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► To cite this version:

Ruben Teixeira Franco, Pierre Buffière, Rémy Bayard. Optimizing agricultural wastes storage before anaerobic digestion: impact of ensiling on methane potential of lignocellulosic biomass. 4th International Conference on Sustainable Solid Waste Management, Jun 2016, Limassol, Cyprus. hal-01692814

HAL Id: hal-01692814

<https://hal.science/hal-01692814>

Submitted on 25 Jan 2018

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Optimizing agricultural wastes storage before anaerobic digestion: impact of ensiling on methane potential of lignocellulosic biomass

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Abstract

Storage requirements for anaerobic digestion feedstock are boosting ensiling research for biogas production purposes. However, so far, studies have been mainly focused on good management practices of easily biodegradable biomass. In this article, effect of ensiling, open-air storage and feedstock moisture on methane potential of an agricultural crop were investigated during six months. Open-air storage presented linear losses of organic matter and energy content along storage, culminating in more than 70% and 90% losses after six months, respectively. For ensiling, lack of accessible sources of carbohydrates hindered acidification, allowing undesired secondary fermentation, which led to non-negligible organic matter losses. Wilting was an ineffective technique for this type of crop, since increased organic matter losses from around 10% to 20% after six months, leading to energy losses. Despite the use of a poorly ensilable biomass, fresh raw material at lower moisture content conserved its methane potential along 6 months of ensiling. Water-soluble phase of methane potential will increase with the ensiling duration. Although this may favor methane production kinetics, higher losses of energy content may occur in case of liquid seepage along storage. Storage optimization of this agricultural waste will be achieved by ensiling raw material without need for wilting and collecting the probable liquid effluent production.

Keywords: Agricultural wastes; Lignocellulosic biomass; Ensiling; Anaerobic digestion; Biochemical methane potential

1. Introduction

Over the last few years, the interest in energy production through anaerobic digestion (AD) of biomass has been increasing due to environmental concerns and political incentives. This renewable energy technology, which is based on the degradation of complex organic matter into a mixture called biogas (mainly composed of CH₄ and CO₂), has today more than 17000 operational plants over Europe [1]. Notwithstanding, AD sector is still known for having a quite fragile financial health. Therefore, in the future a focus on energy efficiency of biogas plants will be crucial. This optimization should concern not only the digester, but also the downstream and upstream systems, *i.e.* the biomass production and its end use.

Biomass storage before AD is an operation that can be potentially optimized. Nowadays, the diversification of biogas sources is quite wide, since energy can be recovered from almost all types of organic waste, fodder or catch/energy crops. Despite the need for a continuous supply of biogas throughout the year, some of these agricultural wastes or crops are seasonally produced. This aspect leads to storage requirements, which in some cases can be of extended durations. Among the preservation technologies that exist today, ensiling appears to be the logical choice to storage biomass

before AD, since it might minimize weight and energy losses.

Ensiling provides a four-step biochemical process based on bacterial fermentation to prevent further degradation. After filling and sealing the silo, an initial aerobic period will occur, in which the oxygen trapped in the system will be consumed for biomass respiration. Once oxygen has been consumed, microorganisms capable of anaerobic growth will compete for available matter and, in suitable conditions, the pH will decrease to approximately 4. Then, maintaining anaerobic conditions and a relatively low pH, minimal enzymatic and microbial activity will occur until feed-out. After unloading the silo for bio-digester feeding, biomass enters once again into aerobic environment. Thereupon, aerobic microorganisms are reactivated, which may spoil the silage.

Given the diversification of critical parameters that influence ensiling and the types of biomass that may need to be stored before AD, ensiling research is still in its early days. Indeed, a recent work [2], which reviews the critical parameters on ensiling for biogas, suggests that current research is mostly undergone with easily biodegradable biomass. Consequently, other types of feedstock, such as agricultural wastes are being left behind.

In order to fill this gap, we have studied a real case of lignocellulosic agriculture waste during six

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months, using different storage approaches. Furthermore, the originality of this work lies in the multi-phase study of methane potential, which allows a deeper understanding about its evolution during storage.

2. Materials and methods

2.1. Raw materials

The substrate used was a sample of a catch crop harvested on 27 May 2015 and used as fodder and agricultural biogas plant feedstock in the Rhône-Alpes region of France (Gaec Béréziat, Les Teppes, 01340 Béréziat, France). Biomass was composed by a mixture of triticale (50% of seed mixture), peas (30% of seed mixture), vicia (10% of seed mixture) and fodder radish (10% of seed mixture) and was chopped at 4cm maximum length at harvesting. Samples were stored at 4°C before further use.

2.2. Storage approach

Storage assays were conducted at laboratory scale to evaluate the impact of ensiling and its dry matter content over time. To evaluate ensiling impact, an aerobic control storage test was also performed. To assess different dry matter content a 6-h wilting was performed outdoors. Good weather conditions were recorded during wilting period: no rainfall occurred and the temperature ranged between 17-24°C. All storage tests were performed in 3.5L airtight round plastic storage drums modified to allow the gas to escape. In order to enable the output of gas produced and at the same time minimizing headspace, silos were filled up to 2.55L with raw material at packing density of 0.7kg/L, the remaining volume being filled with gravel, using a geotextile membrane to separate it from biomass. Silo sealing was different depending on the storage method tested. For ensiling assays, proper plastic lid and rubber ring were used and its airtightness was reinforced with silicone sealant. For aerobic storage purposes no cover was used and silo was left air-open. Once sealed, silos were stored in a temperate room at 25°C for a defined period of days. Storage duration varied between 3, 14, 30, 90 and 180 days.

2.3. Chemical analysis

At the end of storage duration, reactors were open and weighed, biomass was homogenized and two samples were taken. One was used for direct analyses on the crude material and other one was leached with water to obtain and analyze water-soluble and particulate phases. Leaching was performed with a 10:1 water/dry mater ratio during 2h under constant bottle rotation. Phase separation was achieved by centrifugation followed by 0.7µm particle size filtration. Finally, particulate was dried

at 70°C until constant weight and ground at 2mm theoretical length. Crude material/water-soluble and particulate samples were stored at 4°C and -20°C, respectively, until use.

Chemical analysis performed depended on the sample studied. Crude material was analyzed for its Total Solids (TS), Volatile Solids (VS) content and biochemical methane potential (BMP). For the water-soluble phase, besides TS/VS content and BMP, pH, water-soluble carbohydrates (WSC), volatile fatty acids (VFA), ammoniacal nitrogen (NH₃-N) and total Kjeldahl nitrogen (TKN) contents were determined. Particulate solid was analyzed for its TS/VS, TKN and cell wall constituents.

TS was measured by oven drying at 105°C during 24h and VS was subsequently determined by combustion in a muffle oven for 2h at 550°C. Since TS/VS contents are underestimated due to volatile compounds loss lost during procedure [3], values were corrected using an equation suggested by Porter and Murray (2001) [4]. pH was measured by a Consort C3020 device with a SP10B pH-electrode. VFA and WSC contents were determined with high performance liquid chromatography (LC Module 1 plus, Waters) equipped with a Supelcogel™ C-610H column (300 x 7.8 mm, Sigma-Aldrich), both refractive index (RID) and UV detectors and operating with H₃PO₄ 0.1%v as solvent (flow rate of 0.5mL/min). WSC content was estimated as the sum of glucose, xylose, galactose, mannose, arabinose and cellobiose and was determined using the UV detector (210nm). Total VFA was calculated as the sum of lactic, formic, acetic, propionic, butyric, valeric and caproic acid contents obtained with the RID detector. Cell wall constituents – cellulose (CEL), hemicellulose (HEM) and lignin (LIG) - were analyzed through Van Soest modified extractions method [5], based on XP U44-162 French standard. TKN and NH₃-N were determined through the procedure described in the NF EN 25663 French standard.

Most of the results for the chemical analysis will be presented in at least one of two ways: based on %VS_{added} or %VS_{original}. VS_{added} relates to the organic matter of the sample analyzed. On the other hand, results based on VS_{original} take into account the loss of volatile solids during storage and allows to study the evolution of the results based on the VS of the raw material.

2.4. Biochemical methane potential tests

Batch anaerobic digestion tests were performed for crude material and water-soluble phase samples, which enabled to follow the evolution of BMP of each chemical compartment during storage.

Tests were conducted in a temperate room at 35°C using glass vessels of 2L for crude material and 0.1L for water-soluble phase. Vessels were filled with about 5g of sample VS, inoculum in way to

keep a substrate/inoculum VS ratio of 0.3-0.8, and a certain volume of a mineral solution to achieve 60% of the total volume of the vessel. The inoculum used (TS 2.4-2.9%wt; VS 1.7-2.0%wt) was a digested sludge originating from the wastewater treatment plant of La Feyssine, Lyon, France. The mineral solution, which contains essential elements to microbial growth and also gives the solution a buffer able to control any pH adjustments, was prepared according to the recommendations of ISO 11734:1995 standard. Once filled, reactors were purged with a N₂/CO₂ mixture (80/20 %v) for about 5 minutes and then sealed and equilibrated at 35°C.

In order to subtract the volume of methane produced by the inoculum from the one originated from the sample, controls with only inoculum and mineral solution were performed for each batch series. All tests were performed in triplicates.

Biogas production was followed by the overpressure generated inside of the reactors using a Digitron precision manometer, being its composition determined using an Agilent 3000 micro gas chromatography with thermal conductivity detector (GC-TCD). Molsieve 5A (14 m length; pore size: 5 Å) and PoraPlotA (10m length; 0.320 mm ID) columns were used as stationary phases for GC-TCD, with Argon and Helium as carrier gases, respectively. Biogas production and composition were analyzed at least 10 times during batch tests and BMP was considered achieved when daily vessel overpressure of controls equalized the sample ones.

3. Results and discussion

3.1. Fresh material chemical characteristics

Feedstock used for storage purposes, which is characterized in Table 1, can be briefly described as hardly biodegradable biomass for ensiling. Two different reasons can support this fact:

- Negligible presence of water-soluble carbohydrates (WSC) in raw materials. Indeed, it is known that silage will be favored by high lactic acid bacteria (LAB) activity, which will acidify the biomass and lead to better BMP preservation. Since LAB ferment naturally occurring sugars in the crop to produce a mixture of acids [6], LAB activity and pH decrease will be directly linked to WSC content of raw material. Therefore, in absence of WSC or any other accessible source of sugars, a slow decrease of pH and a poorly ensiled material must be expected.
- Hard accessible (hemi-) cellulose content, due to a strong lignin protection. Cellulose and hemicellulose are the main sources of energy in AD, since they can represent more than half of biomass organic matter and they can be directly fermented in sugars upon hydrolysis. Even if it has already been shown that at least

hemicellulose can in part be accessed and degraded during ensiling [7], this must always be preceded by lignin depolymerization or structure modification. This step may require prolonged storage durations to take place. Thereupon, with high lignin content crops and considering ensiling soft operating conditions, where temperature or fungi effect may be negligible, hemicellulose will not be readily accessible in large quantities to promptly acidify and stabilize silage.

Table 1 – Chemical composition of initial and wilted biomass

| | Initial | 6h-wilted |
|------------------------|---------|-----------|
| pH | 7.20 | 7.23 |
| TS^a | 18.2 | 23.4 |
| VS^a | 16.2 | 21.0 |
| WSC^b | 0.15 | 0.15 |
| VFA^b | 4.0 | 2.1 |
| HEM^b | 13.2 | 16.4 |
| CEL^b | 40.2 | 39.6 |
| LIG^b | 18.2 | 16.8 |

Results presented as ^a % of total sample weight; ^b % VS

Regarding wilting impact, a 6h period allowed the increase of TS content from 18% to 23%. Besides moisture decrease, wilting favored a decrease of total VFA content in biomass. This should be related either with their evaporation or degradation and may have an important impact on biomass energy content. Finally, wilting should not impact the fibers content due to its short dry period and low temperature. Differences found in results, especially for hemicellulose, may be explained by the weak accuracy attributed to the fibers analysis when testing not perfectly homogenous samples.

3.2. Impact of storage on biomass fermentation

Effects of storage conditions and duration on silage quality are summarized in Table 2.

Aerobic storage (TS18%A) led to silage spoilage from only 3 days of storage. Since LAB never managed to impose itself and control fermentation, pH remained in neutral zone. Then, with open air environment, aerobic microorganisms were able to freely proliferate. This aerobic activity resulted in several organic losses: more than 10% after 3 days, reaching almost 75% after 180 days. Part of these losses were related to structural carbohydrates degradation, since more than 85% of cellulose content was lost after 180 days of storage. Constant deterioration of NTK content along storage time also indicates huge losses and modifications of organic matter for aerobic conditions.

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

| Condition ^a | Storage duration (days) | VS losses | Chemical characteristics ^b | | | | | | | | | | |
|------------------------|-------------------------|-----------|---------------------------------------|-----------------|-----------------|-----------------|------------------|------------------|---------------------------|------------------|------------------|------------------|------------------|
| | | | pH | LA ^c | AA ^c | BA ^c | VFA ^c | WSC ^c | NH ₃ -N (%TKN) | TKN ^d | HEM ^d | CEL ^d | LIG ^d |
| TS18%E | 3 | 0.4 | 5.02 | 4.6 | 3.8 | 0.0 | 14.1 | 0.5 | 10.7 | 2.8 | 13.7 | 36.1 | 10.2 |
| | 15 | 0.8 | 5.22 | 2.5 | 4.2 | 3.1 | 14.0 | 0.4 | 23.2 | 2.0 | 12.6 | 35.3 | 7.5 |
| | 30 | 5.5 | 5.37 | 0.1 | 5.9 | 4.4 | 15.6 | 0.2 | 37.7 | 2.4 | 11.5 | 35.8 | 7.6 |
| | 90 | 8.5 | 5.41 | 0.1 | 4.9 | 5.4 | 14.9 | 0.2 | 44.1 | 2.3 | 8.7 | 30.7 | 10.5 |
| | 180 | 9.8 | 5.25 | 0.0 | 5.6 | 6.2 | 16.5 | 0.1 | 46.0 | 2.2 | 10.8 | 33.9 | 10.5 |
| TS23%E | 3 | 0.7 | 5.87 | 2.8 | 3.8 | 0.0 | 9.5 | 0.2 | 10.0 | 3.2 | 16.6 | 34.4 | 12.5 |
| | 15 | 7.4 | 5.97 | 0.7 | 4.9 | 3.1 | 11.4 | 0.2 | 25.8 | 2.7 | 13.4 | 31.2 | 10.5 |
| | 30 | 21.7 | 5.54 | 0.1 | 8.8 | 3.5 | 14.5 | 0.1 | 38.0 | 2.4 | 11.3 | 26.7 | 8.8 |
| | 90 | 20.0 | 5.22 | 0.0 | 9.3 | 3.7 | 16.1 | 0.1 | 42.1 | 2.4 | 7.7 | 25.2 | 10.0 |
| | 180 | 19.8 | 5.39 | 0.0 | 8.9 | 4.0 | 17.2 | 0.0 | 46.3 | 2.2 | 11.1 | 26.5 | 9.7 |
| TS18%A | 3 | 14.1 | 6.66 | 1.2 | 2.4 | 0.0 | 6.5 | 0.2 | 6.5 | 2.2 | 14.3 | 32.3 | 14.7 |
| | 15 | 32.4 | 8.08 | 0.0 | 1.1 | 0.7 | 2.2 | 0.1 | 20.7 | 2.3 | 13.1 | 26.3 | 10.6 |
| | 30 | 44.3 | 8.18 | 0.0 | 2.5 | 0.9 | 5.6 | 0.0 | 25.0 | 1.8 | 8.7 | 21.0 | 8.9 |
| | 90 | 68.8 | 9.12 | 0.0 | 0.1 | 0.0 | 0.3 | 0.1 | 1.7 | 0.9 | 3.5 | 8.9 | 8.4 |
| | 180 | 78.6 | 10.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.2 | 1.7 | 0.7 | 2.1 | 4.6 | 5.5 |

^a TS18%E represents the biomass ensiling with original TS content; TS23%E concerns the ensiling of 6h-wilted biomass and; TS18%A symbolizes the open-air storage of the original raw material

^b LA stands for Lactic Acid, AA for Acetic Acid and BA for Butyric Acid; ^c results based on % VS_{added}; ^d results based on % VS_{original}

Concerning ensiling, non-negligible VS losses occurred, especially during the first month of storage. Wilting (TS23%E) was not effective on preventing organic matter deterioration, leading to around 20% of VS losses after 6 months of storage. Unlike, ensiled fresh material (TS18%E) restricted organic matter losses on 10% for the same storage duration. Organic matter losses can be in part explained by restricted acidification of biomass. Indeed, it is well established that if a stable, low pH silage is not achieved, clostridial activity will be encouraged and a secondary fermentation will occur, resulting in organic matter and energy losses [6]. For both ensiling conditions, the necessary acidity for efficient silage, the so-called critical pH value, was never achieved (4.10-4.35 for the TS range used [8]). This suggests a major importance of the content of WSC or another source of readily available carbohydrates on the first moments of ensiling. Their presence or not will define the quantity of available substrate for LAB activity and then the degree and rate of acidification and biomass stabilization. Moreover, higher VS losses of wilted material may be related to a quite slower acidification than fresh biomass storage. In fact, wilted material took more than a month to get a low stable pH while fresh biomass took only 3 days to achieve a pH around 5. Therefore, it can be suggested that the faster the acidification will be, the lower the subsequent biodegradation and VS losses.

To get a deeper knowledge about the acidification mechanisms and their relation with the organic matter losses, an evaluation of the major fermentation products should be made. In these tests,

similar patterns over storage time were recorded for both ensiling conditions. In the early days of ensiling, fermentation was controlled by LAB, as mainly lactic and acetic acid were produced. The presence of both compounds suggest equally homo- and heterofermentative LAB activity. Then, somewhere between 3 and 15 days, lactic acid began to be consumed and butyrate to be produced. This indicates that secondary fermentation occurred through undesired clostridial activity. In fact, clostridial fermentation is mainly based on sugars and lactic acid consumption as energy source via similar pathways, producing not only butyric acid but also carbon dioxide and hydrogen [6]. This will result on VS losses and more important energy losses, since hydrogen will escape to the environment. Furthermore, secondary fermentation is associated with losses of acidity, as it can be confirmed by pH increase from 3 to 15 days of ensiling. This is explained by the fact that butyric acid is a much weaker acid than lactic acid and since only one mole of butyrate is produced from two moles of lactate [6].

Increasing NH₃-N contents along storage were observed for ensiling. This can be explained by proteolytic clostridia activity, which will be favored by perturbations on silage acidification. The proteolytic clostridia can selectively ferment amino acids and amines mainly into ammonia and carbon dioxide. Besides nutrients spoilage, proteolytic clostridia may have an impact on energy losses. In fact, although ammonia should be present in the form of soluble ammonium ions at current pH and temperature values, its liquid-gas equilibrium may

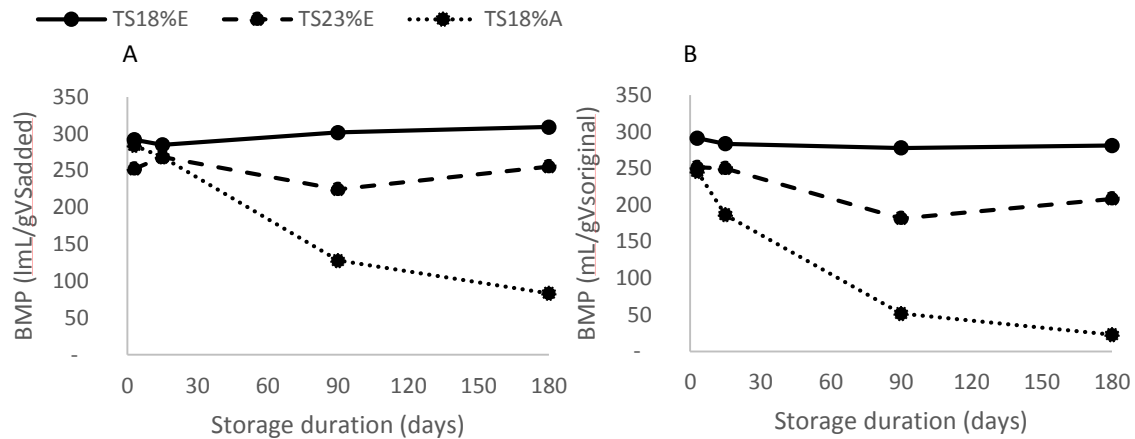


Figure 1 - Global BMP evolution over storage duration. A: methane potential based on VS_{added}; B: methane potential based on VS_{original}, therefore considering storage losses. TS18%E represents the biomass ensiling with original TS content; TS23%E concerns the ensiling of 6h-wilted biomass and; TS18%A symbolizes the open-air storage of the original raw material

allow a continuous slight fraction to pass in the gas phase and that subsequently to escape into the environment. This idea can be corroborated while analyzing TKN evolution along ensiling duration. This element shows a clear decline over storage time when related to the original VS, reaching around 25% of relative losses after 6 months. This shows that part of organic nitrogen or ammonia fraction was lost into the atmosphere, which may result in BMP losses.

From 15 days of storage and until the end of the tests, butyric acid and ammonia-nitrogen increase, and acetic acid remained stable, which strongly suggest a clostridial activity.

Regarding moisture impact on ensiling evolution, results showed that fermentation was not more restricted for all microorganisms in wilted crop. Indeed, while lactic acid production was less extensive for higher TS ensiled biomass, butyric acid contents and subsequently saccharolytic clostridial activity were higher. Concerning proteolytic clostridia activity, no difference was found depending on the ensiling condition. In contrast, several authors [9–12] have shown that higher TS contents delay bacterial growth, especially clostridia due to their particularly sensitivity to water content, leading to a more restricted and well succeeded fermentation. These contradictory results may be explained through differences on readily biodegradable carbohydrates availability in the beginning of ensiling among the tests. For instance, Borreani et al. [9] tested crops with high WSC contents, meaning that even if lower LAB activity was recorded, high carbohydrates content allowed a strong biomass acidification. At the same time, in our tests, as very low WSC content was present, LAB activity was dependent on the activity of other microorganisms, such as enzymes that allow, for instance, hemicellulose hydrolysis. Since it seems

fair to assume that these microorganisms will also have more restricted activity at higher TS contents, less substrate will be available to LAB. Thereupon, acidification will be less effective and conditions will be more suitable for clostridia proliferation. In brief, these results suggest that, for the TS range studied, WSC availability will be more vital to avoid clostridial activity than wilting.

As a final point, despite the lack of WSC in raw materials, biomass is acidified in both ensiling conditions, reaching up to 16% of VFA in the organic biomass. The evolution of carbohydrate sources over storage duration give us hints about the origin of substrates that were acidified. Looking at the evolution of the WSC, there is always a glimmer of these compounds along silage, indicating that it was continuously produced and consumed. With regard to the structural carbohydrates in biomass, there is a degradation tendency of not only the hemicellulose but also cellulose over the 6 months of ensiling. This suggests that in case of absence of WSC, anaerobic microorganisms will get access to sources of structural carbohydrates by hydrolysis even when they are protected by high lignin content. However, in such cases the acidification will be less effective, due to difficulties of accessibility of these compounds and the time required for their partial hydrolysis. Finally, breakdown of cellulose was much more evident in the wilted silage, explaining the source of the largest losses VS during storage.

3.3. Evolution of BMP over storage duration

3.3.1. Methane potential values

Bio-chemical methane potential values varied from 292-309 L/kgVS_{added} for ensiled fresh material (TS18%E), 253-256 L/kgVS_{added} for wilted silage (TS23%E) and 292-83 L/kgVS_{added} for open-air

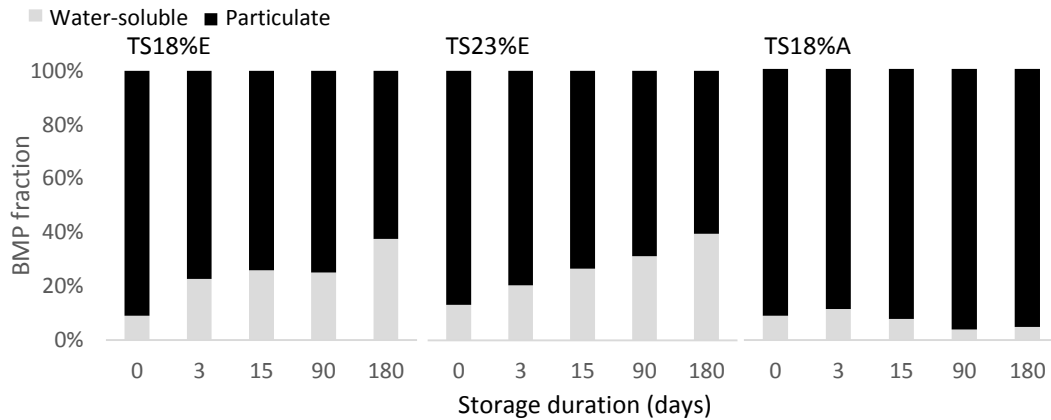


Figure 2 – BMP multi-phase analysis for the different conditions along storage duration. TS18%E represents the biomass ensiling with original TS content; TS23%E concerns the ensiling of 6h-wilted biomass and; TS18%A symbolizes the open-air storage of the original raw material

storage (TS18%A) (Figure 1). Increasing values of BMP based on VS_{added} for silages suggest that ensiling may enhance bio-chemical accessibility of biomass. It can also be observed that remaining organic matter of TS18%E have higher methane potentials than other conditions regardless of storage duration.

Since BMP based on VS_{added} follow similar trends of VS losses according to the storage conditions, methane potentials based on the original organic matter were even more divergent among the studied conditions (Figure 1). This reflects the major importance of VS loss in determining the preservation of BMP, as it was stated in a previous work [13]. Therefore, while considering VS losses during storage, results evidence a major advantage in using ensiled fresh material than wilted silage or open air storage. Part of TS23%E BMP losses seem to be related with the wilting process itself, once before storage wilted biomass had already less 13% of BMP than the fresh one. Moreover, even after 6 months of ensiling, 18%TS biomass presented similar BMP values based on $VS_{original}$ to the raw material (291-279 L/kg $VS_{original}$ before and after 6 months of ensiling, respectively). Therefore, it can be stated that, even if a poorly ensilable biomass is used, conservation of its whole energy content for prolonged storages can be achieved if suitable ensiling conditions are used. The reason for that may reside on the fact that gains in bio-chemical accessibility will compensate organic matter losses during storage.

3.3.3. Solid/liquid fractions

BMP multi-phase analysis over storage duration (Figure 2) present distinct results depending on the storage method used. For open-air storage, particulate phase of BMP was constantly predominant, representing 89-96% of the total methane potential. This may be explained by the fact

that water-soluble phase is only a transition step until VS be completely degraded under aerobic environment. Concerning ensiling, there is a clear trend of increasing water-soluble BMP phase during storage. Both ensiling condition exhibit the same behavior, with the water-soluble phase to range, depending on the storage duration, between 7-38% of the total BMP for fresh ensiled material and 12-40% for wilted silage. This suggest positive and negative aspects. On one hand, this means that the more easily biodegradable fraction will increase over storage, which normally will have a positive effect on methane production kinetics. As a result, for prolonged storage, optimization of AD retention time for this feedstock may be achieved. On the other hand, the increasing probability of BMP losses through effluent production over storage duration can be pointed as one drawback. Indeed, authors agree that liquid effluent or seepage losses will happen when biomass is ensiled too wet, due to high silo compaction rates [6,14]. However, when wilting is harmful to the crop as in the present tests, higher TS content may not be the solution for this issue. Without a doubt, it may be preferable to ensile biomass with low TS content, with one single condition: that potential liquid effluent is recovered and used as feedstock for AD. In the latter one, dry matter and energy losses may occur if effluent is not promptly introduced in AD digester. To avoid that, additional installation for effluent collection and its transportation until the digester should be anticipated.

4. Conclusions

Ensiling was proven to be the logical choice for biomass preservation before biogas production. Lack of accessible sources of carbohydrates hindered acidification, allowing undesired secondary fermentation, which led to non-negligible organic matter losses. This outcome had more impact for

higher dry matter biomass, suggesting that wilting is an ineffective technique for this type of crop. Despite the use of a poorly ensilable biomass, fresh raw material at lower VS content conserved its methane potential along 6 months of ensiling. This suggests that for this condition, gains in bio-chemical accessibility will neutralize organic matter losses during storage. Finally, water-soluble phase of methane potential will increase with the storage duration. Although this may favor methane production kinetics, higher losses of energy content may occur in case of liquid seepage. Therefore, effluent production should be avoided whenever possible or collected and immediately added to the anaerobic digester.

Acknowledgements

Rúben Teixeira Franco held a doctoral fellowship from the Rhône-Alpes region. The authors thank the DEEP laboratory team, including David Lebouil, Hervé Perier-Camby, Nathalie Dumont and Richard Poncet for the given support during the tests. We are grateful to Franck Barra for his permanent availability for discussion and raw material supply. Mathilde Hardier and SUEZ are also acknowledge for the inoculum provided for the BMP tests.

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