Ensiling for biogas production: Critical parameters. A review

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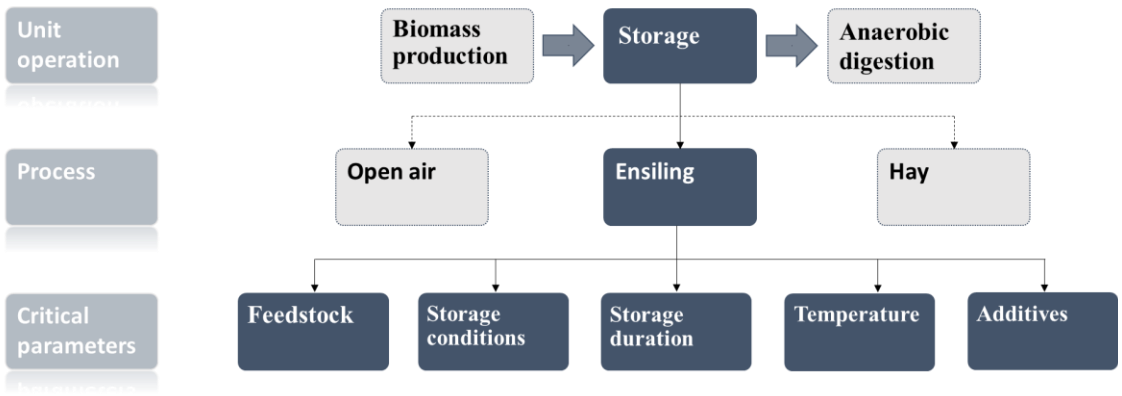
Graphical abstract



Abstract

In order to meet the legislative demands of new energy policy, investment in anaerobic 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 optimized not only at the level of the process, but also down and upstream, in which biomass storage prior to digestion is included. Ensiling is a commonly used and promising techniques to store wet biomass before anaerobic digestion. This article reviews the crucial parameters for ensiling agricultural wastes and crops for biogas production, as source properties, storage management and duration, temperature or additives. According

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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 well. Good silage preservation will occur for feedstocks with low moisture content, high accessible carbohydrates and low buffering capacity. High packing density and reduced particle size will contribute to minimize energy losses during ensiling. Additives are 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 suggest that under specific conditions, ensiling may increase methane potential despite 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 questions still unresolved, this article highlights the good management practices of substrates for biogas production.

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

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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.

Abbreviations

AD, anaerobic digestion; BC, buffering capacity; BMP, biochemical methane potential; Ho, homofermentative; He, heterofermentative; LAB, lactic acid bacteria; NH_3-N, ammoniacal nitrogen; TS, total solids; VS, volatile solids; WSC, water soluble carbohydrates

1. Introduction

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

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

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

Regarding the storage types, three main categories should be mentioned. The first one is 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 biodegradation.

Concerning seasonally produced resources such as crops and wastes, two main storage and preservation technologies have been adapted for methane production purpose: hay and silage systems. Hay storage consists on field drying, inhibiting detrimental microbial activity, followed by the use of large round bales stored outdoors [4]. Even though this system minimizes both labor and storage costs, it leads to high losses in terms of dry matter that may reach up to 60% [5], it is restricted to crops that can dry quickly and uniformly. This technique can be limited by rainfall during harvest [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 of anaerobic growth (for instance, lactic acid bacteria - LAB, enterobacteria, clostridia and yeasts) begin to proliferate and compete for the available organic matter. The first days are critical for the success or failure of the fermentation [8]. If the conditions are suitable, LAB will produce lactic acid for several weeks, decreasing the pH to around 4.0.
- Stabilization phase: the anaerobic conditions are maintained with a decreasing fermentative activity, the pH remains stable wherein minimal enzymatic and microbial activity will occur until feed-out period.
- Feed-out: after unloading the silo for transportation or bio-digester feeding, biomass enters once again into aerobic environment. Thereupon, aerobic microorganisms are reactivated, which may spoil the silage and lead up to 15% of absolute energy losses [9].

As can be seen, ensiling process is quite dynamic, through several successive stages, with competitive environments and microorganisms. Control of biochemical processes and growth of different microorganisms seems therefore rather important, in order to obtain a good silage quality, ready to provide the maximum energetic yield in the anaerobic digester. For instance, energy losses due to respiration, secondary fermentation, effluent production or aerobic deterioration may occur. These phenomena 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 20% and methane potential can be conserved almost entirely or even increase in some cases [7,10–12].

The biochemistry and microbiology principles of ensiling, and more generally the 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 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 purposes, the main objective is to save - or eventually increase, the maximum amount of 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.

2. Influence parameters

2.1. Feedstock

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

2.1.1. Source

Silage can be made from a large variety of biomass. However, its success will rely on several biochemical characteristics of the source of energy. Besides moisture content (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 methane production 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).

Besides the direct impact on the course of ensiling, feedstock source and its biochemical characteristics will influence the impact of other critical parameters over storage. For instance, as can be seen in Figure 2, Herrmann *et al*. [7] showed that the evolution of methane yield over ensiling time strongly depends on the feedstock. This has also been shown for other biomass crops by Lehtomäki [10] and Pakarinen *et al*. [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 additives [7,10,11,18,25] and for the temperature [10].

Although it is clear that biochemical characteristics of raw material are one of the most crucial parameters in ensiling, optimization of storage performance through feedstock choice may not be always possible. Indeed, several restrictions related to 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 for ensiling and AD, the use of wastes or catch crops is preferred in some countries like France, due to political and ethical issues. Conversely, in boreal conditions, energy 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].

2.1.2. Particle Size

Methane fermentation through AD is clearly affected by feedstock's particle size, as 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 available specific surface area and to the reduction of both degree of polymerization and cellulose crystallinity [31]. An identical influence is expected during ensiling, since particle size reduction may lead to faster LAB fermentation and therefore to less organic matter losses. Indeed, Herrmann *et al.* [32] indicates that chopping at harvest as a mechanical treatment reduces the particle size for enhanced manageability of crop material and for better process conditions at ensiling and feeding.

Concerning the validation of the benefits by chopping ensiling raw materials, Gordon *et al*. [33], Herrmann *et al*. [32] and Haag *et al*. [18] presented different results and conclusions. According to the early work [33], based on an ensiling study of alfalfa as forage for animal feed purposes, lower particle size silage were characterized by lower pH, NH_3-N and butyric acid content, and higher lactic acid content. These results suggest that silages with lower particles sizes could present a higher BMP, as the chemical indicators show a better crop preservation. In fact, as reviewed by McDonald *et al*. [8], if a stable and low pH silage is not achieved, clostridial activity will be encouraged and a secondary fermentation will occur. This clostridial fermentation is mainly based on sugars and lactic acid consumption as energy source *via* similar pathways, producing butyric acid, carbon dioxide and hydrogen (Table 1). Furthermore, butyric acid is a much weaker acid than lactic acid. In addition, one mole of butyrate is produced from two moles of lactate. These two effects lead to an increase of the pH and 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 through amino acids and amides fermentation, Table 1. Finally, this clostridial fermentation will reduce the BMP, as energy will be lost through CO_2 and H_2 production. It will also lead to nitrogen loss in the gaseous phase by ammonia formation.

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

Contrasting with the data presented above, Haag *et al*. [18] presented a study with 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 smaller chopping length in both cases. For maize silage, lower methane yield might be 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 part, due to high dry matter losses during the ensiling of the crop with smaller particle size. These results suggest that, despite the accessibility gains usually attributed to a reduced particle size, other biochemical phenomena may affect the BMP of chopped crops. However, this discussion was not detailed by the authors.

Regarding the optimization of chopped length size for ensiling purposes, Mohd-Setapar *et al*. [34] suggested that this subject has been poorly investigated and most studies were performed using pre-defined particle size crops. Nevertheless, Herrmann *et 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, 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 the cases the benefits due to higher methane production by further chopping length reduction did compensate the additional cost of size reduction.

2.1.3. Moisture

The effect of moisture in silage has been extensively studied in the last decades, 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, leading to a more restricted fermentation and therefore, influencing silage preservation. However, different levels of tolerance to dryness are noticed among the involved microorganisms. For instance, clostridia are known to be particularly sensitive to water availability and require wet conditions for active development. In counterpart, LAB are able to ferment biomass at a wide range of TS [8]. Borreani *et al*. [36] evidenced this fact through a series of silage experiments using field pea, faba bean and white lupin at 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 other hand, the saccharolytic clostridial fermentation exponentially decreased as TS 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 NH_3-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.

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, Mahmoud *et al*. [38] and Wilkinson [39] evidenced a decreasing clostridial activity for feedstock with higher TS content with whole crop maize and comfrey silages, respectively. For these latter studies, silage preservation was particularly successful, as 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 fermentation was not restricted for all microorganisms in higher TS crops. Although 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 the same substrates. Haigh and Parker [41] published a work on ryegrass and white clover mixture silages; they found that, despite a higher content of NH_3-N, higher lactic acid fraction among all acids and lower TS losses were obtained for these crops. This 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 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 in Table 2.

Together with the benefits for organic matter preservation, higher total solid content may prevent leachate formation during ensiling. Indeed, several authors as Bastiman [42], Sutter [43] and Zimmer [44] have proposed correlations to predict the behavior of effluent production (Figure 4). In the works by Bastiman [42] and Zimmer [44], similar quadratic derived equations were obtained, in which negligible leachate formation occurs above around 25% of TS. On the other hand, Sutter [43] used a linear adjustment 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 parameters on the effluent production, such as the feedstock, the use of additives, the surface pressure applied, the silo height or the mechanical pre-treatment before ensiling [45]. Besides affecting nutrient and energy losses, effluent production can lead to 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, one must take into account that the recovery of leachate might be complicated. Thus, both for forage or biogas production purposes, effluent production should be avoided and a particular attention has to be paid to the adjustment of the moisture content.

In several cases, the dry matter content of the feedstock is low. Indeed, techniques for 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 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 duration is controlled. In fact, several authors as Borreani *et al*. [36], Carpintero *et al*.

[46], Dawson *et al*. [20] and McEniry *et al*. [21] have compared wilted and un-wilted feedstock chemical characteristics before ensiling. McEniry *et al.* [21] performed a 6h wilting of grass and observed an increase of TS content from 20.1% to 26.5%, with no particular effect on the other chemical properties, such as cell wall composition or water soluble carbohydrates (WSC). The same conclusions were obtained by Borreani *et al*. [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 17.3%, respectively, without other significant modifications on chemical composition. Identically, Carpintero *et al.* [46] worked with ryegrass-clover, in which a 6h pre-wilting allowed the increase of dry matter content from to 17.3% to 34.9%, without affecting the composition.

The same authors also performed a pre-wilting of 48h, in order to achieve a higher TS 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 (NH_3-N) from 1.2 to 2.1g/kg of total nitrogen. Likewise, Dawson *et al*. [20] studied field wilting durations of 28 and 52h for perennial ryegrass and reported an impact of wilting on silage chemical characteristics, particularly on the pH and the buffering capacity. These results suggest that even if higher solid contents may be achieved with prolonged crop wilting, other chemical changes beyond water evaporation might occur leading to organic matter degradation. Therefore, short duration field wilting should be preferably considered when biomass preservation is required during water evaporation. However, evaluation of wilting only through drying duration should be performed with caution. Depending on the 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 of radiation 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].

Contrary to the aforementioned advantages, the few studies that evaluated the impact of TS content in the BMP showed inconclusive results. Pakarinen *et al*. [11] have studied, during six months, grass and ryegrass silage for biogas production purposes. 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 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 of fresh 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, 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 matter losses and BMP.

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.

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.

2.2.1. Presence of air

Oxygen is usually considered as a spoiling agent in a process that needs to achieve 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 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 performed to confirm the impact of aerobic conditions. Indeed, Garcia *et al*. [49] performed aeration tests on alfalfa silage with air rates of 320 mL/d for 21 days at laboratory scale. They reported higher pH and NH_3-N content, and lower lactic acid presence in aerated silage. Even though, the authors did not report a negative effect of aeration on lignocellulosic biomass conversion during storage. At field scale, Langston *et al*. [50] studied air impact on orchard grass and alfalfa silage by pumping air for 5 to 6 hours after filling the silo. They observed high temperatures in the aerated silage as a result of organic matter bio-oxidation and, subsequently, pH, butyric acid and NH_3-N increased, while LAB fermentation was less extensive.

Even though many practices applied during ensiling are intended to prevent the contact with air, the impact of oxidation losses can be observed in four different stages: 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% during ensiling.

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 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 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].

2.2.2. Density

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 associated to a lower porosity, lower amounts of air, and to slower oxygen flows in silage, thereby reducing losses due to aerobic spoiling [56]. These statement have been 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 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/m^3 and showed that higher ones had a positive effect on lactic acid production and enzymatic digestibility for sugar beet pulp inoculated with LAB.

Similarly, Zheng *et al.* [59], used 480, 720 and 960 kg/m^3 packing densities for beet pulp ensiling. They concluded that the higher density provided better silage quality, mainly due to higher lactic acid production.

Neureiter *et al.* [15] investigated not compressed and not tightly sealed whole crop maize silage, and obtained higher pH, higher weight losses and lower lactic acid content than compressed biomass during 44 and 119 days. The impact of these storage conditions on biogas production was only important at 119 days, from which lower density silage were presenting 20% less BMP than control tests.

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

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

2.3. Storage duration

Storage duration is a quite variable parameter, which may depends on the seasonality 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 these supply chain restrictions and not due to its potential impact on the preservation of 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 storage. They obtained good quality silages in both cases; despite a slight increase of 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 fresh whole crop maize one, but after the more prolonged duration it was 22% above the 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.

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 prolonged storage on methane yield for some crops [7]. However, differences between 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 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] study, original biomass was already fairly accessible, as evidenced by the original low lignin range (2.9-6.7%) and by the hemicellulose degradation during storage. Besides that, no noticeable reduction of lignin content was recorded.

In counterparts to these positive results, Pakarinen *et al*. [11] showed that for a maximum 180 days of ensiling, either with original grass/ryegrass, or with wilting periods, or with addition of starters, BMP decreased with storage duration. For grass 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 more than 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 properties of the feedstock used that will define a positive or negative impact on it.

Nevertheless, no coherent correlation between the feedstock source and the impact of the storage time on silage BMP can be proposed, as only a limited number of substrates has been investigated in the literature. Thus, future work on testing the ensiling duration impact on a wider range of substrates is encouraged. This may allow the optimization of the storage duration depending on the chosen feedstock, *i.e.*, to practice prolonged 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 the substrate used. However, for now ensiling should be considered as only a material 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 ensiling has been proved to be advantageous, should be taken in consideration.

2.4. Temperature

Regardless of the location chosen for ensiling, large temperature variations are expected since the silo is usually submitted to ambient temperature. Taking the example of temperate climates, as the Mediterranean one, minimum and maximum temperatures of 0 to 40°C might be attained, respectively. Eventually, these variations can have a real impact on the ensiling course. As a consequence, bacterial growth rates will be different 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 temperatures on the hydrolysis of complex organic compounds [62].

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 showed that higher pH and NH_3-N concentration, residual WSC and lower lactic to acetic acid ratio were obtained for silages ensiled at the highest temperature. All this data suggest that, at 40°C, fermentation was more extensive, reflecting reduced silage quality at the end.

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

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

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

This latter assumption is made taking into account the results published by Lehtomäki [10], who suggested that very low ensiling temperatures do not necessarily lead to subsequent higher methane yields. Depending on the feedstock and on the type of additives used, different effects of temperature on BMP were obtained after 6 months storage. In certain cases, higher BMP by fresh weight were obtained for 5°C storage, but the same was also verified for 20°C ensiling under other conditions. Therefore, tests on the effect of low temperatures on ensiling with different feedstock may be advised. This might allow to verify the existence or not of a hydrolysis barrier at low temperatures that may be unfavorable for the expression of the energy content of some crops during storage.

Despite lower ensiling temperatures appear to favor in most cases the preservation of 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 should probably overcome the benefits from monitoring silo temperature. Even though, some management practices could be encouraged to prevent silage damaging from extreme conditions. For instance, heat transfer by thermal radiation and long duration storage under extremely hot environments may be avoided whenever possible.

2.5. Additives

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 widespread since then. Their first known utilization was in the early twentieth century, through the addition of molasses [8]. In this primordial utilization, the aim was to ensure silage preservation through LAB fermentation enhancement. Also during this period, the utilization of mineral acids for fast acidification of crops began to be practiced. With the evolution of research, diversification of additives increased, as several groups of fermentation stimulants, aerobic deterioration inhibitors, nutrients and absorbents began to be used [8]. Given the general approval of the benefits of additives 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 chemical silage additives is commercially available [13].

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.

2.5.1. Fermentation stimulants

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

On the one hand, both carbohydrate sources and enzymes increase the content of biodegradable material directly and indirectly, respectively. Carbohydrate sources, as molasses and sugars, will introduce additional substrate for LAB, whereas enzymes, such as cellulase or xylanase, will produce additional fermentable sugars from the cell 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 strains convert hexose into lactic acid *via* the Embden-Meyerhof pathway, being at the same time unable to ferment pentoses [65]. In opposite, heterofermentative LAB are able to ferment both hexoses and pentoses, producing lactic acid but also acetic acid, ethanol and carbon dioxide. Since acetic acid is weaker than lactate and since side products are formed, lower dry matter and energy gain should be expected for heterofermentative bacteria addition. Consequently, most of the commercial starters consist of homofermentative LAB. However, heterofermentative bacteria are not discarded since they provide great aerobic stability and are expected to limit BMP losses after feed-out.

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

The data from grass and whole crop maize are in agreement with the statements made by Kalač [23] about the use of additives. The author claims that additives are not 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 unwilted alfalfa, clovers or some grasses. Therefore, it can be suggested that, if a better BMP preservation is reached by using stimulants for grass silage (in which, for instance, WSC content will strongly depend on the stage of growth or species used), a more pronounced effect should be expected for poorly ensilable crops. In other words, while ensiling biomass with low WSC content, low LAB, high moisture and high BC, the use of stimulants may help the fermentation to be carried out more efficiently.

Finally, beyond the primary effect of the feedstock used, the kind of stimulant also has a significant impact on the course of silage. For example, in the case of whole crop 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 stimulants and insufficient amount of results in the literature, it seems impossible to develop a consistent comparison between fermentation stimulants.

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].

Regarding the comparison between the types of inhibitors, the use of organic acids 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 interfere less in the organic matter degradation and in the formation of other side products. On the opposite, if a mineral acid like sulfuric acid is used, the sulfur fraction in the silage will increase, which will then logically lead to biogas production during AD with a higher content of undesirable H_2S. It is likely for these reasons that researchers have preferred to study the impact of formic acid, as a model of organic acids, than sulfuric acid or other mineral acid.

Despite being the most studied inhibitor, few and discordant works on the impact of formic acid on BMP was noticed. For instance, Pakarinen *et al*. [12] worked with forage maize, hemp and fairy bean, using formic acid with concentrations of 0.5% and 1%w/w in ensiling and found that acidification not only preserved the original WSC, but increased their amount compared with the fresh crop. However, in comparison with control tests, formic acid addition resulted in silages with lower BMP in almost all 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 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.

On the opposite, Lehtomäki [10] and McEniry *et al*. [21] observed coherent higher methane yields using similar concentrations of formic acid on grass. Furthermore, the results of Lehtomäki [10] showed that there was a 30% relative increase of the BMP 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 addition may have led to a greater accessibility of the plant cell wall constituents. In fact, addition of dilute or concentrated acid to biomass is used as pre-treatment before AD or enzymatic hydrolysis to render carbohydrates sources more accessible. Several 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 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, given the boost it can give in terms of accessibility of the material for AD and in preservation (by instant pH drop), of crops with low WSC content. In order to sustain these suggestions, more studies on this topic are advised in the future.

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.

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

Search for efficient additives has been one of the main priorities for ensiling researchers in recent years, with focus on fermentation stimulants and inhibitors. 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 poorly ensilable 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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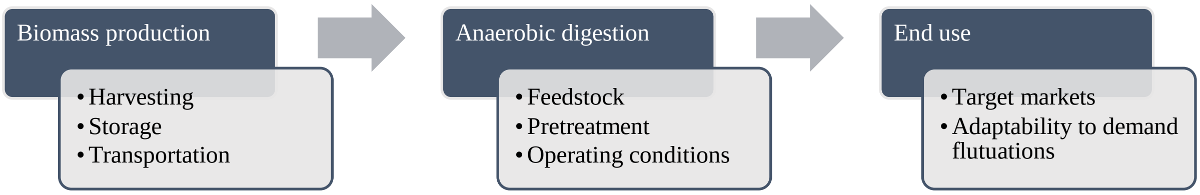

Figure 1 – Simplified supply chain of biogas production with examples of optimization points.

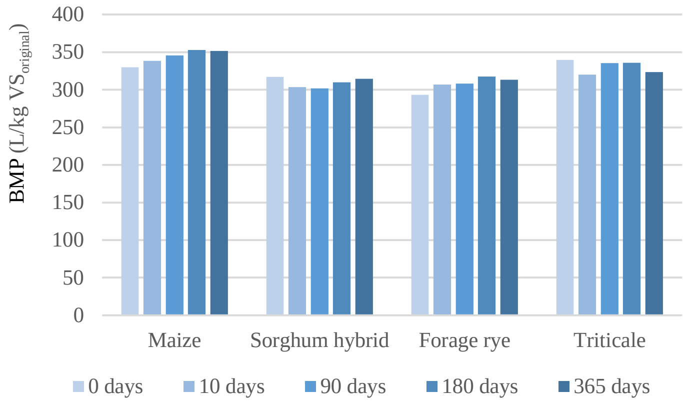

Figure 2 – Methane formation of whole crop maize, sorghum hybrid, forage rye and triticale influenced by storage duration. BMP is based on the organic matter of fresh biomass, which takes into account VS losses during storage (adapted from Herrmann *et al.* [7]).

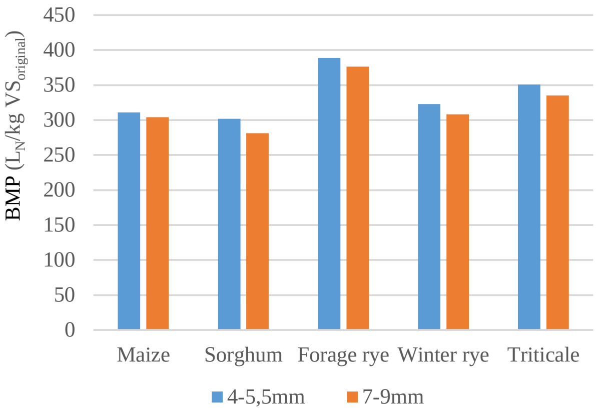

Figure 3 – Effect of farm-scale particle size reduction on methane formation of ensiled crop feedstock. BMP is based on the organic matter of fresh biomass, which takes into account VS losses during storage (adapted from Herrmann *et al.* [32]).

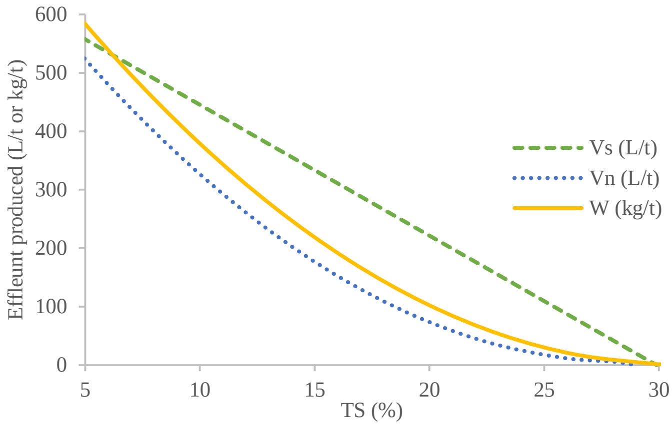

Figure 4 – Effluent production as function of total solids content of ensiled crop. *Vs* is the volume of effluent produced by unit mass of silage [43], *Vn* is the volume of effluent produced by unit mass of herbage [42] and *W* is the weight of effluent produced by unit mass of herbage [44].

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

Saccharolytic clostridial fermentation
$Glucose \rightarrow Butyric\ acid + 2\ CO_2 + 2H_2$
$2\ Lactic\ acid \rightarrow Butyric\ acid + 2\ CO_2 + 2H_2$
Proteolytic clostridial fermentation
$Lysine \rightarrow Acetic\ acid + Butyric\ acid + 2NH_3$
$Alanine + 2\ H_2O \rightarrow Acetic\ acid + 2NH_3 + CO_2$

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

Table 3 – Impact of packing density of silage on total solids losses (adapted from 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% 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
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	

Table 5 – Relative impact of main fermentation stimulants on silage methane yield (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 *et al.* [18]
Whole crop maize	He [a]	90	1% [b]	Haag *et al.* [18]
Whole crop maize	Ho	90	-4% [c]	Herrmann *et al.* [7]
Whole crop maize	Ho+He	90	-5% [c]	Herrmann *et al.* [7]
Whole crop maize	Ho	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 *et al.* [16]
Whole crop maize	Ho+He 2	49	6% [d]	Vervaeren *et al.* [16]
Whole crop maize	Ho+He+Enzymes	49	5% [d]	Vervaeren *et al.* [16]
Whole crop maize	Ho+He+Yeasts+Fungi	49	11% [d]	Vervaeren *et al.* [16]
Grass	Ho	90	12% [d]	Lehtomäki [10]
Grass	Enzyme	90	19% [d]	Lehtomäki [10]
Grass	Ho	110	5% [d]	McEniry *et al.* [21]
Grass	He	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	Ho	90	-11% [b]	Haag *et al.* [18]
Amaranth	He	90	-14% [b]	Haag *et al.* [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 *et al.* [7]
Forage rye	Ho+He	90	3% [c]	Herrmann *et al.* [7]
Triticale	Ho+He	90	2% [c]	Herrmann *et al.* [7]

[a] Ho and He stand for homofermentative and heterofermentative LAB, respectively.

[b,c,d] Based on methane yields expressed in: [b] m^3 by ton of VS added to AD; [c] in m^3 by ton of original VS; [d] in m^3 by ton of fresh biomass.