Evolution of chemical composition and gas emission from aged pig slurry during outdoor storage with and without prior solid separation

V. Moset[*ab], M. Cambra-López[b], F. Estellés[b], A.G. Torres[b], A. Cerisuelo[a]

[a] Animal and Technology Research Center (CITA-IVIA). Pol. Esperanza 100, 12400. Segorbe, Castellón, Spain.

[b] Institute of Animal Science and Technology. Universitat Politècnica de València (UPV), Cno. de Vera 14, 46022. Valencia, Spain.

Abstract. Chemical composition and gas emissions from two types of pig slurry were evaluated: the liquid fraction of mechanical solid-liquid separated slurry (SS), and raw slurry (RS). The slurry was obtained at the end of a pig fattening period and stored in 100 l vessels for 15 weeks simulating outdoor storage conditions. During this period, representative samples were taken and analysed for chemical composition. Methane, carbon dioxide, ammonia, water vapour and nitrous oxide emissions were recorded. The results showed a high biological degradation during the first five weeks of outdoor storage in SS and RS slurries, as a result of an increase in the dissolved chemical oxygen demand, volatile fatty acids and carbon dioxide emission observed in this period. However, methanogenic activity was not evident until week 6 of storage in both slurries, confirmed by the volatile fatty acids accumulation and the negligible methane emissions during the first five weeks of storage. The results showed that differences in the initial slurry organic matter content, influenced by solid separation process, affects the evolution pattern of the

[*] Corresponding author: Tel.: +34 96 471 21 15; Fax: +34 96 471 02 18; E-mail address: moset_ver@gva.es (V. Moset)

organic matter degradation and that the storage time can considerably affect the biodegradability of organic matter in pig slurry.

Keywords: chemical composition, gas emission, aged pig slurry, solid-separation, storage conditions.

Nomenclature

ADF	Acid detergent fibre, g kg^{-1} [FM]
ADL	Acid detergent lignin, g kg^{-1} [FM]
C_{in}	Gas concentration in the input, mg m^{-3}
COD_d	Dissolved chemical oxygen demand, g l^{-1}
C_{out}	Gas concentration in the output, mg m^{-3}
E	Gas emission rates, mg h^{-1}
F	Airflow rate m^3 h^{-1}
FM	Fresh matter
NDF	Neutral detergent fibre, g kg^{-1} [FM]
OM	Organic matter
RS	Raw slurry
SS	Separated slurry after solid separation
TKN_d	Dissolved Kjeldhal nitrogen, g kg^{-1} [FM]
TKN_t	Total Kjeldhal nitrogen, g kg^{-1} [FM]
TS	Total solids, g kg^{-1} [FM]
VFA	Volatile fatty acids, g l^{-1}
VS	Volatile solids, g kg^{-1} [FM]

1. Introduction

The anaerobic degradation of organic matter (OM) takes place during the storage of animal slurries like in any anoxic and rich in OM environment such as rice paddies, the rumen or the hind gut of monogastrics. This is a complex process in which different groups of bacteria interact to convert OM into carbon dioxide (CO_2) and methane (CH_4). Primarily, hydrolytic enzymes from the fermentative bacteria convert complex polymeric biomass (polysaccharides, proteins, lipids, etc.) into their respective monomeric constituents (sugars, amino acids, fatty acids, etc.). The acidogenic fermentative bacteria transform these monomers into H_2, CO_2 and volatile fatty acids (VFA). The VFA are then converted by the acetogenic bacteria into acetic acid, which is the main product utilised by the methanogenic bacteria, the last group of bacteria which is established in the anaerobic degradation process (Angelidaki et al. 1999).

During animal slurry storage, all of these bacterial groups coexist in equilibrium with other groups responsible for processes such as aerobic degradation of OM (Moller et al., 2004), nitrogen nitrification, denitrification and urea mineralisation (Cortus et al., 2008). This high bacterial activity results in the emission of gases related with climate change and detrimental environmental effects such as ammonia (NH_3) and greenhouse gases (CO_2, CH_4 and nitrous oxide, N_2O). Besides gas emissions, bacterial fermentation processes can also lead to a reduction not only in the fertiliser value of manure due to nitrogen losses (Muck and Steenhuis, 1982), but also as energy value to produce biogas due to fermentable OM losses (Moller et al., 2004).

Storage conditions, slurry composition and age are key influencing factors in the performance of these bacteria. Storage conditions affect the anaerobiosis degree of the

slurry, limiting the establishment of anaerobic versus aerobic bacteria. Furthermore, slurry composition affects the establishment of bacteria in the slurry not only because some components, as nitrogen and biodegradable carbon are sources of energy for them, but also because, as stated Fangueiro et al. (2008), the higher contents of OM, especially solids with low density such as fibres, could facilitate more anaerobic conditions and thus a better development and establishment of anaerobic bacteria. Therefore, treatments such as solid-liquid separation where high contents of fibres are separated from liquid to solid phases could have a relevant effect on anaerobic conditions and thus on CH_4 emissions.

Slurry composition depends not only on well known factors such as diet or slurry management (Cahn et al., 1997, Béline et al., 1999, Panetta el al., 2006) but also on its age. The OM in slurry is formed by degradable and non-degradable volatile solids, during storage, the degradation of the most degradable OM by bacterial activity causes an increases in fibrous content in the slurry (Sommer et al. 2004), since this fraction is unaffected by bacterial activity. In addition, during the degradation of slurry there is an accumulation of compounds as metabolic products of the fermentative bacteria (such as VFA) and mineralisation products of nitrogen as NH_3 and N_2O (Béline et al., 1998). Consequently, gas emissions derived from aged slurry are expected to differ over time from these obtained from fresh slurry, thereby affecting its subsequent management.

Monitoring gas emissions and slurry composition during storage might help elucidate the variation of bacterial activity with time. Methane emission is produced only by anaerobic bacteria and NH_3 is produced in the mineralisation of organic nitrogen. However, CO_2 is produced by anaerobic and aerobic bacteria and is also related with urea mineralisation.

There are several works reported in the literature in which fresh slurry is monitored for gas emission and composition over time at different temperatures (Béline et al., 1997, Moller et al., 2004; Sommer et al., 2007) identifying temperature and slurry composition as the most influencing factors affecting gas emission. However, gas emissions and slurry composition in aged slurry stored over long periods in warm temperature conditions (> 20°C) have been studied to a lesser extent and this could provide useful information to develop best management practices to reduce environmental impact caused during aged slurry storage. This information is particularly relevant in Mediterranean counties, such as Spain, where the management of pig slurry consists of a pre-storage below slatted floor during the fattening period (3-4 months) and a further outdoor storage occurs until the slurry is applied to agricultural land. In this context, mechanical solid separation treatment techniques are often applied to reduce the capacity of the outdoor storage lagoons and facilitate slurry transport and field application.

The aim of this study was to monitor gas emissions (CH_4, CO_2, N_2O, NH_3 and H_2O) and the chemical composition of two types of aged fattening pig slurry during 15 consecutive weeks under summer conditions, and to study the effect of initial slurry chemical composition on these parameters by applying the mechanical solid separation process.

2. Material and methods

2.1.Experimental setup

Pig slurry from a complete fattening period (19 weeks) carried out with 128 female pigs (initial weight 20.85 ± 2.80 kg), was obtained from the Animal and Technology Research Centre (CITA) in Segorbe, Castellón, Spain. The animals were fed a diet containing, on average, 2,425 kcal net energy kg^{-1}, 15.1% crude protein, 5.8% crude fat and 3.9% crude

fibre. Animals were housed in whole-slatted pens. At the end of the fattening period, the slurry under the pit was mixed in order to avoid stratification and a representative sample (2,000 l) was taken. Approximately half of the total amount of collected slurry was immediately subjected to a mechanical solid separation process via a mechanical screen separator, with a screen pore diameter of 0.5 mm, commonly used in commercial farms. This slurry was designated separated slurry (SS). The rest was not modified and remained as raw slurry (RS).

For each treatment three 100 l polyethylene vessels were filled with slurry until they reached 80% of their total capacity. A headspace of 130 mm was left between the slurry surface (0.104 m^2) and the top of each vessel. During 15 consecutive weeks in summer, vessels were stored in a roofed space. Slurry and ambient temperature were continuously registered using dataloggers (*HOBO®U12-013,* Onset Computer Corporation, MA, USA).

2.2.Chemical analyses

At the beginning of the experiment, and fortnightly, a representative sample of the slurry from each vessel was taken. The samples were collected using a device for layered liquids sampling (Eijkelkamp©, Eijkelkamp Agrisearch Equipment BV, Germany) that allows sampling the complete vertical profile of the slurry without agitation. After collection, the samples were homogenised and the pH was measured with a pH meter (Crison Basic 20+, Crison, Barcelona, Spain). After pH measurements, samples were frozen at –30ºC.

Total solids (TS), volatile solids (VS), total and dissolved Kjeldhal nitrogen (TKN_t and TKN_d), and dissolved chemical oxygen demand (COD_d) were determined according to APHA (2005). Volatile fatty acids concentration was determined by gas chromatography

following the method described by Jouany (1982) with the addition of an internal standard (4-metil valeric). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to the Van Soest procedure (Van Soest, 1991). The nitrogen and fibre content were only determined in weeks 0, 9 and 15.

2.3.Gas emissions

From the filling of the vessels and during the 15 weeks of storage, gas emissions were measured treating the vessels as dynamic chambers. The gas measurements were performed weekly (3 days per week). On each measuring day, the emissions from two vessels, one from SS treatment and one from RS treatment, were registered during 24 h. During week 11 of the study, no gas measurements were conducted due to equipment malfunctioning.

The dynamic chambers were set up by sealing the vessels containing the slurry with hermetic lids. Three holes were left in the lid for air inlet by depression (inlet holes). The air was sucked from each headspace by a pump (38 l min^{-1} and 7.5 kPa (outlet), Ilmivac, Ilmenau, Germany). Inlet and outlet holes were on opposite sides of the lid to promote air mixing in the headspace. When vessels were not being measured for gas emissions, they remained open to simulate natural conditions in outdoor storage.

Inlet and outlet concentrations of CH_4, CO_2, N_2O, water vapour (H_2O) and NH_3 were analyzed every two hours using a photoacoustic gas monitor (INNOVA1412, Air Tech Instruments, Ballerup, Denmark).

The airflow rate was measured daily in the outlet using a flow meter (Aalborg instruments and Controls INC., NY, USA) and modified if necessary to keep concentrations in the measuring range of the equipment; therefore, airflows in this study ranged from 0.30 $m^3 h^{-1}$ at the beginning and 1.03 $m^3 h^{-1}$ at the end of the measuring period.

140 *2.4.Calculations and data analyses*

141 Gas emission rates (E, mg h^{-1}) were calculated by multiplying the airflow rate times the
142 difference between the gas concentrations in the output and input holes of each vessel for
143 each measured gas, using Eq. (1).

144 $$E = F \times (C_{out} - C_{in}) \quad (1)$$

145 where: F is the airflow rate through the vessel (m^3 h^{-1}), C_{out} is the gas concentration in the
146 output (mg m^{-3}), and C_{in} is the gas concentration in the input (mg m^{-3}).

147 The evolution of slurry chemical composition and gas emission at different moments over
148 the storage period was analysed using a repeated measures analysis (PROC MIXED) of
149 SAS® (2001). The relationship between chemical parameters and pH was studied using a
150 correlation analysis (PROC CORR) of SAS®.

151 **3. Results**

152 Fig. 1 shows the evolution of the hourly environmental and slurry temperature pooled by
153 treatment. Environmental temperature showed a clear diurnal fluctuation at hourly
154 intervals. However the environmental temperature during the experiment was similar
155 among weeks, except for the final week (from week 13 to 15) in which a decrease of the
156 environmental temperature was observed. The average environmental temperature recorded
157 24.9 ± 2.90 ºC, ranged from 15.3 ºC to 30.32 ºC. As regards the slurry temperature, these
158 diurnal fluctuations were less marked than for environmental temperature, being the
159 average slurry temperature equal to 23.9 ± 1.85 ºC, ranged from 18.9 ºC and 26.2 ºC.

160 *3.1.Effect of storage time on slurry chemical composition*

161 Fig. 2 shows the evolution of TS and VS in SS and RS over the 15-week storage period.
162 Raw slurry showed a higher content of TS ($p < 0.05$) and VS ($p < 0.01$) than SS slurry at the

beginning and throughout the storage period. At the beginning of the storage period (week 1), the concentration of TS and VS were 31.3±1.93 g kg^{-1} in SS and 37.1±1.93 g kg^{-1} in RS, for TS; and 27.1±1.68 g kg^{-1} in SS and 35.1±1.68 g kg^{-1} in RS, for VS. At the end of the storage period (week 15), the differences in TS and VS between treatments increased ($p<0.001$) compared with those observed at the beginning of the storage period ($p<0.05$). Regarding TS and VS evolution over the 15-week period, both TS and VS concentration showed a marked decrease during the first three weeks of storage, being this especially relevant for VS. From this point onwards, TS and VS concentration remained constant or slightly increased, showing an increment in both slurries by the end of the study.

Table 1 shows the chemical composition of manure in terms of TKN and fibrous components on weeks 0, 9 and 15 of the study. As for TS and VS, nitrogenous compounds (TKN_t and TKN_d values) in RS were higher than those obtained for SS throughout the storage period. However, the differences between treatments were only statistically significant at the end of the storage period (week 15) and only in the case of TKN_t. Concerning TKN_t and TKN_d evolution, both of them showed a slight decrease over the storage period.

Regarding fibre content, NDF and ADF were significantly higher in RS compared to SS slurry on weeks 9 ($p <0.05$) and 15 ($p <0.001$) of the study. On week 15, the ADL content was also significantly higher ($p <0.001$) in RS than in SS. Over the storage period, NDF, ADF and ADL concentrations decreased from week 0 to week 9 and increased from week 9 to 15 of the storage period in both treatments, this increase was more pronounced in RS compared to SS.

185 Fig. 3 shows the evolution of the COD_d in the SS and RS slurries. During the first three
186 weeks of storage, the COD_d content increased in RS being the COD_d levels in week 3 and 5
187 significantly higher in RS than in SS ($p<0.05$). Thereafter COD_d decreased reaching the
188 minimum values in week 13 of storage. After week 13, there was a similar increase in
189 COD_d content in both slurries, RS and SS.
190 Fig. 4 shows the total VFA content and the individual VFA (acetic, propionic, butyric and
191 isobutyric acids) concentration during the storage period. As for the COD_d, total VFA
192 content in the slurry increased within the first three weeks of the storage period in RS and
193 until the fifth week in SS slurry. An increase in total VFA was observed on week 11 for
194 RS. The VFA content was higher in RS than in SS at the beginning of the storage period (p
195 <0.05). Acetate evolution showed a similar trend than the total VFA, also peaking in week
196 11 in RS. During the first 11 weeks of storage, acetate comprised approximately 50% of the
197 total VFA in both slurries, declining thereafter until 38% in RS and 32% in SS at the end of
198 the storage period.
199 There were no statistically significant differences between treatments in the evolution of
200 propionate until the end of the experimental period (week 15). Propionate followed the
201 same trend as total VFA during the first nine weeks in both slurries, thereafter its
202 concentration in both slurries increased, contrary to total VFA evolution, being higher in
203 RS compared to SS slurry during almost the whole storage period. At the end of the storage
204 period propionate comprised 57% in RS and 62% in SS of the total VFA. Concerning
205 butyrate, its concentration increased during the first three weeks of storage and decreased
206 thereafter reaching negligible levels. The values for butyrate obtained for RS were higher
207 than those obtained for SS during almost all the storage period. However, the concentration

208 of isobutyrate increased during the first 9 weeks (SS) and 11 weeks (RS) in the storage
209 period, and decreased thereafter.
210 Fig. 5 shows the evolution of the pH of both slurries. Contrary to total VFA, the pH of both
211 slurries decreased during the first three weeks and increased thereafter until week 15. There
212 were differences between treatments in weeks 9 and 11 of the study, being the pH in SS
213 slurry significantly higher than that of RS ($p < 0.05$) at these moments.
214 When pH was correlated with VFA it was obtained that VFA content explained 80% of the
215 variation in pH ($R^2=0.80$, $p<0.001$) and the relationship between these two variables, in the
216 range of the pH variation in this experiment, was linear and negative, indicating that the
217 higher levels of VFA the lower pH values.
218 *3.2.Effect of storage time on gas emissions*
219 The emissions of H_2O and N_2O over the storage period were similar and followed a similar
220 pattern among them (data not shown). The minimum emission rates of H_2O and N_2O were
221 recorded at the beginning of the storage period and the maximum levels were observed in
222 week 10 for both gases (H_2O: RS = 86.41 g h^{-1} m^{-2} and SS = 81.46 g h^{-1} m^{-2} and N_2O: RS =
223 1.98 mg h^{-1} m^{-2} and SS =1.59 mg h^{-1} m^{-2}). Only during week 3, were there statistical
224 significant differences between treatments ($p < 0.05$), being N_2O and H_2O emissions higher
225 in RS than in SS slurry (H_2O: RS = 49.58 g h^{-1} m^{-2} and SS = 32.64 g h^{-1} m^{-2} and N_2O: RS
226 =1.64 mg h^{-1} m^{-2} and SS = 0.94 mg h^{-1} m^{-2}).
227 Fig. 6 shows the evolution of the weekly average CO_2 and NH_3 emissions over the 15-week
228 storage period. During the first three weeks of storage, there was an increase in CO_2
229 emission in RS being CO_2 emission in weeks 2 and 3 higher ($p < 0.001$) in RS than in SS.
230 The maximum CO_2 emission rate was observed in week 10 in both slurries (RS = 11.18 g h^{-}

231 ^{1} m^{-2} and SS = 9.92 g h^{-1} m^{-2}). In week 12, CO_2 emission was again higher ($p < 0.001$) in RS
232 than in SS slurry.
233 Ammonia emission increased with time showing emission rates of 0.2-0.3 g h^{-1} m^{-2} at the
234 beginning of the storage period and approximately 0.4 g h^{-1} m^{-2} at the end. Differences in
235 NH_3 emissions between treatments were found in week 3, in which NH_3 emission was
236 higher ($p<0.05$) in RS than in SS slurry.
237 The evolution of the weekly average CH_4 emissions over the 15-week storage period is
238 shown in Fig. 7. Methane emission was very low during the first six weeks of storage in
239 both treatments, however during this period statistical significant differences ($p < 0.05$)
240 were observed, being CH_4 emission higher in RS than in SS slurry. From week 6 onwards,
241 CH_4 emission increased in both slurries. The maximum measured CH_4 emission was
242 reached before in SS slurry than in RS slurry. Maximum measured CH_4 emission was
243 reached in week 10 for SS (3.08 g h^{-1} m^{-2}) and in week 12 for RS (4.72 g h^{-1} m^{-2}).
244 Fractions of C-CH_4 emissions to total carbon emission [C-CH_4/(C-CO_2+C-CH_4)] were also
245 calculated. The C-CH_4(C-CO_2+C-CH_4) ratio during the peak of CH_4 production (week 10-
246 12) increased from 0.12 to 0.50 in SS and from 0.12 to 0.54 in RS.

247 **4. Discussion**

248 The anaerobic degradation of OM from the initial breakdown of organic polymers to the
249 production of CH_4 is a long process that comprises different stages. Our results support the
250 stages defined by Angelidaki et al. (1999), where OM is fermented by the acidogenic and
251 acetogenic bacteria leading first to the formation of intermediate VFA and finally to the
252 production of CH_4.

In our study, during the first stages of the storage period (first five weeks), there was a relative transformation of the more degradable OM into soluble OM as shown by the decrease in TS, VS, NDF, ADF and ADL concentrations and the increase in COD_d, VFA concentration and CO_2 emission during this period. Then, COD_d and VFA concentration decreased coinciding with the increase in the CH_4 production in both slurries as the final step of the anaerobic OM degradation.

Similar trends been observed in other studies when pig fresh slurry was used. Moller et al. (2004) found a similar increment of total VFA content during the first weeks of storage in pig slurry stored at 20ºC followed by an increment in CH_4 emission and a drop of VFA concentration. However, our results show further differences in the OM degradation process between the solid-separated (SS) and the non-separated (RS) slurries. The COD_d is usually used as an indicator of the degree of OM degradation, since during the first steps of the degradation process; fermentative bacteria hydrolyse and convert the suspended solids into dissolved solids to obtain a continuous food supply for their growth (Zhu et al. 2000). These dissolved solids (composed of soluble organic compounds) are represented by the COD_d content. The higher COD_d content observed in RS in week 3 in our results compared with SS, might indicate a higher hydrolytic bacteria activity at the beginning of the storage period in RS compared to SS. These results could be related to the higher OM content of RS compared to SS. In fact, the OM concentration is one of the most relevant parameters in the kinetics of its degradation (Vavilin et al., 1996; Vavilin and Angelidaki, 2004). In addition, as suggested by Fangueiro et al. (2008), the higher OM content in RS slurry, especially the higher fibre content, may have promoted better anaerobic conditions in this slurry and thus enhanced anaerobic bacteria establishment.

The hypothesis that there is a higher bacterial activity in RS during the first weeks is also supported by the higher CO_2 emission at this moment in RS compared with SS slurry. The two main sources of CO_2 emission from slurry are the microbial degradation of OM and the urea mineralisation process by the enzyme urease, which also leads to NH_3 volatilization (Cortus et al., 2008). The higher CO_2 emission rates observed in RS compared to SS in week 3 could have been related with these two processes. As stated above, this could be explained by a higher hydrolytic, acidogenic and acetogenic activity, as shown by the increase in COD_d and VFA during OM degradation during the first three weeks of storage, but also by a higher rate of organic nitrogen mineralisation and denitrification, as shown by a higher NH_3 and N_2O emission in RS at this time (week 3).

The initial VFA content in both slurries was higher compared to values reported in the literature (Moller et al., 2004) in which fresh slurry was used. However, in this study the maximum VFA, which was reached on weeks 3 (RS) and 5 (SS), was lower than that obtained in the works in which fresh slurry was used, probably due to the lower content of biodegradable OM in aged pig slurry as regards to fresh slurry. Concerning the individual VFA, at the beginning of the OM degradation process, acetate was the main VFA produced in both slurries. However, at the end of the storage period, the production of propionate was higher, especially in the RS slurry. Accumulations of propionate in slurry storage have been observed also by other authors such as Moller et al. (2004) and Nozhevnikova et al. (2000). These authors suggested that, in outdoor storage conditions, propionate is accumulated as an intermediate product because it is degraded at a lower rate than butyrate and acetate.

297 Concerning gas emission, CO_2 emissions obtained in this work were in a similar range that
298 those obtained by Dinuccio et al. (2008) for a liquid fraction and untreated pig slurry stored
299 at 25ºC (5-15 mg CO_2 h^{-1} m^{-2}). However, the NH_3 emission obtained by Dinuccio et al.
300 (2008) in the liquid fraction and in the untreated pig slurry at 25ºC was slightly higher
301 (300-700 mg NH_3 h^{-1} m^{-2}) that those obtained in this work, probably because these authors
302 used fresh pig slurry. As stated by Béline et al., (1998) a large part of the nitrogen organic
303 is mineralised during the first two weeks of storage in fresh slurries, therefore low and
304 stable NH_3 emissions over time are expected in aged slurries instead of the observed
305 increase in NH_3 emissions during the storage period obtained in this study. However, this
306 increase could be related with the increment in the pH of both slurries because as stated
307 Muck and Steenhuis (1982) and Canh et al. (1998) the pH of the slurry is one of the most
308 important factors influencing NH_3 emission.
309 N_2O emission obtained in this work was lower compared than those obtained by Amon et
310 al. (2006) using untreated pig slurry at 10ºC. However, it was similar to that obtained by
311 Dinuccio et al. (2008) at 25ºC. These authors registered negligible N_2O emission in
312 untreated slurry and in liquid phase slurry; and significant N_2O emission only in the solid
313 fraction during the first 25 days of the storage period.
314 Our results showed that CH_4 was not emitted from pig slurry until week 6 after the slurry
315 was removed from the storage pit. This delay in CH_4 emission detected in the present study
316 has been observed in other studies (Moller et al., 2004; Sommer et al., 2007). The
317 equilibrium of methanogenic bacteria is generally achieved more slowly than the
318 equilibrium of the rest of bacterial populations that inhabit the slurry (Vavilin and
319 Angelidaki 2004). Additionally, Vavilin and Angelidaki (2004) also suggested that the slow

320 growth of methanogenic bacteria may be related to the formation of specific bacterial
321 morphological aggregates or flocks. In the present study, aged slurry which could
322 presumably have already established methanogenic bacteria was used. This could have
323 accelerated the production of CH_4. However, an important delay in the production of CH_4
324 was observed, probably due to the changes in slurry conditions from the pit under slatted
325 floor and the tanks, together with the vigorous mixing of the slurry at the beginning of the
326 study to promote homogenisation. These changes could have disrupted the anaerobic
327 conditions presumably already established under slatted floor and the structure of the
328 bacterial flocks, thus delaying the onset of methanogenic activity.
329 The understanding of the CH_4 emission pattern during aged slurry storage is useful in order
330 to recommend a maximum period for outdoor storage to prevent significant losses of CH_4,
331 applicable to Mediterranean conditions where aged pig slurry is stored generally without
332 covers during long periods. From our results, the recommended time of storage in summer
333 time in order to minimise CH_4 losses from aged fattening pig slurry to the atmosphere
334 could be established between 30 to 35 days (week 4 to 5). This recommendation could be
335 applicable in those slurry management systems which consist on a pre-storage below slatted
336 floor during the whole fattening period followed by outdoor storage until its application to
337 agricultural land. This is specially the case in those areas where the use of livestock manure
338 as fertiliser is restricted to specific periods of the year (i.e. vulnerable areas under the
339 European Nitrates Directive, 91/676/EC), and therefore slurry is stored in outdoor storage
340 lagoons for long periods of time. Moreover, under the European Nitrates Directive storage
341 lagoons must have a minimum storage capacity of 3-4 months. During this time, and taking
342 into account the results obtained in the present study, major CH_4 emissions to the

343 atmosphere could be expected. According to our results, in storage periods longer than five
344 weeks, the use of gas collection systems in such storage installations to avoid CH_4 losses
345 could be recommended. Although a wide range of management systems are used for pig
346 rearing and slurry handling worldwide, our results are valuable to characterise the evolution
347 of aged slurry, representative to a large extent of outdoor storage in Mediterranean areas
348 and in those cases where pre-storage under pits is expanded throughout the whole of the
349 fattening period and is not mixed with slurries from animals that are in other physiological
350 states.
351 The results obtained in this study concerning $C\text{-}CH_4(C\text{-}CO_2+C\text{-}CH_4)$ ratio show that, under
352 our experimental conditions, during the peak of CH_4 emission, decomposition of OM was
353 dominated by methanogenic microbial community and thus, at this time, the biogas
354 produced could be used as energy source. However, the $C\text{-}CH_4/[C\text{-}CO_2+C\text{-}CH_4]$ ratio
355 during the peak of CH_4 production obtained in the present study (0.50 - 0.54) was lower
356 compared to other experiments. Sommer et al. (2007) obtained a ratio between 0.50 - 0.65
357 during the CH_4 production peak and Moller et al. (2004) obtained a ratio between 0.60 -
358 0.70. This difference could be attributable to the use of aged slurry in our study. Sommer et
359 al. (2007) and Moller et al. (2004) worked with fresh slurry, however the slurry used in this
360 work was obtained after 19 weeks of storage under the pit. The VS biodegradability in the
361 slurry after long pre-storage times is lower than that of the fresh slurry because the
362 degradable *vs.* non-degradable fraction increases with the age of the slurry (Sommer et al.
363 2004).

364 **5. Conclusions**

From our results concerning 15-week storage period in summer conditions of two types of aged fattening pig slurry: separated slurry (SS) and raw slurry (RS), we can conclude that:

- There is relevant transformation of the more degradable OM into soluble OM during the first weeks of aged fattening pig slurry storage. This transformation is more pronounced in the slurry with a higher initial OM concentration (RS) than in separated slurry (SS), indicating a higher hydrolytic, acidogenic and acetogenic activity, as well as higher rate of urea mineralisation and nitrogen denitrification rate at the beginning of the storage period in RS than in SS.
- In aged fattening pig slurry stored under Mediterranean summer conditions, the establishment of all bacterial groups involved in the anaerobic degradation process does not occur until week 5, shown in our results by the VFA accumulation and the negligible CH_4 emission during the first five weeks of storage in both treatments.
- Slurry storage time and thus, the age of the slurry can decrease the biodegradability of OM, since the non-degradable fraction of OM increases over storage time. Storage time can considerably affect the biodegradability of organic matter in pig slurry.

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453 standard error (n =3). The statistical differences between treatments are marked as
454 follows: *** p <0.001, **p <0.01 and *p <0.05
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456 Fig. 3. Evolution of the dissolved chemical oxygen demand (COD_d) of the
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Fig. 6. Emissions of CO_2, and NH_3 from separated (continuous line and▲) and raw slurry (dotted line and ×). All registrations are average from 12 observations from three vessels, error bars indicate standard error. The statistical differences between treatments are marked as follow: *** p <0.001, **p <0.01 and *p <0.05. Missing data on week 11 are due to equipment malfunction.

Fig. 7. Emissions of CH_4 from separated (continuous line and▲) and raw slurry (dotted line and ×). All registrations are average from 12 observations from three vessels, error bars indicate standard error. The statistical differences between treatments are marked as follow: *** p <0.001, **p <0.01 and *p <0.05. Missing data on week 11 are due to equip malfunction.

Table 1. Chemical manure composition from separated and raw aged fattening pig slurries in outdoor storage conditions at different storage times in high temperature conditions.

Storage time (weeks)		0	9	15
Total Kjeldhal Nitrogen g kg^{-1} [FM]	Separated Slurry	3.5	3.2	2.9
	Raw Slurry	4.1	3.6	3.5
	SEM	0.23	0.23	0.23
	Significance	ns	ns	$p<0.05$
Dissolved Kjeldhal Nitrogen g kg^{-1} [FM]	Separated Slurry	2.6	2.5	2.2
	Raw Slurry	3.1	2.8	2.6
	SEM	0.18	0.18	0.18
	Significance	ns	ns	ns
Neutral Detergent Fibre g kg^{-1} [FM]	Separated Slurry	4.23	2.22	4.12
	Raw Slurry	6.34	5.31	10.6
	SEM	0.761	0.761	0.761
	Significance	ns	$p<0.05$	$p<0.001$
Acid Detergent Fiber g kg^{-1} [FM]	Separated Slurry	1.50	0.754	1.62
	Raw Slurry	2.44	2.12	4.52
	SEM	0.314	0.314	0.314
	Significance	ns	$p<0.05$	$p<0.001$
Acid Detergent Lignin g kg^{-1} [FM]	Separated Slurry	0.60	0.32	1.76
	Raw Slurry	0.83	0.78	3.3
	SEM	0.153	0.153	0.153
	Significance	ns	ns	$p<0.001$

FM: Fresh matter
SEM: standard error (n =3)
ns: no significant differences between treatments ($p >0.05$)

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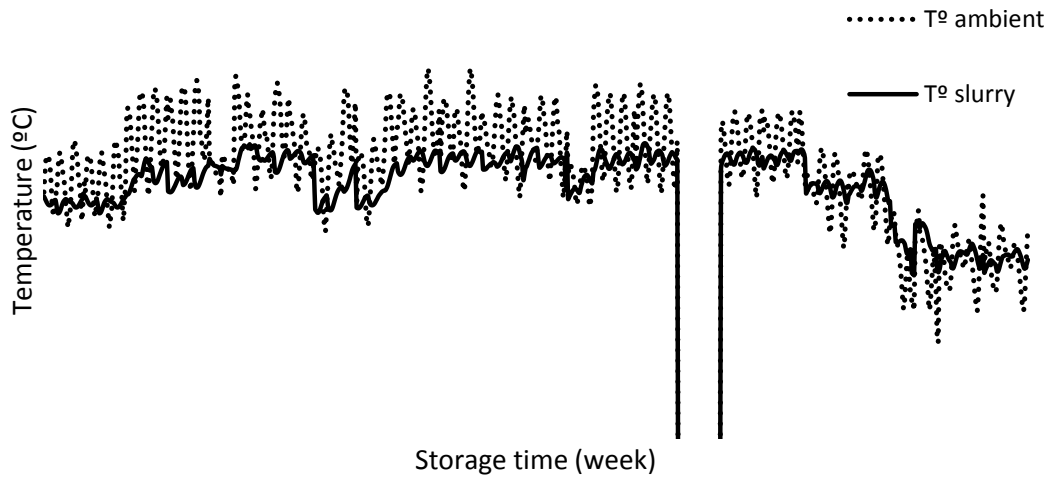

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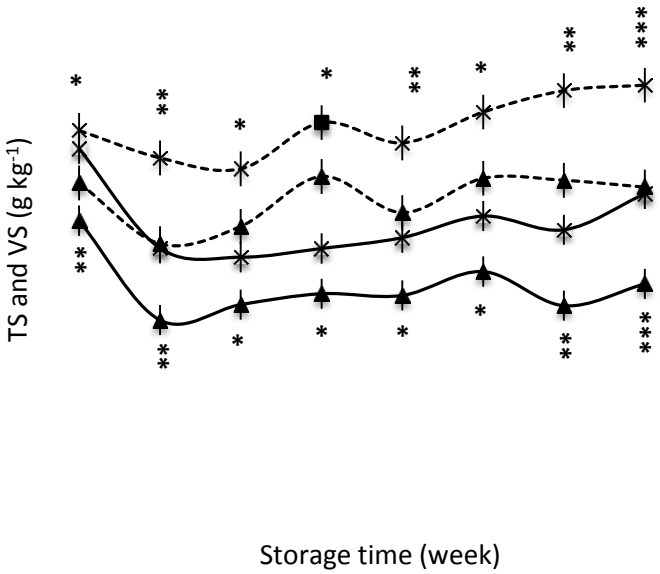

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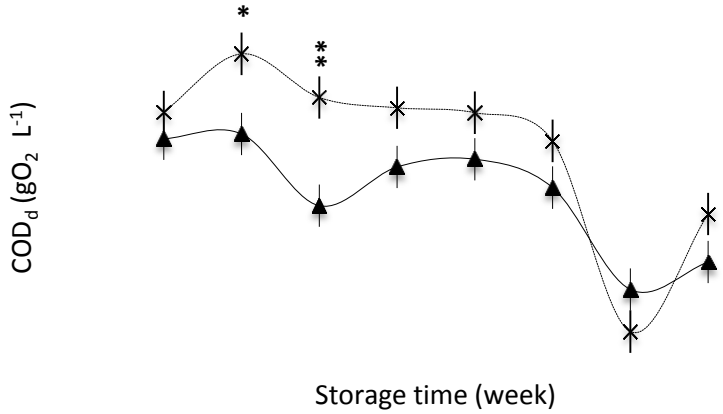

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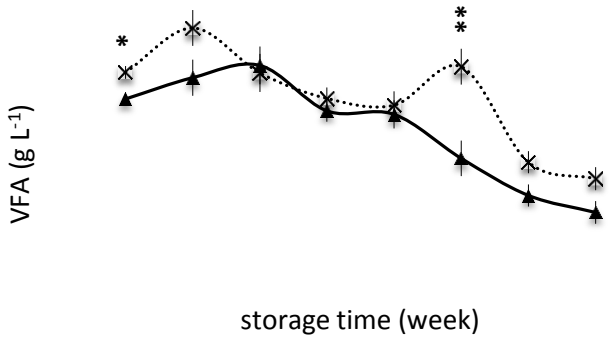

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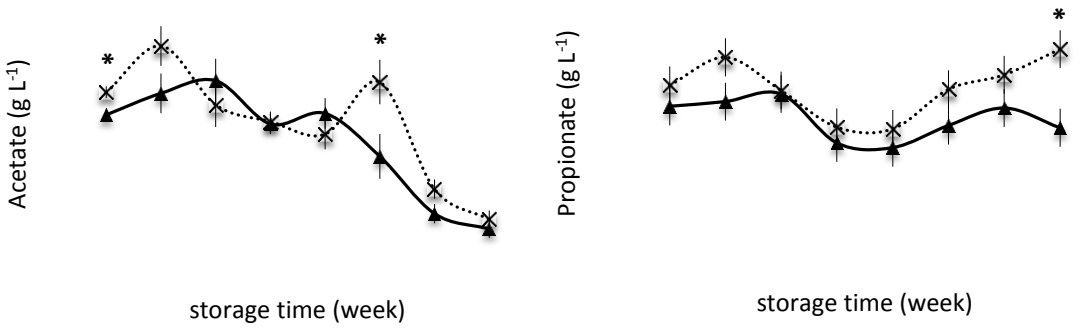

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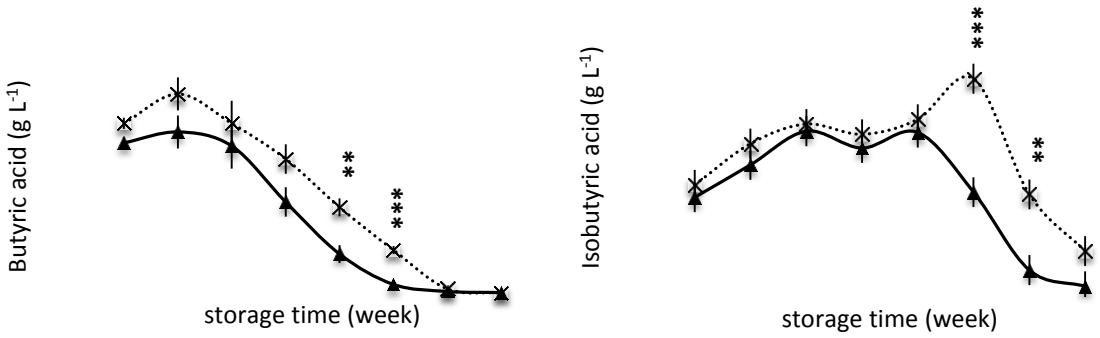

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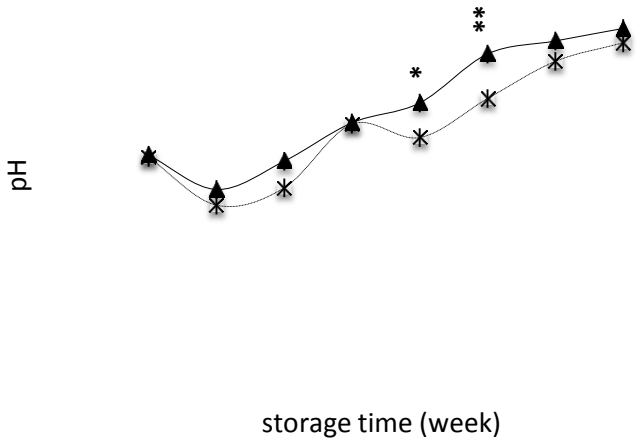

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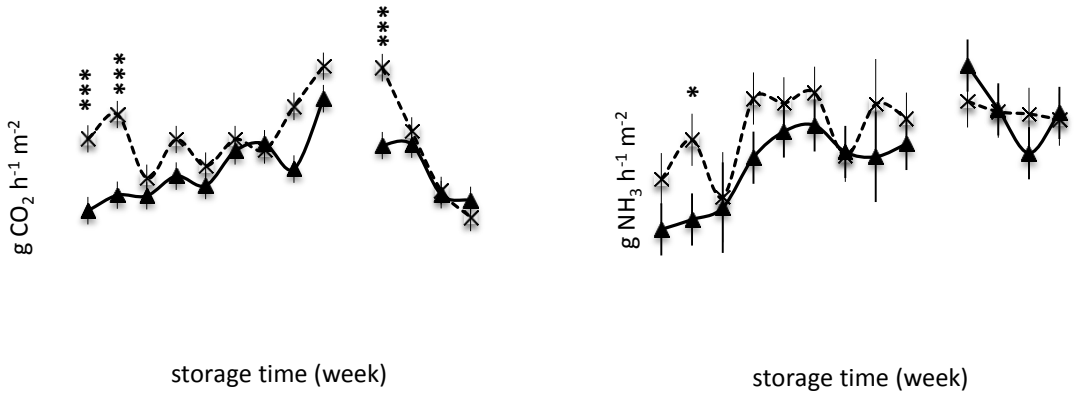

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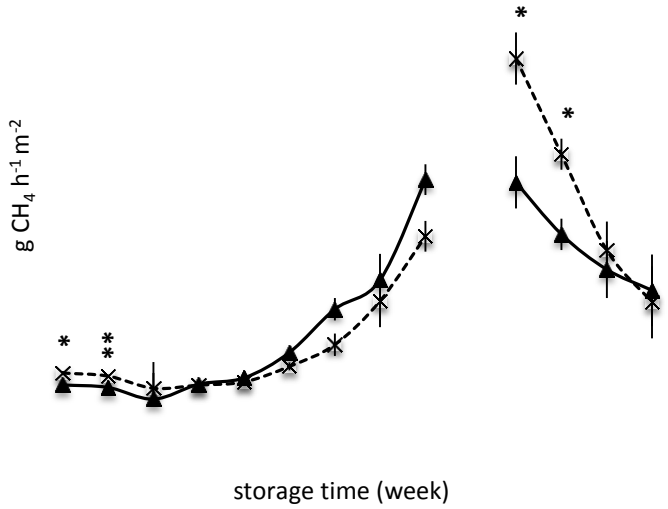

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