How to preserve the energy potential of organic residues during storage? Focus on anaerobic digestion

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Abstract

This work presents a set of long-term storage assays at laboratory scale for two different types of catch crops and cattle manures. Comparison between the impact of ensiling and open-air storage techniques on organic matter and energy preservation was performed. Effects of co-ensiling with cattle manure and several additives on silage quality were assessed as well. Aerobic storage led to methane potential losses of more than 80% after 3 months of storage for catch crop assays and around 74% for cattle manure after 4 months. Higher energy recovery rates were obtained after ensiling, strongly depending on the nature of the organic residue used. For both catch crops, at least 96% of methane potential was preserved after 3 months. In contrast, single-handedly cattle manure lost 46% of its methane potential during long-term ensiling. Conservation of cattle manure was successfully enhanced through co-ensiling with fermentation stimulants and inhibitors. The best storage performance was obtained while combining cattle manure with wheat straw and glucose at high concentrations, for which methane potential was fully conserved even after 4 months of co-ensiling. These results highlight a major advantage in using ensiling rather than open-air storage for these organic residues. Moreover, the use of precursors of organic acids as co-substrates improved silage quality of non-adapted biomass for ensiling, such as cattle manure. This work contributes to the optimization of biomass preservation before anaerobic digestion, which will have a major impact on the methane yield of agricultural biogas plants.

Keywords: Cattle manure; Catch crop; Storage; Ensiling; Anaerobic digestion; Methane potential

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1. Introduction

Anaerobic digestion (AD) is accepted worldwide as a promising energy production technology for a green and sustainable future. This process is based on the biochemical conversion of complex organic matter under anaerobic conditions to a gaseous mixture mainly composed of CH_4 and CO_2, called biogas. Today, there are more than 17 000 operational plants over Europe [1] producing a biogas capable of generate heat, electricity or vehicle fuel.

Since energy can be recovered from nearly all types of organic matter, there is a wide diversity of feedstock that is used for biogas production. However, contrary to the need for a continuous supply of biogas throughout the year, some of these agricultural residues or crops are seasonally produced. Therefore, seasonal raw materials have to be stored for long periods before AD, which may have a strong impact on the energy potential of biomass.

Open air-storage and ensiling are two methods commonly used for biomass conservation before AD. The first one is mostly applied for agricultural wastes, due to the simplicity and low cost of the operation. However, open-air storage facilities are important source of ammonia and odor emissions [2] and should lead to substantial energy losses. These drawbacks can be mitigated if an efficient ensiling is carried out. According to Herrmann *et al.* [3], ensiling lead to full conservation of biochemical methane potential (BMP) of specific crops even after 1 year.

Ensiling is an acidification-based process typically divided in four phases [4–7]. Right after filling and sealing the silo, biomass respiration occurs due to the presence of oxygen trapped in the system. Once oxygen has been depleted, ensiling passes to an anaerobic fermentation phase. If the conditions are suitable, lactic acid bacteria (LAB) will produce lactate from water-soluble carbohydrates (WSC) for several weeks, decreasing the pH to around 4.0. Maintaining anaerobic conditions and a relatively low pH, minimal enzymatic and microbial activity occur until feed-out. After unloading the silo for AD feeding, biomass enters once again into aerobic environment, which may spoil the silage.

Although the fact that ensiling is pointed out as the logical choice to store wet biomass, to the best of our knowledge, there are currently no studies aiming the comparison between the impacts of open-air storage and ensiling on the conservation of the energy potential of different raw materials. Moreover, ensiling is neither been applied nor studied by researchers for some agricultural wastes as manure, mainly due to its unsuitable chemical characteristics for ensiling.

This work brings together a compilation of long-term storage assays at laboratory scale for two different types of catch crops and cattle manures. First, a comparison between open-air storage and ensiling was established during at least 3 months at laboratory scale. Furthermore, long-term assays of co-ensiling of cattle manure with several additives were performed. Finally, this work contributes to the optimization of biomass preservation before AD, which will have a major impact on the methane yield of agricultural biogas plants.

2. Materials and methods

2.1. Feedstocks

Two different types of catch crop and fresh cattle manure, which were collected on an agricultural site in the Rhône-Alpes region of France (Gaec Béreyziat, Les Teppes, 01340 Béréziat, France), were used as raw materials. Catch crop 1 (or “winter” catch crop – CC1) was a mixture of triticale, peas, vicia and fodder radish, and it was chopped at 4 cm maximum length at harvesting. Catch crop 2 (or “summer” catch crop – CC2) was a mixture of sunflower, sorghum, peas, vicia and *Trifolium alexandrinum*, and it was chopped to theoretical particle size of 8 mm before use. Cattle manure 1 (CM1) and Cattle manure 2 (CM2) were collected from the same site but on different seasons of the year. Samples were stored at 4°C before further use.

Catch crop 1, Catch crop 2 and Cattle manure 1 were tested for both open-air storage and ensiling. Besides that, co-ensiling assays were performed for both cattle manures with the following treatments:

- Cattle manure 1: wheat straw (CM1+WS)
- Cattle manure 2: wheat straw (CM2+WS); wheat straw and formic acid (20g/kg) (CM2+WS+FA); wheat straw and glucose (100g/kg) (CM2+WS+G)

Description of feedstocks used in storage experiments are summarized in Table *1*.

2.2. Storage approach

Laboratory trials were performed in 3.5 L airtight round plastic storage drums. In order to enable the output of the gas produced and at the same time minimizing headspace, silos were filled up to 2.55 L with raw material at packing density of 0.7 kg/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. Then, silos were weighed and placed in a controlled-temperature room at 25±2

°C. Storage duration was 3 months for catch crop assays and 4 months while using cattle manure.

2.3. Chemical analysis

At the end of storage, reactors were opened and weighed, biomass was homogenized and two samples were taken. The first one was used for direct analyses on the crude material: total solids (TS) content, volatile solids (VS) content and biochemical methane potential (BMP). The other one was mixed with water in order to measure the pH. This leaching test was performed with a 10:1 water/dry mater ratio during 2 h under constant bottle rotation. Identical sampling procedure and analysis were performed for biomass prior to storage.

TS content was measured by oven drying at 105 °C during 24 h and VS was subsequently burned for 2 h at 550 °C. Since TS/VS contents are underestimated due to the loss of volatile compounds during the drying tests [8], the measures were corrected using the volatilization coefficients at 100 °C suggested by Porter and Murray [9]. pH was measured by a Consort C3020 device with a SP10B pH-electrode.

Table 1 - Storage conditions and treatments applied to feedstocks

Condition	Raw material	Co-substrate [a]	Storage method		TS (%) [b]	VS (%) [b]
			Open-air	Ensiling		
CC1	Catch crop 1	-	Yes	Yes	18.2 ± 0.3	16.2 ± 0.4
CC2	Catch crop 2	-	Yes	Yes	10.1 ± 0.1	8.4 ± 0.2
CM1	Cattle manure 1	-	Yes	Yes	12.8 ± 0.1	10.2 ± 0.1
CM1+WS	Cattle manure 1	Wheat Straw	-	Yes	18.8 ± 0.4	15.9 ± 0.3
CM2+WS	Cattle manure 2	Wheat Straw	-	Yes	19.2 ± 0.1	16.6 ± 0.1
CM2+WS+FA	Cattle manure 2	Wheat Straw + Formic Acid (2%)	-	Yes	20.4 ± 0.1	18.0 ± 0.1
CM2+WS+G	Cattle manure 2	Wheat Straw + Glucose (10%)	-	Yes	24.9 ± 1.4	22.6 ± 2.4

Data presented as: [a] % of feedstock ; [b] % of total sample weight

BMP tests were conducted in a temperate room at 35 °C using glass vessels of 2 L. Vessels were filled with 5 g VS of sample, inoculum in way to keep a substrate/inoculum VS ratio of 0.5 and a certain volume of a mineral solution to achieve 60% of the total volume of the vessel. The inoculum used (TS 2.3-3.3%wt; VS 1.5-2.2%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 N_2/CO_2 mixture (80/20%v) for about 5 minutes, sealed and equilibrated at 35 °C. Blanks with only inoculum and mineral solution were performed for each batch series in order to correct the BMP from residual methane production of the inoculum. All tests were performed in triplicates. Biogas production was determined by pressure measurement using a Digitron precision manometer. Biogas was released when the pressure exceeded 1200 hPa. Gas composition was analyzed using an Agilent 3000 micro gas chromatography with thermal conductivity detector (GC-TCD). Molsieve 5A (14 m length; pore size: 5 Å) and PoraPlot A (10 m 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 7 times during the incubation and BMP was considered achieved when daily vessel overpressure of controls equalized the sample ones. The BMP tests followed the recommendations provided by Holliger *et al.* [10].

3. Results and discussion

3.1. Effects of storage method

3.1.1. pH values

Lower pH values were obtained using ensiling as storage method, Fig. 1. This was especially marked for catch crop silages. Indeed, Catch crop 1 had pH values of 5.4 and 9.1 after 3 months of ensiling and aerobic storage, respectively. In parallel, pH of Catch crop 2 after storage period was 5.6 for ensiling and 9.6 for aerobic treatment. This suggests that catch crops passed through substantial fermentation and accumulation of organic acids during ensiling. This was expected, since these raw materials typically have high content of easily accessible carbohydrates, which are used as substrates for LAB fermentation.

However, pH values of silages were somewhat higher than those obtained by other authors for efficient lactate silages [3,11–23]. Therefore, secondary fermentation should have occurred for catch crop silages through undesired clostridial activity.

Saccharolytic 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 [5]. This will lead to VS and energy losses. Moreover, clostridial activity is associated with losses of acidity. 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 [5]. Low TS content of catch crop (Table *1*) should have boosted secondary fermentation. Indeed, clostridia are known to be particularly sensitive to water availability and require wet conditions for active development [5]. According to Borreani et al. [11] clostridial fermentation exponentially decrease as TS content increase, being negligible from 30% of total solids. In our work, TS content of feedstock was quite below this value, so that clostridia activity was not inhibited.

Regarding storage of Cattle manure 1, pH values of biomass were 8.4 and 9.8 after 4 months of ensiling and aerobic treatment, respectively. The fact that pH of silage was above neutrality indicates that there was no significant accumulation of organic acids. Absence of WSC and high content of strong basic buffer components in raw material can explain the lack of acidity of cattle manure silage.

Finally, pH values of open-air stored biomass (9.1-9.8) were always higher than the respective raw material (6.4-7.9 – results not shown). Therefore, hydrogen-consuming reaction must have occurred, which evidences that aerobic microbial population spoiled biomass.

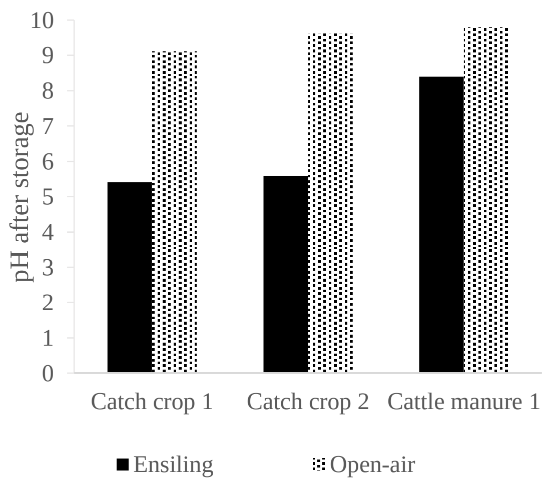

Fig. 1 – pH value after 3 months of storage for Catch crop 1 / Catch crop 2 and 4 months for Cattle manure 1

3.1.2. Organic matter and energy conservation

The effects of open-air storage and ensiling on the conservation of biomass are summarized in Fig. 2.

For Catch crop 1 the use of ensiling instead of open-air storage reduced organic matter losses from around 69% to 10%. In the case of Catch crop 2, 68% of VS was lost during aerobic storage, which was reduced to 26% for ensiling. This discrepancy between methods was even more pronounced for the energy conservation during storage. On the one hand, after 3 months of storage in presence of air, both catch crops lost more than 80% of their original BMP. This demonstrates the harmful effect of an aerobic environment not only on the energy content of the remaining biomass but also on its biochemical accessibility. One the other hand, at least 96% of catch crop's original BMP was conserved after ensiling. Therefore, low TS content of feedstock did not impede the full conservation of its energy content for prolonged periods of ensiling. This allow two different considerations. First, it is conceivable that clostridial activity did not led to substantial energetic damage. In contrast, even if BMP damage and instability was caused by secondary fermentation, this was overcome by gains in biochemical accessibility during ensiling. In fact, according to several authors [12,21,24], a partial degradation of (hemi-) cellulosic compounds occurs during ensiling. Since these polymers are not be fully biodegradable in mesophilic AD [25], their hydrolysis may induce a BMP increase.

Even though confined storage improved cattle manure preservation, substantial losses still occurred. Indeed, after 4 months, organic matter losses only decreased from 53% to 40% and BMP losses from 74% to 46% in ensiling rather than outdoor storage. Ensiling inefficiency for cattle manure should be related with the lack of acidification during the storage period. Since acid conditions were not achieved, silage did not escape from the pH range where damaging microorganism (*e.g.* methanogens) were active. Furthermore cattle manure use in this tests had low TS content (Table *1*), which should have increased the microbial activity. Therefore, cattle manure was not anaerobically stabilized and high VS and energy losses occurred through biogas production during storage.

3.2. Effects of a co-ensiling approach

Ensiling of cattle manure with several co-substrates was investigated at laboratory scale in order to optimize energy conservation of the raw material during prolonged periods of storage. In the previous assays, two of the major issues related with cattle manure preservation were: low TS content and; absence of organic substrates like WSC for LAB fermentation. Wheat straw addition was used to decrease water availability of cattle manure. Formic acid and Glucose were tested as direct and indirect sources of acidification. These two latter compounds are high added-value products and they were tested only as molecule models of a real co-substrate. For instance, the use of glucose may be replaced in field application by a substrate with high WSC content, such as molasses.

3.2.1. pH values

The impact of co-substrates addition on the pH value of cattle manure before and after 4 months of ensiling is presented in Fig. 3.

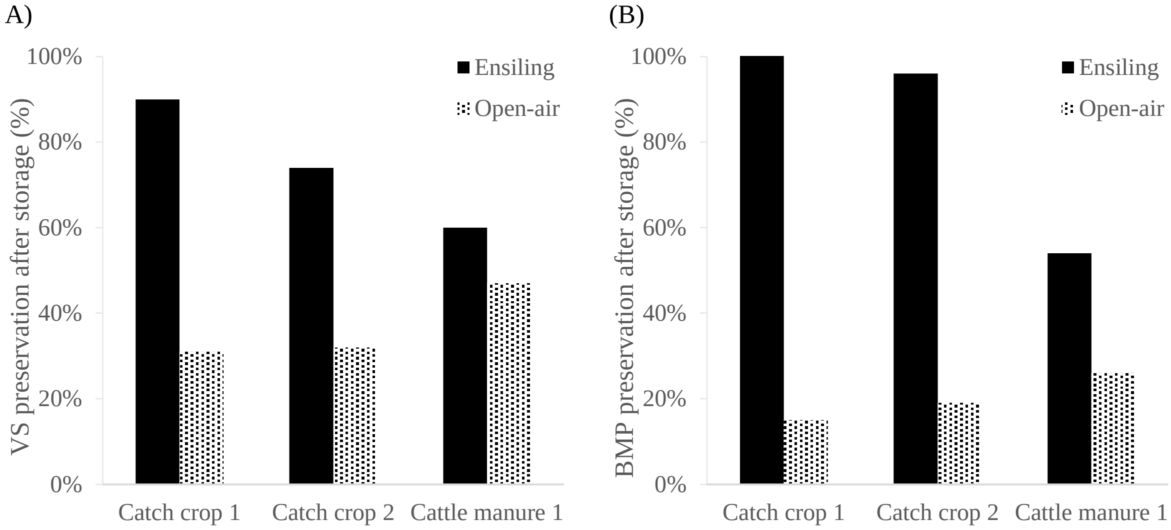

Fig. 2 - Organic matter (A) and BMP (B) losses after 3 months of storage for Catch crop 1 / Catch crop 2 and 4 months for Cattle manure 1. BMP losses are related to the differences between values on a $VS_{original}$ basis, therefore taking into account storage loss

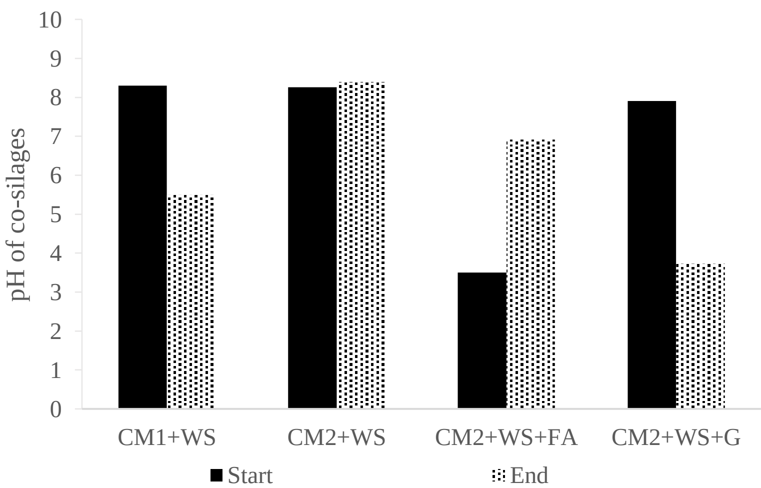

Fig. 3 - pH value before and after 4 months of co-ensiling assays. CM1+WS stands for Cattle manure 1 with wheat straw; CM2+WS for Cattle manure 2 with wheat straw; CM2+WS+FA for Cattle manure 2 with wheat straw and formic acid; CM2+WS+G for Cattle manure 2 with wheat straw and glucose

Wheat straw addition had different impacts on the fermentation of each Cattle manure. Regarding cattle manure 1 (CM1+WS), pH value decreased from 8.3 before storage to 5.5 after 4 months of co-ensiling. This indicates that fermentation occurred and that there was an accumulation of organic acids in co-silage. Yet, wheat straw was mainly composed of fibers and so, its addition should not have contributed to the increase of WSC content in feedstock. Therefore, silage acidification must have been a result of wheat straw impact on TS increase and, consequently, on the limitation of methanogen activity during anaerobic storage. Furthermore, it is suggested that wheat straw addition has reduced the buffer capacity of the raw material at neutral pH value, which facilitated acidification.

In opposite, pH value of Cattle manure 2 was stable during co-ensiling with wheat straw (8.3-8.4). This evidences that straw was not an infallible asset for the conservation all types of Cattle manure though ensiling. In fact, bacterial populations can be influenced by the characteristics of manure [26], which according to Marañón *et al.* [27] may depend on the type of cattle, animal's diet, as well as, on the time of the year. In our experiments, Cattle manure 1 was collected on March 2016, while Cattle manure 2 was from June 2016. Thus, it is possible that, for instance, the activity of methanogen population was predominant in Cattle manure 2, or at least in higher concentration than in Cattle manure 1. In that case, even if organic acids were still produced, they were quickly converted into biogas, hindering biomass acidification and preservation.

Lower pH was observed either for formic acid or glucose addition to CM2+WS after storage. For the formic acid condition, pH was 3.5 before storage and it increased to 6.9 at the end of 4 months of ensiling. The partial loss of acidity demonstrates that formic acid addition was not enough to inhibit fermentation and its consumption.

Co-ensiling of Cattle manure 2 with both glucose and wheat straw led to extensive acidification of biomass. Indeed, pH value was 7.9 for CM2+WS+G feedstock and deceased to 3.7 after 4 months of storage. The fact that the pH was that low indicates that lactic acid was produced in large quantities in the early days of ensiling and that microbial activity was stopped, thus preventing clostridial fermentation. Moreover, this shows that fresh cattle manure had a strong LAB population. Therefore, one may conclude that lack of WSC in raw material was the major barrier for the conservation of cattle manure through the ensiling process.

3.2.2. Organic matter and energy conservation

Results presented in Fig. 4 evidence the strong correlation between biomass acidification and both VS and BMP preservation during anaerobic storage.

Wheat straw addition to Cattle manure 1 enhanced ensiling performance: 92% and 98% of its original organic matter and energy content (BMP) were conserved after 4 months. These were remarkable results for a feedstock that is not naturally adapted for ensiling conditions. Furthermore, this indicates that a pH value around 5.5 may be enough to prevent substantial methanogenic activity for long-term storage of cattle manure (with around 20% of TS content).

Unlike, severe losses occurred for Cattle manure 2 with only wheat straw addition. For this condition, 42% and 67% of its original organic matter and BMP were lost after 4 months, respectively. This evidences the important activity of degrading microorganisms for neutral pH conditions in ensiling medium.

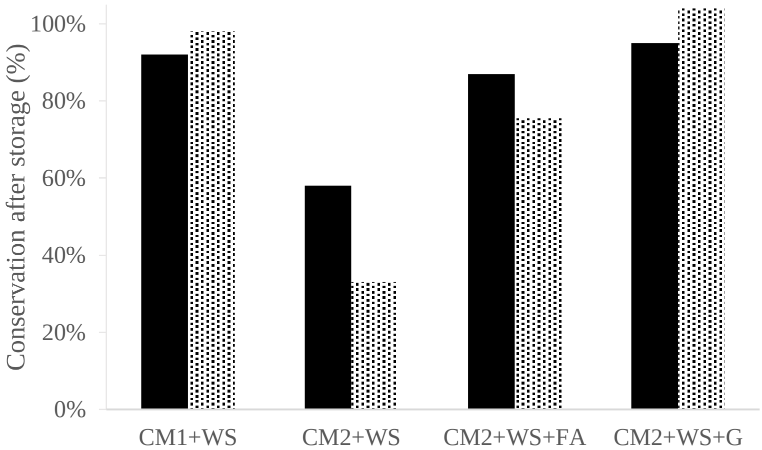

Fig. 4 - BMP and organic matter preservation of co-ensiled cattle manure after 4 months. CM1+WS stands for Cattle manure 1 with wheat straw; CM2+WS for Cattle manure 2 with wheat straw; CM2+WS+FA for Cattle manure 2 with wheat straw and formic acid; CM2+WS+G for Cattle manure 2 with wheat straw and glucose. BMP balance takes into account storage losses

Hence, wheat straw addition cannot be seen as a full guarantee to great biomass and energy conservation during long-term ensiling for all types of cattle manure.

Silage quality of Cattle manure 2 was improved with the use of direct and indirect sources of acidification. Regarding formic acid assays (CM2+WS+A), biomass conservation was enhanced to 87% and 75% of the initial VS and

BMP content, respectively. However there were still non-negligible losses for this condition. This should be linked the loss of acidity during ensiling and probable formic acid consumption previously discussed. In parallel, co-ensiling of cattle manure with both glucose and wheat straw showed outstanding results. As a matter of fact, 95% of original VS was preserved and no loss of BMP was observed until the end of the 4 months. This demonstrates that optimal conservation of energy content of cattle manure during storage may be achieved through co-ensiling with a substrate containing high concentration of available carbohydrates and strong TS content.

4. Conclusions

Energy losses during storage of organic residues were limited through ensiling. Even though, the efficiency of anaerobic storage strongly relied on chemical characteristics of each raw material. On the one hand, full conservation of methane potential occurred for ensiling of catch crops during 3 months. On the other hand, ensiling of single-handedly cattle manure led to 46% loss of its original energetic content after 4 months. Lack of easily available substrate for fermentation, high water availability and non-adapted buffering capacity were some of cattle manure potential features that hindered biomass preservation. For this latter feedstock, our work also revealed that the use of additives may prevent biomass spoilage and lead to full energetic conservation during long-term ensiling. Finally, co-ensiling with a substrate containing high concentration of available carbohydrates appears to be the most resourceful method to optimize cattle manure storage before biogas production. These outcomes may contribute to enhance economics of agricultural biogas plants.

Acknowledgements

Rúben Teixeira Franco held a doctoral fellowship from the Rhône-Alpes region. This work has been undertaken within the SAM project (Stockage Avant Méthanisation - Storage Before AD) funded by ADEME (# 1506C0038). 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 acknowledged for the inoculum provided for the BMP tests.

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