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Ensiling for biogas production: critical parameters. A review Rúben Teixeira Franco, Pierre Buffière, Rémy Bayard^{*} Université de Lyon, INSA Lyon, LGCIE-DEEP, EA4126, F-69621 Villeurbanne cedex,

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7 Abstract

4

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 fully established technology. So as to remain competitive, anaerobic digestion should be 10 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 crucial parameters for ensiling agricultural wastes and crops for biogas production, as 14 source properties, storage management and duration, temperature or additives. According 15

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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 will define the course of ensiling and the impact of other parameters during storage as 17 well. Good silage preservation will occur for feedstocks with low moisture content, high 18 accessible carbohydrates and low buffering capacity. High packing density and reduced 19 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 application should be more appropriate for poorly ensilable biomass. Finally, evidences 22 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 literature in terms of impact of different conditions in the course of ensiling and the 25 26 questions still unresolved, this article highlights the good management practices of substrates for biogas production. 27

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

30 Highlights

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

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.

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

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

69 biodegradation.

Concerning seasonally produced resources such as crops and wastes, two main 70 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].

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

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

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

• Anaerobic fermentation: once oxygen has been depleted, the microorganisms capable 90 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 microbial activity will occur until feed-out period. 98 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]. In contrast, under efficient silage systems, organic matter losses can be limited below 110 111 20% and methane potential can be conserved almost entirely or even increase in some cases [7,10–12]. 112

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

the literature [4,8,13]. On the other hand, although these references are quite important

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

biogas production are not exactly the same: in the first case, protein digestibility,

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.

To our best knowledge, the critical parameters in ensiling for biogas production has not been reviewed earlier. This article examines several points of influence for silage of biogas crops, taking into account the answers stated by the literature and questions that remain unclear. The objectives of this study are to outline good storage practices of 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)

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

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

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

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

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

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

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

168 Besides the direct impact on the course of ensiling, feedstock source and its biochemical characteristics will influence the impact of other critical parameters over 169 170 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 influence parameters can be found in the literature; for instance, concerning the use of 174 additives [7,10,11,18,25] and for the temperature [10]. 175

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 able to be used for ensiling. For instance, even if maize whole plant is an ideal biomass 180 181 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 grow on soil of poor quality with low nutrient input [29]. 184

185 2.1.2. Particle Size

Methane fermentation through AD is clearly affected by feedstock's particle size, as 186 187 it interferes in the kinetics of complex substrates hydrolysis [30]. Normally, methane production is enhanced by particle size reduction, mainly due to the increase of the 188 189 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 pH, NH₃-N and butyric acid content, and higher lactic acid content. These results 199 200 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 pathways, producing butyric acid, carbon dioxide and hydrogen (Table 1). Furthermore, 205 206 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 proteolytic clostridia activity, which will mainly produce ammonia and carbon dioxide 209

through amino acids and amides fermentation, Table 1. Finally, this clostridial
fermentation will reduce the BMP, as energy will be lost through CO₂ and H₂
production. It will also lead to nitrogen loss in the gaseous phase by ammonia
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 reducing storage losses. In addition, they indicated that, in general, reducing chopping 219 220 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 feed-out by enabling higher silage densities and minimizing air introduction. 223 Contrasting with the data presented above, Haag et al. [18] presented a study with 224 225 silage of whole crop maize and amaranth, in which no benefit was found by reducing the chopping length from 8 to 1 mm. Lower methane yields were obtained for the 226 227 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 crop. In the case of amaranth crop silage, this reduction of the methane yield can be, in 229 230 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 crops. However, this discussion was not detailed by the authors. 233

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 length, in connexion with their first study on whole crop maize, sorghum, forage rye, 238 239 winter rye and triticale crops. They reported that chopping crops to particles sizes of 7 to 8 mm are recommended for high methane formation. However, in only one third of 240 241 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 by McDonald et al. [8]), is that a higher total solids (TS) content delay bacterial growth, 246 247 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 only a small decrease of lactic acid production with increasing crop dry matter. On the 254 255 other hand, the saccharolytic clostridial fermentation exponentially decreased as TS 256 increased, being negligible at 30% of total solids.

Moreover, a more restricted proteolytic clostridial fermentation at lower moisture
content was observed by the same authors, testified by a lower level of NH₃-N
production. Therefore, better preserved crops will be expected from higher total solids,
since lower organic matter losses would occur due to the limitation of undesirable
microbial growth.

262 Likewise, Nash [37] worked with grass/clover herbage silage and showed that nutrient losses were much lower in crops with higher dry matter content. Similarly, 263 264 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, respectively. For these latter studies, silage preservation was particularly successful, as 266 267 an increase of lactic acid production was verified for crops with lower moisture level. In contrast, Han et al. [40] published a work with cup-plant silage suggesting that the 268 269 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, lactic acid production and proteolytic clostridial activity were identified to be higher on 271 272 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 NH₃-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 impact on dry matter losses will be overlapped by the increase of lactate fraction in the 276 277 total acids.

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

silage, or the critical pH value, which is function of the total solids content, as shown inTable 2.

283 Together with the benefits for organic matter preservation, higher total solid content may prevent leachate formation during ensiling. Indeed, several authors as Bastiman 284 [42], Sutter [43] and Zimmer [44] have proposed correlations to predict the behavior of 285 286 effluent production (Figure 4). In the works by Bastiman [42] and Zimmer [44], similar quadratic derived equations were obtained, in which negligible leachate formation 287 288 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 differences among the correlations might be explained by the influence of other 290 291 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, losses may be avoided by using the effluent itself as co-feedstock on AD [4]. However, 295 296 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 resource during ensiling. Field wilting prior to ensiling is the most common method to 301 302 achieve higher TS contents for biomass crops [8]: it is inexpensive and it enables water evaporation with little effect on the remaining chemical characteristics if wilting 303 duration is controlled. In fact, several authors as Borreani et al. [36], Carpintero et al. 304

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 filed pea, faba bean and white lupin, as their dry matter content increased from 48.2% to 61.8%, from 23.7% to 29.5% and from 14.2% to 311 312 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 to 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 and an increase of the released ammonia nitrogen (NH₃-N) from 1.2 to 2.1g/kg of total 318 nitrogen. Likewise, Dawson et al. [20] studied field wilting durations of 28 and 52h for 319 320 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. Therefore, short duration field wilting should be preferably considered when biomass 324 325 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

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

As alternative to open air wilting, more complex and expensive treatments, such as chemical desiccation and thermal treatment, can be proposed [8]. Regardless the method used, the water weight to be transported from the field to the silo and after ensiling to

AD will be lower, reducing both transportation and processing costs [36,47].

of TS content in the BMP showed inconclusive results. Pakarinen *et al.* [11] have

Contrary to the aforementioned advantages, the few studies that evaluated the impact

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

not enhanced the BMP, mainly due to higher VS losses during ensiling. For ryegrass,

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

influence the wilting impact on BMP. However, no further conclusions can be drawn,

since the authors did not follow the WSC content, BC or even cell wall constituents offresh material and silage.

More recently, McEniry *et al.* [21] observed that a 6h-wilted grass produced silage with a more restricted fermentation, a higher fraction of lactic acid in the total

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

al. [11], no differences were detected between the wilted and control grass on the dry

349 matter losses and BMP.

334

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

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

357 2.2. Storage conditions

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

362 2.2.1. Presence of air

Oxygen is usually considered as a spoiling agent in a process that needs to achieve 363 364 anaerobic conditions, where LAB can proliferate [6,8,9]. In fact, air causes silage deterioration since it favors the activity of aerobic microorganisms, as heterotrophic 365 366 bacteria, yeasts and molds [48]. Besides the theoretical and macroscopic evidences of oxygen detrimental action on silage, some laboratory and field scale studies have been 367 368 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 presence in aerated silage. Even though, the authors did not report a negative effect of 371 372 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.

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

deterioration at feed-out [8]. According to Egg *et al.* [4], absolute energy losses from

aerobic degradation after storage (feed-out) can reach up to 15%, and up to 10% duringensiling.

383 Concerning the aerobic degradation during ensiling, 99.5% of the oxygen can be

depleted after 30 minutes [51], and an anaerobic environment will be shortly reached.

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

may result in BMP losses (4.5% decrease after 49 days of storage) and would

dramatically increase the risk of aerobic spoilage at feed-out.

For silo loading or feed-out, these losses can be reduced by minimizing the process duration Nevertheless, in some cases, constraints for wilting, transportation or feed-out rates may affect time efficiency. In these circumstances, aerobic stability of silage must be taken in consideration to avoid major losses, as a result of increased activation of aerobic microorganisms. For instance, Plöchl *et al.* [52] observed important TS and BMP losses for whole crop maize silage after only 4 days of air-exposure at feed-out. Similarly, McEniry *et al.* [21] found a decrease by 8.7% for the specific BMP of grass

silage after 8 days of air-exposure at silo opening. Likewise, Herrmann *et al.* [19]

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,

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

It is thus essential to avoid conditions that may lead to aerobic deterioration at any
stage of ensiling. Appropriate silo construction, prompt sealing and high feed-out rates
are thus good management practices required to prevent energy losses due to aerobic
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 preservation, due to its influence on organic matter oxidation. A higher density is 407 associated to a lower porosity, lower amounts of air, and to slower oxygen flows in 408 409 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, Ruppel [57] worked with alfalfa silage for 180 days and found a relation between the 411 412 density and the silage total solid losses, presented in Table 3. Zheng et al. [58] tested silage packing densities of 460, 690 and 920 kg/m³ and showed that higher ones had a 413 positive effect on lactic acid production and enzymatic digestibility for sugar beet pulp 414 415 inoculated with LAB.

Similarly, Zheng *et al.* [59], used 480, 720 and 960 kg/m³ packing densities for beet
pulp ensiling. They concluded that the higher density provided better silage quality,
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 lower423 density silage were presenting 20% less BMP than control tests.

425 allow greater silo capacity, which subsequently leads to lower unit costs of storage

Another advantage shared by different authors is that high bulk densities for ensiling

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

424

Storage duration is a quite variable parameter, which may depends on the seasonality 436 437 of some crops and wastes, or on specific feeding requirements of downstream anaerobic digester. For these reasons, ensiling duration is often defined by taking into account 438 439 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 instance, by Neureiter et al. [15], who tested whole crop maize for 44 and 119 days 441 442 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 increase of BMP was observed. After 44 days, silage BMP was 17% lower than the 444 fresh whole crop maize one, but after the more prolonged duration it was 22% above the 445

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

451 Comparatively, a study for whole crop maize, sorghum hybrid, forage rye and triticale, during 10, 90, 180 and 365 days, showed an apparent positive effect of 452 453 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 limit of accuracy of BMP tests). The higher BMP over time is usually attributed to 455 456 higher bio-accessibility of plant cell wall constituents, which in certain cases can compensate the losses in terms of dry matter [11]. However, in Herrmann et al. [7] 457 458 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 that, no noticeable reduction of lignin content was recorded. 460 461 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

storage time and the BMP. In both studies, cumulative losses in methane yield of morethan 30% after 180 days were observed.

Therefore, two main conclusions may be highlighted: i) there is a real influence of 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. In brief, much can still be done concerning storage time optimization depending on 476 477 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 possible. Exceptions as for sugar beet pulp or whole crop maize, in which prolonged 479

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 rates are known to increase with temperature, in part due to the strong impact of 488 temperatures on the hydrolysis of complex organic compounds [62]. 489 490 Concerning ensiling, many studies have been performed at constant temperature at

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

494	showed that higher pH and NH ₃ -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 of view. In fact, expenses related to energy spending, maintenance and equipment 524 525 should probably overcome the benefits from monitoring silo temperature. Even though, 526 some management practices could be encouraged to prevent silage damaging from extreme conditions. For instance, heat transfer by thermal radiation and long duration 527 528 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 years ago for forage production purposes and they have become increasingly 531 532 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 ensure silage preservation through LAB fermentation enhancement. Also during this 534 535 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 several groups of fermentation stimulants, aerobic deterioration inhibitors, nutrients and 537 538 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 production and diversification. Therefore, currently a wide range of biological and 540 chemical silage additives is commercially available [13]. 541

It is well recognized that the use of additives arose for the forage production and innovations in the field were thus mainly oriented towards the production of quality animal feed [15]. Nevertheless, several commercial products, among the categories presented in Table 4, can be potentially used to enhance the properties of biogas plant feedstock. From this list, two groups are highlighted in the work done by the researchers 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 ensiling, as their benefits for the preservation of crops are generally recognized and they 550 551 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 inoculants should be listed. Their application affects the preservation process in 554 555 different ways and, therefore, they are often combined into a commercial mix, so that their modes of action could complement each other. 556

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].

In contrast, the addition of LAB inoculants will increase the lactic acid bacterial population of silage. With a higher count of LAB, the lactic acidification in the initial stage of preservation is expected to be faster [23]. Depending on the fermentation 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

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 changing the type of stimulant [15,16]. However, due to the wide variety of available 599

stimulants and insufficient amount of results in the literature, it seems impossible todevelop a consistent comparison between fermentation stimulants.

602 2.5.2. Fermentation inhibitors

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

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

Despite being corrosive and difficult to handle, the use of acid in silage remains justified by other factors. One is the fact that these additives are more likely than LAB to restrict proteolysis, due to instantaneous lowering of silage pH. Furthermore, their effectiveness is more reliable since, unlike biological additives, it is not based on the activity of living microorganisms. This means that the content of carbohydrate sources may become unimportant and so, clostridial secondary fermentation can be more easily 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 does not introduce undesirable chemical elements in silage, suggests that these additives 623 624 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 AD with a higher content of undesirable H₂S. It is likely for these reasons that 627 628 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 insignificants changes in chemical composition of biomass for 4 and 8 months were 637

found. The same type of results were obtained by Lehtomäki [10], while using formic
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 due to formic acid degradation given its low concentration, it is possible that acid 644 645 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], increase of accessible surface area and lignin structure alteration [68]. Consequently, the 649 650 use of formic acid as additive appears to be appropriate for, at least, some types of grass silage. This additive should be even more interesting for poorly ensilable biomass, 651 given the boost it can give in terms of accessibility of the material for AD and in 652 653 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**

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

will occur at relatively low moisture contents, high accessible carbohydrates content,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

of methane potential of grass silage. This effect should be more pronounced for poorlyensilable biomass.

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

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

899 points.





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



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



Figure 4 – Effluent production as function of total solids content of ensiled crop. *Vs* is
the volume of effluent produced by unit mass of silage [43], *Vn* is the volume of
effluent produced by unit mass of herbage [42] and *W* is the weight of effluent produced
by unit mass of herbage [44].

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

Saccharolytic clostridial fermentation

 $Glucose \rightarrow Butyric \ acid + 2 \ CO_2 + 2H_2$

2 Lactic acid \rightarrow Butyric acid + 2 CO₂ + 2H₂

Proteolytic clostridial fermentation

Lysine \rightarrow Acetic acid + Butyric acid + $2NH_3$

Alanine + 2 $H_2O \rightarrow Acetic \ acid + 2NH_3 + CO_2$

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	Total solids (%)	рН
	15	4.10
	20	4.20
	25	4.35
	30	4.45
	35	4.60
	40	4.75
	45	4.85
	50	5.00
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998Table 2 – Critical pH value in function of silage TS content (adapted from Kalač [23]).

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
^a Calculated assuming a content of 25	5% of total solids.	

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

-	Category	Examples	Intended mode of action
-	Formontation	LAB	Encourage lactic fermentation by supply
	stimulants	Sugars	of substrate bacteria or enzymes
	stimulants	Enzymes	
-	Fermentation	Formic acid	Reduction of pH of silage to restrict
	inhibitors	Mineral acids	microbial growth
-		Dried sugar beet	Reduce dry matter loss and pollution of
	Absorbents	pulp	water by effluent
		Straw	
-	Aerobic	LAB	Control the deterioration of silage on
	deterioration	Propionic acid	exposure to air
	inhibitors	r topionie acid	exposure to an
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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	Но	90	-4% ^C	Herrmann et al. [7]
Whole crop maize	Ho+He	90	-5% ^c	Herrmann et al. [7]
Whole crop maize	Но	119	-23% ^d	Neureiter et al. [15]
Whole crop maize	Ho+He	119	-18% ^d	Neureiter et al. [15]
Whole crop maize	Amylase	119	-4% ^d	Neureiter et al. [15]
Whole crop maize	Clostridium tyrobutyricum	119	7% ^d	Neureiter et al. [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 et al. [16]
Grass	Но	90	12% ^d	Lehtomäki [10]
Grass	Enzyme	90	19% ^d	Lehtomäki [10]
Grass	Но	110	5% ^d	McEniry et al. [21]
Grass	Не	110	12% ^d	McEniry et al. [21]
Grass	Sucrose	110	8% ^d	McEniry et al. [21]
Grass	Ho+Enzyme	180	1% ^d	Pakarinen et al. [11]
Ryegrass	Ho+Enzyme	180	8% ^d	Pakarinen et al. [11]
Amaranth	Но	90	-11% ^b	Haag <i>et al</i> . [18]
Amaranth	Не	90	-14% ^b	Haag <i>et al</i> . [18]
Sugar beet tops	Но	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 et al. [7]
Triticale	Ho+He	90	2% ^C	Herrmann <i>et al</i> . [7]

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^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 m^3 by ton of fresh biomass.