

151 saponified) were evaluated in terms of biodegradability, specific methane yield (SMY)
152 and methane production rate (MPR), all of these parameters obtained through a series of
153 BMP tests. At this stage, 3 mixtures (M1w, M2w, M3w) and 8 individual ABP winter
154 fractions were assayed.
155 Secondly, the individual and combined effects of saponify the fatty fractions and
156 varying the blood content of mixtures over SMY and MPR, obtained by BMP tests,
157 were studied following a 2^2 full factorial design applied to the summer samples. Two
158 factors, saponification, and blood content were evaluated at 2 different levels. For that
159 purpose, the mixtures M4s, M5s, M6s, and M7s were prepared and the 8 individual
160 ABP summer fractions were also assayed. The analysis of variance (two factor ANOVA
161 test), performed by R project software, was used to assess the effect of individual
162 factors and the interactions between them taking SMY, MPR and lag phase as response
163 variables.

164 **2.3. Pre-treatment**

165 From the anaerobic digestion process perspective, the compulsory hygienisation
166 methods, including particle size reduction, can also be considered as pre-treatments. The
167 ABP fractions were individually sampled in the slaughterhouse, then shredded with a
168 meat mincer (Fama model FTS127; Eurocort, Spain), except SL, BL and MA, to obtain
169 a particle size of 12 mm that complied with EU regulation requirements (European
170 Community, 2011). Sampled ABP fractions belonged to category 2 and 3, but once
171 mixed, any ABP mixtures belonged to category 2 according to the ABP regulation; in
172 this work, pressure sterilisation pre-treatment was applied to fit the hygienisation rule
173 (European Community, 2009). Once pre-treated, all materials were characterised and
174 submitted to a BMP assay.

175 Individual homogenised ABP fractions (except FF, AF, MA, and SL) and whole
176 mixtures M1w, M3w, M4s and M5s were submitted to a pressure sterilization process at
177 133 ºC and 2 bars for 20 minutes, using a high pressure temperature reactor
178 (Zipperclave Pressure Vessel model; Iberfluid Instruments, Spain).
179 For saponified mixtures (M2w, M6s and M7s), corresponding fatty fractions were
180 mixed and then saponified. The corresponding non-fatty fractions were sterilised and
181 then saponified, and the sterilised materials were then mixed. The saponification was
182 conducted under similar conditions to the sterilisation process (133 ºC and 2 bars of
183 absolute pressure for 20 min) but with the addition of alkali. Here, the potassium
184 hydroxide solution (KOH, 85 % purity grade in a 32 %w/w concentration) was added in
185 stoichiometric excess (0.09 g-KOH gVS-1) as proposed by (Battimelli et al., 2009).

186 **2.4. Biochemical methane potential test**

187 The BMP test of individual ABP fractions and mixtures was run at 37 ºC in triplicate
188 according to (Angelidaki and Sanders, 2004) (Soto et al., 1993). The corresponding
189 material and inoculum were placed in glass vials with a working volume of 500 mL
190 (total volume 1200 mL), fitting an initial concentration of 5 g_{COD} L^{-1} and 5 g_{VS} L^{-1},
191 respectively. The inoculum was collected in a mesophilic sewage sludge anaerobic
192 digester (WWTP-Llagosta, Barcelona, Spain) with an hydraulic retention time ranging
193 from 45-55 days. Sice the ABP studied are characterised by high TAN loads, the
194 inoculum used can be considered appropriate for allowing slow growers such as
195 hydrogenotrophic methanogens and homo-acetogenic bacteria, all of which are
196 involved in the syntrophic pathway activated under high TAN levels (Schnurer et al.,
197 2011).
198 Bicarbonate was also added to BMP vials to keep a pH 8. After closing vials ensuring
199 airtight conditions, the headspace were bubbled with N_2 gas to displace air so as to

achieve an anaerobic environment. Control vials, without substrate, were prepared similarly adding only inoculum; consequently, the average gas produced in controls was subtracted to calculate the net biogas production (Angelidaki et al., 2009). A total of 1800 µL of gas volume was extracted in order to monitor cumulative methane and carbon dioxide quantity inside vials, which was determined by gas chromatography (Varian CP-3800 unit; Hayesep packed column (Q 80/100 Mesh; 2 m x 1.8" x 2.0 mm SS) thermal conductivity detector; Varian, USA) (Angelidaki et al., 2009). Gas volume was normalized at temperature 273.15 K and pressure 100 kPa (Strömberg et al., 2014). The content of individual volatile fatty acids (VFA; acetic, propionic, i-butyric, n-butyric, i-valeric, n-valeric, i-caproic and n-caproic acids) per vial at the end of the assay was determined by gas chromatography (Varian CP-3800 unit; packed column (Q 80/100 Mesh; 2 m x 1.8" x 2.0 mm SS) and flame ionization detector; Varian, USA) (Rodríguez-Abalde et al., 2011b).

Biodegradability (BD) was expressed as the percentage of the initial COD content (COD_0) transformed at the end of the assay into methane, VFA and new biomass, according to equation 2 (adapted from (Angelidaki and Sanders, 2004) (Soto et al., 1993)).

Eq. 2. $$BD = A + \frac{Y_A}{(1-Y_A)} * \left(A - 100 * \frac{COD_{VFA}}{COD_O}\right) + \frac{Y_M}{(1-Y_M)} * M$$

Where, A is the methanisation index (% COD_0) M the acidification index) (% $COD_{VFA+CH4}$ to COD_0 and COD_{CH4} to COD_0), Y_A, Y_M, are acetogenic and methanogenic biomass yields (0.064 and 0.028 g g-1, respectively); COD_0, COD_{VFA} are the initial total COD and the final total VFA, expressed in COD equivalent (g_{COD} L^{-1}), concentrations. The experimental specific methane yield (SMY, NL_{CH4} kg_{COD}^{-1}) data was fitted to a modified Gompertz model (equation 3) (Zwietering et al., 1990) (Strömberg et al.,

2015) to obtain the maximum methane production rate and the lag-phase time by a non-linear least square regression analysis.

Eq. 3. $BMP_t = R \cdot exp\left(-exp\left(\frac{\mu}{R}\ (\lambda - t) + 1\right)\right)$

Where, μ is the maximum methane production rate ($NL_{CH4}\ kg_{COD}^{-1}\ d^{-1}$); R is the maximum methane yield ($NL_{CH4}\ kg_{COD}^{-1}$) and λ is the lag phase (d^{-1}).

The kinetic parameters were determined using Solver tool of Excel (Microsoft), using the minimization of the root mean square error (RMSE) (Strömberg et al., 2015) (equation 4) between the adjusted and experimental data for methane production yield. Coefficient of determination R^2 (equation 5) was also calculated to evaluate the Gompertz model; if the kinetic adjustment reveals R^2 close to 1 and a low RMSE, the model selected was successful.

Eq.4 $RMSE = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \overline{y_i})^2}{n}}$

Eq. 5 $R^2 = 1 - \frac{\sum_{i=1}^{i=n}(y_i - \overline{y_i})^2}{\sum_{i=1}^{i=n}(y_i - \hat{y})^2}$

Where, y_i is the experimental value for the sample i, whereas $\overline{y_i}$ is the predicted value by the model, $\hat{y}$ is the mean and n the number of samples taken along each BMP test.

2.5. Energy calculation

The experimental SMY from BMP test was used to estimate the total methane production (TMP; $Nm^3_{CH4}\ y^{-1}$) in the slaughterhouse with equation 6.

Eq.6 $TMP = 0.83 \cdot (MY/1000) \cdot S$

Where 0.83 MY is the specific methane yield of the ABP mixture ($NL_{CH4}\ t^{-1}$), and S is the total quantity of ABP ($tABP\ year^{-1}$) that was estimated considering that an average live weight animal of 100 kg generates an average carcase weight of 80 kg $kg\text{-}animal^{-1}$ in the facility after processing operations (European Commission, 2005). The 0,83 factor is a BMP to full scale correlation coefficient (Bishop et al., 2009). It should be

noted that this conversion was found not to be statistical significant in some cases that could result in biogas over prediction so specific further investigations will be needed. For the mixtures without blood (M3w, M4s, M6s) blood weight was extracted from the average carcase weight.

The gross energy production (Ge, MWh) was estimated by equation 7.

Eq.7 $Ge = \eta \cdot TMP \cdot LCV \cdot 3.6 \cdot 10^{-3}$

Where ŋ is the efficiency electricity-biogas factor (90%, assuming 35% for electricity and 55 % for heat), LCV is the low calorific value of methane (37 MJ $m^{-3}{}_{CH4}$), and $3.6 \cdot 10^{-3}$ is a conversion factor (MJ- MWh^{-1}).

The biogas net energy production (NEP, MWh) was calculated by subtracting the energy consumed by the biogas facility (Be, MWh) from Ge (MWh). The energy demand in the biogas plant Be, for ABP valorisation with a heat & power configuration, (Be; MWh) was estimated by equation 8 (Ware and Power, 2016) that includes the required energy for pumping, grinding, hygienisation, and maintenance of operational mesophilic temperature (38 ºC) inside the biogas reactor (Angelidaki and Sanders, 2004).

Eq.8 $Be = S \cdot \left(1.5.10^{-3} + C_w \cdot \Delta T \frac{4.182}{3{,}6.10^3}\right)$

Where 1.5 is the energy consumption (1.5 kWh t-$substrate^{-1}$ (*Redunit System Combinations, Vogelsang GmbH & Co. KG*, 2018) for grinding, to homogenise and reduce the particle size <50 mm, and pumping; C_w is the specific mean calorific value of animal by-products (0.76 kcal kg^{-1} $°C^{-1}$) (Fellows, 2009), ΔT is the temperature difference (between 17 ºC or mean annual ambient temperature in Barcelona (AEMET, 2014) and 133 ºC); $\frac{4.182}{3{,}6.10^3}$ is a conversion factor (kcal to MWh). It was assumed that an integrated energy scheme (whereby the temperature of the digester is maintained

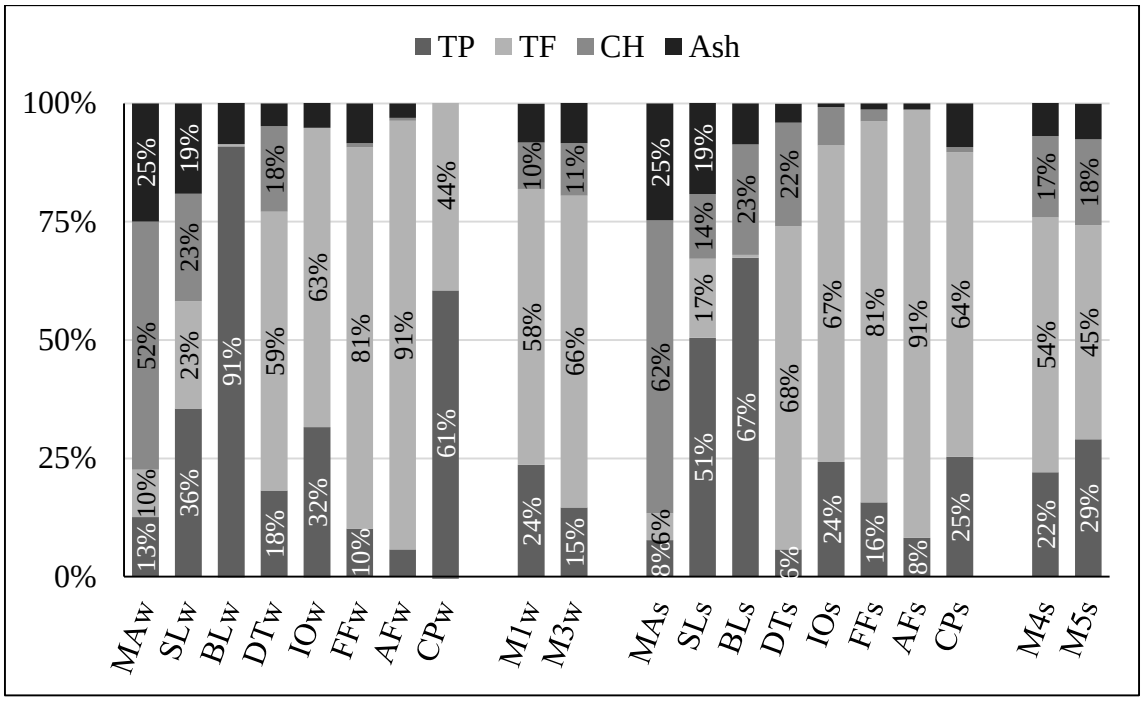
without an additional energy supply as energy applied in sterilisation would be sufficient in maintaining a temperature of 37ºC inside the digester).

3. Results and Discussion

3.1. Typical ABP mixture

The compositional profile of individual ABP fractions and mixtures corresponding to the summer and winter sampling campaigns is shown in Figure 1. The characteristics of individual fractions and mixtures are presented in Table 1 and in Table 2, respectively. Mixtures M2w and M7s; M1w and M5s; M3w and M4s are equivalent but represent different periods of the year (M1w-M3w sampled in winter and M4s-M7s sampled in summer). Based on the amount of each ABP fraction generated and registered in the slaughterhouse, average winter and summer mixtures were created, to include (M1w, M2w, M5s, M7s) or not (M3w, M4s. M6s) blood fraction. M1w and M5s or typical slaughterhouse winter and summer mixtures (without saponification pre-treatment) had the highest protein content, while M3w and M6s or sterilised mixtures without blood, presented the lowest protein content (due to the exclusion of blood) and the highest fat. According to the results, BL presence in the mixtures increased the TP content of winter and summer mixtures (+38% and +25%, respectively; Figure 1).

Figure 1. Compositional profile of pre-treated individual ABP fractions (on a dry matter basis), as well as winter mixtures with or without blood (M1w, M3w) and summer mixtures with or without blood (M5s, M4s). Note: Only those contents ≥10 % TS have been indicated in the graph.



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294 The winter mixtures presented higher TS content (37-45 vs 33-35% for winter and
295 summer, respectively; Table 2) and higher fatter profile than summer mixtures (58-66
296 vs 45-54 %TS for winter and summer, respectively). Summer mixtures contained a
297 slightly higher CBH than winter mixtures. Seasonal effects (on farm animal production
298 conditions as aeration renewal or temperature, climate conditions, etc.) have been
299 previously reported on swine performance, affecting animal growth rate, feed intake or
300 meat quality (dry matter content; water holding capacity; fatty acids profile and
301 tenderness) (Rodríguez-Sánchez et al., 2011). Also, some management practices, such
302 as storage conditions inside the slaughterhouse, explained that blood collected in
303 summer had a lower TS and higher TAN content, or that lower quantities of fatty wastes
304 were available in winter due to a variation of the total fat content of ABP fractions
305 corresponding to internal organs.
306 Regarding individual ABPs in both seasons (Table 1, Figure 1), BL, CP, and SL, were
307 identified as protein-rich materials (TP >30 %TS). Blood presented the lowest fat
308 content and consequently the highest protein index, same tendency as (Hejnfelt and

309 Angelidaki, 2009) reported, being considered as the main source of nitrogen. Also,
310 clipping parts (CP) showed a significantly seasonal variation in their fat and protein
311 content, because their generation did not follow a constant trend and varied widely
312 from pig to pig. Among all ABP identified as rich fat materials (AF, FF, IO, DT), AF
313 has the highest TS value (67 and 77 % in winter and summer, respectively), followed by
314 FF (58 and 54 % in winter and summer, respectively), IO (44 and 38 %TS in winter and
315 summer, respectively) and DT (31 and 35 %TS in winter and summer, respectively). A
316 tendency towards gradual decrease was noticed for fatty materials. These fat-rich
317 materials (>60 %TS) had minor content of proteins and ashes, much the same as
318 values reported for slaughterhouse wastes in general (Hejnfelt and Angelidaki,
319 2009)(Battimelli et al., 2009). MA and SL added the highest carbohydrate content,
320 similar to previously reported by (Hejnfelt and Angelidaki, 2009).
321 **Table 1.** Characteristics and BMP results of ABP fractions corresponding to winter and
322 summer slaughterhouse activity. Notes: summer ABP is highlighted with a grey
323 background. *Submitted to sterilisation. **Submitted to saponification and subsequent
324 sterilisation. Shown data are average values (n= 3).

Parameter	Units	MA	SL	BL*	CP*	DT*	IO*	FF**	AF**
COD	g kg-1	640.2	384.5	398.5	736.0	745.8	1176.9	1576.6	1526.3
		546.2	460.8	521.6	710.6	883.7	867.8	1293.8	1823.7
TS	% wet weight	36.0%	20.7%	19.3%	27.0%	30.7%	44.3%	57.6%	66.9%
		31.7%	22.2%	31.6%	32.6%	35.3%	38.3%	54.2%	76.7%
VS	g kg-1	322.3	182.2	183.3	257.2	292.1	435.7	575.4	621.7
		251.3	199.6	304.7	299.7	335.3	375.0	529.3	764.4
TKN	g kg-1	8.3	15	29.8	27.8	11.2	23.8	11.2	6.5
		5.1	20.0	43.9	14.2	8.3	18.1	14.7	10.3
TAN	g kg-1	1.1	3.3	1.7	1.7	2.2	1.4	1.9	0.3
		1.2	2.1	9.8	1.0	5.0	3.3	1.0	0.3
Weight	% wet weight	6.4%	21.3%	21.7%	1.6%	22.2%	0.3%	6.9%	19.7%
Ratio		5.2%	23.3%	18.4%	2.9%	28.3%	20.8%	0.8%	0.4%
BD	%COD	66.5%	55.0%	60.8%	62.2%	82.5%	87.8%	92.0%	124.2%
Yield	$NL_{CH4}\ kg_{COD}^{-1}$	230±97	195±15	213±7	221±8	334±15	315±4	393±21	419±43
Rate	$NL_{CH4}\ kg_{COD}^{-1}\ d^{-1}$	38	52	79	67	81	91	92	106
λ	d	0.00	0.98	0.00	0.00	2.10	2.05	2.99	1.93
R^2		0.997	0.998	0.994	0.999	0.999	0.999	0.999	1.000
RMSE		4.24	2.90	5.36	2.08	3.67	3.91	3.64	2.01

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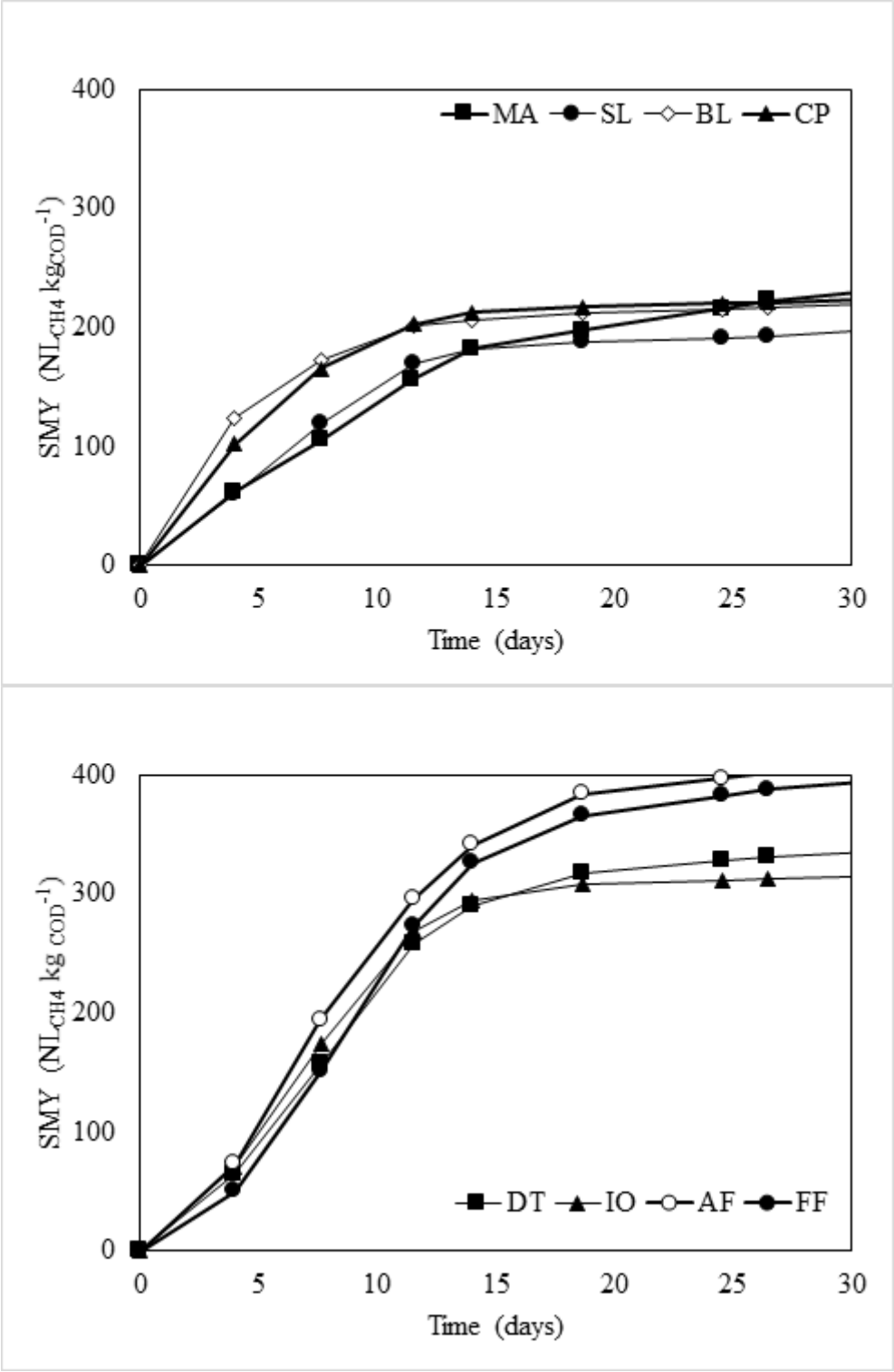
Cumulative methane production of the individual hygienised ABP fractions were determined (Table 1, Figure 2), featuring yields inside the range of previously reported yields (275-348 $NL_{CH4}\,kg_{COD}^{-1}$ (Ortner et al., 2014)), indicating appropriateness as substrates for anaerobic digestion. Mathematical adjustment of experimental data provided good fits (R^2 ranges from 0.994 to 0.999 and RMSE from to 2.0-5.4; Table 1). Consequently, Gompertz Model was reasoned as a good fit for the biogas production of the ABP fractions and mixtures considered (Strömberg et al., 2015). Regarding cumulative curves, L-shape curve shown by protein-rich fractions, as BL or CP indicates that the organic degradable matter was easily hydrolysed with an almost negligible lag phase (Figure 2a). This curve type indicates good availability and degradability. However, despite its evolution, both BL and CP reached the lowest methane yield and biodegradability (<65% COD). This is an example of medium-rapidly degradable feedstock with a relatively medium potential methane yield, taking into account that in a COD basis the theoretical maximum methane production is 0.35 $NL_{CH4}\,kg_{COD}^{-1}$ (Angelidaki and Sanders, 2004). A similar trend was observed for SL and MA though with a slightly lower rate and with slightly higher biodegradability.

342 **Figure 2.** Cumulative methane production relative to initial Chemical Oxygen Demand for

343 ABPs fractions. (a) Protein-rich fractions. (b) Fat-rich fractions.



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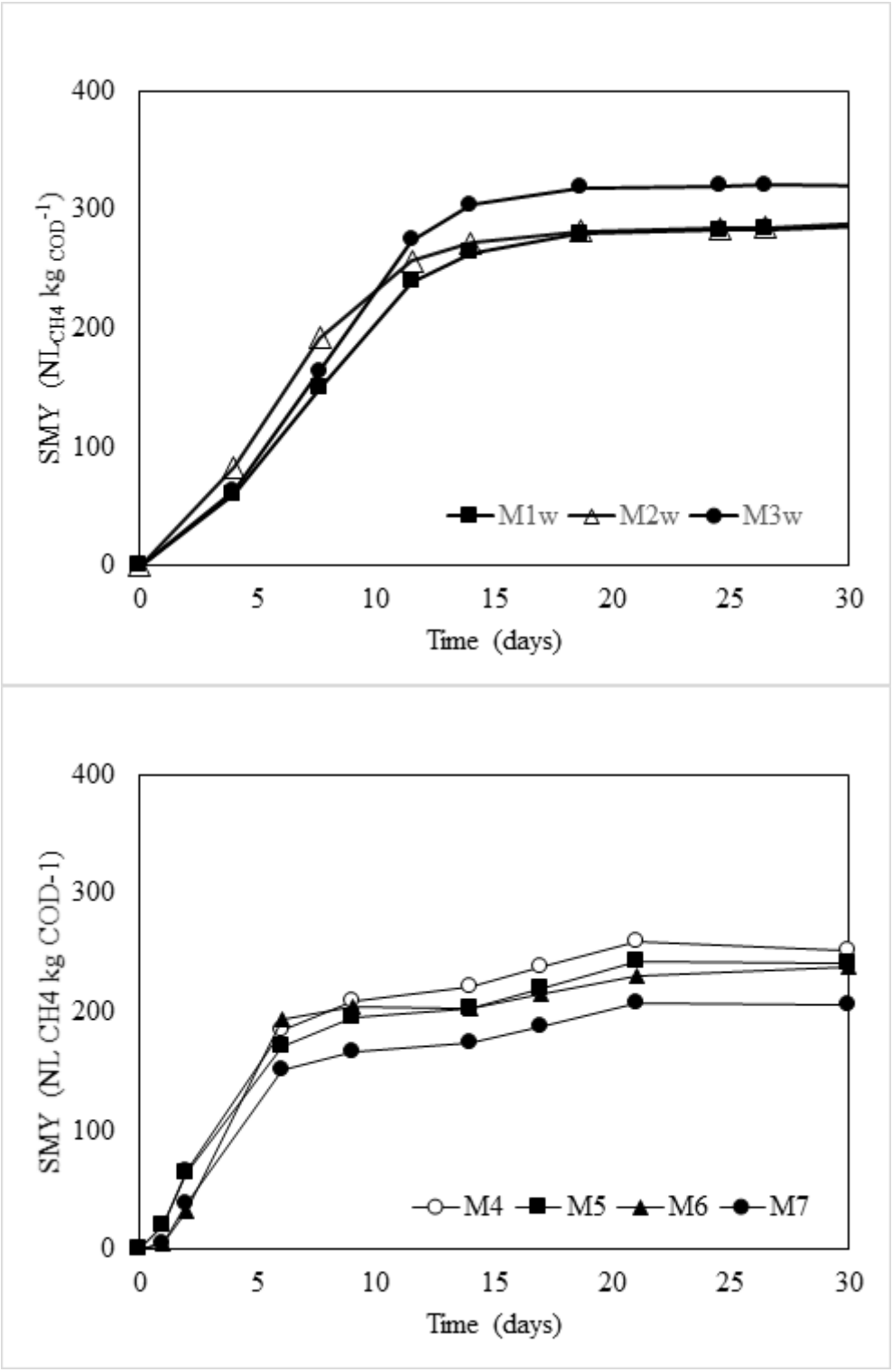
Winter mixtures (M1w-M3w) featured methane yields ranging from 288 to 320 NL_{CH4} kg_{COD}^{-1} (Figure 3, Table 2), showing a pattern more like that of fatty-ABP fractions than protein-rich (Figure 2b). The results described herein demonstrate that saponification and blood exclusion caused a decrease in lag phase as well as a slight increase in SMY and methane rate. At the end of the experiment, the total VFA content was analysed, and only acetic acid was present but in low levels (<0.1 mg L^{-1}). The attained SMY were even higher than the theoretical methane yield, estimated as the proportional sum of individual. This result could be explained by a synergic effect due to the dilution of nitrogen content caused by the mixing of all the ABP fractions (Ortner et al., 2014).

355 **Table 2.** ABP winter and summer mixtures: summary of applied pre-treatment and
356 characterization. Notes: *Saponification of only fatty fractions. Values are given as
357 means (n= 3). Letters "w" and "s" denote winter and summer sampling campaigns.
358 Abbreviations: Ster, sterilisation; Sap, saponification.

Mixture		M1w	M2w	M3w	M4s	M5s	M6s	M7s
Pre-treatment	Ster	Yes	No	Yes	Yes	Yes	No	No
	Ster & Sap*	No	Yes	No	No	No	Yes	Yes
Blood	Presence	Yes	Yes	No	No	Yes	No	Yes
Parameter	units							
COD	g kg-1	1245.7	1127.1	943.9	874.8	866.3	832.3	865.8
TS	% ww	44.9%	38.1%	36.8%	34.9%	35.1%	32.3%	32.3%
VS	g kg-1	436.7	358.3	358.3	339.0	310.4	308.7	283.9
CBH	g kg-1	167.3	148.4	311.4	96.4	72.3	66.2	45.9
TP	g kg-1	97.4	73.1	54.7	70.6	92.9	70.6	92.9
TF	g kg-1	169.9	139.9	257.8	172.2	145.0	172.1	145.1
BD	% COD	82.3%	81.9%	93.0%	69.7%	72.9%	74.6%	70.4%
SMY	$NL_{CH4}\,kg_{COD}^{-1}$	287.8	290.8	320.5	258.5	237.9	241.9	218.2
Rate	$NL_{CH4}\,kg_{COD}^{-1}\,d^{-1}$	80.3	90.4	94.7	120.6	210.9	115.6	142.2
λ	d	2.2	1.5	1.3	0.3	1.5	0.1	0.9
R2		0.998	1.000	0.993	0.992	0.989	0.984	0.983
RMSE		4.3	1.8	0.0	10.4	9.9	11.3	10.5

359

360 **Figure 3.** Cumulative methane production relative to initial Chemical Oxygen Demand of ABP
361 mixtures. (a) winter mixtures (M1w, M2w, M3w) and (b) summer mixtures (M4s, M5s, M6s
362 and M7s).



363

Fatty-ABP fractions IO, DT, FF, and AF had an initial delay between 2-3 days which could be attributed to the initial VFA and LCFA accumulation, as proposed by (Salminen et al., 2000b), but these fatty fractions reached high values of SMY, almost the theoretical maximum biogas yield of 350 $NL_{CH4}\,kg_{COD}^{-1}$ (Angelidaki and Sanders, 2004), and biodegradability (>80 %COD). For lower and medium lipid concentrations, the inhibition could be attributed mainly to LCFA rather than VFA affecting syntrophic methanogenesis (Cirne et al., 2007). (Battimelli et al., 2009) found that the initial production (day 2) could be increased by adding ethanol as a co-substrate. Despite an initial delay, their methane rate (>315 $NL_{CH4}\,kg_{COD}^{-1}$) was also higher than that of protein-rich ABP fractions. The methane production pattern, with sigmoidal-type curves, and as obtained in this work was coherent with others previously reported for lipid rich wastes (Cirne et al., 2007), such as poultry slaughterhouse waste (Salminen et al., 2000b) or fatty slaughterhouse wastes (Battimelli et al., 2009).

Regarding saponification, M2w or sterilised-saponified mixture (FF and AF were saponified, but ABP fractions percentages were the same as M1w) offered less fat content after saponification: the TF content dropped from 170 to 140 g kg^{-1} in M1w and M2w, respectively. Taking M1w as the reference mixture, M2w showed shorter lag phase (-31%), higher methane rate (+12 %) but similar SMY to M1w. (Battimelli et al., 2009) have investigated the effect of saponification, not in the mixture but in grease alone, and observed an increase in production reaching 90% of maximum SMY; meanwhile M2w yield reached 83% of the maximum potential. Therefore, the data of this work indicates that saponification enhanced the initial methane rate but not biodegradability, as concluded by (Hejnfelt and Angelidaki, 2009) , although the saponified ABP materials (FF, AF) in M2w represented a low percentage in the mixture. Consequently, to better assess the positive effect of saponification pre-

389 treatment, the winter ABP fractions BMP tests were carried out by adding to more
390 saponified fat ABP fractions: DT and IO.
391 Since TAN is a well-known inhibitor in anaerobic digestion (Chen et al., 2008), the
392 reduction of its content is expected to diminish this inhibitory effect. TAN reduction is
393 evidence in the mixtures in which the blood was not included .In M1w and M3w BMP
394 experiments where the BL content of mixtures varied from 0 to 19% wet weight (for
395 M3w and M1w-M2w, respectively), on a range of values including data recorded at the
396 slaughterhouse. The mixture without blood, M3w, presented higher SMY compared to
397 M1w and M2w (13.4 and 12,9 % higher, respectively). Since nitrogen is a well-known
398 inhibitor in anaerobic digestion, the inhibitory effect is expected to be reduced when the
399 nitrogen level in the feedstock is lower. A waste dilution of 5 % have proved to
400 improved methane yields for ABP fractions (Hejnfelt and Angelidaki, 2009). However,
401 the exclusion of blood in the mixtures increases fat concentration which can enhance the
402 negative effects of lipids in biogas production. This fact explains the longer lag phase,
403 typical for rich lipid material (Battimelli et al., 2009).

404 **3.2. Combined effects of saponified fat and nitrogen content**

405 Methane production of summer mixtures M4s, M5s, M6s, and M7s is shown in Figure
406 3b. As well as for individual ABP and for mixtures M1w- M3w, the modified Gompertz
407 model provided good fits with R^2 ranging 0.983 - 0.992 and RMSE from 9.9 - 11.3. A
408 similar methane curve pattern is observed in all cases, leading to a biodegradability >70
409 %COD (Table 2) but up to 30 % lower than mixtures M1w and M3w. This can be
410 attributed to the protein content since summer ABP fractions contained less fat content
411 and more protein than in winter (Figure 1). This could also explain the change in the
412 curve pattern of summer mixtures compared to winter mixtures, since M1w-M2w

presented a sigmoidal shape, whereas the M4s-M7w curve is more like an L-shaped curve as for rich ABP materials.

Since fat is normally attributed to inhibition of early stage degradation (Cirne et al., 2007), the lower fat content also could explain why M4s, M5s, M6s, and M7s, presented lag phases up to eight times lower than the corresponding lag-phase of M1w - M3w. As well as for M1w-M3w mixtures, the lag phase decreased in the saponified mixtures compared with the non-saponified mixtures. Anova test for lag phase as a variable response reveals that only the saponification factor is statistically significant. So, saponification improves the initial methane rate delay caused by fat rich ABP fractions.

The lowest SMY was obtained for M7w or mixture with blood and saponified fat, while the highest SMY was obtained for M4w or mixture without blood and non-saponified fat. Although saponified mixtures had lower SMY than those non-saponified mixtures (-10% and -15%), the saponification pre-treatment improved the initial methane rate regarding the corresponding non-saponified mixtures, which is confirmed by the Anova test taking lag phase as response variable (Table 3). Anova test also revealed that both factors, blood and saponification, significantly affected SMY (p-values<0.05) and this effect was higher for the saponification factor, as p-value reveals. However, for M2w mixture compared to M1w the difference was lower than for the equivalents M4s-M7s (0.5 and 15.3, respectively). This difference could be attributed to a lower lipid content in M4s-M7s than in M1w-M3w (Figure 1) and for the fact that M5s and M7s include more saponified ABP fractions (DT, IO, AF, FF) than M2w (only FF and AF). Despite the proved effect of saponification and blood content on SMY, the interaction between blood and saponification has proved not to be statistically significant.

438 **Table 3**. ANOVA test with SMY, methane rate and lag phase as response variables and

439 blood and saponification as factors.

Response	Factor	p-value
SMY	Blood	0.028
	Saponification	0.024
	bl & sap	0.199
Rate	Blood	0.042
	Saponification	0.153
	bl & sap	0.197
Lag phase	Blood	0.153
	Saponification	0.041
	bl & sap	0.457

440
441

Regarding methane rate, the highest value was achieved by the mixture M5s (210 $NL_{CH4}\,kg_{COD}^{-1}\,d^{-1}$) and the lowest value was for mixture M6s (115 $NL_{CH4}\,kg_{COD}^{-1}\,d^{-1}$). In fact, mixture M5s achieves 80 % of the total SMY in the first week of the BMP assay, whereas for the mixture M6s, this degradation level is attained within 2 weeks (day 14). Saponification pre-treatment improved lag phase of both mixture with and without blood but not the methane rate. Anova test taking methane rate as variable response, revealed that only the blood factor is statistically significant. This fact is supported by the previous BMP for M1w, M2w and M3w.

3.3. Energy balance

The energy balance was assessed to evaluate the feasibility of a full-scale biogas plant (Table 4). The energy consumption in slaughterhouse facilities is mainly associated with refrigeration and water heating, and ranges 110-760 kWh t pig-carcase^{-1} (European Commission, 2005) due to seasonal and daily energy demand fluctuations (Ortner et al., 2015). In this work, a capacity of 500,000 animal year-1 was selected as case study. For this case, taking an average energy consumption of 435 kWh t carcase^{-1}, as well as a mean carcase weight and an ABP generation ratio of 80 kg-carcase animal^{-1} and 20 kg-ABP animal^{-1}, respectively, the average energy demand of the slaughterhouse is 17,944 MWh y^{-1}.

Mixtures M1 to M7 represented different periods of year (winter and summer, respectively), with saponification and no saponification pre-treatment and blood inclusion (Table 2). Based on experimental data, TMP with these mixtures ranges from 2.68 - $3.57 \cdot 10^6$ and 1.71 – $1.97 \cdot 10^6$ $Nm^3CH_4\,y^{-1}$ in winter and in summer, respectively, which means that gross energy (Ge) is between 15,281 and 33.034 MWh y^{-1}. The energy demand of the biogas facility (Be) ranges from 1,025 to 1,230 MWh y^{-1} depending on the blood inclusion or exclusion, being equivalent to a 4 - 7 %Ge, while

467 the NEP was estimated as 23,925 – 31,804 and 14,796 – 17,010 MWh y^{-1} in winter and

468 in summer, respectively (Table 4).

469

Table 4. Energy balance from the biogas plant in which biogas is generated by the slaughterhouse mixtures studied. TMP was estimated as SMY (Table 2) multiplied by 0.83 or conversion factor for full scale performance.

Parameter	Units	M1w	M2w	M3w	M4s	M5s	M6s	M7s
Slaughterhouse capacity	animal y^{-1}				500,000			
Energy demand slaughterhouse Ed	MWh y^{-1}				17,944			
Total methane production TMP $\cdot 10^{-3}$	Nm^3_{CH4} y^{-1}	3,571.3	3,264.1	2,679.3	1,877.0	1,971.9	1,710.4	1,881.6
Gross energy production Ge	MWh y^{-1}	33,034	30,193	24,950	17,362	18,240	15,821	17,405
Energy demand of the biogas plant Be	MWh y^{-1}	1,230	1,230	1,025	1,025	1,230	1,025	1,230
Net energy production NEP	MWh y^{-1}	31,804	28,963	23,925	16,337	17,010	14,796	16,175
Energy recovery using NEP	% Ed	183	166	138	94	98	85	93

Considering comparable proportional mixtures M1w and M5s, with the only difference being the season, the demanded energy consumption of the slaughterhouse would be covered by a 183% and 98% in winter and in summer, respectively. Regarding the effect of saponification, mixtures M2w in winter and M6s or M7s in summer, the percentage of energy self-consumption would be 166% and 93 % in summer and in winter, respectively.

Including a biogas plant in the slaughterhouse has both an economic and environmentally positive impact. The fact is that reduction of fossil fuel consumption for electricity and thermal energy generation, reduces or even drives out the purchase cost as well as the related GHG emissions. Ortner et al., (2015) found that GHG emissions were reduced by 79% with a 63 % saving in annual costs at full industrial scale. Some researchers in different countries have estimated the potential of biogas production from slaughterhouse ABP and farm animal manures. Abdeshahian et al., (2016) research revealed potential energy production of 7.12 % of total energy consumption in Malaysia, whereas Ware and Power, (2016) have estimated in Ireland that the biogas produced in abattoirs could produce 1.63 % of the energy demand of the industrial sector .

From a full-scale point of view, both saponification and blood exclusion reduce the net energy production. Despite that, the saponified (M2w, M6s-M7s) and the blood exclusion mixtures (M3w, M4s, M6s) presented higher SMY than the typical winter and summer mixtures M1w, M5s, saponifying fats and blood exclusion decrease COD and blood exclusion reduce the total amount of available ABP mixture. However, the change in methane producing rate caused by the 3 factors considered in this study (blood, saponification pre-treatment and seasonal effect) has a considerable effect on the anaerobic digester unit volume and subsequently on their cost. Since the digester unit is the most expensive unit in a biogas plant (Cuadros et al., 2011), this point must be considered in the economic viability of biogas projects. Techno-economic viability of a biogas project depends not only in the projected energy savings but also on the operating and financing costs (Karellas et al., 2010).

4. Conclusions

Saponification of rich fat rich fractions and blood presence in ABP slaughterhouse mixture was studied through batch assays. Results showed high energy potential for the mixtures studied despite the fat content and ammonia loads. Rich fat ABP have been proved to have a negative impact on methane production rates, specifically in initial biodegradation rates, as was concluded for longer lag phases. Saponification of fat rich ABP fractions in the slaughterhouse mixture, slightly improved in initial degradation rates leading to a lower lag phases. On the other hand, blood, which is associated principally with high ammonia loads, negatively affected methane yield in the two sampling periods considered (winter and summer). So, when blood was not included in the ABP slaughterhouse mixtures, methane yield improved. The statistical analysis of all the mixtures studied taking saponification and blood as factors, with methane yield, methane rate and lag phase as response variables, revealed that interaction between

both factors was not statistically significant. However, the effect of blood was significant for SMY and methane rate whereas saponification was also proven to affect SMY and lag phase.

The energy balance revealed that the slaughterhouse facility can cover 85-100 % of the energy demand by generating energy from biogas through a combined heat and power plant unit. The share of energy cover depends on the season, the inclusion or exclusion of blood and the saponification of fat rich ABP fractions.

Appendix. Abbreviations

A acidification index

ABP animal by-products

AF abdominal fat

ANOVA analysis of variance

Be energy consumed by the biogas facility

BD Biodegradability

BMP biochemical methane potential

BL blood

CBH total carbohydrate

COD chemical oxygen demand

CP clipping parts

DT digestive tract fat

FF fillet fat

Ge gross energy production

IO internal organs

λ lag phase

LCV low calorific value

M methanisation index

M1w winter ABP mixture

M2w winter ABP mixture with saponified fat

M3w winter ABP mixture without blood

M5s summer ABP mixture

M6s summer ABP mixture without blood and saponified fat

M7s summer ABP mixture with saponified fat

MA pig manure

MPR methane production rate

NEP net energy production

μ maximum methane production rate

org-N organic nitrogen

TAN total ammonia nitrogen

TF total fat

TKN total Kjeldahl nitrogen

TMP total methane production

TP total protein content

TS total solids

s subindex for “summer”

RMSE root mean square error

SL sewage sludge

SMY specific methane yield

VFA volatile fatty acids

VS volatile solids

w subindex for winter

Y biomass yield

Acknowledgments

Ana Otero and Mafrica S.A. thanks the support of the Agency for Management of University and Research Grants (AGAUR) of the Catalan Government (2018 DI 074). Mafrica S.A. thanks the support by European Innovation Partnership for Agricultural productivity and Sustainability (EIP-AGRI) trough Operating Group (GO) call of the Catalan Department of Livestock Agriculture and Fisheries (DARP). IRTA thanks the financial support of CERCA Program (Generalitat de Catalunya) and INIA (Spanish Government) through the research project PIONER (ref. RTA2015-00093-00-00).

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