



HAL
open science

Ensiling for biogas production: Critical parameters. A review

Ruben Teixeira Franco, Pierre Buffière, Rémy Bayard

► **To cite this version:**

Ruben Teixeira Franco, Pierre Buffière, Rémy Bayard. Ensiling for biogas production: Critical parameters. A review. *Biomass and Bioenergy*, 2016, 94, pp.94 - 104. 10.1016/j.biombioe.2016.08.014 . hal-01692853

HAL Id: hal-01692853

<https://hal.science/hal-01692853v1>

Submitted on 25 Jan 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

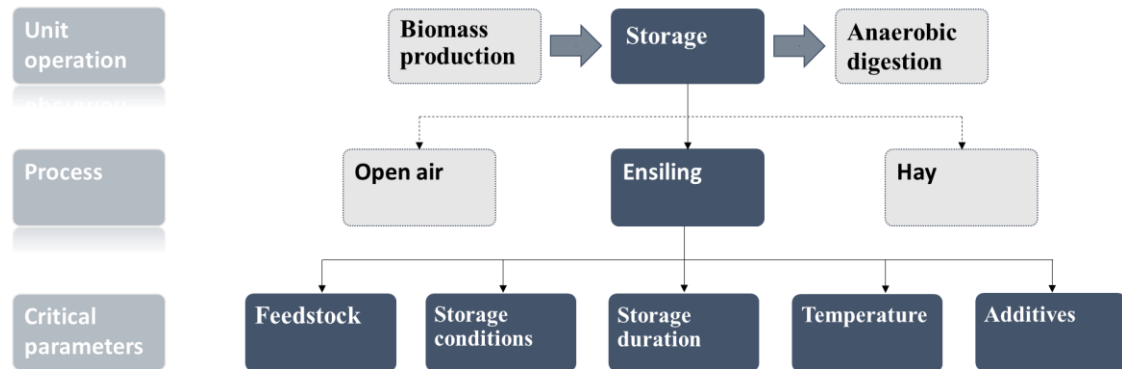
L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Ensiling for biogas production: critical parameters. A review**

2 Rúben Teixeira Franco, Pierre Buffière, Rémy Bayard*

3 *Université de Lyon, INSA Lyon, LGCIE-DEEP, EA4126, F-69621 Villeurbanne cedex,*
4 *France*

5 **Graphical abstract**



7 **Abstract**

8 In order to meet the legislative demands of new energy policy, investment in anaerobic
9 digestion and biogas production has increased in recent years, making it a versatile and
10 fully established technology. So as to remain competitive, anaerobic digestion should be
11 optimized not only at the level of the process, but also down and upstream, in which
12 biomass storage prior to digestion is included. Ensiling is a commonly used and promising
13 techniques to store wet biomass before anaerobic digestion. This article reviews the
14 crucial parameters for ensiling agricultural wastes and crops for biogas production, as
15 source properties, storage management and duration, temperature or additives. According

* Corresponding author. Tel.: +33(0) 4 72 43 87 53

E-mail addresses: ruben.teixeirafranco@insa-lyon.fr (R. Teixeira Franco),
pierre.buffiere@insa-lyon.fr (P. Buffière), remy.bayard@insa-lyon.fr (R. Bayard)

16 to the reported findings in the bibliography, feedstock and its biochemical characteristics
17 will define the course of ensiling and the impact of other parameters during storage as
18 well. Good silage preservation will occur for feedstocks with low moisture content, high
19 accessible carbohydrates and low buffering capacity. High packing density and reduced
20 particle size will contribute to minimize energy losses during ensiling. Additives are
21 widely used but are not always an asset for methane potential conservation and their
22 application should be more appropriate for poorly ensilable biomass. Finally, evidences
23 suggest that under specific conditions, ensiling may increase methane potential despite
24 non-negligible organic matter losses during storage. Exposing the answers given by the
25 literature in terms of impact of different conditions in the course of ensiling and the
26 questions still unresolved, this article highlights the good management practices of
27 substrates for biogas production.

28 *Keywords: Biomass crops; Agricultural wastes; Storage; Ensiling; Anaerobic*
29 *digestion; Methane potential*

30 **Highlights**

- 31 • Biochemical properties of feedstock will define the course of ensiling.
- 32 • Good preservation requires low silage moisture, high water-soluble carbohydrates
33 content and low buffering capacity.
- 34 • High packing density and reduced particle size minimize energy losses.
- 35 • Additives should be a potential asset for preservation of poorly ensilable biomass.
- 36 • Ensiling may be used as methane potential booster before anaerobic digestion.

37 **Abbreviations**

38 AD, anaerobic digestion; BC, buffering capacity; BMP, biochemical methane
39 potential; Ho, homofermentative; He, heterofermentative; LAB, lactic acid bacteria;
40 NH₃-N, ammoniacal nitrogen; TS, total solids; VS, volatile solids; WSC, water soluble
41 carbohydrates

42 **1. Introduction**

43 Taking into account political and environmental concerns, investment in bioenergy
44 production has been intensified and diversified over the past twenty years [1].
45 Considering recent studies [2], biogas production through anaerobic digestion (AD) is
46 one of the renewable energies that is being considered and developed, from which it is
47 believed that at least one quarter of all bioenergy can be originated. Besides the more
48 than 14 000 biogas plants in Europe at the end of 2013 (corresponding to 13 380 ktoe of
49 primary energy production) [2] and its fast growth over the last years, biogas is
50 currently the only technologically fully established renewable energy source that is
51 capable of producing heat, steam, electricity and vehicle fuel [3].

52 Nevertheless the segment's continuing growth over the last years, the focus in energy
53 efficiency of biogas plants will be crucial in the future [2], as it will be for the remaining
54 actors of the energy sector. For biogas production, this optimization can not only get the
55 AD process, but also the downstream and upstream systems, i.e. the biomass production
56 and its end use.

57 Biomass storage before anaerobic digestion, as presented on Figure 1, is one point
58 that can be potentially optimized. Nowadays, the diversification of AD inputs is quite
59 wide, as energy can be recovered from almost all types of organic wastes, forages or
60 catch/energy crops. Otherwise, although the need for continuous feeding of biogas
61 plants throughout the year, some of these agricultural/industrial wastes or crops are
62 seasonally produced, leading to storage requirements, in some cases even of extended
63 durations.

64 Regarding the storage types, three main categories should be mentioned. The first one
65 is open air storage, mostly used for agricultural wastes as animal manure, since it is

66 non-expensive and regularly produced, normally with no need of prolonged storage.
67 Despite everything, even during small periods, open air storage can lead to substantial
68 losses in terms of methane potential, due to air-material contact and aerobic
69 biodegradation.

70 Concerning seasonally produced resources such as crops and wastes, two main
71 storage and preservation technologies have been adapted for methane production
72 purpose: hay and silage systems. Hay storage consists on field drying, inhibiting
73 detrimental microbial activity, followed by the use of large round bales stored outdoors
74 [4]. Even though this system minimizes both labor and storage costs, it leads to high
75 losses in terms of dry matter that may reach up to 60% [5], it is restricted to crops that
76 can dry quickly and uniformly. This technique can be limited by rainfall during harvest
77 [6].

78 Contrasting with the physical transformations in hay systems, ensiling provides a
79 biochemical process based on the preservation under an anaerobic environment, using
80 bacterial fermentation to prevent further degradation. This process has been used to
81 preserve forages for animal feed during centuries. It minimizes weight and energy
82 losses if well succeeded and therefore, appears as a promising technique for storage of
83 wet biomass before methane production.

84 Ensiling can be divided in four phases, according to the main biochemical and
85 microbiological transformations occurring during the process [4,6,7]:

86 • Initial aerobic period: after filling and sealing the silo, biomass respiration occurs due
87 to the presence of oxygen trapped in the system. Respiration continues during several
88 hours, consuming sugars and producing carbon dioxide and water, until all oxygen is
89 removed.

- 90 • Anaerobic fermentation: once oxygen has been depleted, the microorganisms capable
91 of anaerobic growth (for instance, lactic acid bacteria - LAB, enterobacteria,
92 clostridia and yeasts) begin to proliferate and compete for the available organic
93 matter. The first days are critical for the success or failure of the fermentation [8]. If
94 the conditions are suitable, LAB will produce lactic acid for several weeks,
95 decreasing the pH to around 4.0.
- 96 • Stabilization phase: the anaerobic conditions are maintained with a decreasing
97 fermentative activity, the pH remains stable wherein minimal enzymatic and
98 microbial activity will occur until feed-out period.
- 99 • Feed-out: after unloading the silo for transportation or bio-digester feeding, biomass
100 enters once again into aerobic environment. Thereupon, aerobic microorganisms are
101 reactivated, which may spoil the silage and lead up to 15% of absolute energy losses
102 [9].

103 As can be seen, ensiling process is quite dynamic, through several successive stages,
104 with competitive environments and microorganisms. Control of biochemical processes
105 and growth of different microorganisms seems therefore rather important, in order to
106 obtain a good silage quality, ready to provide the maximum energetic yield in the
107 anaerobic digester. For instance, energy losses due to respiration, secondary
108 fermentation, effluent production or aerobic deterioration may occur. These phenomena
109 can lead to up to 40% methane loss if inappropriate management practices are used [9].
110 In contrast, under efficient silage systems, organic matter losses can be limited below
111 20% and methane potential can be conserved almost entirely or even increase in some
112 cases [7,10–12].

113 The biochemistry and microbiology principles of ensiling, and more generally the
114 major parameters for forage silage in view of animal feed, are already well described in
115 the literature [4,8,13]. On the other hand, although these references are quite important
116 to understand the biochemical phenomena during ensiling, the extrapolation to biogas
117 production purposes must be cautious. In fact, the aim of silage for animal feed and
118 biogas production are not exactly the same: in the first case, protein digestibility,
119 palatability and dry matter intake are of prime interest [4], while for biogas production
120 purposes, the main objective is to save - or eventually increase, the maximum amount of
121 carbon that can be transformed into methane.

122 To our best knowledge, the critical parameters in ensiling for biogas production has
123 not been reviewed earlier. This article examines several points of influence for silage of
124 biogas crops, taking into account the answers stated by the literature and questions that
125 remain unclear. The objectives of this study are to outline good storage practices of
126 substrates before AD and point out next steps on ensiling research.

127 **2. Influence parameters**

128 *2.1. Feedstock*

129 Whether discussing ensiling or AD, the choice of input is a factor of great
130 importance, since it affects all biochemical and microbiological interactions during the
131 process. Within this selection, there are several parameters to be regulated, namely its
132 source, particle size or water content.

133 *2.1.1. Source*

134 Silage can be made from a large variety of biomass. However, its success will rely
135 on several biochemical characteristics of the source of energy. Besides moisture content
136 (discussed in further detail in chapter 2.1.3), water soluble carbohydrates (WSC)

137 content, buffering capacity (BC) and epiphytic microflora of feedstock will play a
138 crucial role on the course of ensiling [8], which should impact storage losses: WSC will
139 be partially fermented into volatile fatty acids (VFA) if LAB are present in sufficient
140 amount on a suitable range of moisture content, which will acidify and stabilize the
141 biomass if it possess a relatively low BC.

142 Normally fulfilling the biochemical requirements, whole crop maize is one of the
143 main investigated crops [7,12,14–19] for energy production purposes. It has a relatively
144 low moisture content, a low BC and an adequate WSC content. It is thus considered as
145 an ideal crop for ensiling [8].

146 Similarly, grass is usually conserved as silage [8]. Although it is commonly used
147 and studied [10,11,20–22], grass chemical characteristics will strongly depend on the
148 species used, the stage of growth or even the climate [8]. For instance, in the late stages
149 of growth, the WSC content of grass tends to decrease, while the cell wall components
150 increase [8]. In this case, fermentation will be slower, retarding the decrease of pH
151 necessary for efficient preservation [23].

152 Other crops are used for ensiling, but to a fewer extent. Other cereals such as
153 sorghum [4,5,7,24] and triticale [7,25] have been investigated. In addition, some crop
154 residues such as sugar beet tops [10,17], corn stalks [26] or agricultural and food
155 processing by-products [27] are also attracting an increasing attention from ensiling
156 researchers in recent years.

157 Since biochemical features diverge among the possible sources of feedstock for
158 ensiling, different impacts on BMP (biochemical methane potential) conservation
159 during storage are expected depending on the biomass used [7,12,17,28]. Zubr [28]
160 worked with different types of plants and found that, despite having produced a silage

161 of excellent quality in all cases after one year storage, ensiling favored the methane
162 production for certain materials, while in others the opposite was found.

163 Likewise, Herrmann *et al.* [7] observed different behaviors during silage among the
164 substrates studied. They showed that the methane yield (calculated relatively to the
165 initial amount of volatile solids i.e., by taking into account the storage losses) increased
166 for whole crop maize and forage rye, while for sorghum hybrid and triticale a slight
167 decrease would be expected (Figure 2).

168 Besides the direct impact on the course of ensiling, feedstock source and its
169 biochemical characteristics will influence the impact of other critical parameters over
170 storage. For instance, as can be seen in Figure 2, Herrmann *et al.* [7] showed that the
171 evolution of methane yield over ensiling time strongly depends on the feedstock. This
172 has also been shown for other biomass crops by Lehtomäki [10] and Pakarinen *et al.*
173 [12]. Other examples concerning the impact of biomass source on several ensiling
174 influence parameters can be found in the literature; for instance, concerning the use of
175 additives [7,10,11,18,25] and for the temperature [10].

176 Although it is clear that biochemical characteristics of raw material are one of the
177 most crucial parameters in ensiling, optimization of storage performance through
178 feedstock choice may not be always possible. Indeed, several restrictions related to
179 geography, environmental policies or AD requirements may limit the range of biomass
180 able to be used for ensiling. For instance, even if maize whole plant is an ideal biomass
181 for ensiling and AD, the use of wastes or catch crops is preferred in some countries like
182 France, due to political and ethical issues. Conversely, in boreal conditions, energy
183 crops used for biogas production need to have good winter hardiness and be able to
184 grow on soil of poor quality with low nutrient input [29].

185 2.1.2. Particle Size

186 Methane fermentation through AD is clearly affected by feedstock's particle size, as
187 it interferes in the kinetics of complex substrates hydrolysis [30]. Normally, methane
188 production is enhanced by particle size reduction, mainly due to the increase of the
189 available specific surface area and to the reduction of both degree of polymerization and
190 cellulose crystallinity [31]. An identical influence is expected during ensiling, since
191 particle size reduction may lead to faster LAB fermentation and therefore to less organic
192 matter losses. Indeed, Herrmann *et al.* [32] indicates that chopping at harvest as a
193 mechanical treatment reduces the particle size for enhanced manageability of crop
194 material and for better process conditions at ensiling and feeding.

195 Concerning the validation of the benefits by chopping ensiling raw materials, Gordon
196 *et al.* [33], Herrmann *et al.* [32] and Haag *et al.* [18] presented different results and
197 conclusions. According to the early work [33], based on an ensiling study of alfalfa as
198 forage for animal feed purposes, lower particle size silage were characterized by lower
199 pH, NH₃-N and butyric acid content, and higher lactic acid content. These results
200 suggest that silages with lower particles sizes could present a higher BMP, as the
201 chemical indicators show a better crop preservation. In fact, as reviewed by McDonald
202 *et al.* [8], if a stable and low pH silage is not achieved, clostridial activity will be
203 encouraged and a secondary fermentation will occur. This clostridial fermentation is
204 mainly based on sugars and lactic acid consumption as energy source *via* similar
205 pathways, producing butyric acid, carbon dioxide and hydrogen (Table 1). Furthermore,
206 butyric acid is a much weaker acid than lactic acid. In addition, one mole of butyrate is
207 produced from two moles of lactate. These two effects lead to an increase of the pH and
208 a loss of silage stability. As a consequence, the conditions will be suitable for the
209 proteolytic clostridia activity, which will mainly produce ammonia and carbon dioxide

210 through amino acids and amides fermentation, Table 1. Finally, this clostridial
211 fermentation will reduce the BMP, as energy will be lost through CO₂ and H₂
212 production. It will also lead to nitrogen loss in the gaseous phase by ammonia
213 formation.

214 Similarly, Herrmann *et al.* [32] worked with several crops as sorghum, forage rye,
215 winter rye, whole crop maize and triticale, presenting favorable results for particle size
216 reduction in the ensiling process. They showed that setting very short chopping lengths
217 before ensiling improved fermentation conditions through additional release of easily
218 fermentable substrates, leading to more extensive lactic acid formation, therefore
219 reducing storage losses. In addition, they indicated that, in general, reducing chopping
220 length enhanced the methane yield based on original volatile solids (VS) content as
221 presented in Figure 3. Finally, the authors suggested that shortening chopping length at
222 harvest can have other advantages, such as, reduction of aerobic deterioration risk at
223 feed-out by enabling higher silage densities and minimizing air introduction.

224 Contrasting with the data presented above, Haag *et al.* [18] presented a study with
225 silage of whole crop maize and amaranth, in which no benefit was found by reducing
226 the chopping length from 8 to 1 mm. Lower methane yields were obtained for the
227 smaller chopping length in both cases. For maize silage, lower methane yield might be
228 explained by the weaker lactic acid formation during ensiling for the 1 mm chopped
229 crop. In the case of amaranth crop silage, this reduction of the methane yield can be, in
230 part, due to high dry matter losses during the ensiling of the crop with smaller particle
231 size. These results suggest that, despite the accessibility gains usually attributed to a
232 reduced particle size, other biochemical phenomena may affect the BMP of chopped
233 crops. However, this discussion was not detailed by the authors.

234 Regarding the optimization of chopped length size for ensiling purposes, Mohd-
235 Setapar *et al.* [34] suggested that this subject has been poorly investigated and most
236 studies were performed using pre-defined particle size crops. Nevertheless, Herrmann *et*
237 *al.* [35] recently published an investigation about the profitability of reducing chopping
238 length, in connexion with their first study on whole crop maize, sorghum, forage rye,
239 winter rye and triticale crops. They reported that chopping crops to particles sizes of 7
240 to 8 mm are recommended for high methane formation. However, in only one third of
241 the cases the benefits due to higher methane production by further chopping length
242 reduction did compensate the additional cost of size reduction.

243 2.1.3. Moisture

244 The effect of moisture in silage has been extensively studied in the last decades,
245 mostly for animal feeding purposes. A commonly shared view in literature (supported
246 by McDonald *et al.* [8]), is that a higher total solids (TS) content delay bacterial growth,
247 leading to a more restricted fermentation and therefore, influencing silage preservation.
248 However, different levels of tolerance to dryness are noticed among the involved
249 microorganisms. For instance, clostridia are known to be particularly sensitive to water
250 availability and require wet conditions for active development. In counterpart, LAB are
251 able to ferment biomass at a wide range of TS [8]. Borreani *et al.* [36] evidenced this
252 fact through a series of silage experiments using field pea, faba bean and white lupin at
253 different dry matter contents. The authors observed that, on the one hand, there was
254 only a small decrease of lactic acid production with increasing crop dry matter. On the
255 other hand, the saccharolytic clostridial fermentation exponentially decreased as TS
256 increased, being negligible at 30% of total solids.

257 Moreover, a more restricted proteolytic clostridial fermentation at lower moisture
258 content was observed by the same authors, testified by a lower level of $\text{NH}_3\text{-N}$
259 production. Therefore, better preserved crops will be expected from higher total solids,
260 since lower organic matter losses would occur due to the limitation of undesirable
261 microbial growth.

262 Likewise, Nash [37] worked with grass/clover herbage silage and showed that
263 nutrient losses were much lower in crops with higher dry matter content. Similarly,
264 Mahmoud *et al.* [38] and Wilkinson [39] evidenced a decreasing clostridial activity for
265 feedstock with higher TS content with whole crop maize and comfrey silages,
266 respectively. For these latter studies, silage preservation was particularly successful, as
267 an increase of lactic acid production was verified for crops with lower moisture level.

268 In contrast, Han *et al.* [40] published a work with cup-plant silage suggesting that the
269 fermentation was not restricted for all microorganisms in higher TS crops. Although
270 acetate and butyrate concentrations were lower for crops with higher dry matter content,
271 lactic acid production and proteolytic clostridial activity were identified to be higher on
272 the same substrates. Haigh and Parker [41] published a work on ryegrass and white
273 clover mixture silages; they found that, despite a higher content of $\text{NH}_3\text{-N}$, higher lactic
274 acid fraction among all acids and lower TS losses were obtained for these crops. This
275 might suggest that, even if proteolytic clostridial activity increase in some cases, its
276 impact on dry matter losses will be overlapped by the increase of lactate fraction in the
277 total acids.

278 When the fermentation is restricted to higher solids content, leading to lower
279 acidification, good preservation can be achieved at higher pH. Thus, a qualifying
280 parameter of preservation of silage can be found on the necessary acidity for efficient

281 silage, or the critical pH value, which is function of the total solids content, as shown in
282 Table 2.

283 Together with the benefits for organic matter preservation, higher total solid content
284 may prevent leachate formation during ensiling. Indeed, several authors as Bastiman
285 [42], Sutter [43] and Zimmer [44] have proposed correlations to predict the behavior of
286 effluent production (Figure 4). In the works by Bastiman [42] and Zimmer [44], similar
287 quadratic derived equations were obtained, in which negligible leachate formation
288 occurs above around 25% of TS. On the other hand, Sutter [43] used a linear adjustment
289 on which minimal values for leachate production are predicted at 30% of TS. The
290 differences among the correlations might be explained by the influence of other
291 parameters on the effluent production, such as the feedstock, the use of additives, the
292 surface pressure applied, the silo height or the mechanical pre-treatment before ensiling
293 [45]. Besides affecting nutrient and energy losses, effluent production can lead to
294 serious problems in terms of water pollution due to seepage. For biogas production,
295 losses may be avoided by using the effluent itself as co-feedstock on AD [4]. However,
296 one must take into account that the recovery of leachate might be complicated. Thus,
297 both for forage or biogas production purposes, effluent production should be avoided
298 and a particular attention has to be paid to the adjustment of the moisture content.

299 In several cases, the dry matter content of the feedstock is low. Indeed, techniques for
300 moisture reduction are used in order to ensure a proper preservation of the original
301 resource during ensiling. Field wilting prior to ensiling is the most common method to
302 achieve higher TS contents for biomass crops [8]: it is inexpensive and it enables water
303 evaporation with little effect on the remaining chemical characteristics if wilting
304 duration is controlled. In fact, several authors as Borreani *et al.* [36], Carpintero *et al.*

305 [46], Dawson *et al.* [20] and McEniry *et al.* [21] have compared wilted and un-wilted
306 feedstock chemical characteristics before ensiling. McEniry *et al.* [21] performed a 6h
307 wilting of grass and observed an increase of TS content from 20.1% to 26.5%, with no
308 particular effect on the other chemical properties, such as cell wall composition or water
309 soluble carbohydrates (WSC). The same conclusions were obtained by Borreani *et al.*
310 [36] after a 6h wilting period of field pea, faba bean and white lupin, as their dry matter
311 content increased from 48.2% to 61.8%, from 23.7% to 29.5% and from 14.2% to
312 17.3%, respectively, without other significant modifications on chemical composition.
313 Identically, Carpintero *et al.* [46] worked with ryegrass-clover, in which a 6h pre-
314 wilting allowed the increase of dry matter content from 17.3% to 34.9%, without
315 affecting the composition.

316 The same authors also performed a pre-wilting of 48h, in order to achieve a higher TS
317 content (46.2%), and obtained a decrease in the WSC content from 213 to 203g/kg TS
318 and an increase of the released ammonia nitrogen (NH₃-N) from 1.2 to 2.1g/kg of total
319 nitrogen. Likewise, Dawson *et al.* [20] studied field wilting durations of 28 and 52h for
320 perennial ryegrass and reported an impact of wilting on silage chemical characteristics,
321 particularly on the pH and the buffering capacity. These results suggest that even if
322 higher solid contents may be achieved with prolonged crop wilting, other chemical
323 changes beyond water evaporation might occur leading to organic matter degradation.
324 Therefore, short duration field wilting should be preferably considered when biomass
325 preservation is required during water evaporation. However, evaluation of wilting only
326 through drying duration should be performed with caution. Depending on the
327 geographical situation of the silo and harvest site, the weather condition will affect the

328 wilting process, changing its efficiency. Thus, the exposure time to sun, the intensity of
329 radiation and the ambient temperature are important data to account for.

330 As alternative to open air wilting, more complex and expensive treatments, such as
331 chemical desiccation and thermal treatment, can be proposed [8]. Regardless the method
332 used, the water weight to be transported from the field to the silo and after ensiling to
333 AD will be lower, reducing both transportation and processing costs [36,47].

334 Contrary to the aforementioned advantages, the few studies that evaluated the impact
335 of TS content in the BMP showed inconclusive results. Pakarinen *et al.* [11] have
336 studied, during six months, grass and ryegrass silage for biogas production purposes.
337 They verified that, despite longer wilting times led to lower fermentative activity, it did
338 not enhanced the BMP, mainly due to higher VS losses during ensiling. For ryegrass,
339 lower VS losses and better BMP was obtained after 48h drying, and an opposite effect
340 was obtained for grass silage. These results suggest that initial feedstock properties will
341 influence the wilting impact on BMP. However, no further conclusions can be drawn,
342 since the authors did not follow the WSC content, BC or even cell wall constituents of
343 fresh material and silage.

344 More recently, McEniry *et al.* [21] observed that a 6h-wilted grass produced silage
345 with a more restricted fermentation, a higher fraction of lactic acid in the total
346 fermentation products and a lower pH than control (without wilting). However,
347 contrasting with good initial indications and the poor results obtained by Pakarinen *et*
348 *al.* [11], no differences were detected between the wilted and control grass on the dry
349 matter losses and BMP.

350 In conclusion to the effect of moisture content, the range of 25-30% TS, which
351 generally leads to a less extensive fermentation, effluent production and TS loss, is not

352 yet proved to be an advantage in terms of BMP conservation. The limited number of
353 significant work on this subject for biogas production purpose is certainly a limiting
354 factor for understanding the phenomena involved and to draw further conclusions. More
355 works on the influence of the TS content and on the wilting / drying procedure on BMP
356 should be encouraged in the next future.

357 2.2. *Storage conditions*

358 Despite storage conditions are mainly related to the selection of storage type to be
359 used, there are factors to be taken into account in ensiling, as the presence of air in the
360 system and density. Despite these aspects are partially linked, they will be now
361 presented separately, in order to clarify the particular features of each one.

362 2.2.1. Presence of air

363 Oxygen is usually considered as a spoiling agent in a process that needs to achieve
364 anaerobic conditions, where LAB can proliferate [6,8,9]. In fact, air causes silage
365 deterioration since it favors the activity of aerobic microorganisms, as heterotrophic
366 bacteria, yeasts and molds [48]. Besides the theoretical and macroscopic evidences of
367 oxygen detrimental action on silage, some laboratory and field scale studies have been
368 performed to confirm the impact of aerobic conditions. Indeed, Garcia *et al.* [49]
369 performed aeration tests on alfalfa silage with air rates of 320 mL/d for 21 days at
370 laboratory scale. They reported higher pH and NH₃-N content, and lower lactic acid
371 presence in aerated silage. Even though, the authors did not report a negative effect of
372 aeration on lignocellulosic biomass conversion during storage. At field scale, Langston
373 *et al.* [50] studied air impact on orchard grass and alfalfa silage by pumping air for 5 to
374 6 hours after filling the silo. They observed high temperatures in the aerated silage as a

375 result of organic matter bio-oxidation and, subsequently, pH, butyric acid and NH₃-N
376 increased, while LAB fermentation was less extensive.

377 Even though many practices applied during ensiling are intended to prevent the
378 contact with air, the impact of oxidation losses can be observed in four different stages:
379 field phase; initial aerobic phase in the silo; air infiltration phase and; aerobic
380 deterioration at feed-out [8]. According to Egg *et al.* [4], absolute energy losses from
381 aerobic degradation after storage (feed-out) can reach up to 15%, and up to 10% during
382 ensiling.

383 Concerning the aerobic degradation during ensiling, 99.5% of the oxygen can be
384 depleted after 30 minutes [51], and an anaerobic environment will be shortly reached.
385 Aerobic deterioration is thus mainly due to air penetration into the silo. While testing
386 whole crop maize silage, Herrmann *et al.* [19] evidenced that air-stress during storage
387 may result in BMP losses (4.5% decrease after 49 days of storage) and would
388 dramatically increase the risk of aerobic spoilage at feed-out.

389 For silo loading or feed-out, these losses can be reduced by minimizing the process
390 duration Nevertheless, in some cases, constraints for wilting, transportation or feed-out
391 rates may affect time efficiency. In these circumstances, aerobic stability of silage must
392 be taken in consideration to avoid major losses, as a result of increased activation of
393 aerobic microorganisms. For instance, Plöchl *et al.* [52] observed important TS and
394 BMP losses for whole crop maize silage after only 4 days of air-exposure at feed-out.
395 Similarly, McEniry *et al.* [21] found a decrease by 8.7% for the specific BMP of grass
396 silage after 8 days of air-exposure at silo opening. Likewise, Herrmann *et al.* [19]
397 found, in some cases, a decrease between 5-19% of methane yields taking into account
398 storage losses for whole crop maize silages, after 7 days exposure to air. In such cases,

399 the use of additives to enhance aerobic stability can be an effective action to prevent
400 energy losses at feed-out [19,21,53–55].

401 It is thus essential to avoid conditions that may lead to aerobic deterioration at any
402 stage of ensiling. Appropriate silo construction, prompt sealing and high feed-out rates
403 are thus good management practices required to prevent energy losses due to aerobic
404 spoiling of the organic matter [4,19].

405 2.2.2. Density

406 Packing density of silage is considered as a crucial parameter for dry matter
407 preservation, due to its influence on organic matter oxidation. A higher density is
408 associated to a lower porosity, lower amounts of air, and to slower oxygen flows in
409 silage, thereby reducing losses due to aerobic spoiling [56]. These statement have been
410 confirmed by different authors, with favorable results for higher densities. Indeed,
411 Ruppel [57] worked with alfalfa silage for 180 days and found a relation between the
412 density and the silage total solid losses, presented in Table 3. Zheng *et al.* [58] tested
413 silage packing densities of 460, 690 and 920 kg/m³ and showed that higher ones had a
414 positive effect on lactic acid production and enzymatic digestibility for sugar beet pulp
415 inoculated with LAB.

416 Similarly, Zheng *et al.* [59], used 480, 720 and 960 kg/m³ packing densities for beet
417 pulp ensiling. They concluded that the higher density provided better silage quality,
418 mainly due to higher lactic acid production.

419 Neureiter *et al.* [15] investigated not compressed and not tightly sealed whole crop
420 maize silage, and obtained higher pH, higher weight losses and lower lactic acid content
421 than compressed biomass during 44 and 119 days. The impact of these storage

422 conditions on biogas production was only important at 119 days, from which lower
423 density silage were presenting 20% less BMP than control tests.

424 Another advantage shared by different authors is that high bulk densities for ensiling
425 allow greater silo capacity, which subsequently leads to lower unit costs of storage
426 [56,60]. Conversely, part of the authors referred above also stated that, in certain cases,
427 a high silage density can be expensive, for instance, due to the requirement for heavy
428 compaction equipment or prolonged compaction time [61]. Thus, an economic analysis
429 must be essential to clarify the impact of packing density on consolidation and storage
430 costs. Altogether, until further notice, higher packing densities may be advised to obtain
431 a better preservation of biomass and BMP.

432 Finally, it has to be noticed that silage density may influence effluent production.
433 Thus, tests to assess the maximum density of water retention are encouraged to be
434 performed before storage, in order to avoid leachate formation.

435 2.3. *Storage duration*

436 Storage duration is a quite variable parameter, which may depends on the seasonality
437 of some crops and wastes, or on specific feeding requirements of downstream anaerobic
438 digester. For these reasons, ensiling duration is often defined by taking into account
439 these supply chain restrictions and not due to its potential impact on the preservation of
440 the biomass. However, ensiling time can often affect silage BMP as demonstrated, for
441 instance, by Neureiter *et al.* [15], who tested whole crop maize for 44 and 119 days
442 storage. They obtained good quality silages in both cases; despite a slight increase of
443 weight losses between 44 and 119 days. The pH remained stable and a significant
444 increase of BMP was observed. After 44 days, silage BMP was 17% lower than the
445 fresh whole crop maize one, but after the more prolonged duration it was 22% above the

446 original one. Likewise, Herrmann *et al.* [19] ensiled whole crop maize for 49 and
447 90 days and observed a 3.5% average increase on BMP for the more extended storage.
448 In the same way, increased methane yields for prolonged storage duration of sugar beet
449 pulps has been observed by Lehtomäki [10], while studying ensiling for 90 and
450 180 days.

451 Comparatively, a study for whole crop maize, sorghum hybrid, forage rye and
452 triticale, during 10, 90, 180 and 365 days, showed an apparent positive effect of
453 prolonged storage on methane yield for some crops [7]. However, differences between
454 fresh and final silage methane yield were never superior to 7% for any case (close to the
455 limit of accuracy of BMP tests). The higher BMP over time is usually attributed to
456 higher bio-accessibility of plant cell wall constituents, which in certain cases can
457 compensate the losses in terms of dry matter [11]. However, in Herrmann *et al.* [7]
458 study, original biomass was already fairly accessible, as evidenced by the original low
459 lignin range (2.9-6.7%) and by the hemicellulose degradation during storage. Besides
460 that, no noticeable reduction of lignin content was recorded.

461 In counterparts to these positive results, Pakarinen *et al.* [11] showed that for a
462 maximum 180 days of ensiling, either with original grass/ryegrass, or with wilting
463 periods, or with addition of starters, BMP decreased with storage duration. For grass
464 ensiling, Lehtomäki [10] also showed an inversely proportional relation between the
465 storage time and the BMP. In both studies, cumulative losses in methane yield of more
466 than 30% after 180 days were observed.

467 Therefore, two main conclusions may be highlighted: i) there is a real influence of
468 ensiling duration on the resulting methane yields and; ii) it will be mainly the chemical
469 properties of the feedstock used that will define a positive or negative impact on it.

470 Nevertheless, no coherent correlation between the feedstock source and the impact of
471 the storage time on silage BMP can be proposed, as only a limited number of substrates
472 has been investigated in the literature. Thus, future work on testing the ensiling duration
473 impact on a wider range of substrates is encouraged. This may allow the optimization of
474 the storage duration depending on the chosen feedstock, *i.e.*, to practice prolonged
475 storage for silages that increase their BMP along the ensiling and *vice versa*.

476 In brief, much can still be done concerning storage time optimization depending on
477 the substrate used. However, for now ensiling should be considered as only a material
478 preservation technique and, hence, storage time should be restricted to the minimum
479 possible. Exceptions as for sugar beet pulp or whole crop maize, in which prolonged
480 ensiling has been proved to be advantageous, should be taken in consideration.

481 2.4. *Temperature*

482 Regardless of the location chosen for ensiling, large temperature variations are
483 expected since the silo is usually submitted to ambient temperature. Taking the example
484 of temperate climates, as the Mediterranean one, minimum and maximum temperatures
485 of 0 to 40°C might be attained, respectively. Eventually, these variations can have a real
486 impact on the ensiling course. As a consequence, bacterial growth rates will be different
487 among the microorganisms present in the system. Among this range, biodegradation
488 rates are known to increase with temperature, in part due to the strong impact of
489 temperatures on the hydrolysis of complex organic compounds [62].

490 Concerning ensiling, many studies have been performed at constant temperature at
491 laboratory scale. However, some authors published results that attest the existence of an
492 impact of temperature for ensiling similar to AD. One of these works was performed by
493 Kim and Adesogan [63], who studied corn storage at 20 and 40°C for 82 days. They

494 showed that higher pH and NH₃-N concentration, residual WSC and lower lactic to
495 acetic acid ratio were obtained for silages ensiled at the highest temperature. All this
496 data suggest that, at 40°C, fermentation was more extensive, reflecting reduced silage
497 quality at the end.

498 Similar conclusions were obtained by Garcia *et al.* [49], who worked with higher
499 temperatures for alfalfa silage. While comparing 38 and 65°C storage, they suggested
500 that higher temperature had a less restricted fermentation and were more susceptible to
501 heat damage in just 21 days of ensiling.

502 Moreover, the same effects were observed for poorly ensilable biomass by Browne *et*
503 *al.* [64], while studying dairy cow manure storage for 26 weeks at 9 and 20°C. They
504 reported a constant higher TS and VS content, and lower pH for ensiling at 9°C.
505 Furthermore, after 26 weeks of storage at 20°C, they verified a subsequent biogas
506 production of around 32% of that stored at 9°C.

507 Thus, according to anaerobic fermentation principles and to most of the results
508 observed in the bibliography, it is necessary to maintain relatively low temperatures in
509 order to have a more restricted fermentation and preserve the silage. However, in some
510 cases, a certain level of temperature may be necessary to overcome the initial barrier of
511 hydrolysis in order to obtain an efficient lactic fermentation.

512 This latter assumption is made taking into account the results published by Lehtomäki
513 [10], who suggested that very low ensiling temperatures do not necessarily lead to
514 subsequent higher methane yields. Depending on the feedstock and on the type of
515 additives used, different effects of temperature on BMP were obtained after 6 months
516 storage. In certain cases, higher BMP by fresh weight were obtained for 5°C storage,
517 but the same was also verified for 20°C ensiling under other conditions. Therefore, tests

518 on the effect of low temperatures on ensiling with different feedstock may be advised.
519 This might allow to verify the existence or not of a hydrolysis barrier at low
520 temperatures that may be unfavorable for the expression of the energy content of some
521 crops during storage.

522 Despite lower ensiling temperatures appear to favor in most cases the preservation of
523 the BMP, the regulation of temperature in a silo is not feasible from an economic point
524 of view. In fact, expenses related to energy spending, maintenance and equipment
525 should probably overcome the benefits from monitoring silo temperature. Even though,
526 some management practices could be encouraged to prevent silage damaging from
527 extreme conditions. For instance, heat transfer by thermal radiation and long duration
528 storage under extremely hot environments may be avoided whenever possible.

529 2.5. *Additives*

530 So as to control the course of ensiling, additives began to be used to about a hundred
531 years ago for forage production purposes and they have become increasingly
532 widespread since then. Their first known utilization was in the early twentieth century,
533 through the addition of molasses [8]. In this primordial utilization, the aim was to
534 ensure silage preservation through LAB fermentation enhancement. Also during this
535 period, the utilization of mineral acids for fast acidification of crops began to be
536 practiced. With the evolution of research, diversification of additives increased, as
537 several groups of fermentation stimulants, aerobic deterioration inhibitors, nutrients and
538 absorbents began to be used [8]. Given the general approval of the benefits of additives
539 by farmers and the specificity of each silage, great interest was attributed to their
540 production and diversification. Therefore, currently a wide range of biological and
541 chemical silage additives is commercially available [13].

542 It is well recognized that the use of additives arose for the forage production and
543 innovations in the field were thus mainly oriented towards the production of quality
544 animal feed [15]. Nevertheless, several commercial products, among the categories
545 presented in Table 4, can be potentially used to enhance the properties of biogas plant
546 feedstock. From this list, two groups are highlighted in the work done by the researchers
547 with a view to biogas production: fermentation stimulants and inhibitors.

548 2.5.1. Fermentation stimulants

549 Fermentation stimulants are the most commonly used additives for agricultural
550 ensiling, as their benefits for the preservation of crops are generally recognized and they
551 are usually non-corrosive and safe to handle. Maybe for this reason, most studies on the
552 use of additives in silage for bioenergy production aims this kind of products. Among
553 the best known stimulants, various types of enzymes, carbohydrate sources or LAB
554 inoculants should be listed. Their application affects the preservation process in
555 different ways and, therefore, they are often combined into a commercial mix, so that
556 their modes of action could complement each other.

557 On the one hand, both carbohydrate sources and enzymes increase the content of
558 biodegradable material directly and indirectly, respectively. Carbohydrate sources, as
559 molasses and sugars, will introduce additional substrate for LAB, whereas enzymes,
560 such as cellulase or xylanase, will produce additional fermentable sugars from the cell
561 wall constituents [8].

562 In contrast, the addition of LAB inoculants will increase the lactic acid bacterial
563 population of silage. With a higher count of LAB, the lactic acidification in the initial
564 stage of preservation is expected to be faster [23]. Depending on the fermentation
565 pathways, LAB can be labeled as homo or heterofermentative. Homofermentative

566 strains convert hexose into lactic acid *via* the Embden-Meyerhof pathway, being at the
567 same time unable to ferment pentoses [65]. In opposite, heterofermentative LAB are
568 able to ferment both hexoses and pentoses, producing lactic acid but also acetic acid,
569 ethanol and carbon dioxide. Since acetic acid is weaker than lactate and since side
570 products are formed, lower dry matter and energy gain should be expected for
571 heterofermentative bacteria addition. Consequently, most of the commercial starters
572 consist of homofermentative LAB. However, heterofermentative bacteria are not
573 discarded since they provide great aerobic stability and are expected to limit BMP
574 losses after feed-out.

575 A summary of the results found in the literature for fermentation stimulants impact on
576 biomass methane yield is shown in Table 5. Both positive and negative impacts were
577 obtained using stimulants additives. The effects seem to depend on the type of crop
578 used. In fact, when testing additives for grass [10,11,21], only positive impact on the
579 BMP were obtained, regardless of the stimulant used. In opposition, negative influence
580 was obtained for crops such as amaranth or sugar beet tops [10,18]. Another interesting
581 case is that of whole crop maize: although representing almost half of the published data
582 on the subject [7,15,16,18], it shows inconclusive results regarding the influence of
583 stimulants on BMP. Beyond the results presented in Table 5 for whole crop maize,
584 Herrmann *et al.* [19] also studied this biomass and observed low effects of stimulants on
585 its BMP during ensiling.

586 The data from grass and whole crop maize are in agreement with the statements made
587 by Kalač [23] about the use of additives. The author claims that additives are not
588 necessary for crops ensiling with a high content of fermentable carbohydrates, such as
589 maize or wilted tetraploid ryegrasses; but that can be useful for other crops, such as

590 unwilted alfalfa, clovers or some grasses. Therefore, it can be suggested that, if a better
591 BMP preservation is reached by using stimulants for grass silage (in which, for instance,
592 WSC content will strongly depend on the stage of growth or species used), a more
593 pronounced effect should be expected for poorly ensilable crops. In other words, while
594 ensiling biomass with low WSC content, low LAB, high moisture and high BC, the use
595 of stimulants may help the fermentation to be carried out more efficiently.

596 Finally, beyond the primary effect of the feedstock used, the kind of stimulant also
597 has a significant impact on the course of silage. For example, in the case of whole crop
598 maize, a relative variation of more than 20% in the methane yield may occur by
599 changing the type of stimulant [15,16]. However, due to the wide variety of available
600 stimulants and insufficient amount of results in the literature, it seems impossible to
601 develop a consistent comparison between fermentation stimulants.

602 2.5.2. Fermentation inhibitors

603 The purpose of using inhibitors for ensiling is to preserve, as much as possible, the
604 original material, preventing its degradation in undesirable compounds and
605 subsequently, minimizing dry matter and energy losses. Their mode of action involves
606 the inhibition of the biological activity of the degrading microorganisms by lowering
607 the pH.

608 Frequently used as additives in the last century by farmers in Europe, mainly through
609 Virtanen's process [8], fermentation inhibitors are mainly applied in the form of mineral
610 and organic acids. Within these groups of compounds, sulfuric and formic acid are the
611 most commonly used, respectively [66]. As for stimulants, fermentation inhibitors are
612 often marketed as a mix of compounds. In this particular case, it is usual to combine
613 acids with fermentation and aerobic deterioration inhibition characteristics [13].

614 Despite being corrosive and difficult to handle, the use of acid in silage remains
615 justified by other factors. One is the fact that these additives are more likely than LAB
616 to restrict proteolysis, due to instantaneous lowering of silage pH. Furthermore, their
617 effectiveness is more reliable since, unlike biological additives, it is not based on the
618 activity of living microorganisms. This means that the content of carbohydrate sources
619 may become unimportant and so, clostridial secondary fermentation can be more easily
620 predicted and avoided [66].

621 Regarding the comparison between the types of inhibitors, the use of organic acids
622 appears to be the most suitable option for biogas production purposes. The fact that it
623 does not introduce undesirable chemical elements in silage, suggests that these additives
624 interfere less in the organic matter degradation and in the formation of other side
625 products. On the opposite, if a mineral acid like sulfuric acid is used, the sulfur fraction
626 in the silage will increase, which will then logically lead to biogas production during
627 AD with a higher content of undesirable H₂S. It is likely for these reasons that
628 researchers have preferred to study the impact of formic acid, as a model of organic
629 acids, than sulfuric acid or other mineral acid.

630 Despite being the most studied inhibitor, few and discordant works on the impact of
631 formic acid on BMP was noticed. For instance, Pakarinen *et al.* [12] worked with forage
632 maize, hemp and fairy bean, using formic acid with concentrations of 0.5% and 1% w/w
633 in ensiling and found that acidification not only preserved the original WSC, but
634 increased their amount compared with the fresh crop. However, in comparison with
635 control tests, formic acid addition resulted in silages with lower BMP in almost all
636 experiments. According to the authors, the reasons for this decrease were not clear, as
637 insignificant changes in chemical composition of biomass for 4 and 8 months were

638 found. The same type of results were obtained by Lehtomäki [10], while using formic
639 acid on sugar beet tops at 0.5% v/w content.

640 On the opposite, Lehtomäki [10] and McEniry *et al.* [21] observed coherent higher
641 methane yields using similar concentrations of formic acid on grass. Furthermore, the
642 results of Lehtomäki [10] showed that there was a 30% relative increase of the BMP
643 immediately after addition of formic acid to grass. As it is unlikely that this increase is
644 due to formic acid degradation given its low concentration, it is possible that acid
645 addition may have led to a greater accessibility of the plant cell wall constituents. In
646 fact, addition of dilute or concentrated acid to biomass is used as pre-treatment before
647 AD or enzymatic hydrolysis to render carbohydrates sources more accessible. Several
648 authors suggest that this may be caused by enhanced hydrolysis of biomass [31,67],
649 increase of accessible surface area and lignin structure alteration [68]. Consequently, the
650 use of formic acid as additive appears to be appropriate for, at least, some types of grass
651 silage. This additive should be even more interesting for poorly ensilable biomass,
652 given the boost it can give in terms of accessibility of the material for AD and in
653 preservation (by instant pH drop), of crops with low WSC content. In order to sustain
654 these suggestions, more studies on this topic are advised in the future.

655 **3. Conclusions**

656 Ensiling is a suitable and promising technique for conservation of biomass for
657 methane production purposes. Among its critical parameters, biochemical
658 characteristics of feedstock should be considered in the first place to the success of the
659 storage process. Besides governing the course of ensiling, it will also play an important
660 role on the impact of other parameters during storage. In brief, good silage preservation

661 will occur at relatively low moisture contents, high accessible carbohydrates content,
662 and low buffering capacity.

663 Combination of reduced particle size and high packing density is also advised to
664 minimize methane potential losses during storage. Moreover, appropriate silo
665 construction, prompt sealing and high feed-out rates are required to prevent aerobic
666 spoiling of silage.

667 Search for efficient additives has been one of the main priorities for ensiling
668 researchers in recent years, with focus on fermentation stimulants and inhibitors.
669 However, until now, additives appear only to have a positive effect on the conservation
670 of methane potential of grass silage. This effect should be more pronounced for poorly
671 ensilable biomass.

672 Finally, some evidences suggest that, under specific conditions, ensiling may
673 increase methane potential even taking into account storage losses. One of the possible
674 explanations is that gains in biochemical accessibility may overcome organic matter
675 losses during storage. Next steps in storage research should confirm the use of ensiling
676 as a pre-treatment for some anaerobic digestion feedstock.

677 **Acknowledgements**

678 Rúben Teixeira Franco held a doctoral fellowship from the Rhône-Alpes region
679 (France). This work has been undertaken within the SAM project (*Stockage Avant*
680 *Méthanisation* – Storage Before AD) funded by ADEME (French Agency for Energy
681 and Environment).

682 **References**

683 [1] P. McKendry, Energy production from biomass (part 1): overview of biomass,
684 *Bioresour. Technol.* 83 (2002) 37–46. doi:Pii S0960-8524(01)00118-3\rDoi

- 685 10.1016/S0960-8524(01)00118-3.
- 686 [2] EurObserv'ER, Biogas barometer, Paris, 2014. [http://www.energies-
687 renouvelables.org/observ-er/stat_baro/observ/baro224_Biogas_en.pdf](http://www.energies-
687 renouvelables.org/observ-er/stat_baro/observ/baro224_Biogas_en.pdf).
- 688 [3] European Biogas Association, Biogas. Simply the best, Brussels, 2011.
689 [http://european-biogas.eu/wp-content/uploads/files/2013/10/EBA-brochure-
690 2011.pdf](http://european-biogas.eu/wp-content/uploads/files/2013/10/EBA-brochure-
690 2011.pdf).
- 691 [4] R. Egg, C. Coble, C. Engler, D. Lewis, Feedstock storage, handling and
692 processing, *Biomass and Bioenergy*. 5 (1993).
693 <http://www.sciencedirect.com/science/article/pii/096195349390009S> (accessed
694 January 28, 2015).
- 695 [5] C. Coble, R. Egg, Dry matter losses during hay production and storage of sweet
696 sorghum used for methane production, *Biomass*. 14 (1987) 209–217.
697 <http://www.sciencedirect.com/science/article/pii/0144456587900485> (accessed
698 January 28, 2015).
- 699 [6] Z.G. Weinberg, G. Ashbell, Engineering aspects of ensiling, *Biochem. Eng. J.* 13
700 (2003) 181–188.
- 701 [7] C. Herrmann, M. Heiermann, C. Idler, Effects of ensiling, silage additives and
702 storage period on methane formation of biogas crops, *Bioresour. Technol.* 102
703 (2011) 5153–5161. doi:10.1016/j.biortech.2011.01.012.
- 704 [8] P. McDonald, A. Henderson, S.J. Heron, *The Biochemistry of Silage*, Second
705 Edi, Chalcombe Publications, Marlow, Bucks, 1991.
- 706 [9] E. Zimmer, Efficient silage systems, in: C. Thomas (Ed.), *Forage Conserv.* 80's,
707 British Grassland Society, Brighton, UK, 1980: pp. 186–197.
- 708 [10] A. Lehtomäki, *Biogas Production from Energy Crops and Crop Residues*, (2006)

- 709 1–91.
- 710 [11] O. Pakarinen, A. Lehtoma, S. Rissanen, J. Rintala, Storing energy crops for
711 methane production: Effects of solids content and biological additive, *Bioresour.*
712 *Technol.* 99 (2008) 7074–7082. doi:10.1016/j.biortech.2008.01.007.
- 713 [12] A. Pakarinen, P. Maijala, S. Jaakkola, F.L. Stoddard, M. Kymäläinen, L. Viikari,
714 Evaluation of preservation methods for improving biogas production and
715 enzymatic conversion yields of annual crops, *Biotechnol. Biofuels.* 4 (2011) 20.
716 doi:10.1186/1754-6834-4-20.
- 717 [13] S.J.W.H.O. Elferink, F. Driehuis, J.C. Gottschal, S.F. Spoelstra, Silage
718 fermentation processes and their manipulation, in: *FAO Electronic Conf. Trop.*
719 *Silage*, 1999: pp. 1–28.
720 [http://www.fao.org/WAICENT/FAOINFO/AGRICULT/AGP/AGPC/gp/SILAG](http://www.fao.org/WAICENT/FAOINFO/AGRICULT/AGP/AGPC/gp/SILAGE/PDF/Paper2.pdf)
721 [E/PDF/Paper2.pdf](http://www.fao.org/WAICENT/FAOINFO/AGRICULT/AGP/AGPC/gp/SILAGE/PDF/Paper2.pdf).
- 722 [14] H. Danner, M. Holzer, E. Mayrhuber, R. Braun, Acetic acid increases stability of
723 silage under aerobic conditions, *Appl. Environ. Microbiol.* 69 (2003) 562–567.
724 doi:10.1128/AEM.69.1.562.
- 725 [15] M. Neureiter, J.T. Pereira, C.P. Lopez, H. Pichler, R. Kirchmayr, Effect of silage
726 preparation on methane yields from whole crop maize silages, in: H. Hartmann,
727 B. Ahring (Eds.), *4th Int. Symp. Anaerob. Dig. Solid Waste*, Copenhagen, 2006.
- 728 [16] H. Vervaeren, K. Hostyn, G. Ghekiere, B. Willems, Biological ensilage additives
729 as pretreatment for maize to increase the biogas production, *Renew. Energy.* 35
730 (2010) 2089–2093. doi:10.1016/j.renene.2010.02.010.
- 731 [17] E. Kreuger, I. Nges, L. Björnsson, Ensiling of crops for biogas production:
732 effects on methane yield and total solids determination, *Biotechnol. Biofuels.* 4

- 733 (2011) 44. doi:10.1186/1754-6834-4-44.
- 734 [18] N.L. Haag, H. Nägele, T. Fritz, H. Oechsner, Effects of ensiling treatments on
735 lactic acid production and supplementary methane formation of maize and
736 amaranth – An advanced green biorefining approach, *Bioresour. Technol.* 178
737 (2015) 217–225. doi:10.1016/j.biortech.2014.08.048.
- 738 [19] C. Herrmann, C. Idler, M. Heiermann, Improving aerobic stability and biogas
739 production of maize silage using silage additives, *Bioresour. Technol.* 197 (2015)
740 393–403. doi:10.1016/j.biortech.2015.08.114.
- 741 [20] L.E.R. Dawson, C.P. Ferris, R.W.J. Steen, F.J. Gordon, D.J. Kilpatrick, The
742 effects of wilting grass before ensiling on silage intake, *Grass Forage Sci.* (1999)
743 237–247.
744 [http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:The+effects+of](http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:The+effects+of+f+wilting+grass+before+ensiling+on+silage+intake#0)
745 [f+wilting+grass+before+ensiling+on+silage+intake#0](http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:The+effects+of+f+wilting+grass+before+ensiling+on+silage+intake#0) (accessed January 28,
746 2015).
- 747 [21] J. McEniry, E. Allen, J.D. Murphy, P. O’Kiely, Grass for biogas production: The
748 impact of silage fermentation characteristics on methane yield in two contrasting
749 biomethane potential test systems, *Renew. Energy.* 63 (2014) 524–530.
750 doi:10.1016/j.renene.2013.09.052.
- 751 [22] C. Herrmann, A. Prochnow, M. Heiermann, C. Idler, Biomass from landscape
752 management of grassland used for biogas production: effects of harvest date and
753 silage additives on feedstock quality and methane yield, *Grass Forage Sci.* (2013)
754 549–566. doi:10.1111/gfs.12086.
- 755 [23] P. Kalač, The required characteristics of ensiled crops used as a feedstock for
756 biogas production: a review, *J. Agrobiol.* 28 (2011) 85–96. doi:10.2478/s10146-

- 757 011-0010-y.
- 758 [24] S. Amer, F. Hassanat, R. Berthiaume, P. Seguin, A.F. Mustafa, Effects of water
759 soluble carbohydrate content on ensiling characteristics, chemical composition
760 and in vitro gas production of forage millet and forage sorghum, *Anim. Feed Sci.*
761 *Technol.* 177 (2012) 23–29. doi:10.1016/j.anifeedsci.2012.07.024.
- 762 [25] Y. Chen, R. Sharma-Shivappa, C. Chen, Ensiling agricultural residues for
763 bioethanol production, *Appl. Biochem. Biotechnol.* (2007) 80–92.
764 doi:10.1007/s12010-007-0030-7.
- 765 [26] S. Menardo, P. Balsari, E. Tabacco, G. Borreani, Effect of Conservation Time
766 and the Addition of Lactic Acid Bacteria on the Biogas and Methane Production
767 of Corn Stalk Silage, *Bioenergy Res.* 8 (2015) 1810–1823. doi:10.1007/s12155-
768 015-9637-7.
- 769 [27] G. Krishna, S. Hun, Effects of chemical compositions and ensiling on the biogas
770 productivity and degradation rates of agricultural and food processing by-
771 products, *Bioresour. Technol.* 142 (2013) 553–561.
772 doi:10.1016/j.biortech.2013.05.018.
- 773 [28] J. Zubr, Methanogenic Fermentation of Fresh and Ensiled Plant Materials,
774 *Biomass.* 11 (1986) 159–171.
- 775 [29] A. Lehtomäki, T. Viinikainen, J. Rintala, Screening boreal energy crops and crop
776 residues for methane biofuel production, *Biomass and Bioenergy.* 32 (2008) 541–
777 550. doi:10.1016/j.biombioe.2007.11.013.
- 778 [30] P. Weiland, Biogas production: current state and perspectives, *Appl. Microbiol.*
779 *Biotechnol.* 85 (2010) 849–60. doi:10.1007/s00253-009-2246-7.
- 780 [31] V.B. Agbor, N. Cicek, R. Sparling, A. Berlin, D.B. Levin, Biomass pretreatment:

- 781 fundamentals toward application, *Biotechnol. Adv.* 29 (2011) 675–685.
782 doi:10.1016/j.biotechadv.2011.05.005.
- 783 [32] C. Herrmann, M. Heiermann, C. Idler, A. Prochnow, Particle size reduction
784 during harvesting of crop feedstock for biogas production I: effects on ensiling
785 process and methane yields, *Bioenergy Res.* 5 (2012) 926–936.
786 doi:10.1007/s12155-012-9206-2.
- 787 [33] C.H. Gordon, H.G. Wiseman, J.C. Derbyshire, W.C. Jacobson, Effect on silage
788 of chopping and bruising the forage, *J. Dairy Sci.* (1958) 1–2.
789 doi:10.3168/jds.S0022-0302(59)90749-0.
- 790 [34] S.H. Mohd-Setapar, N. Abd-Talib, R. Aziz, Review on Crucial Parameters of
791 Silage Quality, *APCBEE Procedia.* 3 (2012) 99–103.
792 doi:10.1016/j.apcbee.2012.06.053.
- 793 [35] C. Herrmann, A. Prochnow, M. Heiermann, C. Idler, Particle size reduction
794 during harvesting of crop feedstock for biogas production II: effects on energy
795 balance, greenhouse gas emissions and profitability, *Bioenergy Res.* 5 (2012)
796 937–948. doi:10.1007/s12155-012-9207-1.
- 797 [36] G. Borreani, A. Revello, S. Colombini, M. Odoardi, R. Paoletti, E. Tabacco,
798 Fermentative profiles of field pea (*Pisum sativum*), faba bean (*Vicia faba*) and
799 white lupin (*Lupinus albus*) silages as affected by wilting and inoculation, *Anim.*
800 *Feed Sci. Technol.* 151 (2009) 316–323. doi:10.1016/j.anifeedsci.2009.01.020.
- 801 [37] M.J. Nash, Partial wilting of grass crops for silage. 2. Experimental Silages,
802 *Grass Forage Sci.* 14 (1953) 107–116.
- 803 [38] S.A.Z. Mahmoud, M.M. Zaki, E.A. Saleh, Factors affecting the microbial and
804 chemical composition of silage: IV. Effect of wilting on maize silage,

- 805 Zentralblatt Fuer Bakteriologie, Parasitenkunde, Infekt. Und Hyg. Zweite
806 Naturwissenschaftliche Abteilung Mikrobiologie, Der Landwirtschaft, Der Technol.
807 Und Des Umweltschutzes. 134 (1979) 34–39. doi:10.1016/S0323-
808 6056(79)80061-0.
- 809 [39] J.M. Wilkinson, A laboratory evaluation of comfrey (*Symphytum officinale* L.)
810 as a forage crop for ensilage, *Anim. Feed Sci. Technol.* 104 (2003) 227–233.
- 811 [40] K.J. Han, K.A. Albrecht, R.E. Muck, D.A. Kim, Moisture effect on Fermentation
812 Characteristics of Cup-Plant Silage, *Asian-Australasian J. Anim. Sci.* 13 (2000)
813 636–640.
- 814 [41] P. Haigh, J. Parker, Effect of silage additives and wilting on silage fermentation,
815 digestibility and intake, and on liveweight change of young cattle, *Grass Forage*
816 *Sci.* 40 (1985) 429–436. [http://onlinelibrary.wiley.com/doi/10.1111/j.1365-](http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2494.1985.tb01774.x/full)
817 [2494.1985.tb01774.x/full](http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2494.1985.tb01774.x/full) (accessed January 28, 2015).
- 818 [42] B. Bastiman, Factors affecting silage effluent production, *Exp. Husbandry*. 31 (1976)
819 40–46.
- 820 [43] A. Sutter, Problem of waste effluent from silage making and feeding of silage,
821 *Eur. Product. Agency Organ. Eur. Econ. Co-Operation*. 307 (1957) 74–82.
- 822 [44] E. Zimmer, The influence of pre-wilting on nutrient losses, particularly on the
823 formation of gas, *Tagungsberichte Der Dtsch. Akad. Fur Landwirtschaft-*
824 *Swissenshafen Zu Berlin*. 92 (1967) 37–47.
- 825 [45] D.I.H. Jones, R. Jones, The effect of crop characteristics and ensiling
826 methodology on grass silage effluent production, *J. Agric. Eng. Res.* 60 (1995)
827 73–81.
- 828 [46] C.M. Carpintero, A.R. Henderson, P. McDonald, The effect of some pre-

- 829 treatments on proteolysis during the ensiling of herbage, *Grass Forage Sci.* 34
830 (1979) 311–315.
- 831 [47] M. Gansberger, L.F.R. Montgomery, P. Liebhard, Botanical characteristics, crop
832 management and potential of *Silphium perfoliatum* L. as a renewable resource for
833 biogas production: A review, *Ind. Crop. Prod.* (2014) 1–11.
834 doi:10.1016/j.indcrop.2014.09.047.
- 835 [48] M.K. Woolford, The detrimental effects of air on silage. A review, *J. Appl.*
836 *Bacteriol.* 68 (1990) 101–116. doi:10.1111/j.1365-2672.1990.tb02554.x.
- 837 [49] A. Garcia, W. Olson, D. Otterby, J.G. Linn, W.P. Hansen, Effects of temperature,
838 moisture, and aeration on fermentation of alfalfa silage, *J. Dairy Sci.* (1989) 93–
839 103. doi:10.3168/jds.S0022-0302(89)79084-6.
- 840 [50] C.W. Langston, H.G. Wiseman, C.H. Gordon, W.C. Jacobson, C.G. Melin, L. a.
841 Moore, et al., Chemical and Bacteriological Changes in Grass Silage during the
842 Early Stages of Fermentation I. Chemical Changes, *J. Dairy Sci.* 45 (1962) 396–
843 402. doi:10.3168/jds.S0022-0302(62)89404-1.
- 844 [51] M.A. Sprague, Oxygen disappearance in alfalfa silage, in: *Proc. 12th Int. Grassl.*
845 *Congr., Moscow, 1974: pp. 651–656.*
- 846 [52] A. Prochnow, M. Heiermann, M. Plöchl, B. Linke, C. Idler, T. Amon, et al.,
847 Bioenergy from permanent grassland--a review: 1. Biogas, *Bioresour. Technol.*
848 100 (2009) 4931–44. doi:10.1016/j.biortech.2009.05.070.
- 849 [53] J.M. Wilkinson, D.R. Davies, The aerobic stability of silage: key findings and
850 recent developments, *Grass Forage Sci.* 68 (2013) 1–19. doi:10.1111/j.1365-
851 2494.2012.00891.x.
- 852 [54] M. Plöchl, H. Zacharias, C. Herrmann, M. Heiermann, a. Prochnow, Influence of

853 silage additives on methane yield and economic performance of selected
854 feedstock, CIGR Ejournal. XI (2009) 1–16.

855 [55] H. Nussbaum, Effects of silage additives based on homo- or heterofermentative
856 lactic acid bacteria on methane yields in the biogas processing, in: XVI Int.
857 Silage Conf., 2012: pp. 452–453.

858 [56] R. Muck, B. Holmes, Factors affecting bunker silo densities, Madison, 1999.
859 [http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.168.3068&rep=rep1&](http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.168.3068&rep=rep1&type=pdf)
860 [type=pdf](http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.168.3068&rep=rep1&type=pdf) (accessed January 28, 2015).

861 [57] K.A. Ruppel, Effect of bunker silo management on hay crop nutrient
862 management. M.S. Thesis, Cornell University, Ithaca, NY, 1992.

863 [58] Y. Zheng, C. Yu, Y. Cheng, R. Zhang, B. Jenkins, J.S. Vandergheynst, Effects of
864 ensilage on storage and enzymatic degradability of sugar beet pulp, Bioresour.
865 Technol. 102 (2011) 1489–1495. doi:10.1016/j.biortech.2010.09.105.

866 [59] Y. Zheng, C. Yu, Y. Cheng, C. Lee, C.W. Simmons, T.M. Dooley, et al.,
867 Integrating sugar beet pulp storage, hydrolysis and fermentation for fuel ethanol
868 production, Appl. Energy. 93 (2012) 168–175.
869 doi:10.1016/j.apenergy.2011.12.084.

870 [60] L.D. Amours, P. Savoie, Density profile of corn silage in bunker silos, Can.
871 Biosyst. Eng. 47 (2005) 21–28.

872 [61] R.E. Muck, P. Savoie, B.J. Holmes, Laboratory assessment of bunker silo
873 density. Part I: Alfalfa and Grass, Appl. Eng. Agric. 20 (2004) 157–164.

874 [62] G. Lettinga, S. Rebac, G. Zeeman, Challenge of psychrophilic anaerobic
875 wastewater treatment, TRENDS Biotechnol. 19 (2001) 363–370.

876 [63] S. Kim, A. Adesogan, Influence of ensiling temperature, simulated rainfall, and

877 delayed sealing on fermentation characteristics and aerobic stability of corn
878 silage, *J. Dairy Sci.* 89 (2006) 3122–3132. doi:10.3168/jds.S0022-
879 0302(06)72586-3.

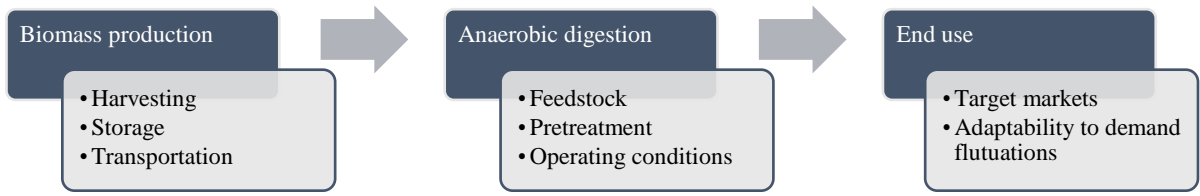
880 [64] J.D. Browne, S.R. Gilkinson, J.P. Frost, The effects of storage time and
881 temperature on biogas production from dairy cow slurry, *Biosyst. Eng.* 129
882 (2014) 48–56. doi:10.1016/j.biosystemseng.2014.09.008.

883 [65] M. Holzer, E. Mayrhuber, H. Danner, R. Braun, The role of *Lactobacillus*
884 *buchneri* in forage preservation, *TRENDS Biotechnol.* 21 (2003) 282–287.
885 doi:10.1016/S0167-7799(03)00106-9.

886 [66] R. Muck, Factors influencing silage quality and their implications for
887 management, *J. Dairy Sci.* 71 (1988) 2992–3002. doi:10.3168/jds.S0022-
888 0302(88)79897-5.

889 [67] A.T.W.M. Hendriks, G. Zeeman, Pretreatments to enhance the digestibility of
890 lignocellulosic biomass, *Bioresour. Technol.* 100 (2009) 10–18.
891 doi:10.1016/j.biortech.2008.05.027.

892 [68] N. Mosier, C. Wyman, B. Dale, R. Elander, Y.Y. Lee, M. Holtzapple, et al.,
893 Features of promising technologies for pretreatment of lignocellulosic biomass,
894 *Bioresour. Technol.* 96 (2005) 673–686. doi:10.1016/j.biortech.2004.06.025.
895
896



897

898 Figure 1 – Simplified supply chain of biogas production with examples of optimization

899 points.

900

901

902

903

904

905

906

907

908

909

910

911

912

913

914

915

916

917

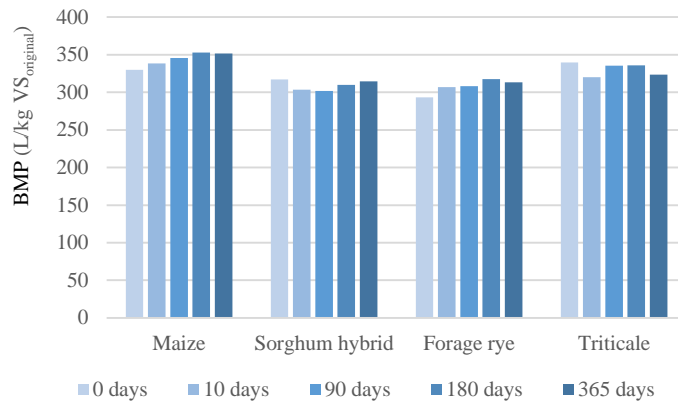
918

919

920

921

922



923

924 Figure 2 – Methane formation of whole crop maize, sorghum hybrid, forage rye and
 925 triticale influenced by storage duration. BMP is based on the organic matter of fresh
 926 biomass, which takes into account VS losses during storage (adapted from Herrmann *et*
 927 *al.* [7]).

928

929

930

931

932

933

934

935

936

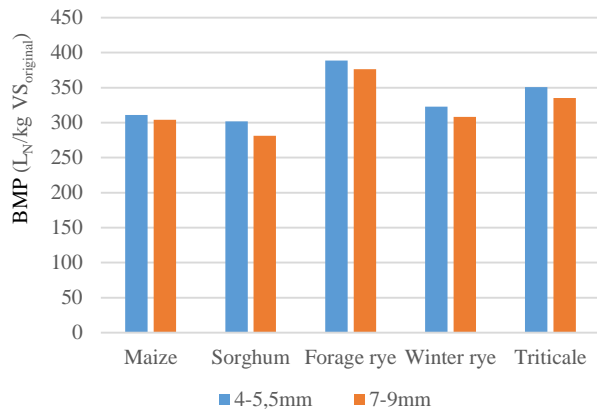
937

938

939

940

941



942

943 Figure 3 – Effect of farm-scale particle size reduction on methane formation of ensiled
 944 crop feedstock. BMP is based on the organic matter of fresh biomass, which takes into
 945 account VS losses during storage (adapted from Herrmann *et al.* [32]).

946

947

948

949

950

951

952

953

954

955

956

957

958

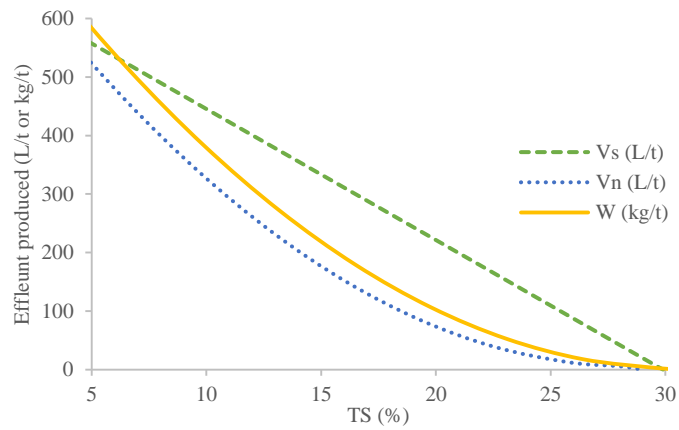
959

960

961

962

963



964

965 Figure 4 – Effluent production as function of total solids content of ensiled crop. V_s is
 966 the volume of effluent produced by unit mass of silage [43], V_n is the volume of
 967 effluent produced by unit mass of herbage [42] and W is the weight of effluent produced
 968 by unit mass of herbage [44].

969

970

971

972

973

974

975

976

977

978

979

980

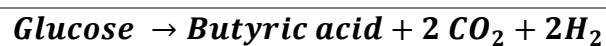
981

982

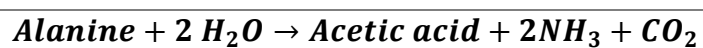
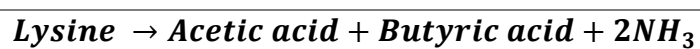
983

984 Table 1 – Examples of clostridial fermentation reactions (adapted from McDonald *et al.*
985 [8]).

Saccharolytic clostridial fermentation



Proteolytic clostridial fermentation



986

987

988

989

990

991

992

993

994

995

996

997

998 Table 2 – Critical pH value in function of silage TS content (adapted from Kalač [23]).

Total solids (%)	pH
15	4.10
20	4.20
25	4.35
30	4.45
35	4.60
40	4.75
45	4.85
50	5.00

999

1000

1001

1002

1003

1004

1005

1006

1007

1008

1009

1010 Table 3 – Impact of packing density of silage on total solids losses (adapted from
1011 Ruppel [57])

Density (kg TS / m ³)	Density (kg/ m ³) ^a	TS losses (%)
160	640	20.2
220	880	16.8
240	960	15.9
266	1064	15.1
290	1160	13.4
350	1400	10.0

1012 ^aCalculated assuming a content of 25% of total solids.

1013

1014

1015

1016

1017

1018

1019

1020

1021

1022

1023

1024

1025 Table 4 – Classification of main silage additives appropriate for biogas production
 1026 purposes (adapted from McDonald *et al.* [8]).

Category	Examples	Intended mode of action
Fermentation stimulants	LAB	Encourage lactic fermentation by supply of substrate, bacteria or enzymes
	Sugars	
	Enzymes	
Fermentation inhibitors	Formic acid	Reduction of pH of silage to restrict microbial growth
	Mineral acids	
Absorbents	Dried sugar beet pulp	Reduce dry matter loss and pollution of water by effluent
	Straw	
Aerobic deterioration inhibitors	LAB	Control the deterioration of silage on exposure to air
	Propionic acid	

1027

1028

1029

1030

1031

1032

1033

1034

1035

1036

1037

1038

1039 Table 5 – Relative impact of main fermentation stimulants on silage methane yield
 1040 (compared with control silages without additives).

Feedstock	Type of additive	Duration (days)	Impact on BMP	Reference
Whole crop maize	Ho ^a	90	-1% ^b	Haag <i>et al.</i> [18]
Whole crop maize	He ^a	90	1% ^b	Haag <i>et al.</i> [18]
Whole crop maize	Ho	90	-4% ^c	Herrmann <i>et al.</i> [7]
Whole crop maize	Ho+He	90	-5% ^c	Herrmann <i>et al.</i> [7]
Whole crop maize	Ho	119	-23% ^d	Neureiter <i>et al.</i> [15]
Whole crop maize	Ho+He	119	-18% ^d	Neureiter <i>et al.</i> [15]
Whole crop maize	Amylase	119	-4% ^d	Neureiter <i>et al.</i> [15]
Whole crop maize	<i>Clostridium tyrobutyricum</i>	119	7% ^d	Neureiter <i>et al.</i> [15]
Whole crop maize	Ho+He 1	49	-12% ^d	Vervaeren <i>et al.</i> [16]
Whole crop maize	Ho+He 2	49	6% ^d	Vervaeren <i>et al.</i> [16]
Whole crop maize	Ho+He+Enzymes	49	5% ^d	Vervaeren <i>et al.</i> [16]
Whole crop maize	Ho+He+Yeasts+Fungi	49	11% ^d	Vervaeren <i>et al.</i> [16]
Grass	Ho	90	12% ^d	Lehtomäki [10]
Grass	Enzyme	90	19% ^d	Lehtomäki [10]
Grass	Ho	110	5% ^d	McEniry <i>et al.</i> [21]
Grass	He	110	12% ^d	McEniry <i>et al.</i> [21]
Grass	Sucrose	110	8% ^d	McEniry <i>et al.</i> [21]
Grass	Ho+Enzyme	180	1% ^d	Pakarinen <i>et al.</i> [11]
Ryegrass	Ho+Enzyme	180	8% ^d	Pakarinen <i>et al.</i> [11]
Amaranth	Ho	90	-11% ^b	Haag <i>et al.</i> [18]
Amaranth	He	90	-14% ^b	Haag <i>et al.</i> [18]
Sugar beet tops	Ho	90	-7% ^d	Lehtomäki [10]
Sugar beet tops	Enzyme	90	-10% ^d	Lehtomäki [10]
Sorghum	Ho+He	90	-1% ^c	Herrmann <i>et al.</i> [7]
Forage rye	Ho+He	90	3% ^c	Herrmann <i>et al.</i> [7]
Triticale	Ho+He	90	2% ^c	Herrmann <i>et al.</i> [7]

1041

1042 ^a Ho and He stand for homofermentative and heterofermentative LAB, respectively.

1043 ^{b,c,d} Based on methane yields expressed in: ^b m³ by ton of VS added to AD; ^c in m³ by ton of original VS;

1044 ^d in m³ by ton of fresh biomass.