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1 **Cattle manure for biogas production. Does ensiling and wheat straw addition**
2 **enhance preservation of biomass and methane potential?**

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6 **Abstract**

7 The effects of ensiling and open-air storage on the conservation of cattle manure were
8 investigated for 120 days at laboratory scale. Impact of co-ensiling with wheat straw
9 was assessed as well. Up to 74% of methane potential was lost during aerobic storage.
10 Negligible energetic losses and ammonia emissions occurred during the first month of
11 anaerobic preservation. However, inadequate chemical properties of fresh cattle manure
12 hindered silage stabilization for extended periods, leading to 46% of methane losses
13 after 4 months. Co-ensiling of cattle manure with wheat straw enhanced biomass
14 acidification, inducing a conservation of more than 86% of the methane potential after 4
15 months. Wheat straw addition suppressed ammonia production and limited the risks of
16 losses through liquid effluent. The current study proves that long-term conservation of
17 cattle manure can be achieved if correct management practices are used, which will
18 undoubtedly improve the performance of biogas plants with this raw material.

19 *Keywords: Cattle manure; Storage; Ensiling; Anaerobic digestion; Methane potential*

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20 **Highlights**

- 21 • Ensiling enhanced biomass and methane conservation of cattle manure
 - 22 • Ammonia emissions during storage are minimized through ensiling
 - 23 • Co-ensiling with wheat straw stabilized biomass at elevated pH value
 - 24 • Wheat straw addition minimized risk of losses through effluent production
 - 25 • Silage with wheat straw conserved at least 86% of methane potential after 4 months
-

26 **Abbreviations**

27 AA, acetic acid; AD, anaerobic digestion; ADF, acid detergent fiber; ADL, acid
28 detergent lignin; BA, butyric acid; BMP, biochemical methane potential; CEL,
29 cellulose; HEM, hemicellulose; LA, lactic acid; LAB, lactic acid bacteria; LIG, lignin;
30 NDF, neutral detergent fiber; NH₃-N, ammonia nitrogen; TKN, total Kjeldahl nitrogen;
31 TS, total solids; VS, volatile solids; WSC, water soluble carbohydrates; VFA; volatile
32 fatty acids

33 **1. Introduction**

34 Several European countries are currently mobilizing for the energy recovery from cattle
35 manure through anaerobic digestion (AD). In France, the annual production of cattle
36 manure is estimated at nearly 87 Mt, which represents around 28.3 MWh of potential
37 primary energy [1]. Nevertheless, due to its production fluctuations and in order to
38 enable a continuous supply of biogas plants throughout the year, cattle manure needs to
39 be stored before use, in some cases for extended periods. For this feedstock, a common
40 practice is the open-air storage before AD. The reason for this is mainly related to the
41 simplicity and low cost of the operation. However, this practice has several drawbacks.
42 Generally speaking, air presence is perceived as a spoiling agent for biomass
43 conservation, leading to important energy losses during long-term storage with aerobic
44 spoilage of easily biodegradable organic fraction. Recent works with different catch

45 crops showed that more than 80% of biochemical methane potential (BMP) was lost
46 after 3 months of storage in the presence of air [2,3]. In the specific case of cattle
47 manure, this reality is further compounded by the fact that open-air storage facilities are
48 important sources of ammonia and odor emissions [4].

49 Regarding conservation methods before AD, ensiling is usually pointed out as the
50 logical choice to store wet biomass. This process is typically divided into four phases,
51 based on different biochemical transformations [5–8]. Right after filling and sealing the
52 silo, biomass respiration occurs due to the presence of oxygen trapped in the system.
53 Once oxygen has been depleted, ensiling passes to an anaerobic fermentation phase. If
54 the conditions are suitable, lactic acid bacteria (LAB) will produce lactate from
55 available carbohydrates for several weeks, decreasing the pH to around 4.0. Maintaining
56 anaerobic conditions and a relatively low pH, minimal enzymatic and microbial activity
57 occur until feed-out. After unloading the silo for AD feeding, biomass enters once again
58 into aerobic environment, which may spoil the silage. While well succeeded, ensiling
59 enhances biomass conservation and minimize energy losses. For instance, Herrmann *et*
60 *al.* [9] observed an average of 2% difference between BMP of fresh and ensiled material
61 after 1-year ensiling of maize, sorghum hybrid, forage rye and triticale.

62 Given the high efficiency of ensiling for other biomass crops, it should be expectable to
63 obtain good preservation of cattle manure using the same method. However, ensiling
64 success is extremely dependent on several biochemical characteristics of feedstock,
65 such as moisture content, water-soluble carbohydrates (WSC) content, buffering
66 capacity and endogenous microflora [6,7]. Indeed, cattle manure does not fulfill the
67 chemical requirements for an efficient ensiling, especially due its lack of WSC. Without

68 those, organic acids cannot be produced in a significant extent and then, the desired
69 level of acidity for silage stabilization would not be achieved.

70 Even if cattle manure does not naturally lead to an optimal ensiling conservation, it is
71 possible that an anaerobic environment reduces organic matter and energy losses during
72 storage. Furthermore, efficiency of cattle manure ensiling may be improved by co-
73 storage with wheat straw. In fact, this co-substrate is considered to have an impact on
74 organic losses of traditional crops ensiling [6]. This may be related to the fact that straw
75 addition decreases the moisture content and microbial concentration of the feedstock.
76 Therefore, straw may act as a delaying agent of bacterial growth, improving energy
77 conservation during storage. Likewise, wheat straw addition to cattle manure should
78 decrease soluble organic matter concentration on the feedstock, which may facilitate
79 silo compaction and prevent energy losses through leachate formation during storage.

80 However, to the best of our knowledge, no study addressing these subjects exists today.

81 This work established a comparison between open-air storage and ensiling of cattle
82 manure during 120 days at laboratory scale. The effects of different degrees of wheat
83 straw addition on silage conservation were also addressed. Storage performance was
84 evaluated, above all, by its impact on BMP conservation of raw material.

85 Notwithstanding, other parameters, such as organic matter losses or eventual gas
86 emissions, were examined during storage as well. This study contributes to the
87 optimization of practices for cattle manure management before biogas production,
88 which will definitively have an impact on the energy recovered downstream.

89 2. Material and methods

90 2.1. Substrates

91 Fresh cattle manure was collected on 10 March 2016 from an agricultural site in the
92 Rhône-Alpes region of France (Gaec Béreyziat, Les Teppes, 01340 Béréziat, France)
93 and it was stored at 4 °C before further use. Fresh raw material had 13% of total solids
94 (TS) content and it was tested in both aerobic and ensiling conditions. In addition, two
95 mixtures of fresh cattle manure and wheat straw were prepared at the laboratory and
96 tested for ensiling. Wheat straw had 10 cm maximum length and it was mixed with
97 cattle manure in order to achieve final TS contents of 19% and 22%, respectively.
98 Storage conditions and description of respective raw materials are summarized in Table
99 1. TS13%E and TS13%A represent the ensiling and open-air storage of fresh cattle
100 manure, respectively. TS19%E corresponds to the co-ensiling of cattle manure and
101 straw with final TS content of 19%. TS22%E is related to the co-ensiling of cattle
102 manure and straw with final TS content of 22%.

103 2.2. Storage approach

104 Laboratory trials were performed in 3.5 L airtight round plastic storage drums. In order
105 to enable the output of the gas produced and at the same time minimizing headspace,
106 silos were filled up to 2.55 L with raw material at packing density of 0.7 kg/L, the
107 remaining volume being filled with gravel, using a geotextile membrane to separate it
108 from biomass. Silo sealing was different depending on the storage method tested. For
109 ensiling assays, proper plastic lid and rubber ring were used and its airtightness was
110 reinforced with silicone sealant. For aerobic storage purposes no cover was used and
111 silo was left air-open. Then, silos were weighed and placed in a controlled-temperature

112 room at 25 ± 2 °C. Storage duration varied between 7, 15, 30 and 120 days. A total of 16
113 (4 operating conditions x 4 storage durations) conservation assays were done.

114 2.3. *Chemical analysis*

115 For each sample time, one silo (per tested condition) was sacrificed. It was opened and
116 weighed, biomass was homogenized and two samples were taken. One was used for
117 direct analyses on the crude material and the other one was mixed with water in order to
118 get two fractions: a water-soluble phase and a particulate phase. This leaching test was
119 performed with a 10:1 water/TS ratio during 2 h under constant bottle rotation. Phase
120 separation was achieved by centrifugation (5000 G; 10 min) followed by 0.7 µm
121 particle size filtration. Finally, the particulate phase was dried at 70 °C until constant
122 weight and ground at 2 mm theoretical length. Crude material/water-soluble and
123 particulate samples were stored at 4 °C and -20 °C, respectively, until use.

124 Crude material was analyzed for its TS content, volatile solids (VS) content and BMP.
125 For the water-soluble phase, besides TS/VS content and BMP, pH, WSC, volatile fatty
126 acids (VFA), total Kjeldahl nitrogen (TKN) and ammonia nitrogen (NH₃-N) fractions
127 were determined. Particulate solid was analyzed for its TS/VS, TKN and cell wall
128 constituents content.

129 TS was measured by oven drying at 105 °C during 24 h and VS was subsequently
130 burned for 2 h at 550 °C. Since TS/VS contents are underestimated due to the loss of
131 volatile compounds during the drying tests [10], the measures were corrected using the
132 volatilization coefficients at 100 °C suggested by Porter and Murray [11]. pH was
133 measured by a Consort C3020 device with a SP10B pH-electrode. WSC, lactic acid and
134 formic acid contents were determined with high performance liquid chromatography

135 (LC Module 1 plus, Waters) equipped with a Supelcogel™ C-610H column (300 x 7.8
136 mm, Sigma-Aldrich), both refractive index (RID) and UV detectors and operating with
137 H₃PO₄ 0.1% v as solvent (flow rate of 0.5 mL/min). WSC content was estimated as the
138 sum of glucose, xylose, galactose, mannose, arabinose and cellobiose and was
139 determined using the UV detector (210 nm). Lactic acid and formic acid contents were
140 obtained with the RID detector. Acetic, propionic, butyric, valeric and caproic acids
141 content were analyzed using gas chromatography (Shimadzu Corp.) equipped with a
142 HP-FFAP fused silica capillary column (30 m x 0.25 mm, Agilent Technologies), a
143 flame ionization detector and using H₂ as carrier gas. Total VFA was calculated as the
144 sum of lactic, formic, acetic, propionic, butyric, valeric and caproic acids. Neutral
145 detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were
146 analyzed through Van Soest and Wine [12] modified extractions method, based on XP
147 U44-162 French standard. **Hemicellulose content (HEM)** was calculated as NDF minus
148 ADF; **cellulose (CEL)** as ADF minus ADL and; lignin (LIG) as approximately equal
149 to ADL. TKN and NH₃-N were determined through the procedure described in the NF
150 EN 25663 French standard.

151 The interest of our experimental procedure is to enable the assessment of the
152 composition and BMP based on the initial mass of product used, since the weight loss
153 was measured. The results for the chemical analysis will thus be presented in two ways:
154 based on % VS_{added} or % VS_{original}. VS_{added} relates to the organic matter of the sample
155 analyzed. The results based on VS_{original} take into account the loss of volatile solids
156 during storage and allows the study of the results based on the VS of the initial material.

157 2.4. *Biochemical methane potential tests*

158 Batch anaerobic digestion tests were performed for crude material and water-soluble
159 phase samples. Tests were conducted in a temperate room at 35 °C using glass vessels
160 of 2 L for crude material and 0.1 L for water-soluble phase. Vessels were filled with 5 g
161 VS of sample, inoculum in way to keep a substrate/inoculum VS ratio of 0.5 and a
162 certain volume of a mineral solution to achieve 60% of the total volume of the vessel.
163 The inoculum used (TS 3.0-3.2% wt; VS 2.0-2.2% wt) was a digested sludge originating
164 from the wastewater treatment plant of La Feyssine, Lyon, France. The mineral
165 solution, which contains essential elements to microbial growth and also gives the
166 solution a buffer able to control any pH adjustments, was prepared according to the
167 recommendations of ISO 11734:1995 standard. Once filled, reactors were purged with a
168 N₂/CO₂ mixture (80/20% v) for about 5 minutes, sealed and equilibrated at 35 °C.
169 Blanks with only inoculum and mineral solution were performed for each batch series in
170 order to correct the BMP from residual methane production of the inoculum. All tests
171 were performed in triplicates.

172 Biogas production was determined by pressure measurement using a Digitron precision
173 manometer. Biogas was released when the pressure exceeded 1200 hPa. Gas
174 composition was analyzed using an Agilent 3000 micro gas chromatography with
175 thermal conductivity detector (GC-TCD). Molsieve 5A (14 m length; pore size: 5 Å)
176 and PoraPlot A (10 m length; 0.320 mm ID) columns were used as stationary phases for
177 GC-TCD, with Argon and Helium as carrier gases, respectively. Biogas production and
178 composition were analyzed at least 7 times during the incubation and BMP was
179 considered achieved when daily vessel overpressure of controls equalized the sample
180 ones. The BMP tests followed the recommendations provided by Holliger *et al.* [13].

181 In order to assess the kinetics of methane production, the experimental data of BMP
182 assays was fitted to the following equation:

$$183 \quad V_{CH_4}(t) = V_{max}(1 - e^{-kt})$$

184 Where, V_{CH_4} corresponds to the volume of methane produced; t is the time of the assay;
185 V_{max} is the maximum volume of methane produced, which was equivalent to the
186 experimental BMP value and; k is the rate coefficient of CH_4 production. Since gas
187 production started almost immediately after reactors were sealed, lag time was not
188 considered in the calculations.

189 **3. Results and discussion**

190 *3.1. Feedstock characterization*

191 Chemical characteristics of raw materials and stored biomass are presented in Table 2.
192 Besides low TS content (12.8%), fresh cattle manure was mainly characterized by a
193 negligible content of WSC and pH value of 7.9. These were unsuitable features for
194 energy conservation through an acidification-based process as ensiling. First of all, as
195 lower TS crops are more unstable and susceptible to deterioration, lower silage pH is
196 required for an efficient ensiling [6]. By analogy with the data published by Kalač [14]
197 for ensiled forage, a critical pH value of around 4.0 is required to preserve fresh cattle
198 manure. In order to achieve such degree of acidification, extensive production of lactic
199 acid should occur in the first days of ensiling [6]. However this would require an
200 adequate level of readily available carbohydrates, *i.e.* high WSC content. In fresh cattle
201 manure, energy sources were mainly concentrated in the form of lignocellulosic
202 compounds. Indeed, cellulose and hemicellulose contents of fresh cattle manure were
203 estimated as 26.8 and 32.5% of organic matter, respectively. Nevertheless, it should not

204 be expected a prompt and wide VFA production from these polymers. Their
205 biochemical accessibility is rather limited by lignin protection (6.1% VS) [15] and
206 depolymerization/hydrolysis reactions should take several weeks to occur in substantial
207 extent.

208 Structure of cell wall constituents changed with wheat straw addition to cattle manure.
209 This was, in fact, the only remarkable effect of wheat straw beyond the increase of
210 TS/VS content. Cellulose content increased to 36.0-37.2% VS after wheat straw
211 addition. In turn, hemicellulose content decreased to 27.5-28.7% VS. No noticeable
212 effect on lignin content was recorded with wheat straw addition.

213 3.2. *Effects of storage method on biomass preservation*

214 3.2.1. Chemical characteristics

215 Aerobic storage was mainly characterized by a pH increase and modifications on
216 organic matter structure, Table 2. Structural carbohydrates were uninterruptedly
217 degraded along the 4 months, culminating in both cellulose and hemicellulose losses of
218 around 60% of their initial content. Furthermore, no accumulation of WSC nor VFA
219 was recorded for this condition. This suggests that the degradation of structural
220 carbohydrates led to severe VS and energy losses. Lignin content did not vary
221 significantly, meaning that no (or limited) delignification occurred during aerobic
222 storage. In addition to these chemical modifications, fungi development was noticeable
223 throughout open-air storage. Therefore, microbial degradation must have occurred
224 through an aerobic metabolism producing carbon dioxide and microbial biomass.

225 Storage in anaerobic conditions of fresh cattle manure led to VFA production and pH
226 decrease until 30 days. Nevertheless, the acidification was slow since only structural
227 carbohydrates were available for fermentation. This should explain the significant

228 reduction of (hemi-) cellulose content during the first month of ensiling. The major
229 components of VFA were acetic and butyric acid, with other small fractions of
230 propionic, valeric and caproic acid. No formation of lactic acid was detected during
231 ensiling. Since the fermentation products of TS13%E are weaker acids than lactic acid,
232 pH drop for stored cattle manure was not as marked as in lactate silages. In addition,
233 between 14 days and 30 days of ensiling, VFA content increased from 6.0% to
234 12.9% VS_{added} but pH value remained stable at 6.9. This evidenced that fresh cattle
235 manure had a strong buffer capacity at neutral pH conditions. Altogether, efficient pH
236 values around 4.0 were not achieved for ensiling of fresh cattle manure and further
237 biomass degradation occurred for prolonged storage periods. Indeed, between 30 and
238 120 days, fermentation profile reversed and more than 90% of VFA were consumed
239 until the end of storage. This caused an increase of pH, which reached a value of 8.4 at
240 120 days. The fact that this pH value were superior to the one of the raw material can be
241 explained by the ammonia production and accumulation during ensiling. Moreover,
242 structural carbohydrates continued to be degraded until the end of the storage. After 4
243 months, 48% and 37% of original hemicellulose and cellulose were biodegraded,
244 respectively. Thus, non-negligible organic matter and energy losses must have occurred
245 for long-term ensiling of fresh cattle manure.

246 Equivalent fermentation profiles were found for TS13%E and co-ensiling conditions in
247 the first month of storage. However, the rate of acidogenesis decreased with wheat
248 straw addition. This may be explained by the fact that wheat straw mostly contains
249 hardly accessible carbohydrates. Moreover, wheat straw addition has decreased the
250 initial microbial concentration and moisture content, which delayed bacterial growth.
251 For instance, it is well known that at least part of silage bacteria have lower activity for

252 higher TS contents [6,16,17]. Nevertheless, wheat straw addition enhanced stability of
253 cattle manure silages. For both TS19%E and TS22%E, pH value decreased from 8.2-8.3
254 before storage to around 5.5-5.6 after 30 days and remained constant until 4 months of
255 ensiling. Additionally, even if VFA production was more restricted for co-ensiling,
256 fermentation had a much higher impact on silage acidification than in TS13%E. This
257 suggests that wheat straw addition reduced the buffer capacity of the raw material at
258 neutral pH value. Despite the apparent stability of wheat straw silages, degradation of
259 structural carbohydrates occurred until the end of the storage. After 4 months, 28% and
260 29% of the sum of original (hemi-) cellulosic compounds were missing for TS19%E
261 and TS22%E, respectively. However, this should not have led to substantial energy
262 losses, since VFA accumulation occurred until the end of storage for both co-silages.

263 The data presented here evidenced that the recommended pH value of around 4.10-4.35
264 for TS content between 15-25% [14] was not applicable for the conservation of cattle
265 manure, or at least for its co-ensiling with wheat straw. It must be remembered that this
266 value of pH is the required acidity to prevent lactate degradation. In our experiments, no
267 lactate was produced during ensiling. This means that either lactic acid was quickly
268 consumed as an intermediate for acidogenesis, or that monosaccharides were degraded
269 by other pathways than LAB fermentation. In any case, the stability of cattle manure did
270 not relied on lactic acid formation but on the accumulation of VFA during storage.

271 Therefore, the critical pH in cattle manure silages should be considered as the required
272 acidity to prevent methanogenic activity and further VFA damage for long-term storage,
273 which was achieved for both TS19%E and TS22%E conditions.

274

275 3.2.2. TKN balance

276 Effects of storage method on total Kjeldahl nitrogen balance are presented in Figure 1.

277 This evolution is related to the TKN content of feedstocks, which was 4.0% VS for fresh
278 cattle manure, 2.7% VS for TS19%E and 2.3% VS for TS22%E.

279 Regarding open-air storage, there was a continuous elimination of TKN, resulting in
280 losses of 58.6% of its original content after 120 days. Mechanisms of degradation can
281 be suggested through analysis of TKN composition along storage. First, there was a
282 considerable decrease of particulate TKN content, which ranged from 52% to 39% of
283 original TKN during the 4 months of aerobic treatment. Major part of particulate loss
284 occurred during the first 7 days of storage, during which the content of the water-
285 soluble phase of organic nitrogen increased. This indicates that some proteins were
286 subject to hydrolysis under aerobic conditions, therefore increasing the amount of
287 organic nitrogen compounds that can be processed by microorganisms capable of
288 nitrogen mineralization and nitrate/nitrite production. However this may only partially
289 explain the original source of TKN losses, due to the limited degradation extent of
290 organic nitrogen in the particulate phase during storage. In fact, the majority of
291 structural modifications of TKN during aerobic treatment occurred in the water-soluble
292 phase. From 7 days of storage and until the end of conservation, the content of organic
293 nitrogen in the liquid fraction decreased, which suggests that the rate of amino acid
294 degradation was significant during aerobic treatment. Even if the previous mechanisms
295 may lead to inorganic nitrogen production, this was not observed during storage.
296 Indeed, ammonia concentration decreased all along storage duration, from
297 1.30% VS_{original} before storage to 0.02% VS_{original} after 120 days of aerobic treatment.
298 This may be explained by the constant perturbations in the $\text{NH}_4^+/\text{NH}_3$ equilibrium (pK_a

299 = 9.25) during conservation. In open-air storage, NH₃ emissions did not have any
300 impact on its concentration in the gas phase, since ammonia produced was instantly
301 diluted in the atmosphere. Thus, in such conditions, nitrogen was considered to be lost
302 through NH₃ production, even at relatively low pH. The rate of ammonia loss improved
303 with time, as pH increased for long periods of aerobic treatment. Furthermore,
304 nitrification reactions may have contributed to the elimination of organic nitrogen in
305 aerobic conditions. This would lead to nitrate and nitrite emissions, which are not
306 measured in the TKN procedure. Considering that all TKN losses led to ammonia
307 emissions, around 40.1 L_{NH₃}/kgVS_{original} were expected to be released into the
308 atmosphere during the 4 months of open-air storage of fresh cattle manure.

309 Ammonia emissions were reduced through ensiling of fresh cattle manure. Indeed, in
310 the first 30 days of ensiling there was a complete preservation of TKN content and at
311 the end of storage only 12% of its original content was lost. This latter value
312 corresponded to a theoretical value of 9.0 L_{NH₃}/kgVS_{original} for gas emissions during the
313 4 months of ensiling. Nevertheless, there was a larger transformation of TKN structure
314 for ensiling than for open-air storage. On the one hand, the organic nitrogen content in
315 both particulate and water-soluble phase decreased almost linearly with time, from a
316 total 68%TKN_{original} before storage to 35%TKN_{original} after 4 months. On the other hand,
317 ammonia content clearly increased along ensiling. In such conditions, TKN degradation
318 must have occurred through two successive reaction mechanisms: hydrolysis of proteins
319 into amino acids and subsequent fermentation in ammonia. These are typical reactional
320 mechanisms that arise during anaerobic fermentation [18] and poor silages (high pH
321 values) [6]. Besides NH₄⁺, these reactions generally lead to other side-products, such as
322 a pool of organic acids, CO₂ and H₂, generating energy losses, regardless of how small

323 they may be. Even if there is a high production of ammonia during ensiling, gas
324 emissions should be avoided as long as the silo is airtight and a low pH medium is
325 present. This explains why degraded nitrogen was stored under the form of NH_4^+ in the
326 first month of ensiling. However, during the last 3 months of storage, pH increased to
327 values near the pK_a of $\text{NH}_4^+/\text{NH}_3$, which led to TKN losses through ammonia
328 production.

329 Co-ensiling of cattle manure with wheat straw did not have a significant impact on the
330 reactional mechanisms related to TKN. However, lower degradation rates of TKN were
331 obtained for TS19%E and TS22%E. In fact, organic nitrogen content (from both
332 particulate and water-soluble phases) was around 70% TKN for all three feedstocks.
333 This amount decreased to 34.8% $\text{TKN}_{\text{original}}$ for TS13%E, 41.4% $\text{TKN}_{\text{original}}$ for TS19%E
334 and 51.9% $\text{TKN}_{\text{original}}$ for TS22%E at the end of storage. These results may be in part
335 linked to the effect of lower growth of proteolytic clostridia for higher TS contents,
336 which has been reported in the literature for other types of silages [6,19,20]. Nitrogen
337 losses were even lower than in TS13%E condition, reaching at the most 2.3% $\text{TKN}_{\text{original}}$
338 during the co-ensiling. This corresponded to theoretical ammonia emissions of 1.2
339 $\text{L}_{\text{NH}_3}/\text{kgVS}_{\text{original}}$ at the end of the storage. Enhanced conservation of nitrogen was
340 related to the stable low pH value obtained during the 4 months for silages with wheat
341 straw.

342 3.2.3. Organic matter losses

343 Higher damage of organic matter was found for the aerobic treatment, as shown in
344 Figure 2. After one month, around 25% of organic matter of open-air stored cattle
345 manure was lost, which ascended to more than 50% after 4 months. This was linked to
346 the constant loss of structural polymers during storage.

347 Silage quality had an important effect on VS conservation during storage. Anaerobic
348 storage of fresh cattle manure limited the organic losses to less than 10% in the first
349 month, the period in which there was an accumulation of VFA. Nevertheless, silage
350 instability of low TS crop aforementioned led to damage of almost 40% of original VS
351 content at the end of the 4 months.

352 Co-ensiling with wheat straw had a positive effect on organic matter conservation. For
353 TS19%E and TS22%E, most part of the VS losses occurred in the first month and
354 corresponded to 5% of original VS. During this period, pH kept decreasing. Therefore,
355 other bacteria populations capable of VS degradation, such as methanogens, must have
356 proliferated under such conditions. Furthermore, fermentation of monosaccharides may
357 lead to several side-products. For instance, reactional mechanisms of acetate or butyrate
358 production include CO₂ and H₂ formation. Besides organic matter damaging, this would
359 cause slight energy losses, as hydrogen would be lost into the environment during silo
360 opening. In addition, nitrogen mineralization contributed to VS losses, although to a
361 minor extent. During the last 3 months of storage, organic matter suffered minimal
362 damage, due to silage stabilization. After 4 months, 92% of original VS was conserved
363 for both co-storage conditions. These are remarkable results regarding silage
364 conservation, since they are equivalent [3,9] or even better [2,21,22] than data found in
365 the literature concerning good quality of crop silage. All things considered, there was a
366 positive effect on the conservation of the biomass inherent to the wheat straw addition.
367 This is in agreement with McDonald et al. [6], who considered straw as an additive for
368 ensiling, due in part to its effect on the reduction of organic matter losses.

369

370 3.3. *Effects of storage method on methane potential*

371 3.3.1. BMP evolution

372 Evolution of methane potential along storage is presented in Figure 3. The results
373 exposed are related to both VS_{original} and VS_{added} , which allows the monitoring of
374 original BMP conservation and eventual modifications on the chemical accessibility of
375 the remaining biomass. BMP of aerobic treatment (TS13%A) ranged from 288 L/kgVS
376 before storage to 157 L/kg VS_{added} and 74 L/kg VS_{original} after 4 months. BMP losses were
377 divided in two distinct chronologic phases. Around half of energetic damage occurred
378 during the first month of storage. This was expected since in first moments of long-term
379 storage, besides the particulate phase, the major part of soluble organic matter should be
380 within reach of aerobic microorganism. In this period, losses followed a linear tendency,
381 equivalent to 1.2% original BMP loss per day. Methane potential losses slowed down
382 somewhere between the second and fourth month and led to 74% of BMP lost at the end
383 of aerobic storage.

384 Energy conservation was improved with ensiling of fresh cattle manure (TS%13E),
385 since constantly higher BMP values during storage were obtained rather than when
386 aerobic assays were carried out. As in the preceding condition, two chronologic phases
387 of BMP evolution were observed for TS13%E. A slight BMP increase was found during
388 the first weeks of storage for low TS silage. Indeed, after 1 month of storage original
389 methane potential was improved by 11%, which is substantially higher than the limit of
390 accuracy of BMP tests. Enhanced biochemical accessibility of feedstock must have been
391 the source of this energetic increment, as BMP related to VS_{added} varied from 288 to 348
392 L/kg VS_{added} during the first month. As previously discussed, during this period,
393 production and accumulation of VFA took place through degradation of (hemi-)

394 cellulosic compounds. These latter polymers are not fully biodegradable in such soft
395 condition as that experienced within the mesophilic AD process [23]. Therefore, their
396 hydrolysis/acidogenesis during ensiling is a reasonable explanation for the BMP
397 increase. This trend did not last until the end of storage, since in the last 3 months BMP
398 of fresh ensiled cattle manure abruptly decreased, either considering organic matter
399 losses or not. Consequently, TS13%E lost 46% of its original methane potential after
400 the end of the 4 months of storage. One can assume that this energetic loss was linked to
401 the consumption of VFA and structural carbohydrates during this period of storage.

402 In order to evaluate the tradeoff between higher energy conservation rates during
403 ensiling and cost increase by silo construction, a financial estimation was performed.
404 For silo construction, a medium practiced cost in France of 40 €/m³ (57 €/ton for a
405 packing density of 0.7 kg/m³) was used. Likewise, a reference value in France of 80
406 €/MWh was applied to the purchase price of additional bio-methane produced. Taking
407 into account the energy conservation rates of TS13%E and TS13%A, methane potential
408 gain through ensiling should be of 172 L/kgVS after a 4-month storage batch. This
409 corresponds to around 6.3 €/ton of additional revenue by ensiling batch. Therefore, the
410 investment made in silo construction should be recovered after 3 years (considering 3
411 storage batches per year). It is important to notice that the real recovery period should be
412 slightly higher, since neither depreciation, nor interest nor other operational costs
413 (compaction, silo covering, biogas treatment, etc.) were considered in this financial
414 estimation.

415 Wheat straw addition had a positive effect on BMP conservation of cattle manure
416 during ensiling in the long term. In fact, minor variations were recorded on the methane
417 potential along the 4 months of storage for both conditions using straw: TS19%E

418 showed BMP values of 277-304 L/kgVS_{added} and 271-287 L/kgVS_{original}; while for
419 TS22%E it were observed 285-321 L/kgVS_{added} and 265-305 L/kgVS_{original}. Although
420 less clear than in previous storage conditions, BMP evolution during storage of both
421 TS19%E and TS22%E can be separated in two periods of time. After one month of
422 storage, BMP related to VS_{added} increased by about 10% and 4% for TS19%E and
423 TS22%E conditions, respectively. This reflects a slight enhancement of biochemical
424 accessibility of raw materials and it should be interrelated with the production and
425 accumulation of VFA in silage from hardly biodegradable carbohydrates during this
426 period. Nevertheless, this effect was counteracted by organic matter losses.
427 Consequently, the improvement of BMP related to original VS was not relevant (within
428 the precision limits). Between the end of the first and fourth month, BMP related to
429 VS_{added} decreased by 3% for TS19%E and 11% for TS22%E. This evidenced that
430 biodegradability of stabilized silages may decrease in long-term storage, which may
431 arise since the so-called biomass stabilization occurred at relatively high pH values. At
432 the end of the 4 months of ensiling, 98% and 86% of feedstock's methane potential was
433 conserved for TS19%E and TS22%E, respectively. This proves that wheat straw
434 addition was in both cases an asset to BMP preservation for long-term ensiling of cattle
435 manure and that use of 19% TS should lead to optimal energy recovery. Furthermore,
436 using the same parameters of the preceding financial estimation, higher energy
437 conservation rates of TS19%E and TS22%E (compared to open-air storage) should lead
438 to 25.7 €/ton and 23.9 €/ton of additional income per storage batch. This indicates that
439 the investment for silo construction should be covered in less than 1 year for efficient
440 ensiling conditions. The price of wheat straw was not included in these calculations,
441 since its energy potential should be recovered in methane production.

442 Finally, our results suggest that there is a strong correlation between fermentation
443 profiles, organic matter conservation and BMP for prolonged storage durations. At the
444 same time, one may take into account that BMP assays may last for about 2 to 3 months
445 to be completed. Therefore, whenever either a quick analysis of several storage
446 conditions is required or only simple approaches are available, organic matter losses
447 determination (*e.g.*, or even total weight loss) may be a resourceful method for
448 qualitative analysis on optimization of storage conditions for a specific feedstock before
449 biogas production.

450 3.3.2. BMP distribution in particulate and water-soluble phase

451 The contribution of the soluble and particulate fraction to the total BMP is presented on
452 Figure 4. During aerobic storage (TS13%A) the water-soluble contribution of BMP
453 varied from 27% before storage to 10% after 4 months. Furthermore, nearly all the
454 water-soluble contribution decreased during the first month of storage. This observation
455 supports the hypothesis earlier debated that higher rate of BMP losses observed during
456 the first weeks of open-air storage was a consequence of the degradation of water-
457 soluble compounds.

458 Minor changes on BMP contributions (between particulate and soluble part) were
459 detected for ensiling of fresh cattle manure (TS13%E) during the first month of storage,
460 with water-soluble phase representing 25-32% of total methane potential. Significant
461 degradation of water-soluble phase only occurred during the last 3 months, decreasing
462 its fraction to 15% of total BMP at the end of storage. These results partially agree with
463 the data afore discussed. On the one hand, a clear increase tendency of water-soluble
464 phase of BMP was expected over the first month of ensiling. This would be the result of
465 the increase of VS soluble fraction along storage duration, which was indeed recorded

466 either by direct measure of VS fractions (result not shown) or by degradation of
467 structural carbohydrates and VFA accumulation. On the other hand, the decrease of
468 water-soluble fraction of BMP in the last 3 months of storage is in line with the loss of
469 almost the totality of VFA that was observed during this period.

470 Analogous evolutions of BMP fractions during ensiling were observed for both degrees
471 of wheat straw addition. For instance, regarding TS19%E condition, water-soluble
472 fraction of BMP increased from 19% prior to storage to 34% after 4 months. This is in
473 agreement with data from fermentation profiles, and confirms that complex energy
474 sources have not only been degraded but also solubilized and conserved in that form for
475 prolonged periods of ensiling. However, this mechanism of energy conservation may
476 not be applicable to all types of cattle manure. In fact, bacterial populations can be
477 influenced by the characteristics of manure [24], which may depend on the type of
478 cattle, animal's diet, as well as, on the time of the year [25]. Therefore, for several types
479 of manures, the methanogens concentration, *e.g.*, may be far superior to the one of the
480 feedstock used in these tests. In such cases, solubilization of particulate would still
481 occur but VFA produced would be degraded due to a higher methanogenic activity.
482 This will weaken ensiling efficiency, as it will lead to substantial energy losses before
483 AD.

484 Moreover, wheat straw addition had an impact on BMP distribution before storage:
485 water-soluble phase of BMP decreased from 25% for TS13%E (and TS13%A) to 20%
486 and 19% for TS19%E and TS22%E, respectively. This was expected since organic
487 matter of wheat straw is mostly under particulate form. Nevertheless, these are
488 promising results for ensiling low TS feedstocks. In field scale silage of standard crops,
489 low TS content should be avoided in order to prevent leachate formation during storage

490 [26–28]. Since part of the BMP of silages are present in water-soluble form, important
491 energy losses may occur due to seepage of eventual liquid effluent production. The use
492 of cattle manure will increase TS content and reduce leachable methane content,
493 therefore decreasing the risk either of effluent production [6] and eventual energy losses
494 that it may entail. Indeed, no production of liquid effluent was recorded during ensiling
495 of cattle manure with wheat straw (result not shown). These outcomes were considered
496 to be representative of a field scale, as cattle manure was ensiled with a commonly used
497 packing density in agricultural silage.

498 3.3.3. Kinetics

499 Besides the maximum value of methane produced, kinetics of BMP assays were
500 characterized by the 1st-order rate coefficient (k) of biomass degradation, Figure 5. This
501 parameter is closely linked to the biochemical accessibility of biomass, hence the
502 interest for biomass conservation purposes. Nevertheless, while testing prolonged
503 storage, different inoculum should be used for part of BMP series. Contrary to methane
504 potential value, modifications on inoculum properties have an impact on the rate of
505 biomass degradation during BMP assays. Therefore, an indirect approach was used to
506 analyze evolution of k values during storage, which consisted in only comparing results
507 between different conditions for the same storage period (BMP tests were performed
508 with the same inoculum).

509 Concerning the feedstocks, higher rate coefficient was found for fresh cattle manure,
510 being the decrease of biochemical accessibility proportional to the amount of wheat
511 straw added. This confirms that the majority of wheat straw VS was hardly reachable,
512 which was previously suggested by the increase of cellulose content and particulate
513 phase of BMP related to straw addition.

514 Storage method had a significant effect on biochemical accessibility of fresh cattle
515 manure. In the first 30 days of storage, the difference between the rate coefficients of
516 the two methods progressively increased, benefiting ensiling. This must have been an
517 outcome of: in first place, the degradation of structural carbohydrates into VFA and
518 their accumulation during this period for TS13%E and; secondly, the important loss of
519 water-soluble compounds in the first month of open-air storage. This scenario was
520 reversed in the last 3 months and the degradation rate of ensiled cattle manure became
521 weaker than the one for the aerobic treatment. On the one hand, this difference cannot
522 be attributed to an increase of the biochemical accessibility for the open-air stored
523 feedstock, since its BMP declined significantly in this period and its water-soluble
524 fraction did not increase. This means that the shift should have been a consequence of
525 VFA degradation for TS13%E, which led, in addition to the energy damaging, to a great
526 loss of accessibility of the organic matter during the 3 last months of storage.

527 Despite different initial values, similar rate coefficients were obtained for TS19%E and
528 TS22%E from 7 days of storage. Furthermore, both conditions with wheat straw
529 presented lower k than TS13%E until 30 days of ensiling. This was explained by the
530 fact that wheat straw led to a more controlled fermentation, and as formerly discussed to
531 scarcer gains related to the BMP kinetics. At the end of storage, TS19%E and TS22%E
532 had far higher degradation rates during the BMP assays than ensiling of fresh cattle
533 manure. This evidenced that wheat straw is an asset on the conservation of great rates of
534 biochemical accessibility for extended periods of ensiling. Restricted fermentation and
535 VFA accumulation in water-soluble phase appear to be the key factors for the success of
536 co-silages with cattle manure and wheat straw.

537 **4. Conclusions**

538 Ensiling led to lower energetic losses and NH₃ emissions than open-air storage during
539 conservation of cattle manure. Yet, chemical properties of fresh cattle manure hindered
540 silage stabilization for extended periods. Co-ensiling of cattle manure with wheat straw
541 enhanced biomass acidification, inducing a preservation of more than 86% of methane
542 potential after 4 months. In addition, wheat straw suppressed ammonia production and
543 limited the risks of energy losses through liquid effluent. In summary, this work
544 demonstrated that long-term conservation of cattle manure can be achieved if correct
545 management practices are used. This will impact the performance of agricultural biogas
546 plants.

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648 Table 1 – Storage conditions

Condition	TS13%A	TS13%E	TS19%E	TS22%E
Approach	Open-air	Ensiling	Ensiling	Ensiling
Wheat straw (%wt)	-	-	8.3%	13.3%
TS (%wt)		12.8 ± 0.1	18.8 ± 0.4	22.4 ± 0.8
VS (%wt)		10.2 ± 0.1	15.9 ± 0.3	17.9 ± 0.7

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665 Table 2 – Chemical characteristics of biomass over storage time (% VS_{added/original})

Condition	Storage duration (days)	Chemical characteristics ^a								
		pH	LA ^b	AA ^b	BA ^b	VFA ^b	WSC ^b	HEM ^c	CEL ^c	LIG ^c
TS13%A	0	7.9	0.0	1.3	0.3	2.1	0.0	32.5	26.8	6.1
	7	7.8	0.0	1.0	0.3	1.7	0.0	26.4	22.5	8.1
	14	7.9	0.0	0.9	0.4	2.0	0.0	24.8	26.1	5.5
	30	8.6	0.0	0.9	0.6	2.2	0.0	20.4	20.6	5.2
	120	9.8	0.0	0.0	0.0	0.0	0.0	13.3	10.3	5.7
TS13%E	0	7.9	0.0	1.3	0.3	2.1	0.0	32.5	26.8	6.1
	7	7.3	0.0	1.6	0.5	3.0	0.0	30.0	26.6	6.3
	14	6.9	0.1	2.8	1.1	6.0	0.0	26.5	24.9	5.8
	30	6.9	0.0	6.7	2.2	12.9	0.0	24.0	20.3	6.2
	120	8.4	0.0	1.1	0.0	1.7	0.0	16.7	16.9	6.1
TS19%E	0	8.3	0.0	1.1	0.2	1.8	0.0	28.7	36.0	6.1
	7	7.2	0.0	2.3	0.5	3.6	0.0	26.6	33.4	6.6
	14	6.5	0.1	2.5	1.0	4.8	0.0	24.8	32.1	5.1
	30	5.6	0.0	4.1	3.0	9.4	0.0	24.5	30.2	5.3
	120	5.5	0.0	7.4	4.2	15.7	0.0	22.0	24.8	4.6
TS22%E	0	8.2	0.0	1.1	0.1	1.8	0.0	27.5	37.2	6.3
	7	6.8	0.0	2.0	0.4	3.1	0.0	26.9	36.7	5.9
	14	6.2	0.0	2.1	0.9	4.0	0.0	27.1	35.8	5.4
	30	5.5	0.0	3.3	2.4	7.5	0.1	27.3	30.8	7.0
	120	5.4	0.0	6.3	4.8	15.5	0.0	21.9	24.8	7.2

^a LA stands for Lactic Acid, AA for Acetic Acid, BA for Butyric Acid, HEM for Hemicellulose, CEL for Cellulose and, LIG for Lignin; ^b results based on % VS_{added}; ^c results based on % VS_{original}

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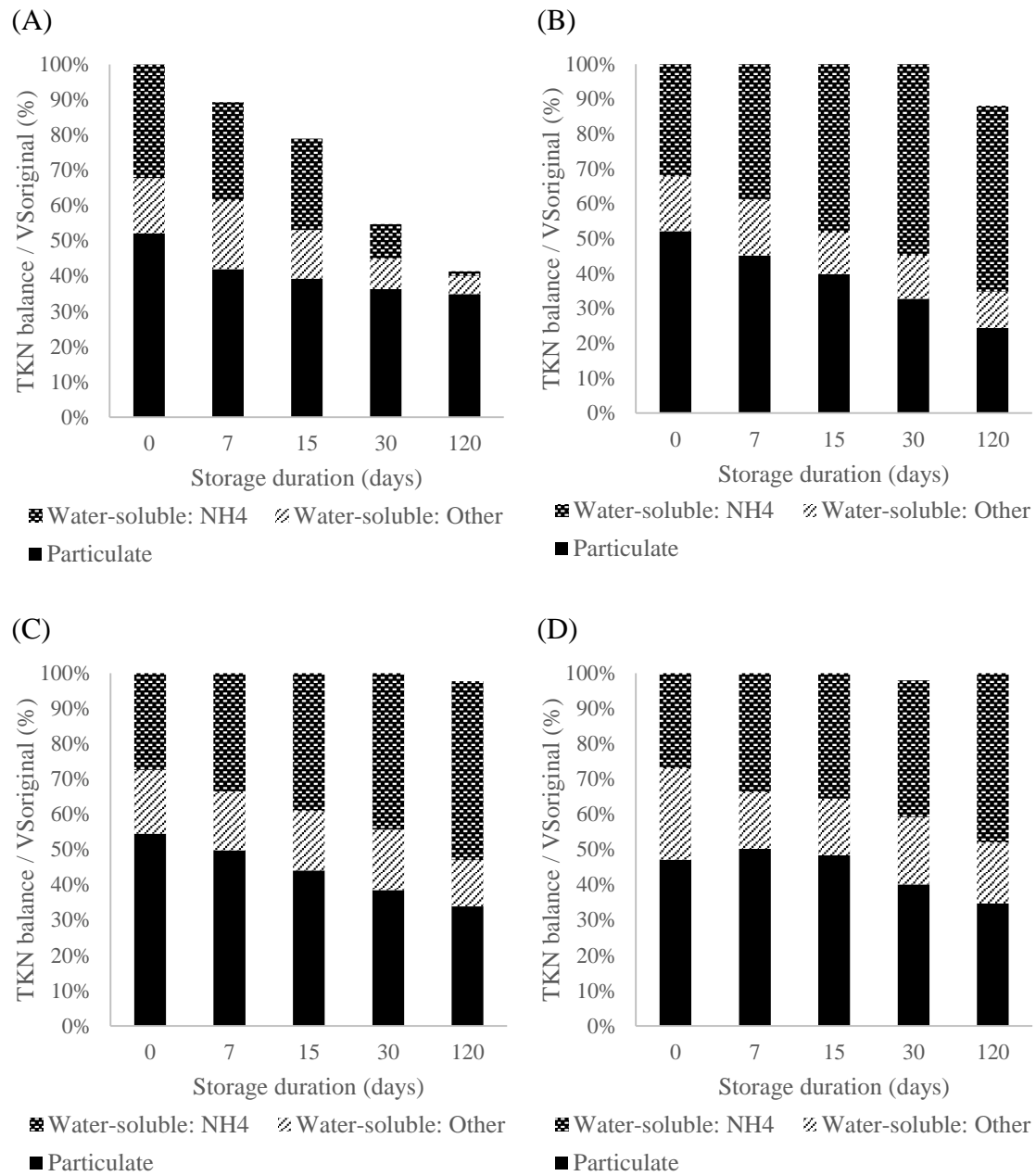
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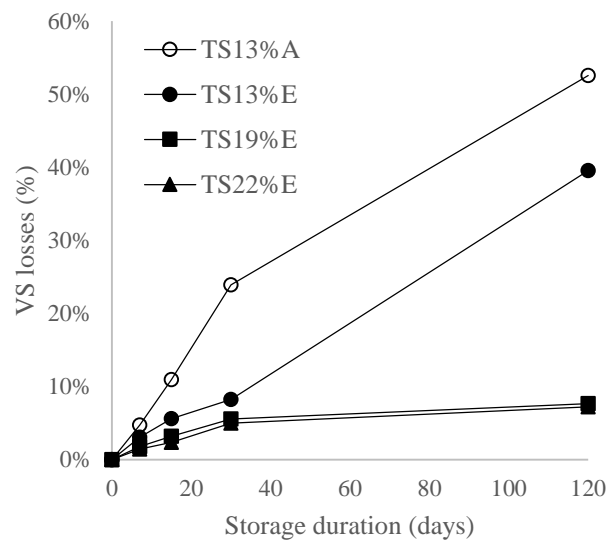
676 Figure 1 – TKN balance for: (A) TS13%A; (B) TS13%E; (C) TS19%E; (D) TS22%E

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682 Figure 2 - Organic matter losses over storage duration

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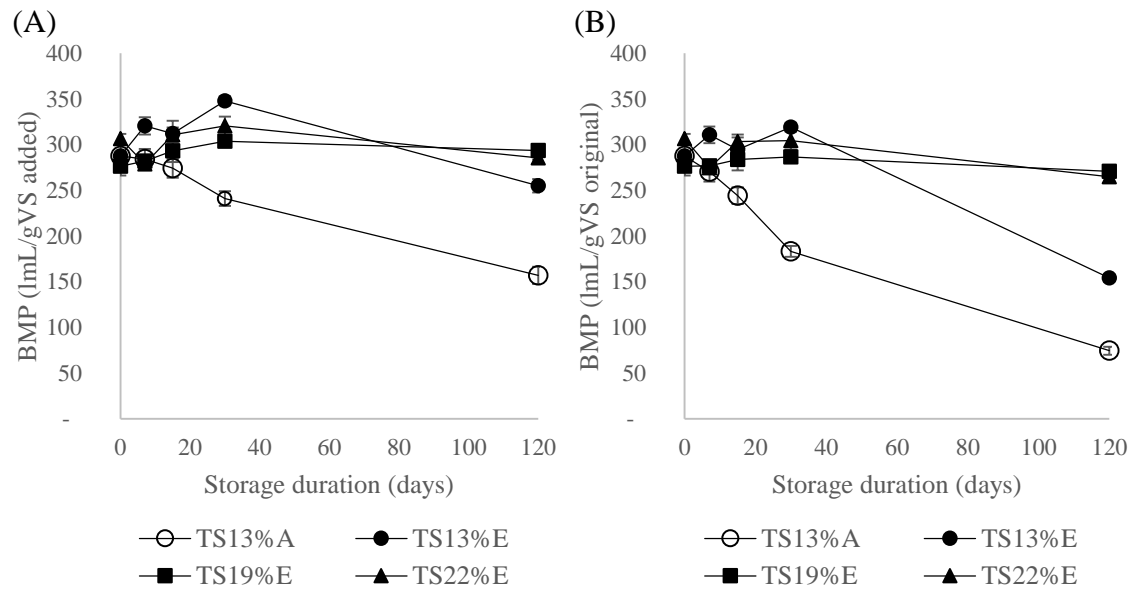
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695 Figure 3 - Global BMP evolution over storage duration. (A): methane potential based on
 696 VS_{added}; (B): methane potential based on VS_{original}, therefore considering storage losses.

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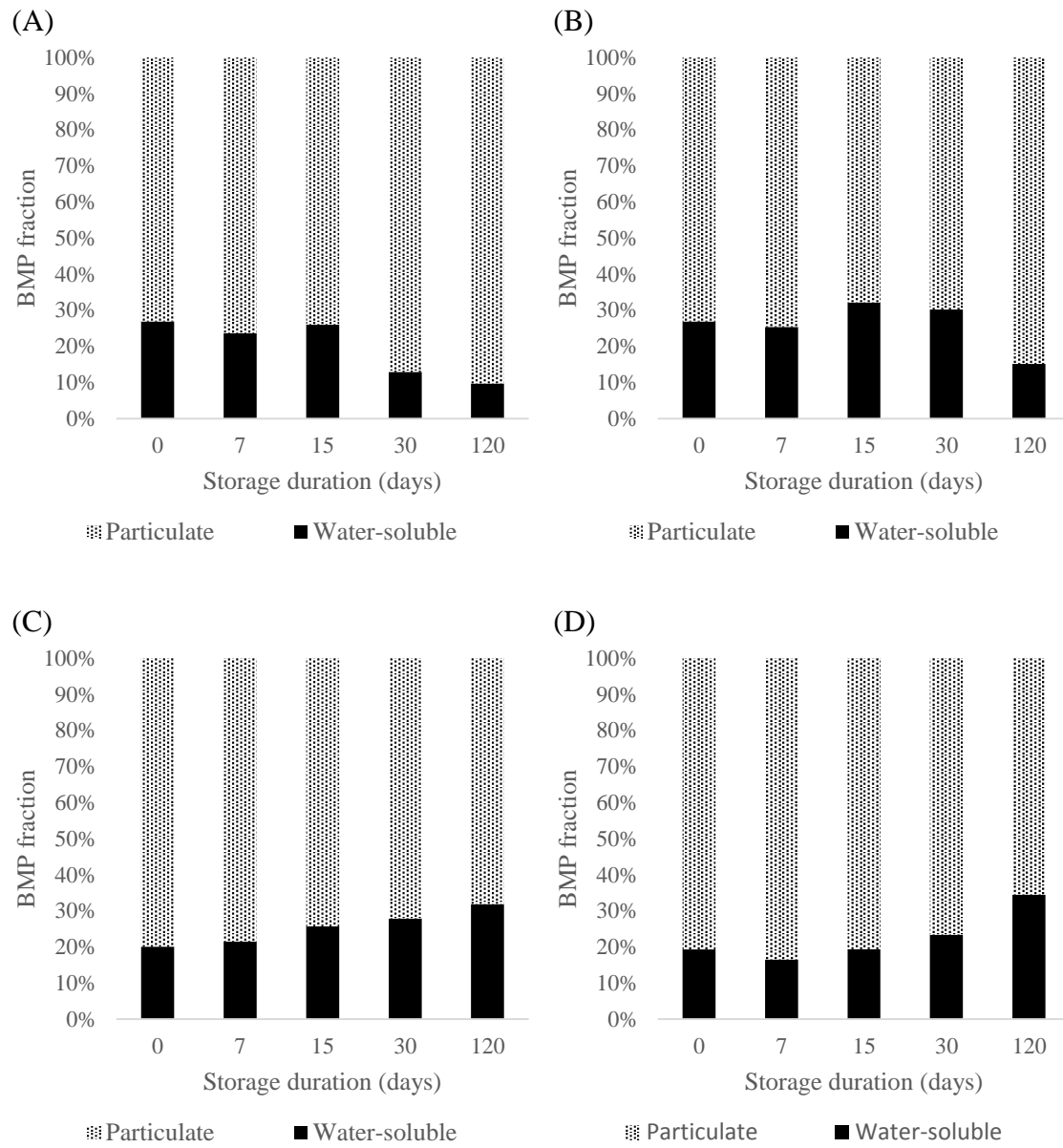
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708 Figure 4 – BMP multi-phase analysis for: (A) TS13%A; (B) TS13%E; (C) TS19%E;
 709 (D) TS22%E

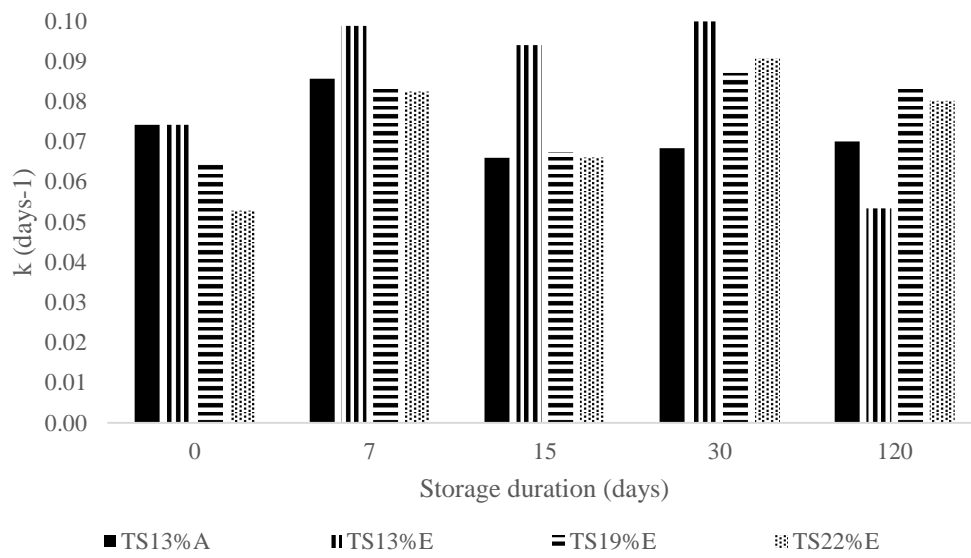
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716 Figure 5 – Rate coefficient (k) of BMP assays over storage time. Standard deviations are
 717 not presented as differences among triplicates were negligible.

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