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Rùben Teixeira Franco

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Optimisation des pratiques de gestion des déchets agricoles en lien avec leur valorisation par méthanisation

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« Quod scripsi, scripsi. »

Pontius Pilate / José Saramago

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RESUME

A l'instar d'autres pays européens, la France se mobilise actuellement pour la valorisation énergétique de ses déchets agricoles par méthanisation. Néanmoins, la production d'une partie de ces ressources n'est pas constante toute au long de l'année. En conséquence, afin de permettre l'approvisionnement continu des installations de biogaz, elles doivent être conservées parfois pendant de longues périodes (plusieurs mois).

Cette thèse porte sur l'optimisation des pratiques de gestion des ressources agricoles avant méthanisation. Différentes conditions de stockage ont été étudiées, en particulier le stockage aérobie ainsi que l'ensilage, dans l'optique de l'amélioration de la conservation du potentiel bio-méthanogène et de l'optimisation de la conversion de la matière organique lors de la méthanisation.

Grâce à une méthode expérimentale originale, la caractérisation multiphasique des déchets à différents stades de leur gestion a été mise en œuvre, à la fois sur les compartiments biochimiques et sur la biodisponibilité du carbone pour la méthanisation. Dans une première partie, la préparation et le stockage de cultures intermédiaires à vocation énergétique ont été étudiés au laboratoire. Les résultats obtenus ont permis de démontrer l'avantage majeur de l'ensilage plutôt que le stockage à l'air libre pour la conservation du pouvoir méthanogène. Deuxièmement, des essais de stockage de longue durée ont été réalisés pour des fumiers de bovin. Ces expériences ont permis de mettre en évidence la mauvaise conservation des fumiers et l'intérêt de l'utilisation de co-substrats pour améliorer la performance du stockage en conditions d'ensilage. Afin d'expliquer les mécanismes majeurs qui se produisent au cours de l'ensilage, une approche de modélisation a été utilisée pour synthétiser et interpréter les résultats. Ces travaux de recherche ont permis d'identifier les bonnes pratiques de gestion pour les intrants de méthanisation agricole afin d'optimiser au mieux la conservation des ressources.

Mots-clés : Biomasse, Déchets agricoles, Cultures à vocation énergétique, Fumier de bovin, Stockage, Ensilage, Méthanisation, Potentiel bio-méthanogène, Modélisation

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ABSTRACT

Like other European countries, France is currently mobilizing for the energy recovery of its agricultural wastes by anaerobic digestion. Nevertheless, the production of these resources undergoes several fluctuations throughout the year. Consequently, in order to allow the continuous supply of biogas plants, these raw materials must be often stored for long periods (several months).

This thesis focuses on the optimization of agricultural wastes management practices before anaerobic digestion. Different storage conditions were studied, notably the open-air storage and ensiling, with the aim of improving the conservation of the biochemical methane potential and optimizing the conversion of organic matter during anaerobic digestion.

An original experimental methodology was developed and implemented for the multiphasic characterization of biomass at different stages of its management and storage, including analysis on the different biochemical fractions and bioavailability of carbon for anaerobic digestion. First, this methodology was applied to the preparation and long-term storage of catch crops. This evidenced a major advantage of using ensiling rather than open-air storage for the preservation of biomass and methane potential. Second, long-term storage trials were conducted for cattle manure. These experiments highlighted the poor conservation of manure and the benefits of co-substrates in the improvement of storage performances under ensiling conditions. To explain the major mechanisms that occur during the ensiling process, a modelling approach was used to synthesize and interpret the results. Finally, this research work led to the identification of good conservation practices for the major feedstocks of agricultural biogas plants.

Keywords: Biomass, Agricultural wastes, Catch crops, Cattle manure, Storage, Ensiling, Anaerobic Digestion, Biochemical methane potential, Modelling

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PRODUCTION SCIENTIFIQUE

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R. Teixeira Franco, P. Buffière, R. Bayard, Optimizing storage of a catch crop before biogas production: impact of ensiling and wilting under unsuitable weather conditions, Biomass and Bioenergy. 100 (2017) 84–91. doi:10.1016/j.biombioe.2017.03.017.

R. Teixeira Franco, P. Buffière, R. Bayard, Cattle manure for biogas production. Does ensiling and wheat straw addition enhance preservation of biomass and methane potential?, *Soumis à Biofuels en Juin 2017*.

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Communications orales en congrès internationaux

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Autres

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LISTE D'ABBREVIATIONS

Abréviations		Français	*Anglais*
-	*[AA]*	Acide acétique	*Acetic acid*
-	*[AD]*	Méthanisation	*Anaerobic digestion*
ADEME	-	Agence de l'environnement et de la maîtrise de l'énergie	*French environment and energy management agency*
-	*[ADF]*	Détergent acide	*Acid detergent fiber*
-	*[ADL]*	Lignine sulfurique	*Acid detergent lignin*
AGV	*[VFA]*	Acides gras volatils	*Volatile fatty acids*
-	*[BA]*	Acide butyrique	*Butyric acid*
-	*[BC]*	Pouvoir tampon	*Buffering capacity*
-	*[CEL]*	Cellulose	*Cellulose*
CLHP	-	Chromatographie liquide à haute performance	*High performance liquid chromatography*
CIVE	-	Culture intermédiaire à vocation énergétique	*Catch crop*
DCO	*[COD]*	Demande chimique en oxygène	*Chemical oxygen demand*
	[DAE]	Equations différentielles et algébriques	*Differential and algebraic equation*
DEEP	-	Déchets Eaux Environnement Pollutions	*Wastes Water Environment Pollutions*
DOSTE	-	Déchets Organiques retour au Sols Traitements et Energie	*Organic Waste back to Soil Treatments and Energy*
-	*[FA]*	Acide formique	*Formic acid*
-	*[FID]*	Détecteur d'ionisation de flamme	*Flame ionization detector*
-	*[GC-TCD]*	Chromatographie gazeuse couplée avec un détecteur par conductivité thermique	*Gas chromatography with thermal conductivity detector*
-	*[He]*	Hétéro-fermentaire	*Heterofermentative*
-	*[HEM]*	Hémicellulose	*Hemicellulose*
-	*[Ho]*	Homo-fermentaire	*Homofermentative*
	[ICP-OES]	Spectromètrie d'émission atomique à plasma induit par haute fréquence	*Inductively coupled plasma optical emission spectrometry*
-	*[LA]*	Acide lactique	*Lactic acid*
-	*[LAB]*	Bactéries lactiques	*Lactic acid bacteria*

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-	*[LIG]*	Lignine	*Lignin*
MH	-	Matière humide	*Total weight*
MS	*[TS]*	Matière sèche	*Total solids*
MV	*[VS]*	Matière volatile	*Volatile solids*
-	*[NDF]*	Détergent neutre	*Neutral detergent fiber*
NH_3-N	*[NH_3-N]*	Ammonium	*Ammonia nitrogen*
NTK	*[TKN]*	Azote Kjeldahl total	*Total Kjeldahl nitrogen*
PAM	-	Prétraitements avant méthanisation	*Pretreatments before anaerobic digestion*
PBM	*[BMP]*	Potentiel bio-méthanogène	*Biochemical methane potential*
-	*[RID]*	Détecteur à indice de réfraction	*Refractive index detector*
SAM	-	Stockage avant méthanisation	*Storage before anaerobic digestion*
-	*[STP]*	Conditions normales de température et de pression	*Standard temperature and pressure*
-	*[WSC]*	Sucres solubles	*Water soluble carbohydrates*
-	*[WWTP]*	Station d'épuration des eaux usées	*Waste water treatment plant*

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INTRODUCTION

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La société est aujourd'hui confrontée à la transition énergétique, considérée comme partie intégrante de la solution pour un développement durable des générations futures. La digestion anaérobie, ou **méthanisation**, est l'une des filières de production d'énergie renouvelable qui a suscité un intérêt croissant au cours des dernières années. Cette filière consiste à réaliser la bioconversion de matières organiques complexes, dans des conditions anaérobies et par réactions successives, en biogaz principalement composé de CH_4 et CO_2. Actuellement, plus de 17 000 installations de production de biogaz sont opérationnelles en Europe [1]. Le biogaz produit peut être valorisé par injection dans le réseau de gaz, converti en électricité ou utilisé comme carburant. Pour ce qui concerne la France, plus de 450 méthaniseurs étaient en exploitation au début de l'année 2016, dont 236 installations de digestion à la ferme [2].

Etant donné que, théoriquement, de l'énergie peut être produite à partir de tous les types de résidus organiques biodégradables, fourrages ou cultures agricoles, il existe actuellement une grande diversification de gisements pour la méthanisation. Dans le contexte français, la digestion anaérobie est principalement considérée comme un **procédé de valorisation des déchets** et la proportion des cultures énergétiques dans l'approvisionnement des installations est limitée à 15% du tonnage total brut des intrants selon la législation actuelle [3]. Parmi les ressources disponibles pour la méthanisation agricole sur le territoire français, le fumier de bovin est la principale ressource à mobiliser pour l'approvisionnement des installations agricoles. La production annuelle de ce résidu sur l'hexagone est d'environ 87 Mt_{MH}, ce qui représente un potentiel énergétique estimé à 28 MWh [4]. Par ailleurs, les cultures intermédiaires à vocation énergétiques (CIVEs) sont largement utilisées pour la production de biogaz. Ces cultures sont réalisées entre deux cultures principales à vocation alimentaire. L'intérêt de la valorisation des CIVEs par digestion anaérobie réside non seulement dans le fait que leur potentiel énergétique est supérieur à celui des effluents d'élevage, mais aussi par le fait qu'elles ne créent pas de compétition d'usage des terres arables et présentent également des qualités agronomiques favorables à la structuration et l'enrichissement du sol.

L'un des enjeux majeurs dans l'optimisation de la filière de méthanisation concerne la gestion des gisements avant la production de biogaz. En effet, avec la nécessité d'un approvisionnement continu des installations de biogaz tout au long de l'année, certaines **biomasses de production saisonnière** doivent être stockées dans la plupart des cas pour des périodes prolongées. Etant donné qu'une partie de ces matrices organiques sont facilement bio-accessibles et biodégradables, le **stockage** à long terme peut entraîner des pertes énergétiques conséquentes et des émissions de gaz à effet de serre. Ainsi, les exploitants et constructeurs d'installations s'interrogent sur le mode de gestion et de préparation de ces déchets avant l'alimentation des digesteurs, cette problématique ayant reçu une attention limitée jusqu'à présent, en particulier pour les résidus d'élevage, liquides ou solides.

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Les travaux de cette thèse ont porté sur l'**optimisation des pratiques de gestion des déchets agricoles en lien avec leur valorisation par méthanisation**. Pour répondre à cette problématique, différentes conditions de stockage ont été évaluées à l'**échelle du laboratoire** à partir de leur impact sur les compartiments biochimiques et sur la biodisponibilité du carbone pour la méthanisation. Deux types de biomasses ont été étudiés : les **CIVEs** et les **fumiers de bovin**. Une approche de **modélisation** a été aussi mise en œuvre pour expliquer les mécanismes majeurs qui se produisent au cours du stockage confiné. Cette thèse s'inscrit dans le cadre du **projet SAM**, Stockage Avant Méthanisation, financé par ADEME (programme DOSTE 2014), dont l'objectif est d'apporter aux agriculteurs et exploitants des conseils techniques sous la forme d'un guide sur les bonnes pratiques de stockage avant méthanisation.

Ce mémoire est divisé en **six chapitres**. Le **premier** chapitre présente un état de l'art des connaissances scientifiques et techniques sur la gestion des déchets avant méthanisation, focalisé sur le stockage confiné ou ensilage. Cette synthèse bibliographique est présentée sous la forme d'un article scientifique, complété par une conclusion précisant le lien entre les questions sans réponse et les objectifs de cette thèse. Dans le **chapitre 2**, la démarche et la méthodologie développées pour les besoins de la thèse sont présentées, y compris la procédure de prélèvement, le choix des analyses et les protocoles opératoires.

Les trois chapitres suivants décrivent les résultats et discussion de cette thèse. Les essais de stockage pour des CIVEs sont présentés dans le **chapitre 3** sous la forme de deux articles scientifiques. Ces travaux expérimentaux ont porté sur la méthode et la durée de stockage, ainsi que l'impact du préfanage[1] et de la teneur en matière sèche sur la conservation de la matière organique et du potentiel énergétique de la biomasse. Le **chapitre 4** est dédié à l'étude d'une matrice difficilement conservable, le fumier de bovin. Des nombreux paramètres d'influence sur la conservation de l'intrant, tels que la teneur en matière sèche ou le co-ensilage par ajout d'additifs, sont ici évalués et présentés sous la forme de trois articles scientifiques. Dans le **chapitre 5**, une synthèse globale regroupe les principaux résultats et tendances observés pour les différents essais de stockage. Cette synthèse a servi de base pour le développement d'un modèle descriptif du stockage en mode d'ensilage, lequel constitue la partie majeure de ce chapitre.

Les conclusions générales et perspectives sont présentées dans la **dernière partie** du mémoire de thèse. Ce dernier chapitre présente les mesures pratiques proposées pour l'optimisation de la gestion des déchets agricoles avant méthanisation, ainsi que les prochaines étapes à suivre dans cet axe de recherche. Finalement, ce chapitre résume la contribution de ces trois années de thèse pour l'optimisation de la filière de la méthanisation dans le secteur de l'énergie et de l'environnement en préconisant le stockage contrôlé des gisements agricoles saisonniers dédiés à la méthanisation agricole.

[1] Le préfanage (*wilting* en anglais) est le terme qui désigne l'opération consistant à laisser les fourrages au sol quelques temps afin qu'ils perdent de l'eau avant l'ensilage.

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1. SYNTHESE BIBLIOGRAPHIQUE

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1.1. Ensiling for biogas production: critical parameters. A review

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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 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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1.1.1. Introduction

Taking into account political and environmental concerns, investment in bioenergy production has been intensified and diversified over the past twenty years [5]. Considering recent studies [6], 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) [6] 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 [7].

Nevertheless the segment's continuing growth over the last years, the focus in energy efficiency of biogas plants will be crucial in the future [6], 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 1-1, 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.

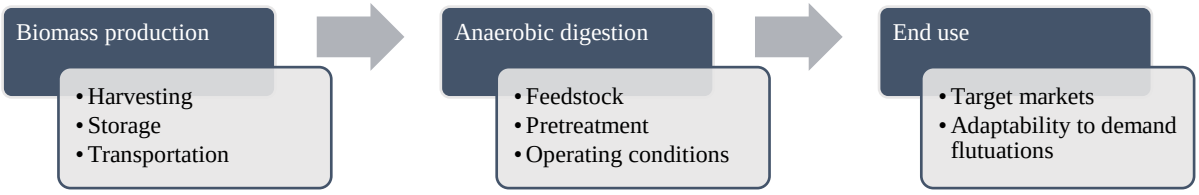

Figure 1-1 – Simplified supply chain of biogas production with examples of optimization points.

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 [8]. Even though this system minimizes both labor and storage costs, it leads to

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high losses in terms of dry matter that may reach up to 60% [9], it is restricted to crops that can dry quickly and uniformly. This technique can be limited by rainfall during harvest [10].

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 [8,10,11]:
- 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 [12]. 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 [13].

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 [13]. 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 [11,14–16].

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 [8,12,17]. 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

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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 [8], 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.

1.1.2. Influence parameters

1.1.2.1. 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.

1.1.2.1.1. 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 1.1.2.1.3), water soluble carbohydrates (WSC) content, buffering capacity (BC) and epiphytic microflora of feedstock will play a crucial role on the course of ensiling [12], 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 [11,16,18–23] 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 [12].

Similarly, grass is usually conserved as silage [12]. Although it is commonly used and studied [14,15,24–26], grass chemical characteristics will strongly depend on the species used, the stage of growth or even the climate [12]. For instance, in the late stages of growth, the WSC content of grass tends to decrease, while the cell wall components increase [12]. In this case, fermentation will be slower, retarding the decrease of pH necessary for efficient preservation [27].

Other crops are used for ensiling, but to a fewer extent. Other cereals such as sorghum [8,9,11,28] and triticale [11,29] have been investigated. In addition, some crop residues such as sugar beet tops [14,21],

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corn stalks [30] or agricultural and food processing by-products [31] 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 [11,16,21,32]. Zubr [32] 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*. [11] 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 1-2).

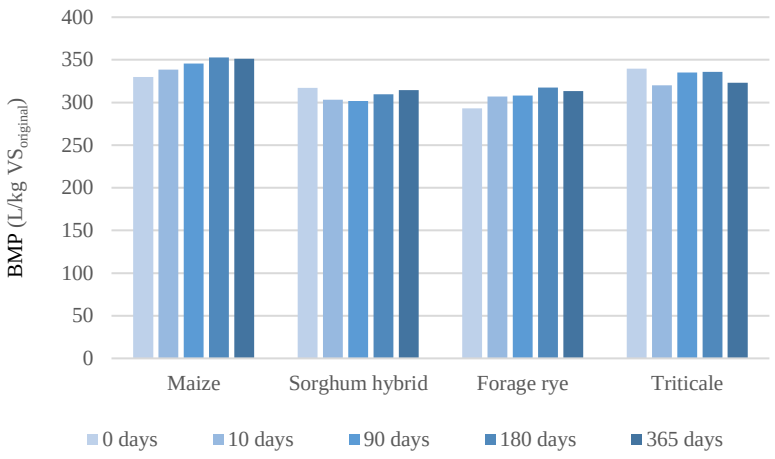

Figure 1-2 – Methane formation of whole crop maize, sorghum hybrid, forage rye and triticale influenced by storage duration. BMP is based on the organic matter of fresh biomass, which takes into account VS losses during storage (adapted from Herrmann *et al.* [11]).

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 1-2, Herrmann *et al*. [11] 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 [14] and Pakarinen *et al*. [16]. 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 [11,14,15,22,29] and for the temperature [14].

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

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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 [33].

1.1.2.1.2. Particle Size

Methane fermentation through AD is clearly affected by feedstock's particle size, as it interferes in the kinetics of complex substrates hydrolysis [34]. 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 [35]. 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.* [36] 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*. [37], Herrmann *et al*. [36] and Haag *et al*. [22] presented different results and conclusions. According to the early work [37], 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*. [12], 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 1-1). 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 1-1. 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*. [36] 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

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chopping length enhanced the methane yield based on original volatile solids (VS) content as presented in Figure 1-3. 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.

Table 1-1 – Examples of clostridial fermentation reactions (adapted from McDonald *et al*. [12]).

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

Contrasting with the data presented above, Haag *et al*. [22] 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.

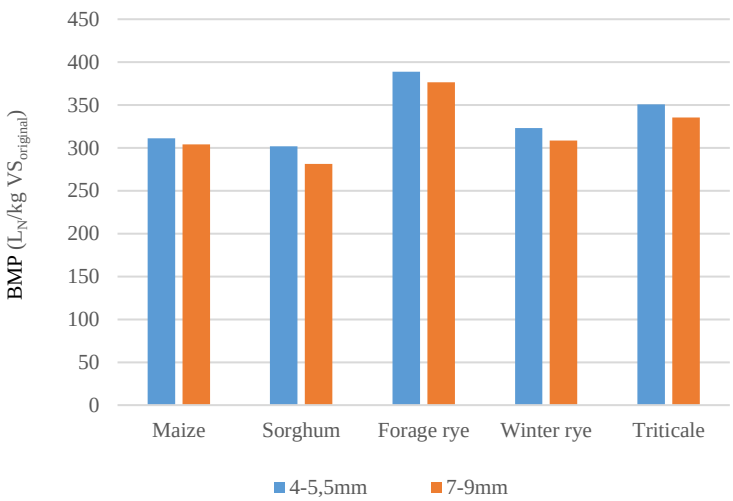

Figure 1-3 – Effect of farm-scale particle size reduction on methane formation of ensiled crop feedstock. BMP is based on the organic matter of fresh biomass, which takes into account VS losses during storage (adapted from Herrmann *et al.* [36]).

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Regarding the optimization of chopped length size for ensiling purposes, Mohd-Setapar *et al*. [38] suggested that this subject has been poorly investigated and most studies were performed using pre-defined particle size crops. Nevertheless, Herrmann *et al.* [39] 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.

1.1.2.1.3. 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*. [12]), 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 [12]. Borreani *et al*. [40] 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 [41] 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*. [42] and Wilkinson [43] 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*. [44] 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 [45] published a work on ryegrass and white clover mixture silages; they found that, despite a higher content of NH_3-N, higher

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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 1-2.

Together with the benefits for organic matter preservation, higher total solid content may prevent leachate formation during ensiling. Indeed, several authors as Bastiman [46], Sutter [47] and Zimmer [48] have proposed correlations to predict the behavior of effluent production (Figure 1-4). In the works by Bastiman [46] and Zimmer [48], similar quadratic derived equations were obtained, in which negligible leachate formation occurs above around 25% of TS. On the other hand, Sutter [47] 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 [49]. 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 [8]. 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.

Table 1-2 – Critical pH value in function of silage TS content (adapted from Kalač [27]).

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

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 [12]: it is

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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*. [40], Carpintero *et al*. [50], Dawson *et al*. [24] and McEniry *et al*. [25] have compared wilted and un-wilted feedstock chemical characteristics before ensiling. McEniry *et al.* [25] 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*. [40] 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.* [50] 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.

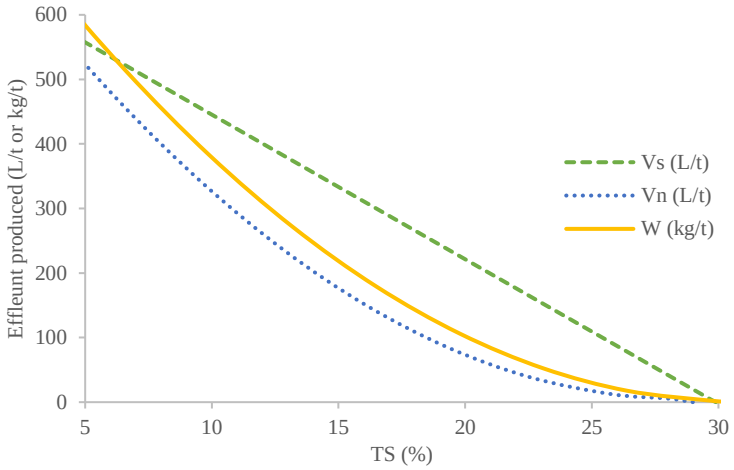

Figure 1-4 – Effluent production as function of total solids content of ensiled crop. *Vs* is the volume of effluent produced by unit mass of silage [47], *Vn* is the volume of effluent produced by unit mass of herbage [46] and *W* is the weight of effluent produced by unit mass of herbage [48].

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*. [24] 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.

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As alternative to open air wilting, more complex and expensive treatments, such as chemical desiccation and thermal treatment, can be proposed [12]. 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 [40,51].

Contrary to the aforementioned advantages, the few studies that evaluated the impact of TS content in the BMP showed inconclusive results. Pakarinen *et al*. [15] 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.* [25] 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.* [15], 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.

1.1.2.2. 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.

1.1.2.2.1. Presence of air

Oxygen is usually considered as a spoiling agent in a process that needs to achieve anaerobic conditions, where LAB can proliferate [10,12,13]. In fact, air causes silage deterioration since it favors the activity of aerobic microorganisms, as heterotrophic bacteria, yeasts and molds [52]. Besides the theoretical and macroscopic evidences of oxygen detrimental action on silage, some laboratory and field scale studies

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have been performed to confirm the impact of aerobic conditions. Indeed, Garcia *et al*. [53] 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*. [54] 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 [12]. According to Egg *et al.* [8], 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 [55], 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.* [23] 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*. [56] 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.* [25] 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.* [23] 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 [23,25,57–59].

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 [8,23].

1.1.2.2.2. 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

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of air, and to slower oxygen flows in silage, thereby reducing losses due to aerobic spoiling [60]. These statement have been confirmed by different authors, with favorable results for higher densities. Indeed, Ruppel [61] worked with alfalfa silage for 180 days and found a relation between the density and the silage total solid losses, presented in Table 1-3. Zheng *et al.* [62] 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.

Table 1-3 – Impact of packing density of silage on total solids losses (adapted from Ruppel [61])

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.

Similarly, Zheng *et al.* [63], 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.* [19] 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 [60,64]. 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 [65]. 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.

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1.1.2.3. 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*. [19], 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.* [23] 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 [14], 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 [11]. 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 [15]. However, in Herrmann *et al.* [11] 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*. [15] 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 [14] 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*.

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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.

1.1.2.4. 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 [66].

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 [67], 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.* [53], 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.* [68], 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 [14], who suggested that very low ensiling temperatures do not necessarily lead to subsequent higher methane

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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.

1.1.2.5. 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 [12]. 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 [12]. 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 [17].

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 [19]. Nevertheless, several commercial products, among the categories presented in Table 1-4, 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.

1.1.2.5.1. 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

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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.

Table 1-4 – Classification of main silage additives appropriate for biogas production purposes (adapted from McDonald *et al.* [12]).

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

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 [12].

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 [27]. 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 [69]. 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 1-5. 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 [14,15,25], only positive impact on the BMP were obtained, regardless of the stimulant used. In

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opposition, negative influence was obtained for crops such as amaranth or sugar beet tops [14,22]. Another interesting case is that of whole crop maize: although representing almost half of the published data on the subject [11,19,20,22], it shows inconclusive results regarding the influence of stimulants on BMP. Beyond the results presented in Table 1-5 for whole crop maize, Herrmann *et al*. [23] also studied this biomass and observed low effects of stimulants on its BMP during ensiling.

Table 1-5 – 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*. [22]
Whole crop maize	He [a]	90	1% [b]	Haag *et al*. [22]
Whole crop maize	Ho	90	-4% [c]	Herrmann *et al*. [11]
Whole crop maize	Ho+He	90	-5% [c]	Herrmann *et al*. [11]
Whole crop maize	Ho	119	-23% [d]	Neureiter *et al*. [19]
Whole crop maize	Ho+He	119	-18% [d]	Neureiter *et al*. [19]
Whole crop maize	Amylase	119	-4% [d]	Neureiter *et al*. [19]
Whole crop maize	*Clostridium tyrobutyricum*	119	7% [d]	Neureiter *et al*. [19]
Whole crop maize	Ho+He 1	49	-12% [d]	Vervaeren *et al*. [20]
Whole crop maize	Ho+He 2	49	6% [d]	Vervaeren *et al*. [20]
Whole crop maize	Ho+He+Enzymes	49	5% [d]	Vervaeren *et al*. [20]
Whole crop maize	Ho+He+Yeasts+Fungi	49	11% [d]	Vervaeren *et al*. [20]
Grass	Ho	90	12% [d]	Lehtomäki [14]
Grass	Enzyme	90	19% [d]	Lehtomäki [14]
Grass	Ho	110	5% [d]	McEniry *et al*. [25]
Grass	He	110	12% [d]	McEniry *et al*. [25]
Grass	Sucrose	110	8% [d]	McEniry *et al*. [25]
Grass	Ho+Enzyme	180	1% [d]	Pakarinen *et al*. [15]
Ryegrass	Ho+Enzyme	180	8% [d]	Pakarinen *et al*. [15]
Amaranth	Ho	90	-11% [b]	Haag *et al*. [22]
Amaranth	He	90	-14% [b]	Haag *et al*. [22]
Sugar beet tops	Ho	90	-7% [d]	Lehtomäki [14]
Sugar beet tops	Enzyme	90	-10% [d]	Lehtomäki [14]
Sorghum	Ho+He	90	-1% [c]	Herrmann *et al*. [11]
Forage rye	Ho+He	90	3% [c]	Herrmann *et al*. [11]
Triticale	Ho+He	90	2% [c]	Herrmann *et al*. [11]

[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.

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The data from grass and whole crop maize are in agreement with the statements made by Kalač [27] 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 [19,20]. 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.

1.1.2.5.2. 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 [12], 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 [70]. 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 [17].

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 [70].

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,

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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*. [16] 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 [14], while using formic acid on sugar beet tops at 0.5%v/w content.

On the opposite, Lehtomäki [14] and McEniry *et al*. [25] observed coherent higher methane yields using similar concentrations of formic acid on grass. Furthermore, the results of Lehtomäki [14] 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 [35,71], increase of accessible surface area and lignin structure alteration [72]. 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.

1.1.3. 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.

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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.

1.1.4. Acknowledgements

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1.2. A Retenir

Cette synthèse bibliographique a permis de confirmer que le stockage peut avoir un impact significatif sur les propriétés de la biomasse et de son potentiel énergétique. Cependant, de nombreuses questions doivent être approfondies. D'abord, nous avons vu que l'ensilage est la technique de stockage permettant de conserver la matière organique de gisements humides avant méthanisation et, que la présence d'oxygène conduit à la biodégradation aérobie de la matière organique. Néanmoins, aucune étude impliquant des comparaisons entre l'impact de l'ensilage et d'autres techniques moins conservatrices (telles que le stockage en plein air) sur l'évolution de la biomasse a été réalisée jusqu'à présent. Cette problématique de stockage optimisé est particulièrement pertinente pour des biomasses qui ne présentent pas les propriétés favorables pour l'ensilage.

En effet, les performances de l'ensilage sont étroitement liées aux caractéristiques biochimiques de la biomasse à stocker. L'une des propriétés qui a une grande influence sur le cours de l'ensilage est la teneur en matière sèche de la biomasse. D'après la littérature scientifique, une teneur élevée en matière sèche est conseillée pour une fermentation plus restreinte et optimisée pendant le stockage. En outre, lorsqu'une diminution de la teneur en eau de la biomasse est souhaitée, le préfanage est la technique la plus couramment utilisée à cet effet. En dépit de ces indications, il n'y a pas de résultats substantiels permettant de confirmer l'avantage d'une teneur en matière sèche plus élevée (et, par voie de conséquence, le bien-fondé du recours au préfanage) pour la conservation du potentiel bio-méthanogène au cours du stockage.

De plus, il semble évident qu'il pourrait y avoir de grandes différences dans la conservation de la matière organique du gisement selon la durée d'ensilage. Cela s'explique par le fait que, pour certaines biomasses, l'ensilage n'est efficace que pour une durée limitée en raison de l'instabilité créée par ses propriétés (notamment des concentrations insuffisantes de sucres accessibles ou une teneur en matière sèche très faible). A notre connaissance, les études où plusieurs conditions de stockage de longues durées ont été testées sont peu nombreuses et, par conséquent, la connaissance de la cinétique des phénomènes qui se produisent pendant l'ensilage est encore très limitée.

Toutefois, la compréhension des mécanismes réactionnels qui ont lieu au cours du stockage confiné a été abordée par plusieurs auteurs dans des approches de modélisation [73,74]. Ces études sont surtout basées sur les phénomènes de fermentations lactique et clostridiale, ainsi que des réactions d'hydrolyse des composés particulaires. Néanmoins, ces approches ne comprennent pas les réactions qui ont lieu au cours de stockages peu performants (tels que l'acétogenèse et la méthanogenèse) et sont encore très éloignées des modèles de méthanisation et de la logique de conservation du potentiel énergétique pour production de biogaz.

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Enfin, l'utilisation d'additifs chimiques et biologiques a été l'axe principal de recherche pour l'ensilage ces dernières années. Malgré cela, les additifs ne contribuent pas toujours à une meilleure conservation du potentiel énergétique. L'utilisation d'additifs semble être particulièrement intéressant pour des matrices difficilement conservables, ce qui doit encore être testé et vérifié dans des recherches futures.

Celles-ci ont été les principales informations acquises à travers de cette étude bibliographique sur l'optimisation de la gestion de la biomasse avant la méthanisation et qui ont servi de base à la définition de la démarche et méthodologie de la thèse, présentée dans le Chapitre 2.

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2. DEMARCHE ET METHODOLOGIE

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2.1. Introduction

L'organisation générale du travail expérimental de thèse et les principes des matériels et méthodes sont présentés dans ce chapitre. Les détails plus complets seront présentés le cas échéant au sein même des chapitres suivants du mémoire, rédigés sous forme d'articles. La démarche de modélisation est présentée séparément dans le Chapitre 5.

La démarche expérimentale de la thèse s'est appuyée sur des essais de stockage à différentes durées de conditionnement. Pour cela, deux matrices de biomasse distinctes ont été utilisées : les CIVEs et le fumier de bovin (Figure 2-1). Ces gisements ont été choisis pour leur importance dans la méthanisation agricole en France, en termes d'abondance sur le territoire français, mais aussi en raison des différentes modalités de gestion utilisées pour chacune de ces ressources. En effet, les CIVEs, comme d'autres cultures agricoles, sont généralement préservées à travers du stockage en ensilage, contrairement aux fumiers de bovin, lesquels sont normalement stockés en tas à l'air libre pour des périodes variables qui peuvent atteindre plusieurs mois.

Figure 2-1 – Types de gisements utilisés dans les expériences. De gauche à droite : CIVE « hiver », CIVE « été », fumier de bovin.

Les gisements, deux types de CIVEs et trois fumiers de bovin, ont été collectés sur une exploitation agricole de la région Auvergne-Rhône-Alpes (Gaec Béreyziat, Les Teppes, 01340 Béréziat, France). La CIVE 1 (ou « hiver ») est constituée d'un mélange de triticale, pois, vesce et radis fourrager, broyée à 4 cm de longueur maximale lors de la récolte. La CIVE 2 (ou « été ») est constituée d'un mélange de tournesol, sorgho, pois, vesce et trèfle d'Alexandrie, broyée à une longueur maximale de 3 cm avant utilisation. Les trois fumiers de bovin ont été collectés sur le même site mais à différentes saisons de l'année. Les échantillons ont été stockés à 4 °C avant utilisation ultérieure.

Le plan général des expériences réalisées dans le cadre de cette thèse est résumé dans le Tableau 2-1.

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Tableau 2-1 – Plan global simplifié des expériences de la thèse.

		CIVE 1	CIVE 2	Fumier 1	Fumier 2	Fumier 3
Partie du mémoire		3.2	3.3	4.2	4.3	4.4
Durée (jours)		3 - 180	7 - 98	7 - 120	7 - 120	7 - 120
Modalité	Aérobie	Oui	Oui	Oui	-	-
	Ensilage	Oui	Oui	Oui	Oui	Oui
Humidité[1]	Préfanage	Courte durée	Longue durée	-	-	-
	Ajout de paille	-	-	Oui	Oui	Oui
Additifs[1]	A. Formique	-	-	-	Oui	-
	Glucose	-	-	-	Oui	Oui
	Amidon	-	-	-	-	Oui

[1]Paramètres testés pour des conditions d'ensilage

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2.2. Approche expérimentale

Les essais de préservation de la biomasse sont réalisés à l'échelle du laboratoire dans des mini-silos en plastique étanches d'un volume utile de 3.5 L équipés d'une sortie de gaz, comme illustré sur la Figure 2 2.

Figure 2-2 - Remplissage des silos et dispositif expérimental.

Les silos sont remplis jusqu'à 2.55 L avec des matières premières à une densité apparente de 0.7 kg/L. Du gravier est utilisé pour remplir le volume restant, en utilisant une membrane géotextile pour le séparer de la biomasse. Pour les essais d'ensilage, les silos sont fermés avec un couvercle en plastique et leur étanchéité à l'air renforcée avec un joint silicone. Pour le stockage aérobie, les silos sont laissés ouverts à l'air. Ensuite, les silos sont pesés et stockés dans une pièce thermostatée à 25±2 °C.

De façon à étudier plusieurs durées de stockage et en même temps de ne pas perturber l'environnement anaérobie des expériences, les prélèvements sont effectués par sacrifice. Ainsi, plusieurs silos sont utilisés pour chaque condition expérimentale. A titre d'exemple, des essais de stockage de fumier ont été préparés pour des durées de 7, 15, 30 et 120 jours. Par conséquent, 4 réacteurs ont été utilisés pour chacune des conditions de stockage.

De plus, afin de suivre la production de gaz pendant les essais de stockage confiné, des compteurs de gaz (MilliGascounter, RITTER) sont connectés à la sortie de gaz des silos. Toutefois, les émissions gazeuses n'ont pas pu être quantifiées en raison des faibles débits de gaz pendant les essais et d'éventuelles micro-fuites dans le circuit. En revanche, l'analyse qualitative du gaz produit a pu être mise en place par des mesures ponctuelles de la composition du gaz par micro-chromatographie en phase gazeuse pour les composés suivants : CH_4, CO_2, H_2, H_2S, N_2 et O_2. Pour plus de détails sur la micro-chromatographie en phase gazeuse, voir la section 2.4.7. Test de Potentiel Bio-Méthanogène.

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2.3. Procédure de prélèvement et analyses

Lorsque la durée de stockage souhaitée est atteinte, les silos sont ouverts et pesés pour déterminer les pertes de matière humide (MH) et de matière organique. L'ensemble de l'échantillon présent dans le silo est collecté, puis mélangé manuellement pour homogénéisation et enfin deux échantillons sont prélevés. L'un est utilisé pour des analyses sur la matière brute et l'autre est mis en contact avec de l'eau afin d'obtenir deux fractions : une phase soluble et une phase particulaire. Ce test de lixiviation est effectué avec un rapport eau/MS de 10:1 pendant 2h sous une agitation constante par retournement (10 rotations/min). La séparation des phases est obtenue par centrifugation (5000 G ; 10 min ; température ambiante) suivie d'une filtration granulométrique de 0.7 µm. Enfin, la phase particulaire est séchée à 70 °C jusqu'à poids constant et broyée à 2 mm de longueur théorique. Les échantillons bruts/solubles d'une part, et particulaires d'autre part, sont stockés à 4 °C et -20 °C, respectivement, jusqu'à leur utilisation ultérieure.

Les analyses réalisées pour chacun des échantillons sont les suivantes :

- **Echantillon brut** : teneur en matière sèche (MS) / matière volatile (MV) ; potentiel bio-méthanogène (PBM)
- **Phase soluble** : MS/MV ; PBM ; pH ; sucres solubles ; acides gras volatils (AGV) ; alcools ; azote Kjeldahl (NTK) et ammonium (NH_3-N) ; demande chimique en oxygène (DCO) ; analyse élémentaire (K, S, P)
- **Phase particulaire** : MS/MV ; NTK ; DCO ; analyse des fibres (méthode Van Soest).

Cette procédure expérimentale, résumée dans la Figure 2-3, permet l'évaluation de la biomasse et de ses différents compartiments chimiques en fonction de la masse initiale du produit utilisé, puisque la perte de masse humide/organique est mesurée. Les résultats d'analyses chimiques seront donc présentés de deux façons dans ce mémoire: à la fois sur la base de $\%MV_{ajoutée}$, c'est-à-dire la matière volatile de l'échantillon tel qu'il a été prélevé après la durée de stockage souhaitée, mais aussi sur la base de $\%MV_{initiale}$, c'est-à-dire celle de l'échantillon avant stockage. D'une part, la $MV_{ajoutée}$ se réfère à la matière organique de l'échantillon analysé. D'autre part, les résultats basés sur $MV_{initiale}$ tiennent compte de la perte de matière volatile pendant le stockage et permettent d'étudier les résultats en fonction de la MV du gisement.

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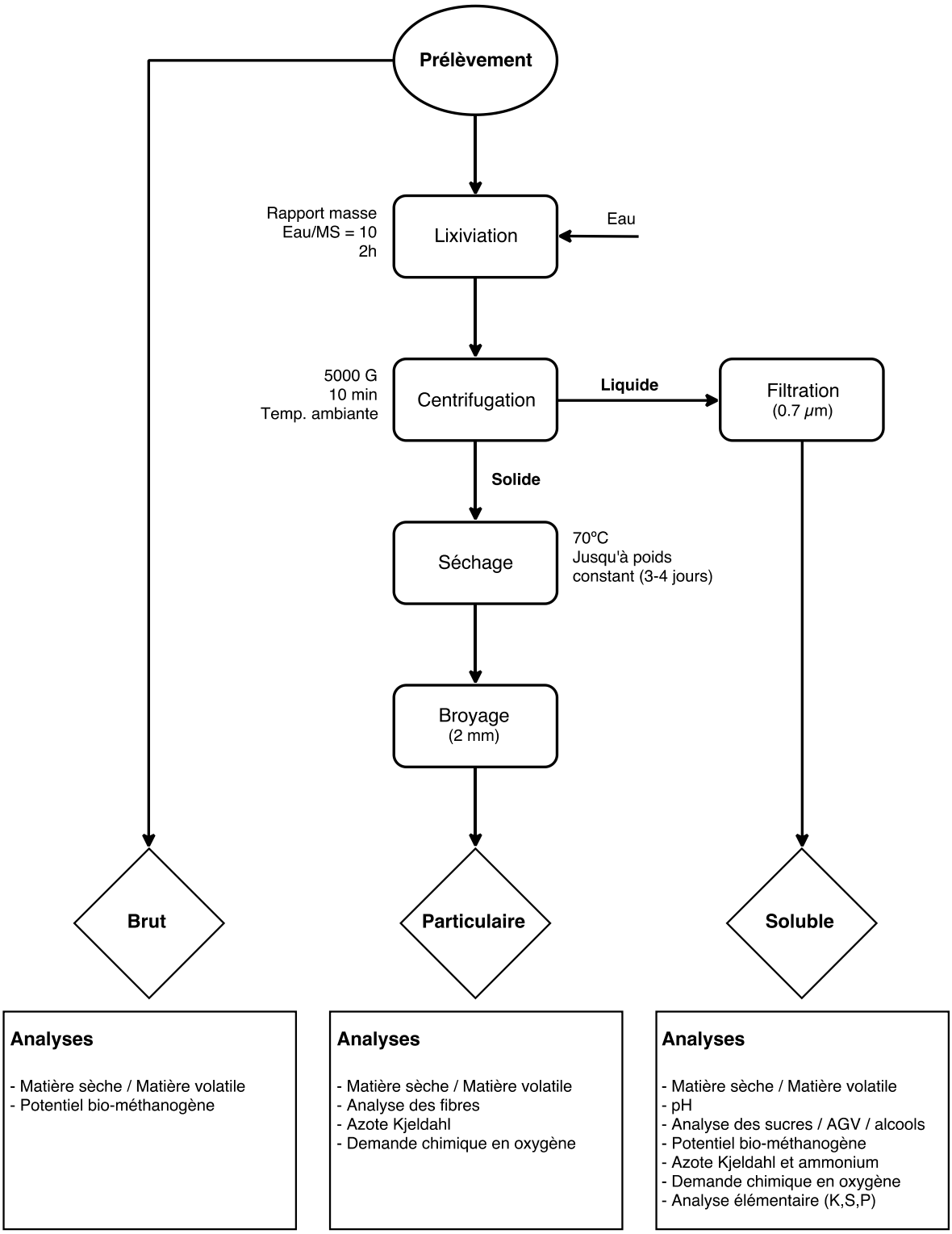

Figure 2-3 - Organigramme de la procédure de prélèvement et analyses.

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2.4. Choix des analyses et protocoles opératoires

2.4.1. Détermination de la Matière Sèche et Matière Volatile et corrections utilisées

Les mesures de MS/MV sont indispensables pour des études liées à la méthanisation, vu que les résultats sont souvent présentés sur la base de ces paramètres. De même, dans le cas du stockage, l'utilisation des données sur la base MS/MV permet le suivi des pertes de matière organique au cours du temps et l'évolution de la biomasse par rapport à la matière organique initiale.

La détermination de la teneur en matière sèche pondérale est effectuée par pesée différentielle, avant et après séchage dans une étuve à 105 °C pendant 24 heures. Ensuite, la teneur en matières volatiles est déterminée par calcination dans un four thermostaté à 550 °C pendant 2 heures, par pesée différentielle.

Figure 2-4 - Echantillons bruts et solubles dans des coupelles en aluminium pour l'analyse MS/MV.

Etant donné que les teneurs de MS/MV sont sous-estimées en ensilage en raison de la perte de composés volatils au cours de la procédure de séchage en étuve [21], les mesures de MS/MV ont été corrigées. Pour cela, les coefficients de volatilisation à 100 °C suggérés par Porter and Murray [75] ont été utilisés et les corrections effectuées selon l'équation suivante :

$$MS_C = MS_{NC} + 0.375\,AL + 0.892\,AGV + 0.975\,Alcools + 0.987\,NH_3 - N \qquad (2.1)$$

Où, MS_C et MS_{NC} désignent les teneurs en matière sèche corrigées et non-corrigées, respectivement ; AL la concentration en acide lactique ; AGV la concentration des acides acétique, formique, butyrique, propionique, valérique et caproïque ; $Alcools$ la teneur en méthanol, éthanol et butanol ; $NH_3 - N$ la teneur en ammonium. Toutes les concentrations sont exprimées en g/g_{MH}. La même équation peut être utilisée pour la correction de la matière volatile, en remplaçant MS_C et MS_{NC} par MV_C et MV_{NC}, respectivement. A titre d'exemple, pour le prélèvement après 28 jours de la condition TS14%E avec la CIVE 2, la teneur en MV_{NC} mesurée était 8.97%, alors que la MV_C après correction avec l'équation 2.1 était 10.5%. Cela représente une sous-estimation de la MV sans correction d'environ 15%, ce qui aurait eu un impact significatif sur les résultats basés sur les mesures de MS/MV, comme le potentiel bio-méthanogène.

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2.4.2. Détermination du pH

Le pH est un paramètre essentiel pour les essais de stockage et sa mesure est indispensable lors de la réalisation d'essais d'ensilage. La mesure du pH est facilement accessible et rapide. Elle permet d'obtenir des indications sur le stade de fermentation et stabilité de la biomasse stockée.

Dans cette thèse, le pH a été analysé pour la phase soluble des échantillons par un appareil Consort C3020 avec une électrode de pH SP10B, comme illustré par la Figure 2-5.

Figure 2-5 – Mesure du pH pour un échantillon soluble après lixiviation.

2.4.3. Analyses des sucres solubles, AGV et alcools

Dans des conditions de stockage, la teneur en sucres solubles de la matière première est l'un des paramètres ayant un impact majeur sur la réussite de l'ensilage. En effet, ce sont généralement ces composés qui vont être dégradés dans les premiers jours de conservation pour la production des AGV (et alcools), entraînant une baisse de pH et la stabilisation de la biomasse. Pour cette raison, la composition en sucres solubles, AGV et alcools de la phase soluble après lixiviation a été suivie pour tous les essais de stockage réalisés.

La teneur en sucres solubles est la somme des concentrations en glucose, xylose, galactose, mannose, arabinose et cellobiose. La concentration en AGV est la somme des concentrations des acides lactique, formique, acétique, propionique, butyrique, valérique et caproïque. Les alcools considérés dans cette analyse sont le méthanol, l'éthanol et le butanol.

Les teneurs en sucres solubles, acide lactique et acide formique sont déterminées par chromatographie liquide à haute performance (CLHP - Module 1 plus, Waters) équipée d'une colonne Supelcogel ™ C-610H (300 x 7.8 mm, Sigma-Aldrich), avec des détecteurs à indice de réfraction (RID) et UV, en fonctionnant avec du H_3PO_4 0,1%(V/V) comme solvant (débit de 0.5 mL/min). D'une part, les sucres solubles sont déterminés à l'aide du détecteur UV (210 nm). D'autre part, les teneurs en acide lactique et en acide formique sont obtenues avec le détecteur RID.

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Les AGV restants - acides acétique, propionique, butyrique, valérique et caproïque – et les alcools (méthanol, éthanol et butanol) sont analysées à l'aide d'une chromatographie en phase gazeuse (Shimadzu Corp.) équipée d'une colonne capillaire en silice fondue greffée dite HP-FFAP (30 m x 0,25 mm, Agilent Technologies), d'un détecteur d'ionisation de flamme (FID) et H_2 comme gaz vecteur.

2.4.4. Analyse des fibres

Les composés structurels de la biomasse peuvent être dégradés au cours du stockage. Cela peut entraîner des gains d'accessibilité da la matière ou, au contraire, des lourdes pertes de carbone.

Dans ces travaux de thèse, l'analyse des fibres a été réalisée par la méthode d'extractions modifiée de Van Soest and Wine [76], selon la norme FD U44-162 [77]. Dans cette procédure, les fractions pariétales sont déterminées par solubilisations successives (extractions séquentielles), par des solvants appropriés, des différentes fractions organiques se comportant à l'extraction comme : les substances solubles, les hémicelluloses, la cellulose et la lignine. Cette analyse séquentielle est d'abord accomplie avec le NDF (*Neutral Detergent Fiber* en anglais), puis avec l'ADF (*Acid Detergent Fiber* en anglais) sur le résidu et finalement avec l'ADL (*Acid Detergent Lignin* en anglais). Le NDF est un détergent neutre qui permet la solubilisation des composés non-structurels de la fraction particulaire de l'échantillon et est composé de sodium dodecyl sulfate ($C_{12}H_{25}NaO_4S$), EDTA-Na ($C_{10}H_{14}N_2Na_2O_8.2H_2O$), tétraborate de sodium décahydraté ($Na_2B_4O_7.10H_2O$), hydrogénophosphate de sodium (Na_2HPO_4) et triéthylèneglycol ($C_6H_{14}O_4$). L'ADF est un détergent acide composé d'hexadécyltriméthylammonium bromure ($C_{19}H_{42}NBr$) et acide sulfurique (H_2SO_4) dilué qui permet l'extraction des hémicelluloses de l'échantillon. De l'acide sulfurique concentré - H_2SO_4 72%(V/V) – est utilisé comme ADL pour l'extraction des celluloses.

Ce protocole est mis en œuvre pour les échantillons particulaires après séchage et broyage avec une prise de masse d'environ 0.5 g et des essais en triplicata. Etant donné que le dispositif d'extraction dispose de 18 emplacements pour des capsules d'extraction, cette procédure permet l'analyse de deux échantillons à la fois.

Après chaque étape d'extraction, les échantillons sont analysés pour leur MS/MV et la masse des résidus d'extraction (NDF, ADF et ADL) est exprimée en pourcentage de la quantité de matière volatile initialement introduite ($\%MV$), selon l'équation suivante :

$$\%NDF\ (ou\ ADF, ADL) = \frac{W_2 - W_3 - W_0}{(W_1 - W_0) \times \%MV} \tag{2.2}$$

Où W_0 est la masse de la capsule sans échantillon ; W_1 est la masse de la capsule avec l'échantillon avant extractions ; W_2 est la masse du creuset contenant la capsule après l'étape d'extraction respective et la procédure de mesure de la MS ; W_3 est la masse du creuset contenant la capsule minéralisée, après la procédure de mesure de la MV.

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Finalement, la teneur en hémicelluloses est calculée comme NDF moins ADF; la cellulose comme ADF moins ADL et ; la lignine comme étant égale à ADL.

2.4.5. Dosage de l'azote Kjeldahl et ammonium

L'évolution de l'azote organique dans les phases particulaires et solubles, ainsi que de l'ammonium dans la phase soluble a été suivie pour les différentes conditions de stockage étudiées. D'une part, cela a eu pour but d'approfondir les connaissances sur les potentielles émissions de gaz à effet de serre pendant le stockage de biomasse avant méthanisation. D'autre part, l'ammonium a été suivi comme un indicateur de l'efficacité de l'ensilage, étant donné que la formation de ce composé est une conséquence de la fermentation protéolytique, laquelle se produit pour des niveaux de pH élevés et entraîne des pertes énergétiques.

Ce dosage a été fait par une méthode type Kjeldahl, selon la procédure décrite dans la norme NF EN 25663 [78]. Cette analyse est basée sur le principe d'une transformation de l'azote en une espèce quantifiable. L'analyse se déroule en trois étapes. La première étape consiste à minéraliser l'azote sous forme d'ammonium (sulfate d'ammonium) par réaction entre l'échantillon et l'acide sulfurique à ébullition (400 °C) en présence d'un catalyseur à base de sélénium et de sulfate de potassium destiné à élever le point d'ébullition du mélange. Ensuite, la solution minéralisée est distillée en présence d'un excès de soude afin de produire de l'ammoniac. Ce produit de réaction est entraîné par de la vapeur d'eau et récupéré par condensation dans un erlenmeyer de récupération. Le NH_3 ainsi condensé réagit avec l'acide borique présent initialement dans l'erlenmeyer. La solution finale est titrée avec HCl : dosage en retour de la forme basique de l'acide borique. Pour la détermination du taux d'ammonium libre, il suffit de distiller l'échantillon sans passer par l'étape de minéralisation.

2.4.6. Détermination de la Demande Chimique en Oxygène

La DCO est la quantité d'oxygène nécessaire à l'oxydation chimique de matières contenues dans un volume de solution. Ce paramètre est largement utilisé en méthanisation, dans la mesure où il est étroitement lié à la matière organique susceptible de produire du méthane en conditions anaérobies. Dans la même logique, le suivi de la conservation de la DCO peut être un indicateur de l'efficacité du stockage des matières premières avant la production de biogaz.

Dans la démarche expérimentale de cette thèse, la DCO a été mesurée pour les phases particulaires et solubles. D'une part, la DCO du particulaire a été mesurée par une méthode Walkley and Black [79] modifiée, inspirée de la norme ISO 14235 [80]. D'autre part, la procédure colorimétrique HACH (méthode 8000) a été utilisée pour déterminer la DCO soluble. Dans les deux méthodes, l'oxydation chimique est effectuée avec du dichromate de potassium en milieu d'acide sulfurique, suivi d'un dosage colorimétrique.

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2.4.7. Test de Potentiel Bio-Méthanogène

Ce test permet d'évaluer le potentiel de production de méthane d'un échantillon solide ou liquide, c'est-à-dire la quantité maximale de méthane générée lors de sa biodégradation en conditions anaérobies optimales. Son intérêt pour les essais de stockage est de permettre d'évaluer la conservation énergétique pour différents modalités de gestion de la biomasse en vue de son utilisation en méthanisation.

Les essais PBM sont effectués pour les échantillons bruts, mais aussi pour les matières solubles après lixiviation. Les tests sont réalisés dans une pièce thermostatée à 35 °C, en utilisant des flacons en verre de 2 L pour les échantillons bruts (Figure 2-6) et 0.1 L pour les fractions solubles. Les flacons sont remplis avec 5 g de MV d'échantillon, inoculum afin de maintenir un rapport de $MV_{substrat}/MV_{inoculum}$ de 0.5 et un certain volume de solution minérale pour atteindre 60% du volume total du flacon. L'inoculum utilisé est une boue digérée provenant de la station d'épuration des eaux usées de La Feyssine, Lyon, France. La solution minérale, qui contient des éléments essentiels au développement microbien tels que certains métaux afin d'éviter des carences en éléments spécifiques et confère également à la solution un pouvoir tampon pour réduire les éventuels écarts de pH, a été préparée conformément aux recommandations de la norme internationale ISO 11734 [81].

Une fois remplis, les réacteurs sont purgés avec un mélange N_2/CO_2 (80/20%V) pendant environ 5 minutes, hermétiquement fermés et ensuite incubés à 35 °C. Des témoins négatifs ayant uniquement de l'inoculum et de la solution minérale sont aussi préparés pour chaque série d'essais, de façon à corriger le PBM à partir de la production résiduelle de méthane de l'inoculum (Figure 2-7). Tous les tests sont réalisés en triplicata.

Figure 2-6 – Flacons des essais PBM hermétiquement fermés dans la pièce tempérée.

La production de biogaz est déterminée par des mesures de pression avec un manomètre de précision Digitron. Le biogaz est libéré lorsque la pression dépasse 1200 hPa. La composition du gaz est analysée par micro-chromatographie gazeuse couplée avec un détecteur par conductivité thermique (GC-TCD), Agilent 3000. Des colonnes Molsieve 5A (14 m de longueur, taille des pores: 5 Å) et PoraPlot A (10 m de longueur, 0.320 mm de diamètre interne) sont utilisées comme phases stationnaires pour la GC-TCD, avec de l'argon et de l'hélium comme gaz vecteurs, respectivement. La production et la composition du biogaz sont déterminées au minimum 7 fois au cours de l'incubation. Le PBM est considérée comme

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atteint lorsque la surpression quotidienne des flacons des témoins est équivalente à celle des flacons avec du substrat. Dans son ensemble, notre protocole de mesure suit les recommandations fournies par le groupe de travail international sur l'harmonisation des protocoles de potentiel bio-méthanogène, Holliger *et al*. [82].

Afin d'évaluer la cinétique de la production de méthane, les données expérimentales des essais de PBM sont ajustées à l'équation suivante:

$$V_{CH_4}(t) = V_{max}(1 - e^{-kt}) \tag{2.3}$$

Où, V_{CH_4} correspond au volume de méthane produit; t est le temps de l'analyse; V_{max} est le volume maximal de méthane produit, qui est équivalent à la valeur de PBM expérimental et; k est la constante de vitesse de la production de CH_4, supposée du premier ordre et confirmée par les analyses (Figure 2-7). Comme la production de gaz débute presque immédiatement après la fermeture des réacteurs, le temps de latence n'a pas été pris en compte dans les calculs.

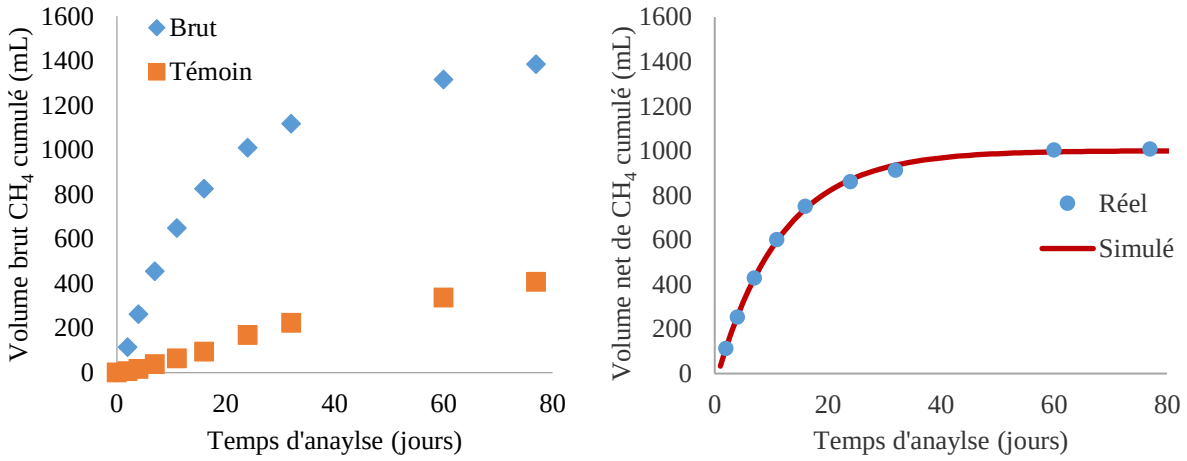

Figure 2-7 – A gauche : volume brut de méthane d'un échantillon et du témoin ; A droite : volume net de méthane produit par un échantillon et simulation avec cinétique du premier ordre.

2.4.8. Analyse K, S et P en solution

L'analyse des éléments K, S et P dans la fraction soluble après lixiviation a été réalisée par spectrométrie d'émission atomique à plasma induit par haute fréquence (ICP-OES), Jobin Yvon Ultima 2. Ces mesures ont eu pour but d'évaluer l'impact des différentes conditions de stockage sur la disponibilité présumée de ces éléments dans la solution.

La méthode utilisée s'inspire de la procédure standardisée ISO 11885 [83] et consiste à mesurer l'émission atomique par spectroscopie optique. Les échantillons sont nébulisés et l'aérosol ainsi produit est transporté dans une torche à plasma où se produit l'excitation. Les spectres d'émission atomique des raies caractéristiques sont produits par un plasma induit par haute fréquence. La lumière

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polychromatique est dispersée par un spectromètre à réseau et l'intensité des raies est évaluée par des détecteurs (photomultiplicateurs). Les signaux des détecteurs sont ensuite traités et contrôlés par un système informatique.

Les résultats de l'analyse élémentaire peuvent être consultés dans les annexes B1-B5, étant donné qu'ils ne sont pas présentés ni discutés dans les articles de la partie « Résultats et Discussion » du mémoire.

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RESULTATS ET DISCUSSION

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3. STOCKAGE DES CULTURES INTERMEDIAIRES A VOCATION ENERGETIQUE AVANT METHANISATION

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3.1. Introduction

Ce chapitre présente les études à l'échelle du laboratoire pour des CIVEs, le premier type de ressource utilisé pour l'étude des conditions de stockage sur la conservation de la biomasse. Les résultats et discussion sont présentés dans deux articles scientifiques, chacun relié à une série d'essais de stockage à long terme. Les principales conclusions à retenir pour l'ensemble des essais avec CIVEs sont présentées dans la dernière section de cette partie.

Dans le premier article, la matière première utilisée a été une CIVE "hiver", constituée d'un mélange de triticale, pois, vesce et radis fourrager. Dans cette série d'expériences, l'impact de l'ensilage, du stockage à l'air libre et d'un préfanage de courte durée sur l'évolution de la matière organique et du potentiel énergétique a été étudié pendant 180 jours. Dans le second article, une CIVE "été", composée d'un mélange de tournesol, sorgho, pois, vesce et trèfle d'Alexandrie a été testée pendant 98 jours. En complément de l'étude des conditions d'ensilage et de la conservation en présence d'air, cette publication s'est intéressée à l'effet du préfanage limité par des conditions météorologiques défavorables, sur la conservation des ressources pendant le stockage.

Les données supplémentaires des analyses qui ne sont pas présentés lors de la discussion des articles scientifiques avec CIVEs sont consultables dans les annexes B1 et B2.

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3.2. 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*

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3.2.1. 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 CH_4 and CO_2), has today more than 17000 operational plants over Europe [84]. 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 [85], 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 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.

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3.2.2. Materials and methods

3.2.2.1. 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 Béreyziat, 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.

3.2.2.2. 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±2°C for a defined period of days. Storage duration varied between 3, 14, 30, 90 and 180 days.

3.2.2.3. 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

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acids (VFA), ammoniacal nitrogen (NH_3-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 [21], values were corrected using an equation suggested by Porter and Murray [75]. 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 H_3PO_4 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 [76], based on XP U44-162 French standard. TKN and NH_3-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.

3.2.2.4. 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 N_2/CO_2 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.

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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.

3.2.3. Results and discussion

3.2.3.1. Fresh material chemical characteristics

Feedstock used for storage purposes, which is characterized in Table 3-1, 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 [12], 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 [16], 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.

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.

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Table 3-1 – 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

3.2.3.2. Impact of storage on biomass fermentation

Effects of storage conditions and duration on silage quality are summarized in Table 3-2.

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 TKN content along storage time also indicates huge losses and modifications of organic matter for aerobic conditions.

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 [12]. 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 [27]). 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

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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.

Table 3-2 – Chemical characteristics of biomass over storage time (%$VS_{added/original}$ unless otherwise specified).

Condition	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[a]	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[a]	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[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 %$VS_{original}$

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 trough 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 [12]. 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 [12].

Increasing NH_3-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,

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although ammonia should be present in the form of soluble ammonium ions at current pH and temperature values, its liquid-gas equilibrium may 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 [40–43] 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.* [40] 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 was 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 was 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 was 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.

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3.2.3.3. Evolution of BMP over storage duration

3.2.3.3.1. 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 storage (TS18%A) (Figure 3-1). 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 3-1). This reflects the major importance of VS loss in determining the preservation of BMP, as it was stated in a previous work [15]. 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/kgVS$_{original}$ 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.

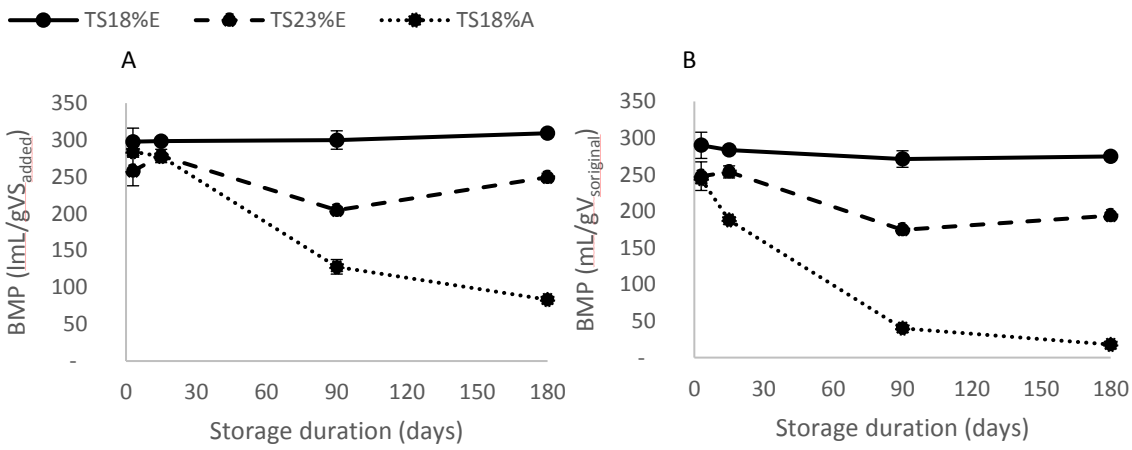

Figure 3-1 - Global BMP evolution over storage duration. A: methane potential based on VS$_{added}$; B: methane potential based on VS$_{original}$, therefore considering storage losses. TS18%E represents the biomass ensiling with original TS content; TS23%E concerns the ensiling of 6h-wilted biomass and; TS18%A symbolizes the open-air storage of the original raw material.

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3.2.3.3.2. Solid/liquid fractions

BMP multi-phase analysis over storage duration (Figure 3-2) 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 [8,12]. 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.

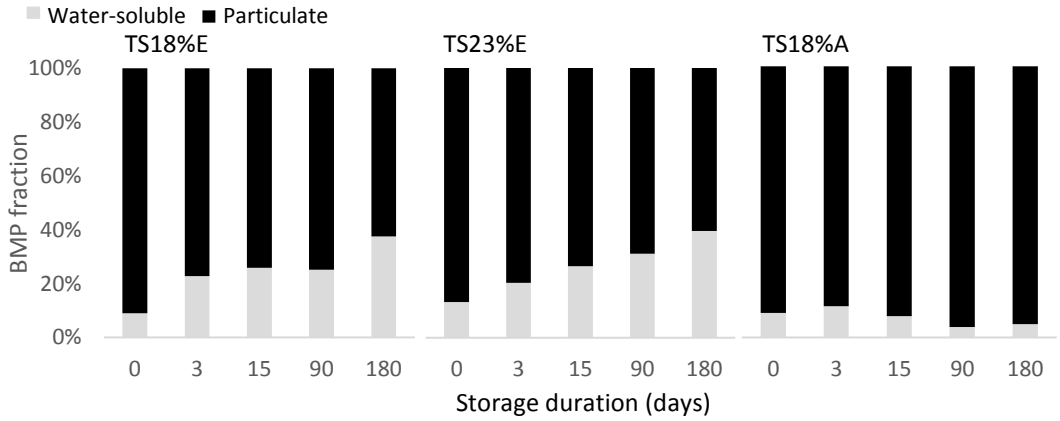

Figure 3-2 – BMP multi-phase analysis for the different conditions along storage duration. TS18%E represents the biomass ensiling with original TS content; TS23%E concerns the ensiling of 6h-wilted biomass and; TS18%A symbolizes the open-air storage of the original raw material.

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3.2.4. 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.

3.2.5. Acknowledgements

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3.3. Optimizing storage of a catch crop before biogas production: impact of ensiling and wilting under unsuitable weather conditions

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Abstract

Ensiling and open-air storage of up to 98 days were assessed for a catch crop in order to identify the best management practices before its use as feedstock for anaerobic digestion. For ensiling, this included the study of the effect of wilting limited by unsuitable weather conditions. Results showed that after 98 days, methane potential of biomass open-air stored corresponded to 18% of the one for the ensiling of the same feedstock. Wilting was inefficient while witnessing unfavorable weather conditions at harvesting. Even if prolonged wilting periods under severe conditions led to decrease of moisture content, it did not provide improvements on long-term conservation of methane potential. For high moisture silage, instability of fermentation was offset by gains in biochemical accessibility. In such conditions, biomass should be promptly ensiled without wilting. Leachate produced during ensiling of high moisture crops should be collected and used for biogas production.

Keywords*: Wilting; Storage; Ensiling; Anaerobic digestion; Methane potential*

3.3.1. Introduction

Seasonality of crops and other agricultural wastes used for biogas production is at the origin of storage requirements before anaerobic digestion (AD). Since biogas production through AD should operate continuously throughout the year, seasonal biomass feedstock needs to be preserved for prolonged periods, which may reach up to 6 months in some cases.

Among the current preservation methods, ensiling emerges as a logical choice to store wet biomass for energy production purposes. Based on biochemical processes, ensiling provides an anaerobic

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environment suitable for bacterial fermentation, which leads to biomass acidification, limiting further degradation and energy losses.

Together with the nature of the feedstock, air presence, storage duration or the use of additives, the control of the moisture content is one of the key parameters for the success of feedstock ensiling before AD [85]. Indeed, it is commonly shared that low moisture contents slow down bacterial growth, leading to a more restricted fermentation and better silage preservation [12,40,42,43]. Furthermore, silages with at least 25% of total solids content (TS) may prevent leachate formation during ensiling [46–48], avoiding potential energy losses due to seepage.

Field wilting (*i.e*. natural air-drying of crops after harvesting) prior to ensiling is the most common method to achieve higher TS contents for biomass crops when moisture content is excessively high. Besides being inexpensive, it may enable water evaporation with little effect on the remaining chemical characteristics if short wilting duration (around 6h) is used [25,40,50]. However, wilting effectiveness is strongly dependent on weather conditions of the harvest site. In fact, natural environmental conditions such as solar radiation, vapor pressure, rainfall and eventually wind speed are correlated with the drying rate of biomass [12]. Consequently, when unfavorable weather conditions are present at harvesting, prolonged wilting durations must be used to obtain the desired TS content. In such cases, it is suggested that other chemical changes beyond water evaporation occur, which might influence ensiling conservation as well. For instance, Carpintero *et al.* [50] observed an impact of 48h wilting on the water-soluble carbohydrates (WSC) and ammonia nitrogen (NH_3-N) content of ryegrass-clover. Likewise, Dawson *et al*. [24] 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. However, results on the impact of prolonged wilting durations on the conservation of the biochemical methane potential (BMP) during ensiling are still scarce and inconclusive. To our best knowledge, only Pakarinen *et al*. [15] have tested this parameter and observed better BMP conservation during ryegrass ensiling after 48h drying, while an opposite effect was obtained for grass silage.

The objective of this study was thus to compare ensiling and open-air storage as methods for preservation of a catch crop before biogas production. For ensiling, the aim was to optimize the management practices of the feedstock when unfavorable weather conditions are present at harvesting. Both fermentative profiles and BMP were monitored at different stages of preservation, which enabled a detailed analysis of the phenomena involved during storage.

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3.3.2. Material and methods

3.3.2.1. Feedstock

Raw material used was a sample of a catch crop harvested on 9 November 2015 from an agricultural site in the Rhône-Alpes region of France (Gaec Béreyziat, Les Teppes, 01340 Béréziat, France). Biomass was composed by a mixture of sunflower, sorghum, peas, *Vicia sp.* and *Trifolium alexandrinum*.

3.3.2.2. Wilting process

Storage preparation procedure is summarized in Figure 3-3. After harvesting, part of the fresh biomass (10.1%TS) was ensiled and the remaining fraction was wilted. Non-suitable weather conditions were recorded during wilting: daily average temperatures ranged between 7.7-14.9°C; extremely cloudy sky along with fog patches. After 72h of wilting, the dried sample had 14.1% of TS content. At this point, part of the 72h-wilted biomass was ensiled and the remaining amount was moved to an air-ventilated stove at 35°C, in order to be able to test a larger range of moisture content in the ensiling assays. After a total of 96h of wilting, the last fraction of biomass (38.7%TS) was removed from the stove, equilibrated to room temperature and then ensiled. Fresh and wilted biomass were chopped to theoretical particle size of 8mm with a rotary shear shredder immediately before storage. This particle size was chosen taking into account the recommendations of Herrmann *et al.* [36,39] for a compromise between high methane conservation rates and low chopping costs. Biomass was manually mixed between each step of storage preparation and samples used for storage assays were taken in representative quantity of raw material, *i.e.* 10%.

3.3.2.3. Storage approach

Storage assays were conducted at laboratory scale to monitor the impact of ensiling and prolonged wilting on biomass conservation. To evaluate ensiling impact, an aerobic control storage test was also performed for the 96h-wilted sample. 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 the silo was left air-open. Once sealed, the silos were weighed and placed in a temperate room at 25±2°C for a defined period. Storage duration varied between 7, 14, 28 and 98 days. A total of 16 (4 operating conditions x 4 storage durations) laboratory silos were used.

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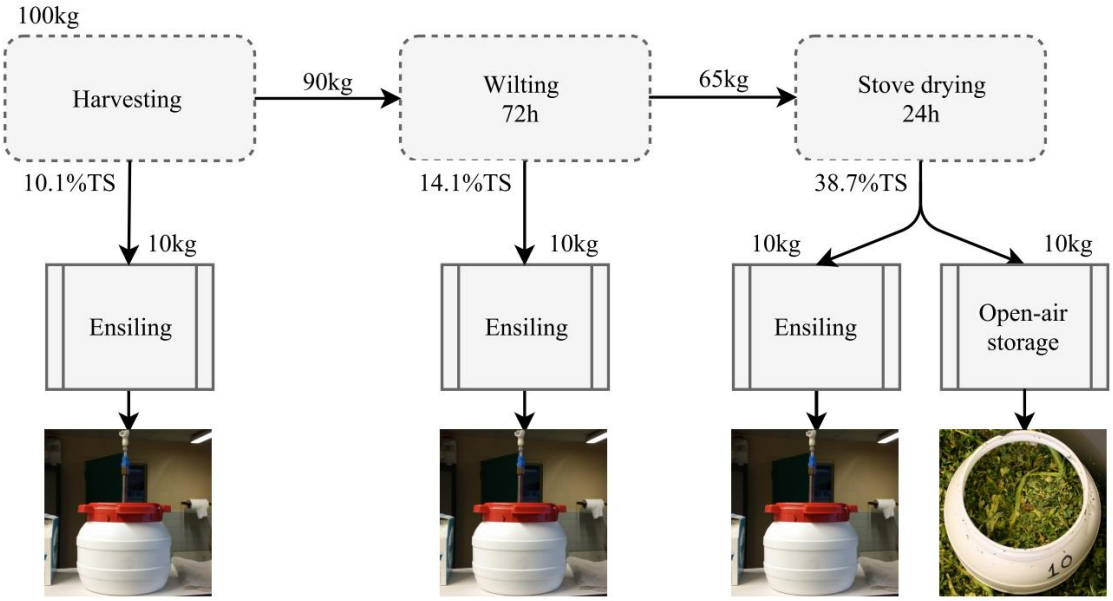

Figure 3-3 - Storage preparation procedure.

3.3.2.4. Chemical analysis

Chemical analysis procedure after sampling is illustrated in Figure 3-4. For each tested duration, one silo (per tested condition) was opened and weighed, biomass was homogenized and two samples were taken. One was used for direct analyses on the crude material and the other one was mixed with water in order to get two fractions: a water-soluble phase and a particulate phase. This leaching test 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, the particulate phase 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.

Crude material was analyzed for its TS content, volatile solids (VS) content and BMP. For the water-soluble phase, besides TS/VS content, pH, WSC, volatile fatty acids (VFA), NH_3-N and total Kjeldahl nitrogen (TKN) contents were determined. Particulate solid was analyzed for its TS/VS, TKN and cell wall constituents content.

TS was measured by oven drying at 105°C during 24h and VS was subsequently burned for 2h at 550°C. Since TS/VS contents are underestimated due to the loss of volatile compounds during the drying tests [21], the measures were corrected using the equation suggested by Porter and Murray [75]. 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 H_3PO_4 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

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determined using the UV detector (210nm). Total VFA was calculated as the sum of lactic, formic, acetic, propionic, butyric, valeric and caproic acids content obtained with the RID detector. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed through Van Soest and Wine [76] modified extractions method, based on XP U44-162 French standard. Hemicellulose content (HEM) was calculated as NDF minus ADF, cellulose (CEL) as ADF minus ADL and lignin (LIG) as approximatively equal to ADL.TKN and NH_3-N were determined through the procedure described in the NF EN 25663 French standard.

The interest of our experimental procedure is, first, to be able to compare the results of composition and BMP to the initial mass of product used since the weight loss is monitored. The results for the chemical analysis will thus be presented in two ways: based on $\%VS_{added}$ or $\%VS_{original}$. VS_{added} relates to the organic matter of the sample analyzed. The results based on $VS_{original}$ take into account the loss of volatile solids during storage and allows the study of the results based on the VS of the initial material.

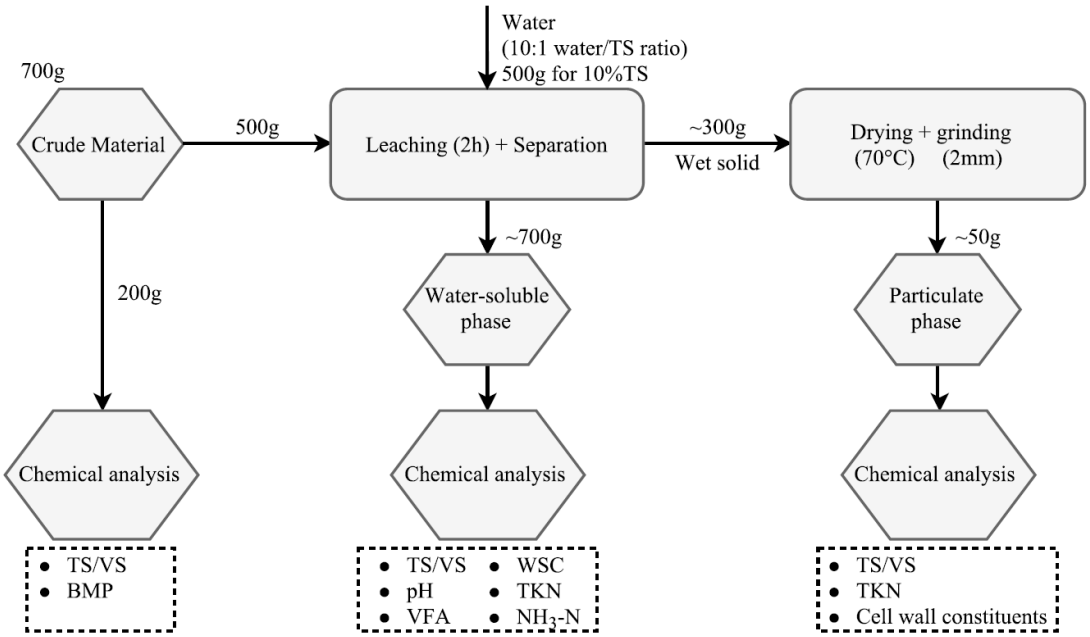

Figure 3-4 – Chemical analysis procedure after sampling.

3.3.2.5. Biochemical methane potential tests

Batch anaerobic digestion tests were performed for crude material samples. Tests were conducted in a temperate room at 35°C using glass vessels of 2L. Vessels were filled with at least $5g_{VS}$ 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: 2.6-3.0%wt; VS: 1.8-2.0%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

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11734:1995 standard. Once filled, reactors were purged with a N_2/CO_2 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 pressure measurement using a Digitron precision manometer. Biogas was released when the pressure exceeded 1200 hPa. Biogas composition was determined 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 8 times during batch tests 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*. [82].

3.3.3. Results and discussion

3.3.3.1. Raw materials characterization

Results of chemical analysis on fresh and wilted biomass before storage are summarized in Table 3-3. Fresh catch crop was characterized by a low level of TS (10.1%) and high WSC content in the organic matter (12.0%VS). These values are important to understand ensiling fermentation, since silage quality will strongly rely on TS and WSC content of feedstock [85]. High WSC level of fresh material should enable a prompt acidification of silage by lactic acid bacteria (LAB) fermentation. However, secondary fermentation will be favored at low TS content if extremely acid environments are not reached. This should preclude biomass stabilization and lead to substantial VS losses.

To avoid the risk of having unstable silages due to low TS content, wilting was performed before storage. As mentioned earlier, natural drying of biomass had a minor effect on the moisture content of feedstock. TS content only increased from 10.1% to 14.1% of total biomass after 72h-wilting. Drying ineffectiveness should be closely linked to the poor weather conditions recorded during wilting process.

At the same time, TS content of 96h-wilted biomass increased to 38.7%, due to the use of an air-ventilated stove at 35°C on the last 24h of drying. Besides, 96h-wilting had a substantial impact on WSC, leading to degradation of more than half of the initial content. This suggest that some drying conditions, including high temperatures and wind speed (which may occur in summer), may lead to important organic matter losses before storage.

Wilting duration also had an impact on pH values: it decreased from 6.4 to 6.2 after 72h-wilting and to 5.8 after 96h-wilting. Nevertheless, the reason for this remained unclear, as VFA contents were stable and almost negligible during wilting. Regarding fibers constituents, prolonged periods of wilting had a

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negative impact on cellulose preservation. Finally, some fluctuations were recorded on NH_3-N and TKN fractions, but no correlation was found between their content and wilting duration.

Table 3-3 – Chemical characteristics of feedstock and impact of wilting.

Biomass	Non-wilted	72h-wilted	96h-wilted
Condition [a]	TS10%E	TS14%E	TS39%E / TS39%A
pH	6.4	6.2	5.8
TS [b]	10.1	14.1	38.7
VS [b]	8.4	11.6	32.3
VFA [c]	0.3	0.4	0.1
WSC [c]	12.0	12.7	5.7
HEM [c]	11.3	10.1	10.5
CEL [c]	20.4	18.3	16.8
LIG [c]	3.8	3.4	3.0
TKN [c]	4.7	5.1	5.1
NH_3-N [d]	2.6	7.0	4.5

[a] TS10%E represents the ensiling of non-wilted biomass; TS14%E concerns the ensiling of the 72h-wilted biomass; TS39%E and TS39%A symbolize the ensiling and open-air storage of the 96-wilted biomass, respectively.
Results presented as [b] % of total sample weight; [c] $\%VS_{original}$, [d] %TKN

3.3.3.2. Storage impact on chemical characteristics

A major advantage is evidenced in using ensiling as biomass conservation method rather than open-air storage, Table 3-4. Regarding aerobic treatment, significant organic matter degradation occurred from the first week of storage: 21.9% and 68.8% of original VS content was lost after 7 and 98 days, respectively. This was a result of degradation of both non-structural and structural carbohydrates and organic nitrogen compounds present in the raw material. In fact, more than 99% of the original WSC content had been consumed after only 7 days of open-air storage. This led to organic matter losses, as no accumulation of VFA was recorded. For the structural carbohydrates, the degradation took place all along storage in aerobic environment as can be observed in Figure 3-5, resulting after 98 days in 78% and 47% losses of original hemicellulose and cellulose contents, respectively. Degradation of organic nitrogen was evidenced by TKN decrease from 5.1 to 3.8%$VS_{original}$ and ammonia nitrogen increase from 4.5 to 46.8%TKN until 28 days of aerobic storage. Moreover, between 28 and 98 days of storage, the NH_3-N and TKN contents dropped dramatically, resulting in around 85% ammonia loss during this period. This showed that, besides organic matter degradation, undesired ammonia emissions into the atmosphere are favored by open-air storage. This should be a result of the impact of a continuous pH increase for aerobic environment, from 5.8 at start to 9.6 after 98 days of storage, on the NH_3-NH_4^+ equilibrium reaction and NH_3 stripping. Additionally, fungi development was detected throughout

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aerobic storage. This suggests that microbial degradation of organic matter took place *via* an aerobic metabolism producing CO_2 and microbial biomass.

Table 3-4 - Fermentation profiles of biomass over storage time (%$VS_{added/original}$ unless otherwise specified).

Condition	Storage duration (days)	VS losses	Chemical characteristics [a]							
			pH	LA[b]	AA[b]	BA[b]	VFA[b]	WSC[b]	NH_3-N (%TKN)	TKN[c]
TS10%E	0	-	6.4	0.0	0.0	0.2	0.3	12.0	2.6	4.7
	7	5.3	4.0	18.9	2.1	0.0	22.1	2.3	8.2	4.7
	14	8.1	4.2	23.1	3.3	0.0	28.2	0.3	12.3	4.7
	28	20.9	6.7	0.5	5.0	6.2	17.6	0.4	23.7	5.0
	98	25.8	5.6	0.0	7.2	8.3	19.5	0.1	40.2	5.6
TS14%E	0	-	6.2	0.0	0.0	0.3	0.4	12.7	7.0	5.1
	7	3.9	4.2	18.9	2.5	0.0	22.4	0.3	12.7	5.3
	14	5.2	4.4	17.9	3.6	0.0	22.6	0.4	15.3	5.3
	28	10.4	5.9	8.1	7.9	0.9	18.6	0.8	24.0	6.0
	98	16.8	7.0	0.0	6.4	5.5	13.6	0.2	37.0	6.2
TS39%E	0	-	5.8	0.0	0.0	0.1	0.1	5.7	4.5	5.1
	7	2.1	4.9	7.7	1.2	0.0	9.4	2.9	11.4	5.7
	14	2.6	4.6	10.8	1.3	0.0	12.5	1.6	13.1	5.4
	28	3.0	4.4	11.0	1.8	0.2	13.6	0.6	12.9	5.5
	98	3.4	4.4	11.6	2.6	1.0	16.2	0.6	12.9	5.5
TS39%A	0	-	5.8	0.0	0.0	0.1	0.1	5.7	4.5	5.1
	7	21.9	8.4	1.5	1.5	0.0	3.3	0.1	34.7	4.7
	14	38.6	9.0	0.6	0.4	0.0	1.1	0.2	40.9	3.8
	28	48.3	9.3	0.4	0.0	0.2	0.7	0.2	46.8	3.8
	98	67.8	9.6	0.0	0.0	0.0	0.0	0.0	14.1	2.0

[a] LA stands for Lactic Acid, AA for Acetic Acid and BA for Butyric Acid; [b] results based on %VS_{added}; [c] results based on %$VS_{original}$

In contrast, during ensiling, biomass is mainly subject to microorganism capable of anaerobic proliferation. For the same raw material used in open-air storage (96h-wilted biomass), ensiling enabled acidification, resulting in pH values between 4.9 after 7 days and 4.4 at the end of storage. A continuous production of lactic acid from available carbohydrates sources during ensiling was behind it. Lactic acid content varied from 7.7%VS_{added} after 7 days and 11.6%VS_{added} after 98 days of storage. Butyric acid production was negligible during ensiling of 96-h wilted biomass. Consequently, silage was stabilized

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by escaping from the pH range where spoiling anaerobic microorganisms are active. This limited VS losses to only 5% of the ones obtained in aerobic environment after 98 days.

Nevertheless, results showed that biomass stabilization through ensiling strongly depended on the TS content. For both fresh and 72h-wilted biomass (low TS contents) faster acidification occurred and after 7 days of ensiling pH was already at 4.0 and 4.2, respectively. This was a result of a more extensive LAB activity for lower TS contents, as lactic acid content was 18.9%VS_{added} after 7 days for these crops. However, from 14 days of ensiling, low TS crops revealed to be instable and fermentation profiles reverse direction. In fact, lactic acid content of fresh ensiled biomass dropped from 23.1%VS_{added} at 14 days to 0.5% VS_{added} and 0%VS_{added} after 28 and 98 days, respectively. Similarly in 72h-wilted silage, lactic acid content decreased from 17.9%VS_{added} at 14 days to 8.1%VS_{added} after 28 days and to nothing at the end of storage. In the same period of time, butyric acid started to be produced in both low TS silages. Final butyrate contents of 8.3%VS_{added} for fresh biomass and 5.5% for 72h-wilted silage were obtained. The degradation of lactic acid and the concomitant production of butyric acid at low TS content indicates that secondary fermentation occurred 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 [12]. Besides the unstable behavior, this will also 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 [12]. Indeed, the pH of fresh ensiled biomass increased from 4.2 at 14 days to 5.6 at the end of storage. Likewise, 72h-wilted silage presented a huge increase of pH from 4.4 at 14 days to 7.0 at 98 days of storage. Major impact of clostridial fermentation on VS losses was also observed at low TS. After 98 days of ensiling, cumulative organic matter losses of fresh and 72h-wilted biomass reached 25.8% and 16.8%, respectively.

The instability of higher moisture crops was also illustrated by an increasing ammonia nitrogen content during ensiling. NH_3-N fraction varied from 12.3%TKN at 14 days to 40.2%TKN at the end of fresh biomass ensiling. Similarly, NH_3-N content of 72h-wilted silage increased from 15.3%TKN to 37.0%TKN in the same period of storage. Proteolytic clostridial activity, which is favored by disturbances on silage acidification, should be in the origin of this increase. This type of 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 if an alkaline pH is reached. Since unstable silage never exceeded a pH value of 7.0, only a small fraction of NH_3-N should be lost into the atmosphere. This is corroborated by the fact that there is no clear decline trend of TKN content during the tests. However, attention must be paid to this issue if pH values of poor silages tends to go towards the alkalinity for even more prolonged storage durations.

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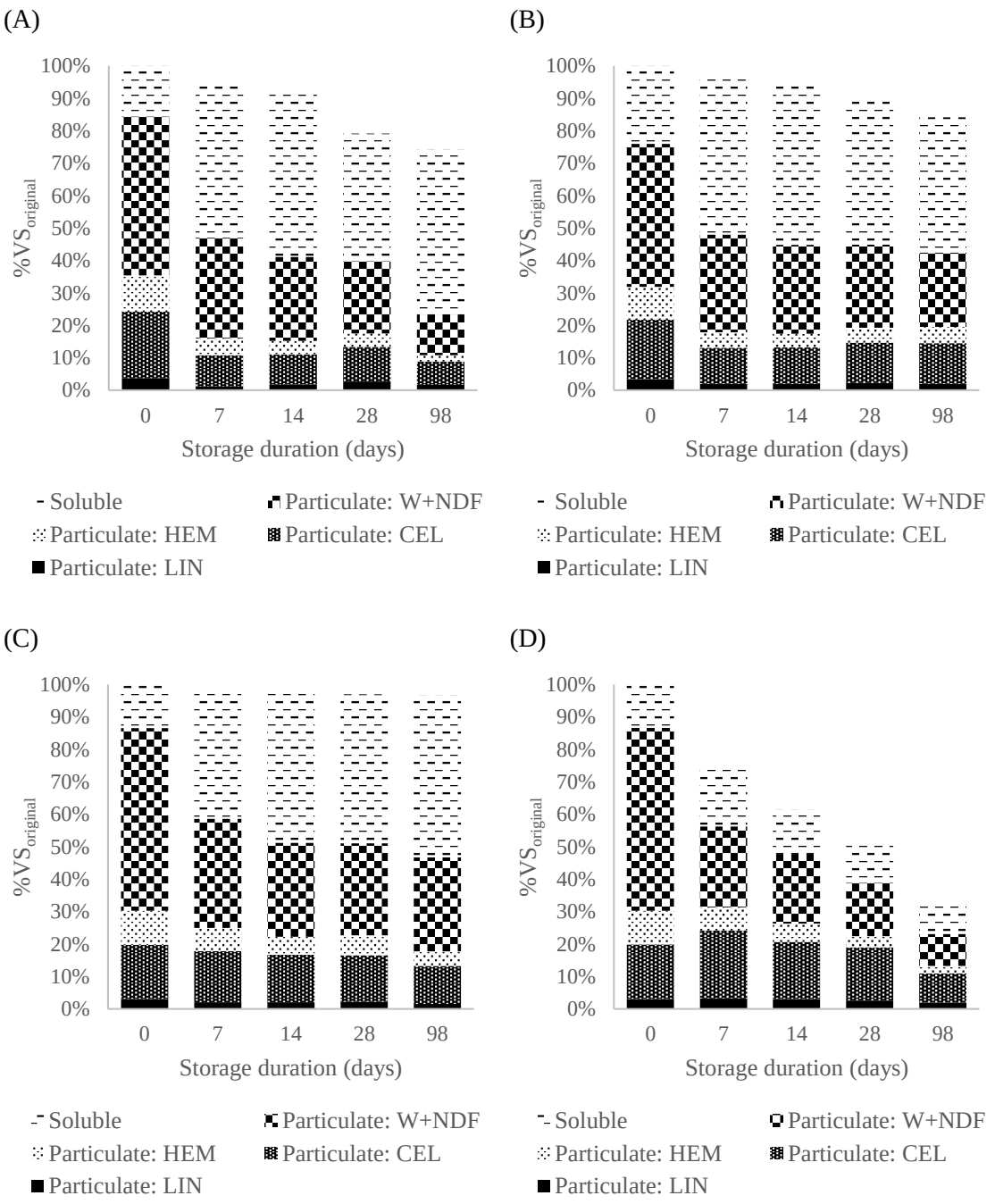

Figure 3-5 – Total volatile solids balance for: (A) TS10%E; (B) TS14%E; (C) TS39%E; (D) TS39%A.

Concerning the substrates used by microorganisms for acidification, the results suggested that substances other than WSC are the original source for organic acids production. This is based on the fact that original WSC content of fresh biomass is largely inferior to the total VFA generated during ensiling. Taking the example of fresh ensiled biomass, 28.2%VS_{added} (26.0%$VS_{original}$ – result not shown) of total VFA were produced after 14 days of storage, from a material with 12.0%VS of original WSC. Additional information about the source of acidification products and phenomena can be found in the VS balance presented in Figure 3-5. Indeed, it can be observed that a great shift on VS structure occurred

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during the first 7 days of ensiling. This corresponds to the period of time where the major part of acidification took place. The major modification was the increase of water-soluble phase of VS, which varied from 13-24%VS before storage to 42-46%$VS_{original}$ after 7 days of ensiling. This was an outcome of the degradation of both non-structural and structural fractions of particulate phase. In fact, besides the reduction of Water+NDF soluble phase of particulate, there was a meaningful breakdown of (hemi-) cellulosic compounds during the first week of ensiling. Lignocellulosic degradation during early acidification may be a result of endogenous enzyme activity. Indeed, while studying grass hemicelluloses ensiling, Dewar *et al*. [86] reported that enzyme activity may contribute to additional sugar availability for fermentation. These additional substrate released by the particulate phase must have been a useful support to VFA production in the first week of ensiling. From 7 days of storage VS structure of biomass evolved according to the stability of silage. For low TS crops (especially for non-wilted biomass), particulate phase of VS continued to be degraded, since pH level turned back into bacterial and enzymatic activity range. For 92h-wilted silage, minor further increase of soluble fraction was recorded. However, we observed a slow but continuous breakdown of lignocellulose carbohydrates for this condition. Since at lower pH levels enzyme activity is considerably reduced, this should be a result of acid hydrolysis by VFA produced during fermentation [12,86]. After 98 days of ensiling for fresh biomass, cumulative breakdowns of 83% and 64% of hemicellulose and cellulose were detected, respectively. For 76h-wilted silage, losses reached 50% for hemicellulose and 32% for cellulose. Finally, for the same ensiling duration 92h-wilted biomass lost 57% of hemicellulose and 30% of cellulose. These results show a greater extent of cell wall breakdown than previously reported results. For instance, Pakarinen *et al*. [16] and Menardo *et al*. [30] found a slight decrease of hemicellulose content during ensiling, while cellulose was not significantly degraded. In turn, other authors observed both hemicellulose and cellulose degradation with ensiling, but always below 10% of the original content [29,62,87]. Discrepancy between these results may be partially explained through differences in cell wall accessibility. Firstly, higher particle size was used in part of literature [16,29,87]. This may influence digestibility of lignocellulosic compounds during ensiling, as particle size reduction is frequently linked to the increase of the available specific surface area and to the reduction of both degree of polymerization and cellulose crystallinity [35]. Furthermore, unlike part of these works [16,29], the lignification degree of the feedstock used in this study was very low, as initial lignin values ranged from 3.8-4.6%VS. Finally, the results of the aforementioned works were mostly presented on a TS/VS_{added} basis. This may carry an underestimation of cell wall breakdown if substantial organic matter losses occurred during ensiling.

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3.3.3.3. Evolution of BMP during storage

Discrepancies between results of open-air and ensiling conservation were even more evident if we compare the evolution of the methane potential (BMP) along storage, Figure 3-6. Methane yields for the aerobic treated sample ranged between 336 and 153 L/kgVS$_{added}$. While taking into account storage losses (L/kgVS$_{original}$), this represented more than 80% of energy losses from 7 to 98 days of storage. On the opposite, the 96h-wilted silage recorded slightly increasing values of BMP during storage, even considering organic matter losses. At the end of the 98 days, the BMP based on VS$_{original}$ of 96h-wilted biomass open-air stored was only 18% of the one for the ensiling of the same feedstock. Nevertheless, aerobic treatment does not appear to be harmful for the methane yield of 96h-wilted biomass in the first week of storage, despite important VS losses. Indeed, similar BMP values based on VS$_{original}$ were obtained for open-air and ensiling of 96h-wilted biomass at 7 days. This was a result of an enhanced biochemical accessibility of aerobic storage in early days, as reflected by the higher BMP referring to VS$_{added}$ for this condition after the first week. Today aerobic pretreatments for biomass are based on the delignification functions of microorganisms such as white rot fungi [88,89]. In this work, besides the use of a feedstock with low lignin content, no biological agent was added. Yet, fungi development that was observed during open-air storage could have the above-mentioned properties. Nevertheless, characterization of microbial biomass produced was not further investigated, since the main goal was to follow BMP evolution along storage.

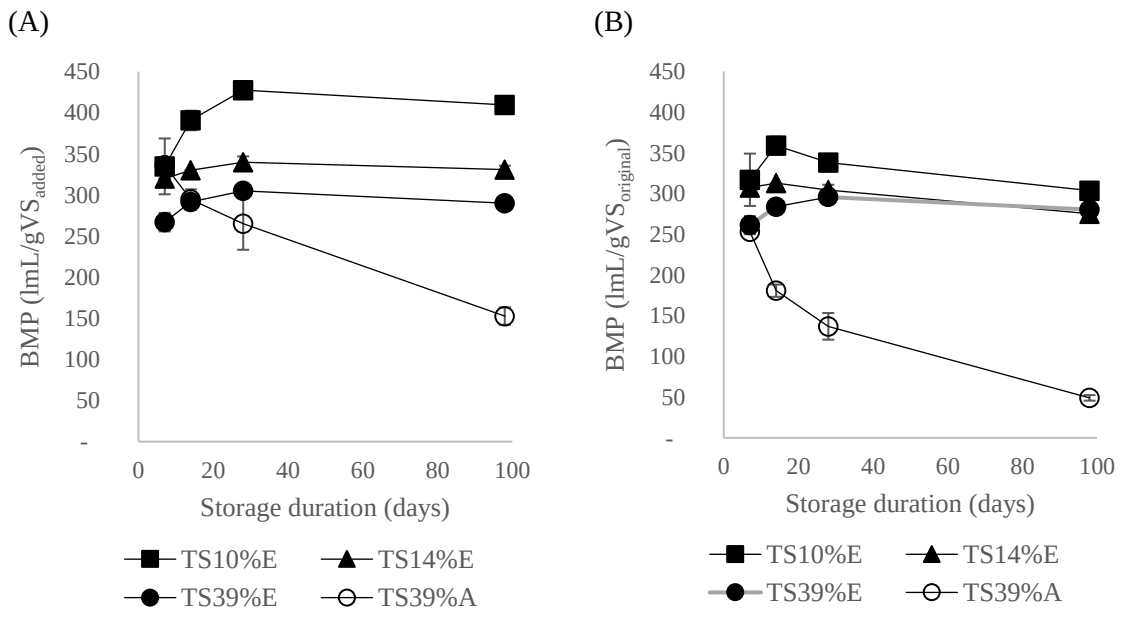

Figure 3-6 - BMP evolution over storage duration. (A): methane potential based on VS$_{added}$; (B): methane potential based on VS$_{original}$, therefore considering storage losses.

Regarding ensiling, long-term storage had little effect on BMP based on VS$_{original}$. Methane potential varied from 317 to 304 L/kgVS$_{original}$ for non-wilted biomass, 307-275 L/kgVS$_{original}$ for 72h-wilted silage

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and 261-280 L/kg$VS_{original}$ for 96h-wilted biomass between 7 and 98 days of ensiling. Nevertheless, it is possible to identify distinct impacts on methane potential for different periods of storage. First of all, it is interesting to notice that, despite higher VS losses, low TS crops had higher BMP values after 7 days of ensiling. This should be a consequence of heat damage during wilting process of TS39% silage. Indeed, as previously mentioned, extremely warm and ventilated conditions used at the end of the 96h-wilting led to important WSC losses. In addition, higher rates of hydrolysis and fermentation of lignocellulosic fractions during the first 7 days of ensiling for low TS crops should have enhanced their biochemical accessibility and therefore their methane potential. Between 7 and 14 days of storage, BMP increased for all silages. This corresponds to a period of organic acid production from structural carbohydrates, which rendered biomass more available for methanogens. The increase was more expressive for TS10% and TS39% silages, for which higher VFA accumulation occurred during this period. After 14 days and until at least the end of first month of storage, BMP based on VS_{added} continued to increase for all silage conditions. However at this time, stability of silage began to play an important role on methane potential conservation. Indeed, while taking into account VS losses, BMP decreased for low TS crops. This evidenced a harmful effect of secondary clostridial fermentation for energy conservation. Between 28 and 90 days of storage, instability of non-wilted and 72h-wilted silage led to losses of BMP based on $VS_{original}$ of around 10%.

Results of BMP based on $VS_{original}$ after 98 days of storage suggest that wilting was incapable of improve energy conservation before and during ensiling. Despite its more constant and easily predictable fermentation pathways, 96h-wilted biomass probably lost part of its methane potential by heat damage (or volatilization) during wilting. Concerning low TS crops, especially fresh ensiled biomass, the gains in biochemical accessibility overcame their instability and VS losses. Moreover, the results suggest that the higher fermentation rates for low TS crops are not inevitably damaging for BMP conservation, especially for shorter storage durations.

In short, the catch crop used in these tests should be promptly ensiled after harvest if unsuitable weather conditions are present for wilting. However, one should take into attention the possibility of leachate production during ensiling of high moisture crops. Concerning these laboratorial assays, liquid effluent production was around 23% of total biomass weight (result not shown) after 98 days of ensiling for fresh biomass (TS10%E). In a field scale silo, packing density should be at least equal to the one used in this work. Therefore, important leachate production should occur while using the same feedstock. Indeed, the early correlations done by Bastiman [46], Sutter [47] and Zimmer [48] predict an effluent production that range from 34 to 45% of the total weight for a silage with 10% of TS. Moreover, results presented in Figure 3-5 show that VS water-soluble fraction will increase along storage duration, resulting in more than 65% of total VS content after 98 days of storage for TS10%E condition. In this case, important losses of methane potential will occur if effluent produced is not collected and promptly added to the anaerobic digester downstream.

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3.3.4. Conclusions

Ensiling is a far more appropriated method than open-air storage for conservation of the catch crop studied before anaerobic digestion. Wilting was inefficient while witnessing unfavorable weather conditions at harvesting. Even if prolonged wilting periods under warmer/dryer conditions led to a decrease of the moisture content, it did not provide improvements on long-term conservation of the methane potential. For high moisture products, instability of fermentation is offset by gains in biochemical accessibility. In such conditions, biomass should be promptly ensiled without wilting. Liquid effluent that will likely be produced, should be collected and used for biogas production.

3.3.5. Acknowledgements

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3.4. A retenir

La première conclusion à tirer des études avec deux types différents de CIVEs est que le stockage dans des conditions aérobies doit être proscrit avant méthanisation. En effet, les matières organiques ont été largement dégradées pendant de longues périodes en présence d'oxygène et, pour cette modalité de stockage, la perte de potentiel de production de méthane des CIVEs a été supérieure à 80% après 3 mois de stockage.

La performance du stockage a été améliorée par l'ensilage : les pertes de matière organique ont été limitées par l'acidification du gisement et la conservation de l'énergie de la biomasse après 3 mois a varié entre 78% et 96% en fonction des conditions testées. Les valeurs les plus élevées de potentiel bio-méthanogène après un stockage de longue durée ont été obtenues pour la matière fraiche ensilée immédiatement après la récolte. Il est important de souligner que ces CIVEs n'ayant pas des propriétés idéales pour l'ensilage – elles avaient notamment des faibles teneurs en sucres libres et matière sèche - de meilleures conservations pendant l'ensilage peuvent être envisagées pour d'autres types de biomasse plus riches en sucres.

Le préfanage, utilisé comme technique pour diminuer la teneur en eau des échantillons, n'a pas fourni un avantage en termes de conservation d'énergie et doit être évité lorsque les conditions météorologiques sont défavorables (faible effet de séchage en raison de l'humidité nocturne). Dans de tels cas, il est préférable d'ensiler la biomasse rapidement après la récolte. Néanmoins, si la teneur en MS est trop faible, une perte de lixiviat riche en carbone organique est probable lors du compactage du silo, dont la quantité augmentera au cours de l'ensilage en raison de la solubilisation de la matière organique. En conséquence, un dispositif de récupération de l'effluent liquide doit être prévu dans le silo, pour qu'il puisse être rapidement injecté dans le méthaniseur, afin d'éviter une fermentation ultérieure de la matière organique soluble et une perte d'énergie. En outre, il a été démontré que même si une faible teneur en MS entraîne une certaine instabilité et une fermentation secondaire pendant l'ensilage, les pertes d'énergie seront limitées, voire contrebalancées par une augmentation des gains de bio-accessibilité pendant le stockage.

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4. STOCKAGE DES FUMIERS DE BOVIN AVANT METHANISATION

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4.1. Introduction

Les résultats et discussion des essais de stockage à l'échelle du laboratoire pour des fumiers de bovin sont présentés dans cette partie sous la forme de trois articles scientifiques, chacun associé à une série distincte d'essais de stockage à long terme. Les principales conclusions à retenir pour l'ensemble des essais avec fumier de bovin sont présentées dans la dernière partie de ce chapitre.

Dans la première étude de cette partie, les effets du stockage confiné (mode ensilage) et du stockage à l'air libre sur la conservation du Fumier 1 et de son énergie ont été étudiés pendant 120 jours. L'impact de différents degrés d'addition de paille de blé (et par conséquent de l'augmentation de la teneur en matière sèche) sur la conservation de la biomasse a également été abordé. Les résultats obtenus suggèrent qu'il peut y avoir un avantage potentiel dans l'utilisation de co-substrats pour l'amélioration de l'ensilage de fumiers de bovin. Ce sujet a été approfondi par un screening d'additifs, effectué au sein du laboratoire DEEP dans le cadre d'un stage de recherche [90], dont les résultats majeurs se trouvent aussi dans l'annexe B6. Cette étude a consisté à évaluer l'influence de l'ajout de plusieurs co-substrats à différentes concentrations sur l'acidification d'un fumier de bovin en conditions d'ensilage, pour une durée maximale de 15 jours. Ces essais ont permis d'identifier l'acide formique (2 $\%_{MH}$) et le glucose (à partir de 4 $\%_{MH}$), comme additifs permettant d'abaisser le pH du fumier de bovin vers 4, valeur souvent considérée comme suffisante pour favoriser la stabilisation de la matière organique lors de l'ensilage.

L'utilité de l'acide formique et du glucose dans la conservation de la matière organique et du potentiel bio-méthanogène du fumier de bovin avec paille de blé (Fumier 2) a été ensuite testée pendant 120 jours en conditions d'ensilage. Les résultats sont présentés dans le deuxième article de ce chapitre. Finalement, le troisième article résume la dernière série d'essais de stockage de fumiers de bovin (Fumier 3), où les effets de l'ajout non seulement de glucose à différentes teneurs en matière sèche, mais aussi d'amidon sur l'évolution de la biomasse, du potentiel énergétique et des émissions gazeuses ont été étudiés pendant 120 jours de co-ensilage.

Les données supplémentaires des analyses qui ne sont pas présentés lors de la discussion des articles scientifiques avec fumier de bovin sont consultables dans les annexes B3, B4 et B5.

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4.2. Cattle manure for biogas production. Does ensiling and wheat straw addition enhance preservation of biomass and methane potential?

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Abstract

The effects of ensiling and open-air storage on the conservation of cattle manure were investigated for 120 days at laboratory scale. Impact of co-ensiling with wheat straw was assessed as well. Up to 74% of methane potential was lost during aerobic storage. Negligible energetic losses and ammonia emissions occurred during the first month of anaerobic preservation. However, inadequate chemical properties of fresh cattle manure hindered silage stabilization for extended periods, leading to 46% of methane losses after 4 months. Co-ensiling of cattle manure with wheat straw enhanced biomass acidification, inducing a conservation of more than 86% of the methane potential after 4 months. Wheat straw addition suppressed ammonia production and limited the risks of losses through liquid effluent. The current study proves that long-term conservation of cattle manure can be achieved if correct management practices are used, which will undoubtedly improve the performance of biogas plants with this raw material.

Keywords*: Cattle manure; Biomass; Storage; Ensiling; Anaerobic digestion; Methane potential*

4.2.1. Introduction

Several European countries are currently mobilizing for the energy recovery from cattle manure through anaerobic digestion (AD). In France, the annual production of cattle manure is estimated at nearly 87 Mt, which represents around 28.3 MWh of potential primary energy [4]. Nevertheless, due to its production fluctuations and in order to enable a continuous supply of biogas plants throughout the year, cattle manure needs to be stored before use, in some cases for extended periods. For this feedstock, a common practice is the open-air storage before AD. The reason for this is mainly related to the simplicity and low cost of the operation. However, this practice has several drawbacks. Generally speaking, air presence is perceived as a spoiling agent for biomass conservation, leading to important energy losses during long-term storage with aerobic spoilage of easily biodegradable organic fraction. Recent works

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with different catch crops showed that more than 80% of biochemical methane potential (BMP) was lost after 3 months of storage in the presence of air [91,92]. In the specific case of cattle manure, this reality is further compounded by the fact that open-air storage facilities are important sources of ammonia and odor emissions [93].

Regarding conservation methods before AD, ensiling is usually pointed out as the logical choice to store wet biomass. This process is typically divided into four phases, based on different biochemical transformations [8,10,12,85]. 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 available carbohydrates 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. While well succeeded, ensiling enhances biomass conservation and minimize energy losses. For instance, Herrmann *et al.* [11] observed an average of 2% difference between BMP of fresh and ensiled material after 1-year ensiling of maize, sorghum hybrid, forage rye and triticale.

Given the high efficiency of ensiling for other biomass crops, it should be expectable to obtain good preservation of cattle manure using the same method. However, ensiling success is extremely dependent on several biochemical characteristics of feedstock, such as moisture content, water-soluble carbohydrates (WSC) content, buffering capacity and endogenous microflora [12,85]. Indeed, cattle manure does not fulfill the chemical requirements for an efficient ensiling, especially due its lack of WSC. Without those, organic acids cannot be produced in a significant extent and then, the desired level of acidity for silage stabilization would not be achieved.

Even if cattle manure does not naturally lead to an optimal ensiling conservation, it is possible that an anaerobic environment reduces organic matter and energy losses during storage. However, to the best of our knowledge, no study addressing this subject exists today.

This work established a comparison between open-air storage and ensiling of cattle manure during 120 days at laboratory scale. The effects of different degrees of wheat straw addition on silage conservation were also addressed. Storage performance was evaluated, above all, by its impact on BMP conservation of raw material. Notwithstanding, other parameters, such as organic matter losses or eventual gas emissions, were examined during storage as well. This study contributes to the optimization of practices for cattle manure management before biogas production, which will definitively have an impact on the energy recovered downstream.

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4.2.2. Material and methods

4.2.2.1. Substrates

Fresh cattle manure was collected on 10 March 2016 from an agricultural site in the Rhône-Alpes region of France (Gaec Béreyziat, Les Teppes, 01340 Béréziat, France) and it was stored at 4 °C before further use. Fresh raw material had 13% of total solids (TS) content and it was tested in both aerobic and ensiling conditions. In addition, two mixtures of fresh cattle manure and wheat straw were prepared at the laboratory and tested for ensiling. Wheat straw had 10 cm maximum length and it was mixed with cattle manure in order to achieve final TS contents of 19% and 22%, respectively. Storage conditions and description of respective raw materials are summarized in Table 4-1.

Table 4-1 – Storage conditions.

Condition	TS13%A	TS13%E	TS19%E	TS22%E
Approach	Open-air	Ensiling	Ensiling	Ensiling
Wheat straw (%wt)	-	-	8.3%	13.3%
TS (%wt)	12.8 ± 0.1		18.8 ± 0.4	22.4 ± 0.8
VS (%wt)	10.2 ± 0.1		15.9 ± 0.3	17.9 ± 0.7

4.2.2.2. 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 varied between 7, 15, 30 and 120 days. A total of 16 (4 operating conditions x 4 storage durations) conservation assays were done.

4.2.2.3. Chemical analysis

For each sample time, one silo (per tested condition) was sacrificed. It was opened and weighed, biomass was homogenized and two samples were taken. One was used for direct analyses on the crude material and the other one was mixed with water in order to get two fractions: a water-soluble phase and a particulate phase. This leaching test was performed with a 10:1 water/TS ratio during 2 h under constant bottle rotation. Phase separation was achieved by centrifugation (5000 G; 10 min) followed by 0.7 µm particle size filtration. Finally, the particulate phase was dried at 70 °C until constant weight and ground

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

Crude material was analyzed for its TS content, volatile solids (VS) content and BMP. For the water-soluble phase, besides TS/VS content and BMP, pH, WSC, volatile fatty acids (VFA), total Kjeldahl nitrogen (TKN) and ammonia nitrogen (NH_3-N) fractions were determined. Particulate solid was analyzed for its TS/VS, TKN and cell wall constituents content.

TS 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 [21], the measures were corrected using the volatilization coefficients at 100 °C suggested by Porter and Murray [75]. pH was measured by a Consort C3020 device with a SP10B pH-electrode. WSC, lactic acid and formic acid 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 H_3PO_4 0.1%v as solvent (flow rate of 0.5 mL/min). WSC content was estimated as the sum of glucose, xylose, galactose, mannose, arabinose and cellobiose and was determined using the UV detector (210 nm). Lactic acid and formic acid contents were obtained with the RID detector. Acetic, propionic, butyric, valeric and caproic acids content were analyzed using gas chromatography (Shimadzu Corp.) equipped with a HP-FFAP fused silica capillary column (30 m x 0.25 mm, Agilent Technologies), a flame ionization detector and using H_2 as carrier gas. Total VFA was calculated as the sum of lactic, formic, acetic, propionic, butyric, valeric and caproic acids. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed through Van Soest and Wine [76] modified extractions method, based on XP U44-162 French standard. Hemicellulose content (HEM) was calculated as NDF minus ADF; cellulose (CEL) as ADF minus ADL and; lignin (LIG) as approximatively equal to ADL. TKN and NH_3-N were determined through the procedure described in the NF EN 25663 French standard.

The interest of our experimental procedure is to enable the assessment of the composition and BMP based on the initial mass of product used, since the weight loss was measured. The results for the chemical analysis will thus be presented in two ways: based on $\%VS_{added}$ or $\%VS_{original}$. VS_{added} relates to the organic matter of the sample analyzed. The results based on $VS_{original}$ take into account the loss of volatile solids during storage and allows the study of the results based on the VS of the initial material.

4.2.2.4. Biochemical methane potential tests

Batch anaerobic digestion tests were performed for crude material and water-soluble phase samples. Tests were conducted in a temperate room at 35 °C using glass vessels of 2 L for crude material and 0.1 L for water-soluble phase. 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

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total volume of the vessel. The inoculum used (TS 3.0-3.2%wt; VS 2.0-2.2%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.* [82].

In order to assess the kinetics of methane production, the experimental data of BMP assays was fitted to the following equation:

$$V_{CH_4}(t) = V_{max}(1 - e^{-kt}) \tag{4.1}$$

Where, V_{CH_4} corresponds to the volume of methane produced; t is the time of the assay; V_{max} is the maximum volume of methane produced, which was equivalent to the experimental BMP value and; k is the rate coefficient of CH_4 production. Since gas production started almost immediately after reactors were sealed, lag time was not considered in the calculations.

4.2.3. Results and discussion

4.2.3.1. Feedstock characterization

Chemical characteristics of raw materials and stored biomass are presented in Table 4-2. Besides low TS content (12.8%), fresh cattle manure was mainly characterized by a negligible content of WSC and pH value of 7.9. These were unsuitable features for energy conservation through an acidification-based process as ensiling. First of all, as lower TS crops are more unstable and susceptible to deterioration, lower silage pH is required for an efficient ensiling [12]. By analogy with the data published by Kalač [27] for ensiled forage, a critical pH value of around 4.0 is required to preserve fresh cattle manure. In order to achieve such degree of acidification, extensive production of lactic acid should occur in the first

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days of ensiling [12]. However this would require an adequate level of readily available carbohydrates, *i.e.* high WSC content. In fresh cattle manure, energy sources were mainly concentrated in the form of lignocellulosic compounds. Indeed, cellulose and hemicellulose contents of fresh cattle manure were estimated as 26.8 and 32.5% of organic matter, respectively. Nevertheless, it should not be expected a prompt and wide VFA production from these polymers. Their biochemical accessibility is rather limited by lignin protection (6.1%VS) [94] and depolymerization/hydrolysis reactions should take several weeks to occur in substantial extent.

Table 4-2 – Chemical characteristics of biomass over storage time (%$VS_{added/original}$).

Condition	Storage duration (days)	Chemical characteristics [a]								
		pH	LA[b]	AA[b]	BA[b]	VFA[b]	WSC[b]	HEM[c]	CEL[c]	LIG[c]
TS13%A	0	7.9	0.0	1.3	0.3	2.1	0.0	32.5	26.8	6.1
	7	7.8	0.0	1.0	0.3	1.7	0.0	26.4	22.5	8.1
	14	7.9	0.0	0.9	0.4	2.0	0.0	24.8	26.1	5.5
	30	8.6	0.0	0.9	0.6	2.2	0.0	20.4	20.6	5.2
	120	9.8	0.0	0.0	0.0	0.0	0.0	13.3	10.3	5.7
TS13%E	0	7.9	0,0	1.3	0.3	2.1	0.0	32.5	26.8	6.1
	7	7.3	0,0	1.6	0.5	3.0	0.0	30.0	26.6	6.3
	14	6.9	0,1	2.8	1.1	6.0	0.0	26.5	24.9	5.8
	30	6.9	0,0	6.7	2.2	12.9	0.0	24.0	20.3	6.2
	120	8.4	0,0	1.1	0.0	1.7	0.0	16.7	16.9	6.1
TS19%E	0	8.3	0,0	1.1	0.2	1.8	0.0	28.7	36.0	6.1
	7	7.2	0,0	2.3	0.5	3.6	0.0	26.6	33.4	6.6
	14	6.5	0,1	2.5	1.0	4.8	0.0	24.8	32.1	5.1
	30	5.6	0.0	4.1	3.0	9.4	0.0	24.5	30.2	5.3
	120	5.5	0.0	7.4	4.2	15.7	0.0	22.0	24.8	4.6
TS22%E	0	8.2	0.0	1.1	0.1	1.8	0.0	27.5	37.2	6.3
	7	6.8	0.0	2.0	0.4	3.1	0.0	26.9	36.7	5.9
	14	6.2	0.0	2.1	0.9	4.0	0.0	27.1	35.8	5.4
	30	5.5	0.0	3.3	2.4	7.5	0.1	27.3	30.8	7.0
	120	5.4	0.0	6.3	4.8	15.5	0.0	21.9	24.8	7.2

[a] LA stands for Lactic Acid, AA for Acetic Acid, BA for Butyric Acid, HEM for Hemicellulose, CEL for Cellulose and, LIG for Lignin; [b] results based on %VS_{added}; [c] results based on %$VS_{original}$

Structure of cell wall constituents changed with wheat straw addition to cattle manure. This was, in fact, the only remarkable effect of wheat straw beyond the increase of TS/VS content. Cellulose content

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increased to 36.0-37.2%VS after wheat straw addition. In turn, hemicellulose content decreased to 27.5-28.7%VS. No noticeable effect on lignin content was recorded with wheat straw addition.

4.2.3.2. Effects of storage method on biomass preservation

4.2.3.2.1. Chemical characteristics

Aerobic storage was mainly characterized by a pH increase and modifications on organic matter structure, Table 4-2. Structural carbohydrates were uninterruptedly degraded along the 4 months, culminating in both cellulose and hemicellulose losses of around 60% of their initial content. Furthermore, no accumulation of WSC nor VFA was recorded for this condition. This suggests that the degradation of structural carbohydrates led to severe VS and energy losses. Lignin content did not vary significantly, meaning that no (or limited) delignification occurred during aerobic storage. In addition to these chemical modifications, fungi development was noticeable throughout open-air storage. Therefore, microbial degradation must have occurred through an aerobic metabolism producing carbon dioxide and microbial biomass.

Storage in anaerobic conditions of fresh cattle manure led to VFA production and pH decrease until 30 days. Nevertheless, the acidification was slow since only structural carbohydrates were available for fermentation. This should explain the significant reduction of (hemi-) cellulose content during the first month of ensiling. The major components of VFA were acetic and butyric acid, with other small fractions of propionic, valeric and caproic acid. No formation of lactic acid was detected during ensiling. Since the fermentation products of TS13%E are weaker acids than lactic acid, pH drop for stored cattle manure was not as marked as in lactate silages. In addition, between 14 days and 30 days of ensiling, VFA content increased from 6.0% to 12.9%VS_{added} but pH value remained stable at 6.9. This evidenced that fresh cattle manure had a strong buffer capacity at neutral pH conditions. Altogether, efficient pH values around 4.0 were not achieved for ensiling of fresh cattle manure and further biomass degradation occurred for prolonged storage periods. Indeed, between 30 and 120 days, fermentation profile reversed and more than 90% of VFA were consumed until the end of storage. This caused an increase of pH, which reached a value of 8.4 at 120 days. The fact that this pH value were superior to the one of the raw material can be explained by the ammonia production and accumulation during ensiling. Moreover, structural carbohydrates continued to be degraded until the end of the storage. After 4 months, 48% and 37% of original hemicellulose and cellulose were biodegraded, respectively. Thus, non-negligible organic matter and energy losses must have occurred for long-term ensiling of fresh cattle manure.

Equivalent fermentation profiles were found for TS13%E and co-ensiling conditions in the first month of storage. However, the rate of acidogenesis decreased with wheat straw addition. This may be explained by the fact that wheat straw mostly contains hardly accessible carbohydrates. Moreover, wheat straw addition has decreased the initial microbial concentration and moisture content, which delayed

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bacterial growth. For instance, it is well known that at least part of silage bacteria have lower activity for higher TS contents [12,73,74]. Nevertheless, wheat straw addition enhanced stability of cattle manure silages. For both TS19%E and TS22%E, pH value decreased from 8.2-8.3 before storage to around 5.5-5.6 after 30 days and remained constant until 4 months of ensiling. Additionally, even if VFA production was more restricted for co-ensiling, fermentation had a much higher impact on silage acidification than in TS13%E. This suggests that wheat straw addition reduced the buffer capacity of the raw material at neutral pH value. Despite the apparent stability of wheat straw silages, degradation of structural carbohydrates occurred until the end of the storage. After 4 months, 28% and 29% of the sum of original (hemi-) cellulosic compounds were missing for TS19%E and TS22%E, respectively. However, this should not have led to substantial energy losses, since VFA accumulation occurred until the end of storage for both co-silages.

The data presented here evidenced that the recommended pH value of around 4.10-4.35 for TS content between 15-25% [27] was not applicable for the conservation of cattle manure, or at least for its co-ensiling with wheat straw. It must be remembered that this value of pH is the required acidity to prevent lactate degradation. In our experiments, no lactate was produced during ensiling. This means that either lactic acid was quickly consumed as an intermediate for acidogenesis, or that monosaccharides were degraded by other pathways than LAB fermentation. In any case, the stability of cattle manure did not relied on lactic acid formation but on the accumulation of VFA during storage. Therefore, the critical pH in cattle manure silages should be considered as the required acidity to prevent methanogenic activity and further VFA damage for long-term storage, which was achieved for both TS19%E and TS22%E conditions.

4.2.3.2.2. TKN balance

Effects of storage method on total Kjeldahl nitrogen balance are presented in Figure 4-1. This evolution is related to the TKN content of feedstocks, which was 4.0%VS for fresh cattle manure, 2.7%VS for TS19%E and 2.3%VS for TS22%E.

Regarding open-air storage, there was a continuous elimination of TKN, resulting in losses of 58.6% of its original content after 120 days. Mechanisms of degradation can be suggested through analysis of TKN composition along storage. First, there was a considerable decrease of particulate TKN content, which ranged from 52% to 39% of original TKN during the 4 months of aerobic treatment. Major part of particulate loss occurred during the first 7 days of storage, during which the content of the water-soluble phase of organic nitrogen increased. This indicates that some proteins were subject to hydrolysis under aerobic conditions, therefore increasing the amount of organic nitrogen compounds that can be processed by microorganisms capable of nitrogen mineralization and nitrate/nitrite production. However this may only partially explain the original source of TKN losses, due to the limited degradation extent

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of organic nitrogen in the particulate phase during storage. In fact, the majority of structural modifications of TKN during aerobic treatment occurred in the water-soluble phase.

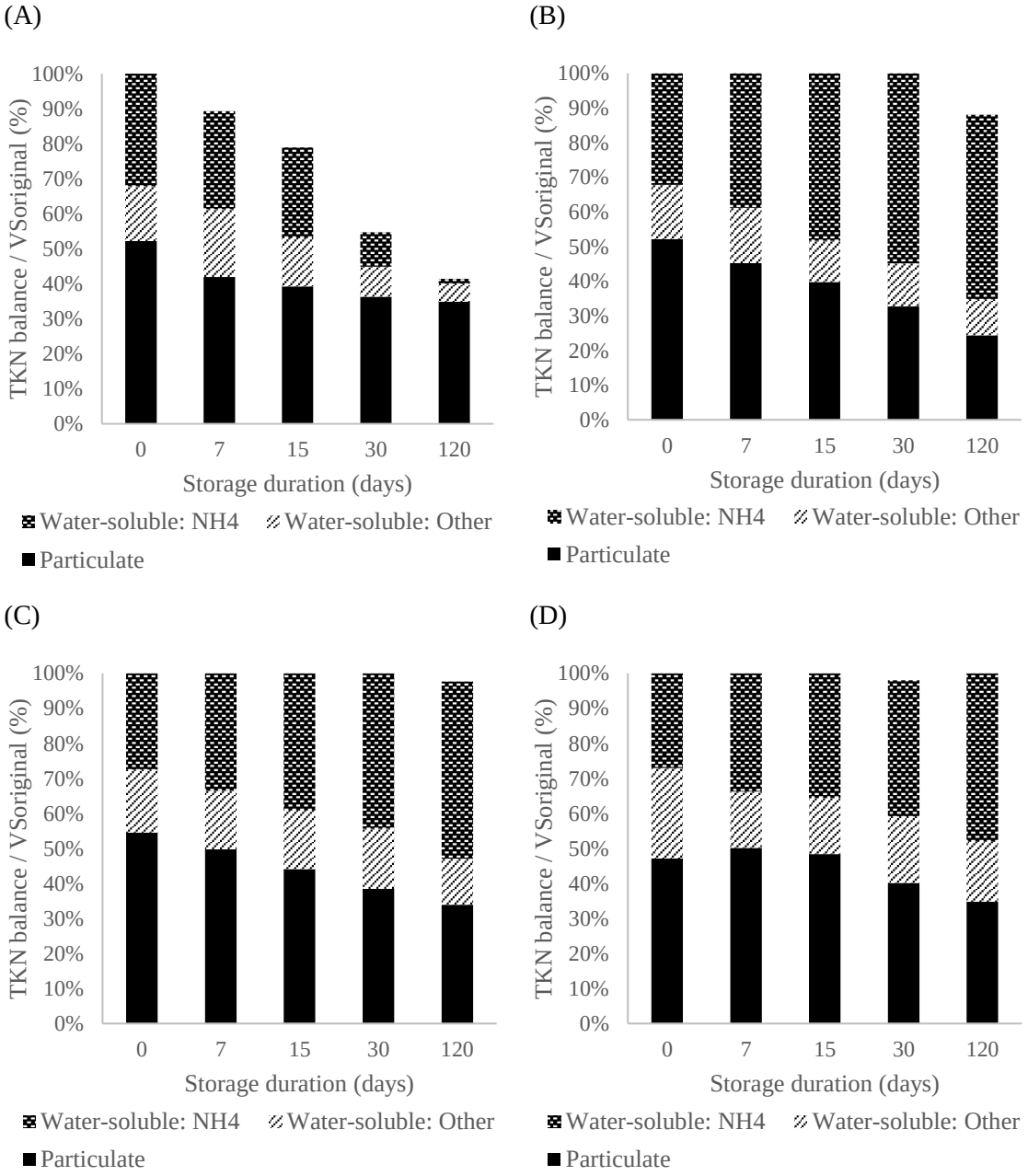

Figure 4-1 – TKN balance for: (A) TS13%A; (B) TS13%E; (C) TS19%E; (D) TS22%E.

From 7 days of storage and until the end of conservation, the content of organic nitrogen in the liquid fraction decreased, which suggests that the rate of amino acid degradation was significant during aerobic treatment. Even if the previous mechanisms may lead to inorganic nitrogen production, this was not observed during storage. Indeed, ammonia concentration decreased all along storage duration, from 1.30%$VS_{original}$ before storage to 0.02%$VS_{original}$ after 120 days of aerobic treatment. This may be explained by the constant perturbations in the NH_4^+/NH_3 equilibrium ($pK_a = 9.25$) during conservation. In open-air storage, NH_3 emissions did not have any impact on its concentration in the gas phase, since

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ammonia produced was instantly diluted in the atmosphere. Thus, in such conditions, nitrogen was considered to be lost through NH_3 production, even at relatively low pH. The rate of ammonia loss improved with time, as pH increased for long periods of aerobic treatment. Furthermore, nitrification reactions may have contributed to the elimination of organic nitrogen in aerobic conditions. This would lead to nitrate and nitrite emissions, which are not measured in the TKN procedure. Considering that all TKN losses led to ammonia emissions, around 40.1 $L_{NH3}/kgVS_{original}$ were expected to be released into the atmosphere during the 4 months of open-air storage of fresh cattle manure.

Ammonia emissions were reduced through ensiling of fresh cattle manure. Indeed, in the first 30 days of ensiling there was a complete preservation of TKN content and at the end of storage only 12% of its original content was lost. This latter value corresponded to a theoretical value of 9.0 $L_{NH3}/kgVS_{original}$ for gas emissions during the 4 months of ensiling. Nevertheless, there was a larger transformation of TKN structure for ensiling than for open-air storage. On the one hand, the organic nitrogen content in both particulate and water-soluble phase decreased almost linearly with time, from a total $68\%TKN_{original}$ before storage to $35\%TKN_{original}$ after 4 months. On the other hand, ammonia content clearly increased along ensiling. In such conditions, TKN degradation must have occurred through two successive reaction mechanisms: hydrolysis of proteins into amino acids and subsequent fermentation in ammonia. These are typical reactional mechanisms that arise during anaerobic fermentation [95] and poor silages (high pH values) [12]. Besides NH_4^+, these reactions generally lead to other side-products, such as a pool of organic acids, CO_2 and H_2, generating energy losses, regardless of how small they may be. Even if there is a high production of ammonia during ensiling, gas emissions should be avoided as long as the silo is airtight and a low pH medium is present. This explain why degraded nitrogen was stored under the form of NH_4^+ in the first month of ensiling. However, during the last 3 months of storage, pH increased to values near the pK_a of NH_4^+/NH_3, which led to TKN losses through ammonia production.

Co-ensiling of cattle manure with wheat straw did not have a significant impact on the reactional mechanisms related to TKN. However, lower degradation rates of TKN were obtained for TS19%E and TS22%E. In fact, organic nitrogen content (from both particulate and water-soluble phases) was around 70%TKN for all three feedstocks. This amount decreased to $34.8\%TKN_{original}$ for TS13%E, $41.4\%TKN_{original}$ for TS19%E and $51.9\%TKN_{original}$ for TS22%E at the end of storage. These results may be in part linked to the effect of lower growth of proteolytic clostridia for higher TS contents, which has been reported in the literature for other types of silages [12,42,43]. Nitrogen losses were even lower than in TS13%E condition, reaching at the most $2.3\%TKN_{original}$ during the co-ensiling. This corresponded to theoretical ammonia emissions of 1.2 $L_{NH3}/kgVS_{original}$ at the end of the storage. Enhanced conservation of nitrogen was related to the stable low pH value obtained during the 4 months for silages with wheat straw.

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4.2.3.2.3. Organic matter losses

Higher damage of organic matter was found for the aerobic treatment, as shown in Figure 4-2. After one month, around 25% of organic matter of open-air stored cattle manure was lost, which ascended to more than 50% after 4 months. This was linked to the constant loss of structural polymers during storage.

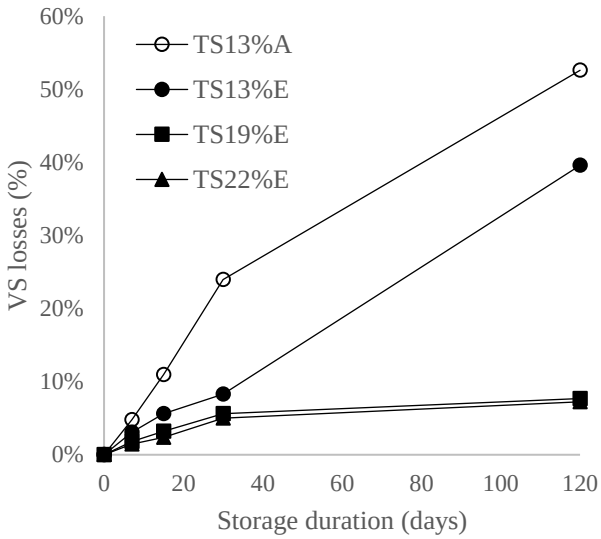

Figure 4-2 - Organic matter losses over storage duration.

Silage quality had an important effect on VS conservation during storage. Anaerobic storage of fresh cattle manure limited the organic losses to less than 10% in the first month, the period in which there was an accumulation of VFA. Nevertheless, silage instability of low TS crop aforementioned led to damage of almost 40% of original VS content at the end of the 4 months.

Co-ensiling with wheat straw had a positive effect on organic matter conservation. For TS19%E and TS22%E, most part of the VS losses occurred in the first month and corresponded to 5% of original VS. During this period, pH kept decreasing. Therefore, other bacteria populations capable of VS degradation, such as methanogens, must have proliferated under such conditions. Furthermore, fermentation of monosaccharides may lead to several side-products. For instance, reactional mechanisms of acetate or butyrate production include CO_2 and H_2 formation. Besides organic matter damaging, this would cause slight energy losses, as hydrogen would be lost into the environment during silo opening. In addition, nitrogen mineralization contributed to VS losses, although to a minor extent. During the last 3 months of storage, organic matter suffered minimal damage, due to silage stabilization. After 4 months, 92% of original VS was conserved for both co-storage conditions. These are remarkable results regarding silage conservation, since they are equivalent [11,92] or even better [14,15,91] than data found in the literature concerning good quality of crop silage. All things considered, there was a positive effect on the conservation of the biomass inherent to the wheat straw addition. This is in agreement with McDonald

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et al. [12], who considered straw as an additive for ensiling, due in part to its effect on the reduction of organic matter losses.

4.2.3.3. Effects of storage method on methane potential

4.2.3.3.1. BMP evolution

Evolution of methane potential along storage is presented in Figure 4-3. The results exposed are related to both $VS_{original}$ and VS_{added}, which allows the monitoring of original BMP conservation and eventual modifications on the chemical accessibility of the remaining biomass. BMP of aerobic treatment (TS13%A) ranged from 288 L/kgVS before storage to 157 L/kgVS_{added} and 74 L/kg$VS_{original}$ after 4 months. BMP losses were divided in two distinct chronologic phases. Around half of energetic damage occurred during the first month of storage. This was expected since in first moments of long-term storage, besides the particulate phase, the major part of soluble organic matter should be within reach of aerobic microorganism. In this period, losses followed a linear tendency, equivalent to 1.2% original BMP loss per day. Methane potential losses slowed down somewhere between the second and fourth month and led to 74% of BMP lost at the end of aerobic storage.

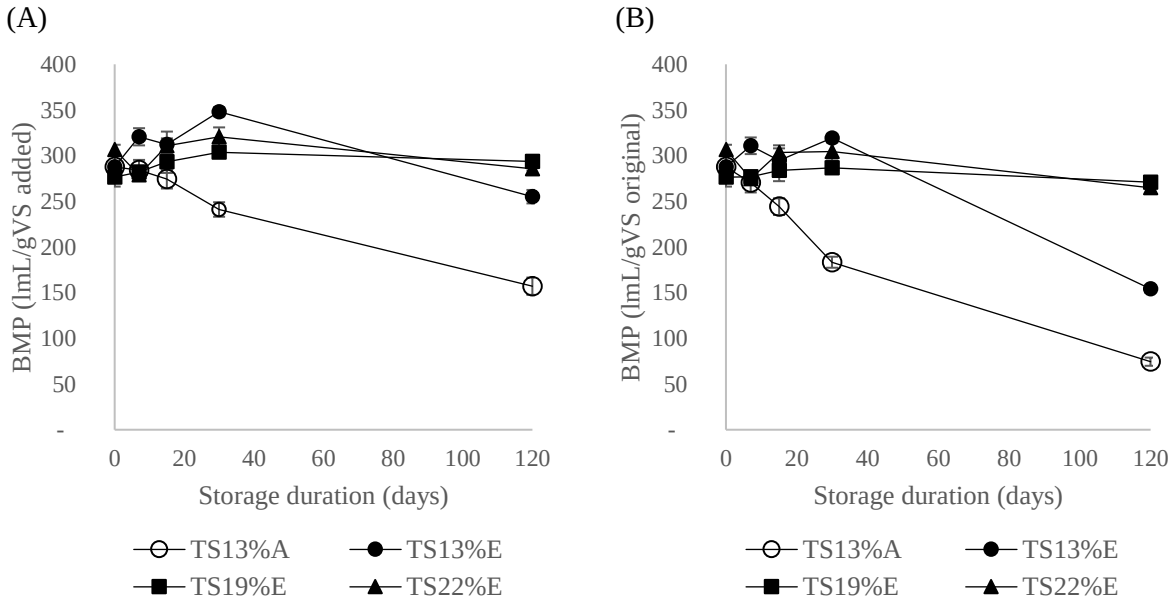

Figure 4-3 - Global BMP evolution over storage duration. (A): methane potential based on VS_{added}; (B): methane potential based on $VS_{original}$, therefore considering storage losses.

Energy conservation was improved with ensiling of fresh cattle manure (TS%13E), since constantly higher BMP values during storage were obtained rather than when aerobic assays were carried out. As in the preceding condition, two chronologic phases of BMP evolution were observed for TS13%E. A slight BMP increase was found during the first weeks of storage for low TS silage. Indeed, after 1 month of storage original methane potential was improved by 11%, which is substantially higher than the limit of accuracy of BMP tests. Enhanced biochemical accessibility of feedstock must have been the source

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of this energetic increment, as BMP related to VS_{added} varied from 288 to 348 L/kgVS_{added} during the first month. As previously discussed, during this period, production and accumulation of VFA took place through degradation of (hemi-) cellulosic compounds. These latter polymers are not fully biodegradable in such soft condition as that experienced within the mesophilic AD process [96]. Therefore, their hydrolysis/acidogenesis during ensiling is a reasonable explanation for the BMP increase. This trend did not last until the end of storage, since in the last 3 months BMP of fresh ensiled cattle manure abruptly decreased, either considering organic matter losses or not. Consequently, TS13%E lost 46% of its original methane potential after the end of the 4 months of storage. One can assume that this energetic loss was linked to the consumption of VFA and structural carbohydrates during this period of storage.

Wheat straw addition had a positive effect on BMP conservation of cattle manure during ensiling in the long term. In fact, minor variations were recorded on the methane potential along the 4 months of storage for both conditions using straw: TS19%E showed BMP values of 277-304 L/kgVS_{added} and 271-287 L/kg$VS_{original}$; while for TS22%E it were observed 285-321 L/kgVS_{added} and 265-305 L/kg$VS_{original}$. Although less clear than in previous storage conditions, BMP evolution during storage of both TS19%E and TS22%E can be separated in two periods of time. After one month of storage, BMP related to VS_{added} increased by about 10% and 4% for TS19%E and TS22%E conditions, respectively. This reflects a slight enhancement of biochemical accessibility of raw materials and it should be interrelated with the production and accumulation of VFA in silage from hardly biodegradable carbohydrates during this period. Nevertheless, this effect was counteracted by organic matter losses. Consequently, the improvement of BMP related to original VS was not relevant (below the precision limits). Between the end of the first and fourth month, BMP related to VS_{added} decreased by 3% for TS19%E and 11% for TS22%E. This evidenced that biodegradability of stabilized silages may decrease in long-term storage, which may arise since the so-called biomass stabilization occurred at relatively high pH values. At the end of the 4 months of ensiling, 98% and 86% of feedstock's methane potential was conserved for TS19%E and TS22%E, respectively. This proves that wheat straw addition was in both cases an asset to BMP preservation for long-term ensiling of cattle manure and that use of 19% TS should lead to optimal energy recovery.

Finally, our results suggest that there is a strong correlation between fermentation profiles, organic matter conservation and BMP for prolonged storage durations. At the same time, one may take into account that BMP assays may last for about 2 to 3 months to be completed. Therefore, whenever either a quick analysis of several storage conditions is required or only simple approaches are available, organic matter losses determination (*e.g.*, or even total weight loss) may be a resourceful method for qualitative analysis on optimization of storage conditions for a specific feedstock before biogas production.

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4.2.3.3.2. BMP distribution in particulate and water-soluble phase

The contribution of the soluble and particulate fraction to the total BMP is presented on Figure 4-4. During aerobic storage (TS13%A) the water-soluble contribution of BMP varied from 27% before storage to 10% after 4 months. Furthermore, nearly all of this decrease occurred during the first month of storage. This observation supports the hypothesis earlier debated that higher rate of BMP losses observed during the first weeks of open-air storage was a consequence of the degradation of water-soluble compounds.

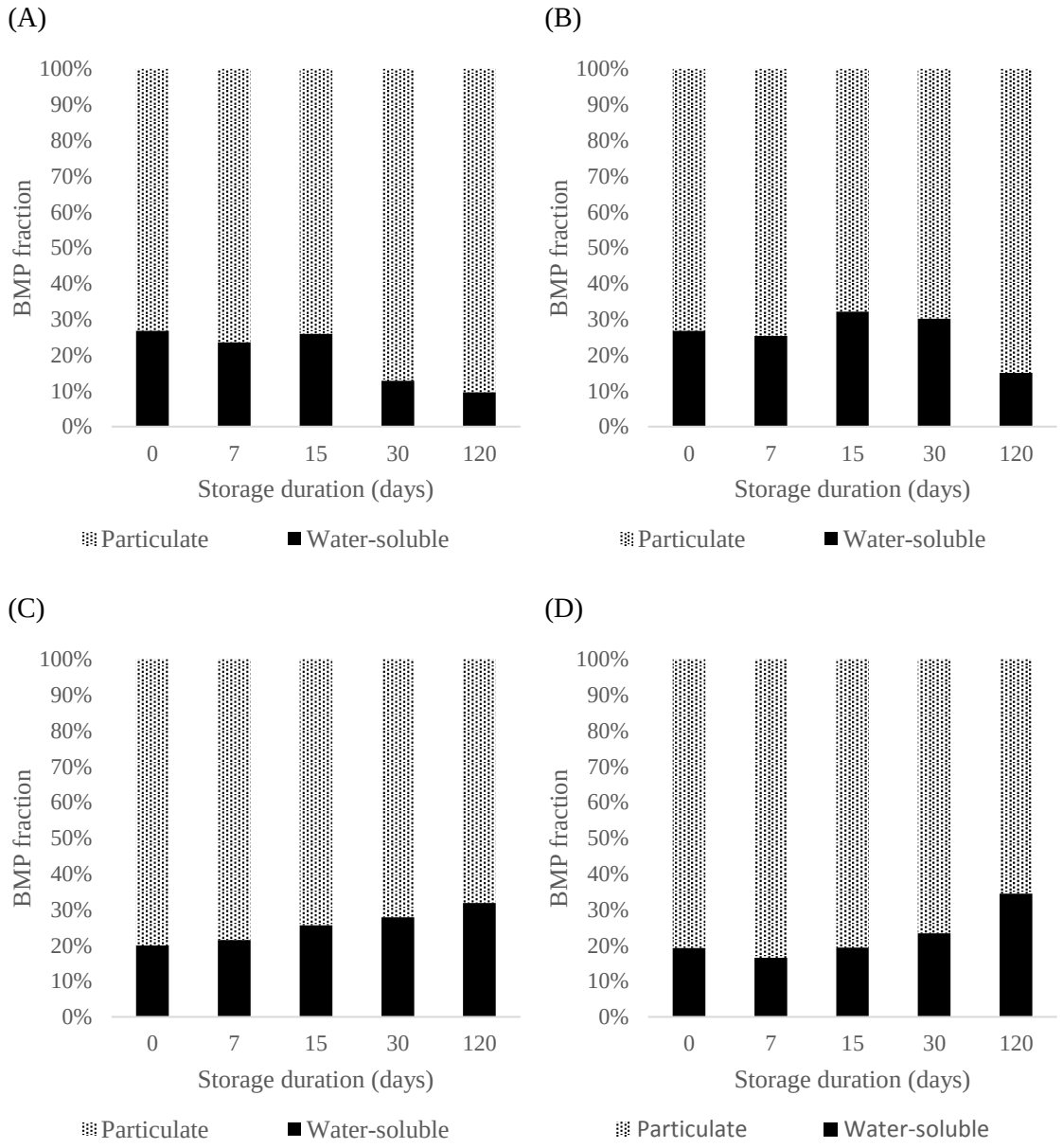

Figure 4-4 – BMP multi-phase analysis for: (A) TS13%A; (B) TS13%E; (C) TS19%E; (D) TS22%E.

Minor changes on BMP contributions (between particulate and soluble part) were detected for ensiling of fresh cattle manure (TS13%E) during the first month of storage, with water-soluble phase representing 25-32% of total methane potential. Significant degradation of water-soluble phase only

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occurred during the last 3 months, decreasing its fraction to 15% of total BMP at the end of storage. These results partially agree with the data afore discussed. On the one hand, a clear increase tendency of water-soluble phase of BMP was expected over the first month of ensiling. This would be the result of the increase of VS soluble fraction along storage duration, which was indeed recorded either by direct measure of VS fractions (result not shown) or by degradation of structural carbohydrates and VFA accumulation. On the other hand, the decrease of water-soluble fraction of BMP in the last 3 months of storage is in line with the loss of almost the totality of VFA that was observed during this period.

Analogous evolutions of BMP fractions during ensiling were observed for both degrees of wheat straw addition. For instance, regarding TS19%E condition, water-soluble fraction of BMP increased from 19% prior to storage to 34% after 4 months. This is in agreement with data from fermentation profiles, and confirms that complex energy sources have not only been degraded but also solubilized and conserved in that form for prolonged periods of ensiling. However, this mechanism of energy conservation may not be applicable to all types of cattle manure. In fact, bacterial populations can be influenced by the characteristics of manure [97], which may depend on the type of cattle, animal's diet, as well as, on the time of the year [98]. Therefore, for several types of manures, the methanogens concentration, *e.g.*, may be far superior to the one of the feedstock used in these tests. In such cases, solubilization of particulate would still occur but VFA produced would be degraded due to a higher methanogenic activity. This will weaken ensiling efficiency, as it will lead to substantial energy losses before AD.

Moreover, wheat straw addition had an impact on BMP distribution before storage: water-soluble phase of BMP decreased from 25% for TS13%E (and TS13%A) to 20% and 19% for TS19%E and TS22%E, respectively. This was expected since organic matter of wheat straw is mostly under particulate form. Nevertheless, these are promising results for ensiling low TS feedstocks. In field scale silage of standard crops, low TS content should be avoided in order to prevent leachate formation during storage [13,46,47]. Since part of the BMP of silages are present in water-soluble form, important energy losses may occur due to seepage of eventual liquid effluent production. The use of wheat straw will increase TS content and reduce leachable methane content, therefore decreasing the risk either of effluent production [12] and eventual energy losses that it may entail. Indeed, no production of liquid effluent was recorded during ensiling of cattle manure with wheat straw (result not shown). These outcomes were considered to be representative of a field scale, as cattle manure was ensiled with a commonly used packing density in agricultural silage.

4.2.3.3.3. Kinetics

Besides the maximum value of methane produced, kinetics of BMP assays were characterized by the 1st-order rate coefficient (k) of biomass degradation, Figure 4-5. This parameter is closely linked to the biochemical accessibility of biomass, hence the interest for biomass conservation purposes. Nevertheless, while testing prolonged storage, different inoculum should be used for part of BMP series.

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Contrary to methane potential value, modifications on inoculum properties have an impact on the rate of biomass degradation during BMP assays. Therefore, an indirect approach was used to analyze evolution of *k* values during storage, which consisted in only comparing results between different conditions for the same storage period (BMP tests were performed with the same inoculum).

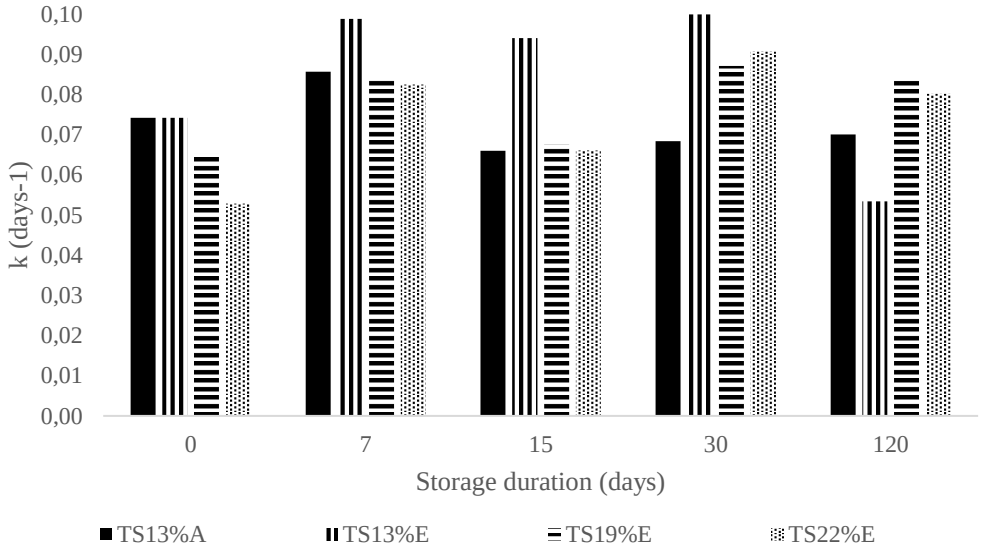

Figure 4-5 – Rate coefficient (k) of BMP assays over storage time. Standard deviations are not presented as differences among triplicates were negligible.

Concerning the feedstocks, higher rate coefficient was found for fresh cattle manure, being the decrease of biochemical accessibility proportional to the amount of wheat straw added. This confirms that the majority of wheat straw VS was hardly reachable, which was previously suggested by the increase of cellulose content and particulate phase of BMP related to straw addition.

Storage method had a significant effect on biochemical accessibility of fresh cattle manure. In the first 30 days of storage, the difference between the rate coefficients of the two methods progressively increased, benefiting ensiling. This must have been an outcome of: in first place, the degradation of structural carbohydrates into VFA and their accumulation during this period for TS13%E and; secondly, the important loss of water-soluble compounds in the first month of open-air storage. This scenario was reversed in the last 3 months and the degradation rate of ensiled cattle manure became weaker than the one for the aerobic treatment. On the one hand, this difference cannot be attributed to an increase of the biochemical accessibility for the open-air stored feedstock, since its BMP declined significantly in this period and its water-soluble fraction did not increase. This means that the shift should have been a consequence of VFA degradation for TS13%E, which led, in addition to the energy damaging, to a great loss of accessibility of the organic matter during the 3 last months of storage.

Despite different initial values, similar rate coefficients were obtained for TS19%E and TS22%E from 7 days of storage. Furthermore, both conditions with wheat straw presented lower *k* than TS13%E until

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30 days of ensiling. This was explained by the fact that wheat straw led to a more controlled fermentation, and as formerly discussed to scarcer gains related to the BMP kinetics. At the end of storage, TS19%E and TS22%E had far higher degradation rates during the BMP assays than ensiling of fresh cattle manure. This evidenced that wheat straw is an asset on the conservation of great rates of biochemical accessibility for extended periods of ensiling. Restricted fermentation and VFA accumulation in water-soluble phase appear to be the key factors for the success of co-silages with cattle manure and wheat straw.

4.2.4. Conclusions

Ensiling led to lower energetic losses and NH_3 emissions than open-air storage during conservation of cattle manure. Yet, chemical properties of fresh cattle manure hindered silage stabilization for extended periods. Co-ensiling of cattle manure with wheat straw enhanced biomass acidification, inducing a preservation of more than 86% of methane potential after 4 months. In addition, wheat straw suppressed ammonia production and limited the risks of energy losses through liquid effluent. In summary, this work demonstrated that long-term conservation of cattle manure can be achieved if correct management practices are used. This will impact the performance of agricultural biogas plants.

4.2.5. Acknowledgements

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4.3. Co-ensiling of cattle manure before biogas production: effects of fermentation stimulants and inhibitors on biomass and methane preservation

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Abstract

The impact of formic acid and glucose addition on the co-ensiling of cattle manure with straw was assessed during 4 months at laboratory scale. Feedstock deprived of additives lost 67% of its methane potential for prolonged ensiling. This was mainly due to the lack of water-soluble carbohydrates and to the high methanogenic activity of cattle manure. The use of co-substrates enhanced biomass and energy conservation during ensiling. The best storage performance was obtained for co-ensiling of cattle manure with glucose (100 g/kg of feedstock). For this condition, lactate production was extensive, which allowed biomass acidification, suppressed ammonia emissions and led to full preservation of methane potential after 4 months. Therefore, in field-scale storage, co-ensiling with a high sugar content (or starch-rich) co-substrate appears to be the most resourceful method to optimize cattle manure preservation. Application of this measure will have a major impact on the methane yield of agricultural biogas plants where cattle manure has to be stored for long periods.

Keywords*: Cattle manure; Additives; Storage; Ensiling; Anaerobic digestion; Methane potential*

4.3.1. Introduction

Cattle manure is a major input for agricultural biogas plants, especially in countries with intensive livestock systems. In France there are currently 267 of these installations [2] and nearly 87 Mt of cattle manure annually produced that may be mobilized for anaerobic digestion (AD) in the near future [4]. However, there are still several constraints slowing down the valorization of cattle manure. This includes the storage requirements and the consequences on the methane potential of cattle manure that it may entail. On the one hand, biogas plants should operate continuously and feedstock should be available

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throughout the year. Yet, cattle manure, such other agricultural wastes and crops, undergoes production fluctuations, which periodically leads to the need of storage during long periods.

Generally speaking, the main issues related to storage of cattle manure are that the current field-scale practices are not optimized for energy production purposes and scientific studies about this subject are scarce. Cattle manure is typically stored outdoors in contact of air, in order to have a simple and low-cost operation. This has several drawbacks: it leads to leachate generation, ammonia and odor emissions [93] and energy losses. Recently, Teixeira Franco *et al*. [99] evidenced that up to 74% of cattle manure's biochemical methane potential (BMP) may be lost during 4 months of open-air storage. Nevertheless, the authors showed that anaerobic storage, or ensiling, may limit energy losses to a maximum of 46% after 4 months.

Traditionally used as fodder conservation method, ensiling is a biochemical process that mainly rely on anaerobic conditions and acidification of the feedstock to be efficient [12,85]. While applied to specific crops, this technique leads to full conservation of the BMP, even after 1 year [11]. However, fresh cattle manure does not satisfy the chemical requirements for an efficient ensiling, whether due to its high moisture, low water soluble carbohydrates (WSC) content, strong basic buffering capacity or endogenous microflora. For instance, without WSC, hetero or homo-lactic fermentation cannot occur and organic acids (especially lactic acid) cannot be rapidly produced in a significant extent, so as to abruptly lower the pH value and inhibit further bacterial activity.

To the best of our knowledge, few studies reported work on cattle manure storage. Teixeira Franco *et al*. [99] have approached the optimization of cattle manure silage before biogas production. The authors evidenced that co-ensiling with wheat straw may enhance methane conservation of cattle manure to more than 86% after 4 months. The decrease of moisture content and the alteration of buffering properties should be in the origin of this improvement. However it is suggested that the mechanisms of energy conservation may not be generalized to other types of cattle manure, due to the variability of the characteristics and of the bacterial population in this feedstock.

The use of additives is considered to be a resourceful method to ensure a good silage quality. Fermentation stimulants and inhibitors are two of the most important silage additives categories among the wide range of biological and chemical products commercially available. The role of fermentation stimulants is to encourage lactic fermentation either by supply of substrate (*e.g.* fermentable sugars), lactic acid bacteria (LAB) or enzymes [12]. In contrast, inhibitors (acids) are also used to prevent biological activity of the degrading microorganisms by lowering the pH of the feedstock. These two types of silage additives are serving as a basis for most of ensiling research in recent years [11,14–16,19,20,22,23,25]. Nevertheless, none of these co-storage studies were performed with cattle manure.

In order to assess the effects of additives on the ensiling of cattle manure with straw, laboratory experiments were conducted for up to 4 months. A fermentation stimulant (glucose) and an inhibitor

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(formic acid) were used as model molecules of co-substrates for ensiling. Besides the evaluation of BMP during storage, the monitoring of fermentative profiles, organic matter losses and potential gas emissions were included in our work. Our objective is to contribute to the improvement of cattle manure management before energy production, which will have a major impact on the ammonia emissions and on the methane yield of agricultural biogas plants.

4.3.2. Material and methods

4.3.2.1. Feedstocks

Fresh cattle manure (10% of total solids (TS) content) was collected on June 1st, 2016 from an agricultural site in the Rhône-Alpes region of France (Gaec Béreyziat, Les Teppes, 01340 Béréziat, France) and it was stored at 4 °C before further use. A mixture of fresh cattle manure and wheat straw was prepared at the laboratory and it was tested for ensiling. Wheat straw with 10 cm maximum length was mixed with cattle manure in order to achieve final TS content of 19%. The TS content of cattle manure with straw was chosen taking into account the recommendations of Teixeira Franco *et al*. [99] for the optimization of cattle manure storage.

Besides the control assay (*i.e.* ensiling without silage additives), the following treatments were applied to cattle manure with straw before filling laboratory silos and being tested for ensiling (Table 4-3): formic acid, 8.2% of volatile solids (VS) content; glucose, 16.6%VS and; glucose, 40.2%VS. Concentrations of silage additives were selected through a short-term screening performed at the laboratory, in which the impact of silage additives on the pH evolution was examined.

Table 4-3 – Ensiling conditions and treatments applied to cattle manure with wheat straw (% of total sample weight).

Ensiling condition	Additive		TS content
	Molecule	Content	
Control	-	-	19.2±0.1
Formic acid	Formic Acid	2.0	20.4±0.1
Glucose 4%	Glucose	4.0	22.3±0.5
Glucose 10%	Glucose	10.0	24.9±1.4

4.3.2.2. Experimental 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

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a geotextile membrane to separate it from biomass. Proper plastic lid and rubber ring were used for silo sealing and its airtightness was reinforced with silicone sealant. Then, silos were weighed and placed in a controlled-temperature room at 25±2 °C. Storage duration varied between 7, 15, 30 and 120 days. A total of 16 (4 operating conditions x 4 storage durations) ensiling assays were performed.

4.3.2.3. Chemical analysis

For each sample time, one silo (per tested condition) was sacrificed. It was opened and weighed, biomass was homogenized and two samples were taken. One was used for direct analyses on the crude material and the other one was mixed with water in order to get two fractions: a water-soluble phase and a particulate phase. This leaching test was performed with a 10:1 water/TS ratio during 2 h under constant bottle rotation. Phase separation was achieved by centrifugation (5000 G; 10 min) followed by 0.7 µm particle size filtration. Finally, the particulate phase was dried at 70 °C until constant weight and ground at 2 mm theoretical length. Crude material/water-soluble and particulate samples were stored at 4 °C and -20 °C, respectively, until analysis.

Crude material was analyzed for its TS content, volatile solids (VS) content and BMP. For the water-soluble phase, besides TS/VS content, pH, WSC, volatile fatty acids (VFA), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN) and ammonia nitrogen (NH_3-N) fractions were determined. Particulate solid was analyzed for its TS/VS, COD, TKN and cell wall constituents. Feedstocks were not analyzed for COD, TKN and NH_3-N content.

TS 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 [21], the data were corrected according to the volatilization coefficients at 100 °C suggested by Porter and Murray [75]. pH was measured by a Consort C3020 device with a SP10B pH-electrode. WSC, lactic acid and formic acid 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 H_3PO_4 0.1%v as solvent (flow rate of 0.5 mL/min). WSC content was estimated as the sum of glucose, xylose, galactose, mannose, arabinose and cellobiose and was determined using the UV detector (210 nm). Lactic acid and formic acid contents were obtained with the RID detector. Acetic, propionic, butyric, valeric and caproic acids content were analyzed using gas chromatography (Shimadzu Corp.) equipped with a HP-FFAP fused silica capillary column (30 m x 0.25 mm, Agilent Technologies), a flame ionization detector and using H_2 as carrier gas. Total VFA was calculated as the sum of lactic, formic, acetic, propionic, butyric, valeric and caproic acids. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed through Van Soest and Wine [76] modified extractions method, based on FD U44-162 standard [77]. Hemicellulose content was calculated as NDF minus ADF; cellulose as ADF minus ADL and; lignin as equal to ADL. TKN and NH_3-N were determined through the procedure

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described in the NF EN 25663 standard [78]. COD of water-soluble phase was determined through the colorimetric HACH procedure (method 8000). COD of particulate phase was measured by a Walkley and Black [79] modified method, based on the NF ISO 14235 international standard [80].

The interest of our experimental procedure is to enable the assessment of the composition and BMP based on the initial mass of product used, since the weight loss was measured. The results for the chemical analysis will thus be presented in two ways: based on $\%VS_{added}$ or $\%VS_{original}$. VS_{added} relates to the organic matter of the sample analyzed. The results based on $VS_{original}$ take into account the loss of volatile solids during storage and allow the study of the results based on the VS of the initial material.

4.3.2.4. Biochemical methane potential tests

Batch anaerobic digestion 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 international standard [81]. 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 8 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*. [82].

4.3.3. Results and discussion

4.3.3.1. Feedstock characterization

Chemical properties of feedstocks are gathered in both Table 4-3 and 4-4. Considering the pH value (8.3) and the TS content (19.2%) of fresh cattle manure with wheat straw, AD microflora should have proliferated during storage. However, energy sources of raw material were mostly structural carbohydrates. Indeed, cellulose and hemicellulose contents of cattle manure were estimated as 36.1 and

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30.6% of organic matter, respectively, while WSC fraction was negligible. This means that biomass acidification during storage should be preceded by hydrolysis of polymers, as in biogas production process [95]. Since hydrolysis reactions take several weeks to occur in substantial extent under ensiling conditions [91], fermentation of sugars will occur slowly. In the meantime, hydrogen-consuming reactions may have occurred, such as proteins mineralization and subsequent NH_4^+/NH_3 equilibrium. Besides, it was detected a rather high VFA content in cattle manure before storage. This allowed two different considerations. First, biomass was already fermenting before storage, which means that cattle manure was not totally fresh. Consequently, this may have impacted growth rates of acidogens and methanogens during ensiling. Furthermore, the fact that high pH and VFA content co-existed in the raw material evidences that cattle manure had strong basic buffer compounds. Altogether, the biochemical properties of the cattle manure used in this work should hinder biomass preservation during ensiling, especially in absence of WSC or organic acids addition.

Table 4-4 - Chemical properties of biomass over ensiling duration (%VSadded/original).

Assay	Period (days)	VS losses (%)	Chemical characteristics [a]									
			pH	LA[b]	AA[b]	BA[b]	FA[b]	VFA[b]	WSC[b]	HEM[c]	CEL[c]	LIG[c]
Control	0	-	8.3	0.0	3.7	0.0	0.0	5.5	0.0	30.6	36.1	8.3
	7	2.5	7.1	0.0	3.4	0.4	0.0	5.7	0.0	28.5	34.1	8.1
	15	5.5	7.7	0.0	2.4	0.6	0.0	5.0	0.0	28.3	31.3	8.5
	30	14.8	8.2	0.0	0.2	0.5	0.0	4.2	0.0	25.1	31.1	9.1
	120	42.0	8.4	0.2	0.0	0.0	0.0	0.2	0.0	14.6	18.7	9.2
Formic acid	0	-	3.5	0.0	1.5	0.0	8.2	10.0	0.0	32.1	32.2	7.3
	7	0.4	3.6	0.4	1.2	0.1	8.8	10.9	0.0	30.5	31.5	6.8
	15	0.6	3.9	0.5	1.2	0.0	8.6	10.5	0.0	29.5	32.0	6.9
	30	0.7	3.8	0.6	1.1	0.1	9.8	11.9	0.0	29.1	32.7	6.2
	120	13.4	6.9	0.1	5.6	1.5	0.0	9.1	0.0	23.2	29.6	7.7
Glucose 4%	0	-	7.9	0.0	1.8	0.0	0.0	2.6	16.6	27.3	30.9	10.5
	7	1.9	4.0	13.5	2.0	0.5	0.0	17.0	4.1	27.1	27.8	6.2
	15	2.5	4.1	15.2	2.0	0.7	0.0	18.9	2.0	24.8	26.2	5.0
	30	3.4	4.1	10.0	2.1	1.8	0.0	15.2	0.0	26.1	27.9	5.4
	120	9.0	5.4	0.0	2.2	3.2	0.0	10.9	0.0	25.6	29.6	6.1
Glucose 10%	0	-	7.9	0.0	1.3	0.0	0.0	1.9	40.2	21.9	23.9	4.6
	7	1.5	4.0	11.2	1.8	0.3	0.0	14.1	34.0	18.2	19.4	4.0
	15	2.3	4.0	14.5	1.8	0.4	0.0	17.9	30.1	17.3	19.9	3.6
	30	2.7	3.8	15.1	2.0	0.6	0.0	18.2	24.2	17.4	19.2	3.5
	120	4.6	3.7	19.5	3.9	1.0	0.0	25.9	18.5	15.5	18.2	3.5

[a] LA stands for Lactic Acid, AA for Acetic Acid, BA for Butyric Acid, FA for Formic Acid, HEM for Hemicellulose, CEL for Cellulose and, LIG for Lignin; [b] results based on $\%VS_{added}$; [c] results based on $\%VS_{original}$

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A proportional increase of TS content was observed with the use of co-substrates. This was expected since additives were used as pure compounds and not in solution. Since the activity of silage bacteria is at least in part vulnerable to changes in TS [12,73,74], this may have influenced the silage process. In the case of formic acid addition, the pH value of the feedstock was affected as well. Indeed, it decreased to 3.5, which is a considerable lower value than the ones recommended by Kalač [27] for lactate silages (4.10-4.20 for TS content of 15-20%) and by Teixeira Franco *et al.* [99] for anaerobic stabilization of cattle manure with wheat straw (pH value of around 5.4-5.6). Concerning raw material with glucose addition, WSC content ascended to 16.6%VS (14.8%TS) and 40.2%VS (36.5%TS) for Glucose 4% and Glucose 10% conditions, respectively. These WSC contents are quite superior than the ones found in the literature for feedstock successfully acidified during ensiling (3-14%TS) [16,22,28,30,100,101]. Thus, efficient acidification should occur for cattle manure treated with glucose if a proper microflora is present, notably in respect of LAB concentration.

4.3.3.2. Effects of storage method on biomass preservation

4.3.3.2.1. Fermentation profiles

Anaerobic storage of cattle manure with wheat straw addition only (Control) did not promote a wide biomass acidification, Table 4-4. In the first 7 days of storage, pH of control still decreased to 7.1. Then, it constantly increased until the end of storage period, reaching a value of 8.4 after 4 months. This can be explained by the evolution of VFA fraction along ensiling duration. Indeed, there was a slight VFA accumulation in cattle manure during the first days of storage, mainly due to the short production of butyric acid. In contrast, the acetate initially present in fresh cattle manure was almost totally lost during the first month of storage. This evidences that the methanogen activity was already present at the beginning of storage and became predominant somewhere between 7 and 15 days of storage, which impeded biomass stabilization. This was confirmed by one-off measures of the composition of the gas produced during these experiments (results not shown): CH_4/CO_2 ratio was already 0.26 after 1 day and 0.61 after 10 days of storage. In the first place, this can be related to the premise that cattle manure was not totally fresh, which may have favored the growth of methanogenic bacteria. In like manner, it is possible that methanogens concentration in raw material was more important than in the cattle manure with wheat straw used by Teixeira Franco *et al.* [99] (which was efficiently acidified during ensiling). In fact, it is known that bacterial populations can be influenced by the characteristics of manure [97] which, according to Marañón *et al.* [98], may depend on the type of cattle, animal's diet, as well as, on the time of the year. In any case, silage stabilization was not achieved for control assay and only trace concentrations of organic acids were detected after 4 months of storage.

Coupled with that, structural carbohydrates content regularly decreased for control silage. After 4 months of storage, hemicellulose and cellulose concentrations were 14.6 and 18.7% of original VS,

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respectively. This represented a degradation of around 50% of total original hemicellulosic and cellulosic compounds. Since no accumulation of WSC nor VFA was recorded during prolonged periods for the control tests, major organic and energy losses should have occurred. Regarding organic matter, losses ascend to 14.8% after 30 days and 42.0% at the end of the storage. An average of 0.35% of organic matter was lost per day during this period.

Co-ensiling with formic acid improved conservation of cattle manure, especially for short periods of storage. During the first 30 days, formic acid was fully conserved and pH persisted below 4.0. In parallel, VFA content increased, which was supposed to be partially caused by lactic acid production. This should be a result of acid hydrolysis of hemicelluloses by the existing organic acids in silage [12,86]. As a matter of fact, hemicellulose content of formic acid silage decreased to 29.1%$VS_{original}$ after 30 days, while cellulose fraction was stable. However, between 30 and 120 days of ensiling the fermentation profiles reversed and both formic and lactic acids were consumed. At the same time, other organic acids were produced, notably acetate and butyrate. Consequently, VFA content decreased from 11.9%VS_{added} after 30 days to 9.1%VS_{added} at the end of storage. Due to that and to the fact that the VFA produced were weaker acids than formate and lactate, the pH value increased to 6.9 after 4 months of ensiling. One hypothesis that can be suggested for the fermentation shift is that the acid pH of silage was only delaying the growth of methanogens. This would explain the slow pH increase in the first month of storage. Then, as pH increased, microbial growth became important and formic acid was further degraded into biogas, which also had an impact on the pH. Consequently, lactic acid must have be converted, *e.g.*, into butyric acid by clostridial fermentation, which is a typical mechanism in poor quality silage [12]. Furthermore, the increased pH should have allowed the enzymatic hydrolysis of structural polymers for prolonged silage periods [86]; and the subsequent fermentation into VFA. This is corroborated by the accentuated decline of structural carbohydrates that was verified in the last 90 days of storage. Indeed, after 4 months of ensiling, hemicellulose and cellulose contents respectively decreased to 23.2 and 29.6% of original VS. Finally, the two different phases of fermentation had an impact on the conservation of organic matter. After 1 month, VS losses were lower than 1%, which ascended to 13.4% at the end of the storage.

Co-ensiling of cattle manure with glucose underwent a strong acidification in the early days of storage. Indeed, pH value of silage was 4.0 for both Glucose 4% and Glucose 10% conditions after 7 days, which was due to the conversion of WSC into lactic acid (Table 4-4). This shows that the concentration of LAB in the original raw material was significant. Consequently, the lack of WSC probably blocked the conservation of cattle manure through the ensiling process. Silage acidification slowed down LAB activity but it was not completely stopped. For this reason, WSC continued to be consumed during the remaining ensiling period. After 15 days of storage, lactic acid concentration was around 15%VS_{added} for both co-silages with glucose addition. After this period, two distinct fermentation pathways were observed on the co-silages experiments with glucose. On the one hand, for Glucose 10% condition,

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WSC was in large excess and lactic fermentation continued until the end of the storage. As a result, after 4 months, pH value of Glucose 10% silage was 3.7 and its lactate concentration rose to nearly 20%VS_{added}. On the other hand, WSC of Glucose 4% assay was extinguished between 15 and 30 days of ensiling. Coupled with that, there was a degradation of lactic acid and a significant increase of butyric acid content during this period. This suggests that the acid pH value (4.1) was unable to avoid clostridial activity since there was no more available substrate for lactate production. This clostridial proliferation led to a secondary fermentation that was mainly based on lactic acid consumption for the production of butyrate, carbon dioxide and hydrogen [12]. Between 30 and 120 days, clostridial fermentation led to full degradation of lactic acid on Glucose 4% silage. Besides butyric acid, other VFA were found, such as caproic and valeric acids (results not shown). Moreover, after 4 months of storage, the pH value of Glucose 4% increased to 5.4. This is explained by the fact that butyrate is a weaker acid that lactate and because this reactional mechanism involves the consumption of two moles of lactic acid for the production of one mole of butyric acid.

Additionally, co-ensiling of cattle manure with glucose enhanced preservation of hemicellulosic and cellulosic compounds. Regarding Glucose 4% silage, degradation of structural carbohydrates was not significant. For Glucose 10%, around 27% of the sum of initial hemicellulose and cellulose fractions were missing. This degradation should be linked with an acid hydrolysis by the VFA produced during ensiling. Nevertheless, this should not have led to energy losses since biomass was extremely well conserved under acid conditions. Consequently, VS losses were limited to 9.0% and 4.6% after 4 months for Glucose 4% and Glucose 10% assays, respectively. The poorer conservation of organic matter for Glucose 4% silage should be connected to the secondary fermentation that was observed for this condition.

4.3.3.2.2. Chemical oxygen demand balance

Effects of ensiling and co-substrates on the COD of crude material and its water-soluble and particulate fractions are exposed in Figure 4-6. The evolution along ensiling time is related to $VS_{original}$ in order to account for biomass weight loss in the COD balance.

The most significant results were obtained for the ensiling of cattle manure without additives. Before storage, around 93% of the total COD was in the particulate phase. This is linked to earlier statements, evidencing that in cattle manure the majority of the energy sources were hardly bio-accessible. Second, there was a clear decline of particulate COD during the experiments, which is explained by the loss of structural carbohydrates. Furthermore, no accumulation of their degradation products in the water-soluble COD was observed. Thus, global COD in the control (without additives) decreased with ensiling time, which surely led to large methane potential losses.

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The use of co-substrates for the ensiling of cattle manure improved the conservation of COD. For this reason, the fluctuations of its composition along duration were minimal, which made it difficult to establish long-term trends in co-ensiling. Regarding formic acid assays, despite the important VS losses recorded for prolonged durations, COD of crude material was stable until 120 days of storage. This can be in part explained by the fact that formic acid has low COD content (0.35 g_{O2}/g), so that its degradation did not cause significant COD decrease. Furthermore, there was an important solubilization of particulate COD in the last 3 months of ensiling. This should correspond to the hydrolysis of structural carbohydrates and subsequent conversion into VFA, as it was seen for the fermentation profiles (subsection 4.3.3.2.1). In addition to the solubilization effect and the potential kinetic gain in AD conditions, this may have caused an amplification of carbohydrates accessibility.

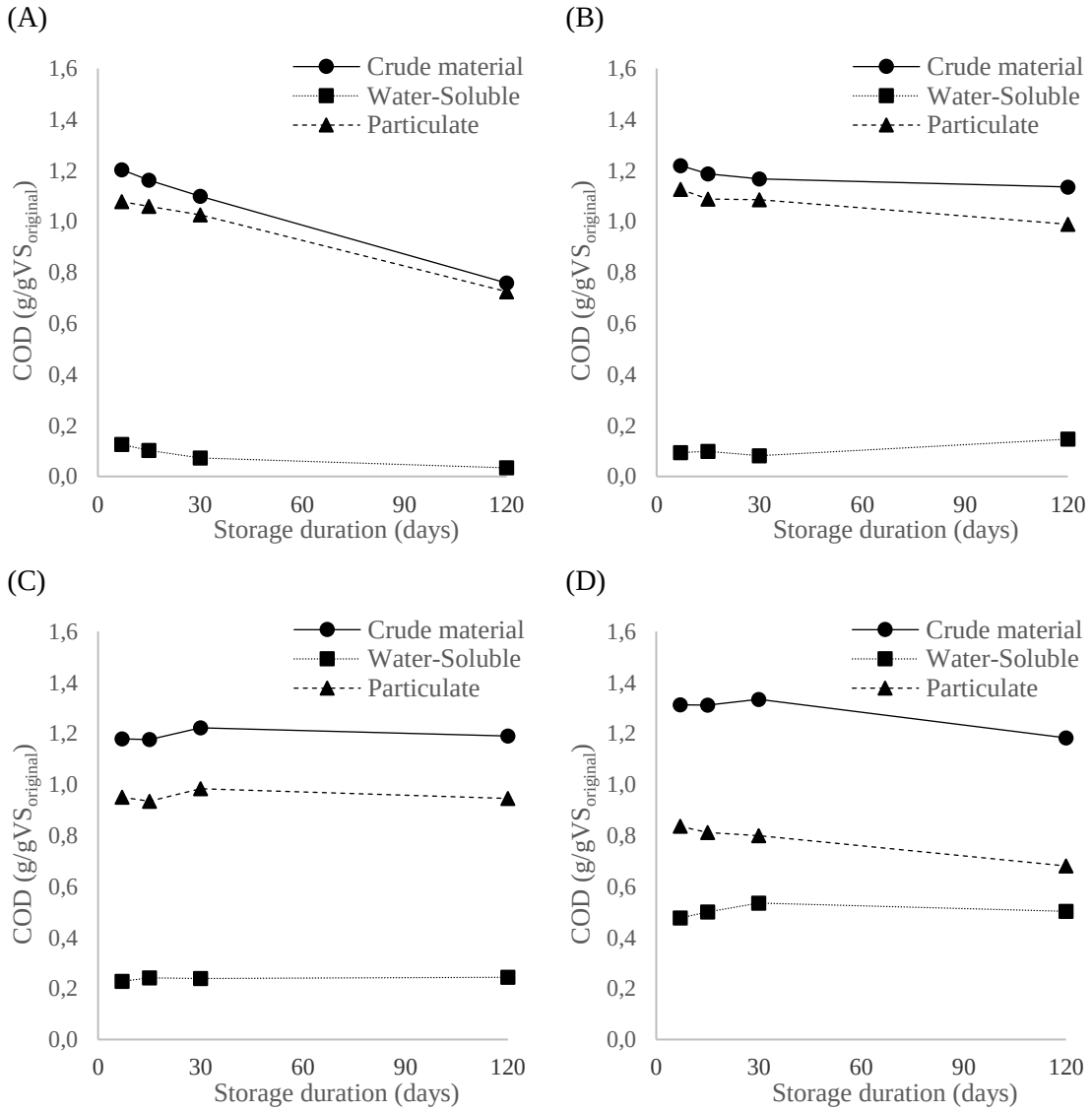

Figure 4-6 – Evolution of COD related to $VS_{original}$ along ensiling period for: (A) Control; (B) Formic acid; (C) Glucose 4%; (D) Glucose 10%. Feedstocks were not analyzed.

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Ensiling of cattle manure with glucose addition had little impact on COD conservation (Figure 4-6). For Glucose 4%, both particulate and water-soluble fractions of COD were stable until the end of the 4 months. This was expected for the particulate phase, since fibers content did not suffer any significant degradation during storage. However, a degradation of soluble COD should have been recorded between 30 and 120 days of storage, since clostridial fermentation led to hydrogen gas formation. The reasons for this inconsistency remain unclear. Concerning Glucose 10% condition, there was a persistent solubilization of particulate COD along the experiment. This can be attributed to the acid hydrolysis of hemicelluloses by the VFA produced during ensiling.

4.3.3.2.3. Total Kjeldahl nitrogen balance

Anaerobic storage of cattle manure with straw (control) led to constant TKN loss during the experiments, Figure 4-7. This can be explained through the analysis of TKN fractions along the storage period. On the one hand, there was a decrease of organic nitrogen in both particulate and water-soluble phases after 4 months of about 12% and 39%, respectively. This loss represented 19% of the total organic nitrogen. This suggests that nitrogen mineralization occurred through two successive mechanisms typically found in anaerobic conditions [12,95]: hydrolysis of proteins into amino acids and subsequent fermentation into ammonia nitrogen. Therefore, NH_3-N concentration should increase under such conditions. However, for control assays it was observed that ammonia content of cattle manure declined with storage time. This can be explained by the fact that pH interruptedly increased to values near the pKa (9.25) of NH_4^+/NH_3. Therefore, although ammonia was produced, its chemical equilibrium led to ammonia losses through gas emissions. After 4 months, around 23% of original TKN was lost for control experiment. If we hypothesize that the TKN was lost by ammonia emissions, around $8.2L_{NH3}/kgVS_{original}$ were expected to be released into the atmosphere during the 4 months of control experiments.

Regarding the formic acid tests, 11% of particulate TKN content was degraded in the 4 months of storage. This may be linked with acid hydrolysis in the first 30 days; and due to the proteolytic clostridial activity (for higher pH values) in the last 3 months of ensiling. Nevertheless, since pH was always below 7.0, the mineralized nitrogen was partially conserved as water-soluble ammonia. At the end of formic acid assays, less than 10% of original TKN was lost into the atmosphere.

For both conditions of cattle manure with glucose addition, no decrease of TKN was recorded until 4 months of ensiling. Yet, as for fermentation profiles, TKN composition of the two glucose experiments evolved differently during storage. In the case of Glucose 10%, there was no degradation of particular TKN and NH_3-N was constant. This shows that silage was extremely stable with glucose addition and that no secondary fermentation occurred. For Glucose 4%, the same trend was observed in the early days of ensiling. However, in the last 3 months there was a decrease of around 18% concerning the particulate TKN and an increase of around 39% for the soluble TKN. This indicates that proliferation

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of proteolytic clostridia occurred for prolonged storage duration. Hence, the amount of co-substrate used in Glucose 4% was not enough to fully stabilize long-term silage of cattle manure with straw.

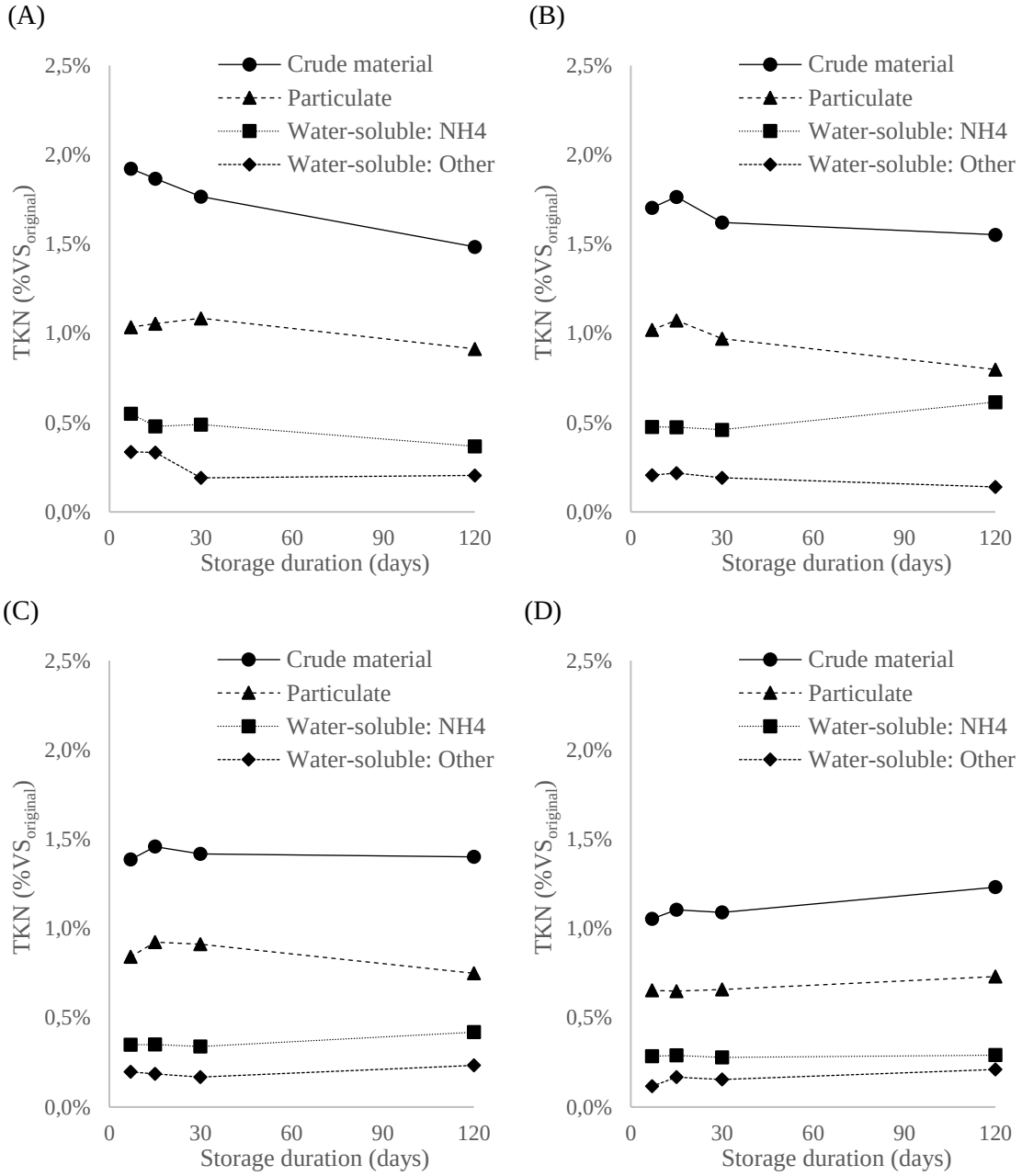

Figure 4-7 – Evolution of TKN related to VSoriginal along ensiling period for: (A) Control; (B) Formic acid; (C) Glucose 4%; (D) Glucose 10%. Feedstocks were not analyzed.

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4.3.3.3. Effects of storage method on methane potential

Control silage showed BMP values of 128-229 L/kgVS$_{added}$ and 75-229 L/kgVS$_{original}$, Figure 4-8. In the first 7 days of control assays, both methane potential based on VS$_{added}$ and VS$_{original}$ were stable. This evidences that neither the energy content nor the biochemical accessibility of cattle manure with straw changed during short-term ensiling, which should be linked with the small fermentation activity and pH decrease that occurred in this period. However, for longer storage durations, the BMP of control was significantly damaged. Indeed, after 30 and 120 days of ensiling, 24% and 67% of original BMP was lost, respectively. This obviously was a consequence of the extensive degradation of structural carbohydrates (without any VFA accumulation), due to the high pH levels.

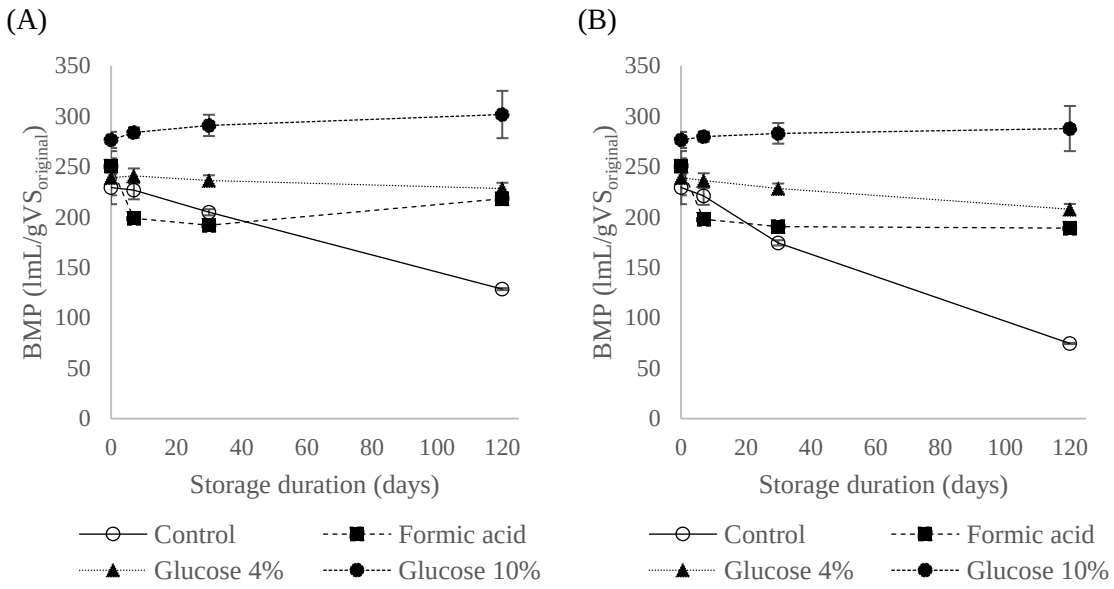

Figure 4-8 - BMP evolution of crude material over storage duration. (A): methane potential based on VS$_{added}$; (B): methane potential based on VS$_{original}$, therefore considering storage losses. BMP for 15 days of ensiling was not determined.

Co-ensiling with formic acid limited the harmful impact of storage on the methane potential of the raw material. In this case, BMP values were 192-250 L/kgVS$_{added}$ and 189-250 L/kgVS$_{original}$ during the 4 months. The major variation of methane potential for formic acid silage occurred in the first 7 days of storage. In this period, around 21% of both BMP related on VS$_{added}$ and VS$_{original}$ was lost. As previously discussed for formic acid assays, organic matter losses were negligible and no noticeable modification of chemical characteristics was observed during this first week. Therefore, this indicates that formic acid addition had an impact on the biodegradability of the biomass in the first days of storage. After that, BMP based on VS$_{original}$ remained constant and global energy losses were limited to 25% at the end of the 4 months. Yet, BMP related to VS$_{added}$ increased by 14% in the last 3 months of storage. Two conclusions can be deduced from these results. First, there was an increment of biochemical accessibility, which can be attributed to the production of VFA from cell wall constituents. In fact,

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structural carbohydrates are not fully biodegradable in mesophilic AD conditions [96]. Therefore, their hydrolysis and fermentation during ensiling may explain the BMP increase in a VS_{added} basis. Second, this gain of accessibility countered the formic acid loss in the last 90 days, so that BMP related to $VS_{original}$ persisted unaltered until the end of the storage.

Higher stability of methane potential was achieved through the co-ensiling of cattle manure with glucose. Indeed, Glucose 4% silage presented BMP values of 228-241 L/kgVS_{added} and 208-238 L/kg$VS_{original}$ along the 4 months of storage. In parallel, for Glucose 10% condition, BMP values were 276-302 L/kgVS_{added} and 276-288 L/kg$VS_{original}$. During the first month of storage, both experiments with glucose had constant methane potential. These results are in line with the data from the fermentation profiles and illustrate the efficiency of lactate production on the energy conservation during ensiling. Concerning Glucose 10% assay, this trend persisted and no loss of BMP was observed until the end of the 4 months. However, an eventual positive impact of hemicellulose hydrolysis and fermentation on the methane potential was not significant inside the precision limits. In contrast, in the remaining 90 days of ensiling, BMP of Glucose 4% progressed differently. For this condition, 13% of the methane potential related to $VS_{original}$ was lost after 4 months. This BMP damage can be explained by the secondary fermentation that led to lactic acid degradation and nitrogen mineralization.

4.3.4. Conclusions

Ensiling of cattle manure with straw at 19%TS led to extensive methane potential losses. The use of co-substrates enhanced biomass and energy conservation during ensiling. In particular, co-ensiling with glucose (100 g/kg of feedstock) encouraged an extensive lactate production. This allowed biomass stabilization, suppressed ammonia emissions and led to full preservation of methane potential after 4 months. Therefore, co-ensiling with a high sugar content substrate appears be the most resourceful method to optimize cattle manure preservation before biogas production. At full scale application this should include a starch-rich residue, or any other sugar-rich agro product.

4.3.5. Acknowledgements

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4.4. Effects of storage conditions, total solids content and silage additives on fermentation profiles and methane preservation of cattle manure before anaerobic digestion

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Abstract

The impacts of storage conditions, total solids content and silage additives were investigated at laboratory scale for cattle manure during up to 4 months. Wheat straw, glucose and starch were used as model molecules of ensiling co-substrates. Single-handedly cattle manure lost 37% of its original methane potential after 4 months of anaerobic storage. This was essentially caused by the low total solids content and by the absence of water soluble carbohydrates in the feedstock. Wheat straw addition decreased moisture content and enabled cattle manure stabilization for a pH of 6.0. In contrast, extensive heterofermentative lactic acid bacteria fermentation occurred during co-ensiling with glucose and the pH quickly dropped to values below 4.0. Starch was used as substrate for fermentation but its hydrolysis limited bacterial activity and acidification. Finally, all the three co-substrates led to minimal methane potential losses after 4 months of storage. Co-ensiling will improve the energy efficiency of agricultural biogas plants that use stored cattle manure as feedstock.

Keywords*: Cattle manure; Additives; Storage; Ensiling; Anaerobic digestion; Methane potential*

4.4.1. Introduction

The growth of the agricultural biogas sector in France is promoting the energy valorization of livestock manure. According to the French Environment and Energy Management Agency, cattle manure potential for biogas production in France may reach around 183 Mt/year by 2030, which could represent an annual energy potential of 40 500 GWh [102].

Despite this expected use as a source of energy, cattle manure is still typically treated as a waste. Indeed, biogas plant operators take very few precautions during its handling and management. In fact, like other

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agricultural wastes or crops, cattle manure is not produced at constant rates all year round. Therefore, in order to ensure a constant supply of raw material for anaerobic digestion (AD) plants, cattle manure must be stored before use, from few days up to several weeks. The use of open-air storage facilities is the most common practice for this type of feedstock. On the one hand, these are simple and low-cost installations. Yet, the contact with air will lead to odor and ammonia emissions [93], biomass damage and energy losses. As reported in our previous work [99], losses of methane potential for cattle manure may ascend to more than 70% after 4 months in presence of air, which can be mitigated through ensiling or confined storage.

Ensiling is a preservation process that depends on the natural fermentation of biomass under hermetically sealed conditions to minimize the contact with air. It can be divided in four successive stages [12,85]. First, aerobic biomass respiration occurs during several hours, converting water-soluble carbohydrates (WSC) in carbon dioxide, water and releasing energy. Once oxygen is depleted, microorganisms capable of anaerobic growth will compete for the substrate. This is the critical stage of ensiling since it depends on the nature and on the biochemical characteristics of the raw material, which may not be easily predicted or controlled. If conditions are suitable, lactic acid bacteria (LAB), naturally present in wet biomass like manure [103], will produce lactate from available WSC, decreasing the pH value to around 4.0. The combination of an anaerobic and acid environment will stabilize the biomass and impede further substantial microbial activity until the feed-out. After unloading the silo, aerobic microorganism are reactivated, which may spoil the silage if prompt AD feeding is not carried out. This storage method is considered to be the most efficient way to preserve wet biomass [12] and leads to full energy conservation for prolonged durations [11,14,21,30,91,92]. However, substantial losses will occur during ensiling of inadequate feedstocks, such as cattle manure. Indeed, we showed on a former work [99] that 46% of fresh cattle manure's biochemical methane potential (BMP) may be lost after 4 months of anaerobic storage. We suggested that the lack of WSC and high moisture content were the causes of ensiling failure.

Co-ensiling of cattle manure with other substrates has been a promising approach to limit the energy loss during storage. According to Teixeira Franco *et al*. [99], wheat straw addition to fresh manure stimulated a conservation of more than 86% of BMP after 4 months of ensiling. Mechanisms related to the increase of total solids (TS) content and reduction of neutral buffering capacity were proposed to explain this improvement. However, wheat straw addition may not be an asset for the preservation of all types of manure. In another of our studies [103], more than 60% of BMP was lost after long-term co-ensiling of manure with straw. We suggested that this was caused by a substantial methanogen activity of cattle manure before storage. In this latter work, the most impressive results were obtained for the use of direct and indirect sources of acidification. In particular, co-ensiling of fresh cattle manure with addition of both wheat straw and high concentration of glucose led to full conservation of methane potential even after 4 months.

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In the present work, the objective was to deepen the knowledge about the best co-storage practices of cattle manure before AD. The effects of ensiling, total solids content and silage additives were evaluated through storage assays at laboratory scale with cattle manure and several co-substrates for up to 120 days. For the impact of TS content, wheat straw was added to the raw material. Moreover, glucose and starch were used as model molecules of fermentation stimulants for the co-ensiling assays. The innovative experimental approach of this study enabled the monitoring of fermentation profiles, fibers structure and conservation of both organic matter and methane potential during several stages of ensiling for all conditions. Finally, this work addresses discussion and conclusions for the improvement of the energy efficiency of agricultural biogas plants that use stored cattle manure as feedstock.

4.4.2. Material and methods

4.4.2.1. Feedstock and storage approach

Fresh cattle manure was collected on October 24th, 2016 from an agricultural site in the Rhône-Alpes region of France (Gaec Béreyziat, Les Teppes, 01340 Béréziat, France) and it was stored at 4 °C before further use. Besides storage of single-handedly cattle manure (CM12%), co-ensiling assays were performed with several co-substrates: wheat straw (10 cm maximum length), anhydrous glucose (D-(+)-glucose for analysis, Chimie-Plus, France) and starch (starch soluble pure, Chimie-Plus, France). Glucose was mixed with water and added as solution to cattle manure. This allowed an approximately constant TS content of feedstock before and after glucose addition. Starch was added as powder due to its very low solubility in water. The concentration of co-substrates was chosen taking into account our previous study concerning the optimization of cattle manure storage [103]. The following treatments were applied to cattle manure before silo filling and starting co-ensiling:

- *CM16%*: 70 g of wheat straw per kg of CM12%;
- *CM21%*: 153 g of wheat straw per kg of CM12%;
- *CM12%+G*: 100 g of glucose per kg of CM12%;
- *CM16%+G*: 100 g of glucose per kg of CM16%;
- *CM21%+G*: 100 g of glucose per kg of CM21%;
- *CM16%+S*: 53 g of starch per kg of CM16%.

Storage conditions are summarized in Table 4-5. All feedstocks were submitted for 30 and 120 days of storage. Additionally, storage durations of 7 and 15 days were tested for co-ensiling assays with either glucose or starch. These extra storage periods were done in order to follow the LAB fermentation that was expected in the early days of ensiling with these latter co-substrates. A total of 22 ensiling assays were performed according to the storage approach exposed in our previously published work [91].

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Table 4-5 - Ensiling conditions and treatments applied to cattle manure.

Condition	Duration (days)	Co-substrate			TS (%) [b]	VS (%) [b]
		Wheat straw [a]	Glucose [a]	Starch [a]		
CM12%	30; 120	-	-	-	11.6 ± 0.1	9.50 ± 0.12
CM16%	30; 120	7.0%	-	-	16.3 ± 0.4	14.2 ± 0.4
CM21%	30; 120	15.3%	-	-	20.9 ± 1.2	18.6 ± 1.2
CM12%+G	7; 15; 30; 120	-	10.0%	-	10.3 ± 0.3	9.04 ± 0.21
CM16%+G	7; 15; 30; 120	7.0%	10.0%	-	15.3 ± 0.3	14.0 ± 0.3
CM21%+G	7; 15; 30; 120	15.3%	10.0%	-	19.8 ± 0.5	18.2 ± 0.4
CM16%+S	7; 15; 30; 120	7.0%	-	5.3%	20.4 ± 0.1	18.4 ± 0.1

Results presented as wt% of: [a] cattle manure; [b] feedstock

4.4.2.2. Chemical analysis

The chemical analysis procedure of Teixeira Franco *et al.* [91] was used with the following modifications. Ethanol and acetic, propionic, butyric, valeric and caproic acids content were analyzed using gas chromatography (Shimadzu Corp.) equipped with a HP-FFAP fused silica capillary column (30 m x 0.25 mm, Agilent Technologies), a flame ionization detector and using H_2 as carrier gas. BMP tests were also performed for water-soluble phase samples using glass vessels of 0.1 L. The inoculum for BMP tests had 2.0-3.0% of TS content and 1.4-2.2% volatile solids (VS) content. In order to assess the kinetics of methane production, the experimental data of BMP assays were fitted to the following equation, since the methane production always followed a 1st-order kinetics:

$$V_{CH_4}(t) = V_{max}(1 - e^{-kt}) \tag{4.2}$$

Where, V_{CH_4} corresponds to the net volume of methane produced (total volume minus volume produced by the blank); t is the time of the assay; V_{max} is the maximum volume of methane produced, which was equivalent to the experimental BMP value and; k is the rate coefficient of CH_4 production. Since gas production started almost immediately after reactors were sealed, lag time was not considered in the calculations.

4.4.3. Results and discussion

4.4.3.1. Feedstock characterization

The data presented in Table 4-5 shows that fresh cattle manure had a quite low TS content (11.6%). This was inadequate for storage due to technical and biological reasons. First, it should cause difficulties in filling and compacting a field-scale silo due to liquid seepage. Second, low TS content leads to excessive bacterial activity in anaerobic conditions and consequently to high potential damage of biomass. The

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major impact of wheat straw addition was the increase of TS/VS content. Indeed, TS content of feedstock was 16.3% and 20.9% after addition of wheat straw in two distinct amounts. Regarding feedstocks for glucose trials, the addition of co-substrate in solution kept the organic matter content stable before ensiling. On the opposite, starch was used in the form of pure compound and so, TS content increased from 16.3% to 20.4% after adding the co-substrate.

Furthermore, fresh cattle manure did not have any detectable content of WSC, Table 4-6. This was an undesired feature, since WSC are the most accessible substrates for acidification purposes during ensiling. Only raw materials with glucose addition had a significant initial sugars content, which varied between 35% and 53%VS. These latter WSC concentrations were significantly higher than the ones found in the literature for feedstocks that were intensely acidified during ensiling [16,22,28,30,100,101]. Therefore, there will be an extensive production of organic acids for co-silages with glucose addition if LAB population is present in substantial amount before storage. Concerning CM16%+S condition, no WSC was observed in the initial sample, since starch is not a water-soluble molecule. However, taking into account the amount of co-substrate used in the preparation of CM16%+S, the theoretical content of starch in this feedstock should be around 27%VS. Considering that a wide breakdown of the glycosidic bonds of this polysaccharide and its subsequent fermentation occur in the early days of storage, the amount of starch used should be enough to ensure a proper biomass acidification. Regarding co-ensiling with only wheat straw, there was no noticeable increase on the initial WSC content. This is explained by the fact that energy sources in wheat straw are mostly concentrated in the cell wall constituents, which is confirmed by the increase of the structural carbohydrates content in CM16% and CM21% feedstock, Table 4-7.

The use of co-substrates did not have a major impact on the pH of raw materials, which ranged from 7.4 to 8.1. However, these pH values were quite higher than the ones of traditionally ensiled crops, such as maize or herbage. For instance, pH values of 5.5-5.8 can be found in the literature for fresh maize [15,20]. Likewise, Neal and Thornley [73] assumed that the pH of herbage is 6.0 before storage. Notwithstanding, there was already an important amount of volatile fatty acids (VFA) present in fresh cattle manure (5.0%VS). This suggests that fresh cattle manure had strong basic buffering components. Moreover, higher production of organic acids should occur during storage of cattle manure in order to reach the same level of acidity of a typical ensiled crop.

Finally, total Kjeldahl nitrogen (TKN) content decreased with the addition of co-substrates. This reflects the absence of nitrogen compounds in the silage additives. Therefore, nitrogen structure was identical for all conditions. Moreover, it is important to notice that ammonia nitrogen (NH_3-N) content was quite high in feedstocks (15-22%TKN). This might result in substantial ammonia emissions if pH moves toward alkalinity during the storage period [99,103].

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Table 4-6 – Fermentation profiles of biomass over ensiling duration (%$VS_{added/original}$ unless otherwise specified).

Condition	Duration (days)	pH	WSC[a]	LA[a]	AA[a]	BA[a]	VFA[a]	Ethanol[a]	NH_3-N (%TKN)	TKN[b]
CM12%	0	7.7	-	-	3.6	0.2	5.0	-	20.8	3.2
	30	6.1	0.1	-	9.9	1.5	14.1	-	37.7	3.4
	120	7.8	0.2	-	4.5	0.5	8.3	-	41.7	3.0
CM16%	0	8.1	-	-	1.9	-	2.5	-	21.5	1.9
	30	5.9	-	-	6.3	1.0	9.0	-	30.3	2.3
	120	5.9	0.1	-	7.1	1.7	11.7	-	33.8	2.4
CM21%	0	8.1	-	-	1.4	-	1.8	-	14.8	1.3
	30	6.0	-	-	4.6	1.0	6.7	-	28.2	1.8
	120	6.0	0.1	-	5.6	1.5	9.3	-	33.2	1.7
CM12%G	0	7.4	52.6	0.1	2.4	-	2.9	-	20.7	1.6
	7	3.8	25.6	17.1	3.0	-	20.7	4.9	18.7	1.6
	15	3.6	-	23.7	3.3	0.3	27.8	13.9	18.3	1.5
	30	3.6	0.1	24.2	3.8	-	28.0	16.6	18.8	1.5
	120	3.8	0.2	17.4	6.9	0.4	25.8	16.6	19.7	1.5
CM16%G	0	7.6	40.5	-	1.8	-	1.8	-	21.1	1.1
	7	4.4	9.5	13.9	2.1	-	16.5	4.6	17.6	1.2
	15	3.7	0.7	17.5	2.2	0.2	20.3	10.0	16.9	1.2
	30	3.6	0.1	18.4	2.5	-	20.8	11.4	16.4	1.2
	120	3.6	0.1	16.4	3.3	-	20.2	11.8	15.9	1.3
CM21%G	0	7.6	35.6	-	1.5	-	1.6	-	20.1	0.9
	7	4.6	9.2	12.9	1.7	-	14.9	5.8	17.1	1.0
	15	3.8	-	13.2	1.7	0.6	15.7	9.5	15.7	1.1
	30	3.9	0.1	13.0	-	-	13.0	10.2	16.8	1.1
	120	5.0	-	-	2.0	5.8	11.2	10.3	22.6	1.1
CM16%S	0	7.9	-	-	1.6	-	2.1	-	15.8	1.6
	7	5.5	-	1.3	3.8	2.1	8.2	0.3	14.8	1.7
	15	5.1	-	-	3.7	3.0	7.9	0.3	16.7	1.7
	30	5.6	0.1	-	4.9	4.6	11.6	-	18.5	1.6
	120	4.8	0.1	-	5.5	5.0	13.9	0.4	21.3	1.6

WSC stands for Water Soluble Carbohydrates, LA for Lactic Acid, AA for Acetic Acid, BA for Butyric Acid, VFA for Volatile Fatty Acids, NH_3-N for Ammonia Nitrogen and TKN for Total Kjeldahl Nitrogen; [a] results based on %VS_{added}; [b] results based on %$VS_{original}$

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Table 4-7 – Impact of ensiling period and co-substrates on fibers composition of cattle manure (%$VS_{original}$).

Condition	Duration (days)	Hemicellulose	Cellulose	Lignin
CM12%	0	28.4 ± 1.1	25.7 ± 1.4	8.5 ± 0.3
	30	21.7 ± 0.6	19.3 ± 1.8	8.9 ± 0.7
	120	16.6 ± 0.5	15.9 ± 1.2	7.9 ± 0.5
CM16%	0	31.1 ± 0.5	36.8 ± 1.5	7.4 ± 0.19
	30	25.5 ± 1.3	26.3 ± 2.6	9.5 ± 0.5
	120	25.0 ± 0.19	22.5 ± 0.20	10.1 ± 0.07
CM21%	0	35.7 ± 0.8	42.6 ± 1.5	8.4 ± 0.15
	30	29.9 ± 0.9	34.2 ± 2.0	8.2 ± 0.24
	120	27.6 ± 0.3	27.6 ± 7.6	9.7 ± 2.6
CM12%G	0	13.7 ± 0.4	13.5 ± 0.8	3.5 ± 0.16
	7	12.5 ± 1.2	12.2 ± 2.0	3.2 ± 0.23
	15	13.0 ± 0.22	13.0 ± 0.5	3.0 ± 0.10
	30	11.7 ± 0.3	12.3 ± 0.4	2.9 ± 0.03
	120	11.6 ± 0.09	11.5 ± 2.5	3.7 ± 0.8
CM16%G	0	18.5 ± 0.5	22.0 ± 0.6	4.4 ± 0.09
	7	16.2 ± 0.24	18.8 ± 1.0	3.7 ± 0.16
	15	17.1 ± 0.4	20.1 ± 0.6	4.2 ± 0.10
	30	17.1 ± 0.4	18.8 ± 1.1	4.3 ± 0.19
	120	16.2 ± 0.3	15.0 ± 1.5	6.7 ± 0.6
CM21%G	0	21.6 ± 0.20	25.5 ± 2.0	4.8 ± 0.3
	7	19.8 ± 0.5	22.4 ± 1.4	4.5 ± 0.24
	15	20.7 ± 0.5	21.7 ± 1.4	6.1 ± 0.3
	30	21.7 ± 0.4	24.3 ± 1.2	5.0 ± 0.21
	120	21.4 ± 0.4	20.7 ± 1.6	7.0 ± 0.13
CM16%S	0	28.0 ± 0.8	32.8 ± 3.2	9.2 ± 0.9
	7	23.0 ± 0.4	23.0 ± 0.3	7.3 ± 0.06
	15	23.6 ± 0.8	23.6 ± 2.9	8.0 ± 0.7
	30	24.8 ± 1.0	23.8 ± 1.8	5.6 ± 0.24
	120	24.2 ± 0.21	22.9 ± 1.4	4.8 ± 0.3

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4.4.3.2. Effects of storage method on biomass preservation

4.4.3.2.1. Fermentation profiles

Fresh cattle manure was acidified during the first month of anaerobic storage (CM12%). Indeed, pH of silage decreased from 7.7 to 6.1 during this period. This was due to accumulation of VFA in biomass, which reach around 14%VS_{added} after one month. However, fermentation profiles of CM12% shifted for longer storage periods: pH value increased to 7.8 and organic acids concentration decreased to 8.3%VS_{added} after 4 months. Considering storage losses, this represented a VFA degradation of around 53% in the last 3 months. The origin of these two distinct fermentation profiles can be attributed to the poor characteristics of fresh cattle manure, notably the lack of WSC and the low TS content. In other words, without WSC in raw material, the degrading bacteria must access the particulate carbohydrates in order to produce VFA. Consequently, acidification will be slower and lactic acid may only be an intermediate in the production of other mixed organic acids. This hypothesis is been implemented in anaerobic digestion models due to the fast biodegradation of lactate in neutral pH values [95]. In our experiments with single-handedly cattle manure, the major component of VFA was acetic acid, with other small fractions of butyric, propionic, valeric and caproic acid. Since these fermentation products are weaker acids than lactic acid, pH drop for single-handedly cattle manure was not as marked as in lactate silages. Thereupon, due to the relatively high pH and water availability, the methanogenic population must have proliferated, leading to VFA consumption in the last months of anaerobic storage. This must have caused severe weight and energy losses for CM12%.

Wheat straw addition to cattle manure led to pH stabilization in the long term. Concerning CM16%, pH decreased from 8.1 to 5.9 in the first 30 days and did not varied until the end of the storage. For CM21%, pH was around 6.0 in the last 3 months of ensiling. This was a result of organic acids accumulation during the 4 months of conservation. Indeed, VFA content in co-silages with wheat straw was 6.7-9.0%VS_{added} after 30 days and 9.3-11.7%VS_{added} at the end of the storage. Stabilization of biomass should be linked to the wheat straw impact on water availability. In fact, it is known that at least part of silage bacteria have lower activity for higher TS contents [12,73,74]. Therefore, it is likely that TS increase caused by wheat straw addition was enough to prevent methanogenic activity for a pH level around 6.0. This is in agreement with the results obtained in our previous work [99], concerning silage stabilization of cattle manure with wheat straw while experiencing a wide organic acid production.

Furthermore, as in stored fresh cattle manure, VFA mix in CM16% and CM21% was mainly composed of acetic and butyric acids. In anaerobic processes, the production of these VFA includes other side-products, such as H_2 [95]. Since hydrogen gas escapes into the environment after silo feed-out, its production during ensiling leads to energy losses. Therefore, even considering that all the degraded carbohydrates were conserved in the form of VFA, some energy loss should have occurred for CM16% and CM21% silages.

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Unlike the previous storage conditions, glucose addition to cattle manure encouraged a strong biomass acidification in the early days of ensiling. Indeed, after 7 days of storage, pH was 3.8, 4.4 and 4.6 for CM12%G, CM16%G and CM21%G, respectively. This was a result of an abundant lactic acid production, which represented already 12.9-17.1%VS_{added} after 7 days of co-ensiling. After that, lactic acid production and pH drop continued until full consumption of WSC. At the end of first months, pH was 3.6-3.8 for co-ensiling trials with glucose addition. This demonstrates that LAB was present in great extent in the raw material and that it was the lack of substrate that hindered silage acidification for single-handedly cattle manure. Moreover, the fact that lactate production was higher for low TS contents evidenced that LAB also had a quite high sensitivity to water availability in biomass. In the last 3 months of storage, minimal pH modifications occurred for CM12%G and CM16%G. In opposite, pH of CM21%G increased to 5.0 after 120 days, concomitantly with the full lactate consumption and butyrate production. This should be a result of a clostridial fermentation, which is a reactional mechanism that occurs in poor quality silages. However, this was unexpected since clostridial activity should be delayed with the decrease of water availability [12,40].

Besides lactic acid, ethanol was also found in substantial concentrations for co-ensiling with glucose: 4.6-5.8%VS_{added} after 7 days, 10.2-16.6%VS_{added} after 30 days. This type of alcoholic fermentation was not recorded in our former study with co-ensiling of cattle manure with glucose [103]. In fact, under ensiling conditions, LAB can ferment a wide range of substrates by several pathways [12]. Using glucose as substrate, LAB can be labeled in two main groups: homofermentative (Ho LAB) and heterofermentative (He LAB). On the one hand, Ho LAB almost exclusively produce lactic acid from glucose. On the other hand, the heterofermentative pathways of LAB fermentation convert glucose in lactic acid, ethanol, carbon dioxide and water. The data presented here evidences that LAB populations significantly vary among cattle manures and that in this work mostly He LAB population was present in fresh cattle manure. Furthermore, the differences of LAB populations among cattle manures will have an important effect on the rate of WSC conversion during ensiling. In fact, if He LAB fermentation is carried out, the amount of glucose consumed should be two times higher in order to achieve the same level of acidity of Ho LAB fermentation, since ethanol is not an hydrogen donor ($pK_a = 16$). This should explain why WSC was consumed much faster in these assays (He LAB fermentation) than in our previous work on Ho LAB fermentation [103].

Starch addition (CM16%+S) improved biomass acidification of cattle manure with wheat straw. Indeed, pH of CM16%+S was already 5.5 after 7 days and 4.8 at the end of the storage. This suggests that starch was used as substrate for VFA production. However, fermentation rates of CM16%+S were lower than while using glucose as co-substrate. After the first week of ensiling, *e.g.*, VFA content was 8.2%VS_{added} for co-silage with starch, while it was 16.5%VS_{added} and 14.9%VS_{added} for CM16%+G and CM21%+G, respectively. Moreover, VFA was mainly composed of acetic and butyric acids during ensiling with starch. Lactate was only detected in the first 7 days of CM16%S and in small amount (1.3%VS_{added}).

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This evidences that starch hydrolysis limited the rate of acidogenesis and impeded lactate accumulation. Furthermore, ethanol was only present in trace concentrations for CM16%+S condition, which indicates that substantial ethanol production through heterofermentative LAB only occurs at low pH conditions (as in co-silages with glucose addition). This is agreement with Ren *et al.* [104], who showed that the optimal pH for ethanol production from refined sugar during anaerobic digestion was below 4.0.

Finally, the results presented in Table 4-6 show that NH_3-N concentration was correlated with the pH of silage. Indeed, there was no significant modification of ammonia-nitrogen content for ensiling conditions with pH lower 5.5 after 7 days (glucose and starch co-silages). In contrast, NH_3-N substantially increased during storage of cattle manure without addition of fermentation stimulants. This was especially true for single-handedly cattle manure, for which ammonia was 20.8%TKN before storage and 41.7%TKN after 4 months of ensiling. For these latter conditions, the increase of ammonia content should be related with the increasing proteolysis for high pH values [12,42,43]. Besides ammonia, proteolytic activity lead to side-products, such as a mix of VFA, CO_2 and H_2 [95], therefore increasing energy losses during storage. Even if degradation of nitrogenous compounds took place in some conditions, no ammonia emissions should have occurred, since TKN content did not significantly decreased during storage. This is explained by the fact that pH never reached the pK_a of NH_4^+/NH_3 (9.25).

4.4.3.2.2. Structural carbohydrates

The data presented in Table 4-7 shows that there was a substantial degradation of structural carbohydrates for all storage conditions. According to Dewar *et al*. [86] and McDonald *et al*. [12], conversion of these polymers during storage may be linked with enzyme activity or acid hydrolysis by organic acids produced in the course of ensiling.

The higher breakdown rates of structural carbohydrates were found for CM12%, CM16% and CM21%. After 30 days of ensiling, around 18-24% of the sum of original (hemi-) cellulosic compounds were missing for the three conditions without addition of fermentation stimulants. At the end of the 4 months, the breakdown reached 40% for CM12%, 30% for CM16% and 29% for CM21%. Therefore, high amount of WSC should have been released by sugar polymers and used as substrate for VFA production in the long term. Since an acid pH was not detected for these conditions, breakdown of (hemi-) celluloses was probably a result of endogenous enzyme activity. Furthermore, it is important to notice that, for CM12% silage, the consumption of structural carbohydrates after 4 months was considerably higher than for the other ensiling conditions. This coincides with the period for which there was a partial degradation of VFA for this condition. Thus, several losses must have occurred for single-handedly cattle manure after 4 months of storage.

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Glucose addition to cattle manure restricted the degradation of structural carbohydrates during ensiling. Indeed, after 4 months, conversion of initial complex sugars was 15% for CM12%+G, 23% for CM16%+G and 11% for CM21%+G. Similarly to the other ensiling conditions, the breakdown products were likely used as substrate for lactate production, since no accumulation of WSC was observed during storage period. Moreover, co-silages with glucose addition mostly had pH values below 4.0. Therefore, the degradation of hemicellulose and cellulose for these conditions may have occurred through acid hydrolysis by lactate produced during storage.

In like manner, the results of fibers analysis for co-ensiling with starch indicates that there was an extensive plant cell wall degradation during storage period. Nevertheless, in this case the damage exclusively occurred in the first week and corresponded to around 24% of the original neutral detergent fiber (NDF) content. This was quite a high value compared to co-ensiling with glucose conditions after one week, or even to CM16% and CM21% after 1 month. Also, this corresponded to a period in which starch should also have been hydrolyzed and fermented into VFA. Consequently, it is possible that NDF losses in the first days of ensiling were partially due to the breakdown of starch and not of (hemi-) cellulosic compounds. In fact, Van Soest *et al*. [105] stated that starch interfere with the analysis of cell wall constituents and increase NDF value. Therefore, further interpretation on the cell wall composition of co-ensiling with starch was discouraged and avoided in this study.

4.4.3.2.3. Conservation of organic matter

The extension of fermentation and stability of silage had a significant effect on the preservation of organic matter over ensiling period, Figure 4-9. As a matter of fact, it was during storage of single-handedly cattle manure (CM12%) that the largest VS degradation occurred. For this condition, VS losses were in average 6.5% per month of storage. This high rate of organic matter spoilage is due to the instability caused by the low TS content of raw material and the subsequent rapid loss of structural carbohydrates.

Reduction of water availability through co-ensiling with wheat straw had a positive impact on the organic matter conservation, especially in the long term. For both CM16% and CM21%, VS losses were minimal and mostly occurred during the first month of storage (Figure 4-9A). After 4 months, more than 94% of organic matter was conserved for co-ensiling of cattle manure with wheat straw. This evidences the importance of a high TS content on the conservation of organic matter while using feedstocks without easily accessible substrate for fermentation. In this case, VS losses should have been caused by the formation of side-products during fermentation, such as CO_2 and H_2.

Likewise, for co-silages with glucose addition, losses of organic matter principally occurred in the early days of storage (Figure 4-9B). However, the range of degradation was higher than in co-ensiling with only wheat straw. After 15 days, original VS conservation reached 86% for CM12%+G and 89% for

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both CM16%+G and CM21%+G. These values are coherent with the amounts of CO_2 and H_2O that should be produced assuming that all original WSC were fermented by heterofermentative LAB. Therefore, no energy losses should have occurred during the first 15 days of co-ensiling with glucose. In addition, between 15 days and the end of ensiling, stability of CM12%+G and CM16%+G blocked further organic matter degradation. On the opposite, VS conservation of CM21%+G decreased from 89% after 30 days to 84% at the end of storage. This should be related to the secondary fermentation that happened for CM21%+G condition during this period.

Ensiling of cattle manure with starch (CM16%+S) can be considered as an intermediate condition in terms of VS preservation. On the one hand, organic matter of CM16%+S was less degraded than the ones of co-silages with glucose addition. This is explained by the weaker heterofermentative LAB activity for the raw material with starch. On the other hand, for CM16%+S, VS conversion was higher than during ensiling without the use of fermentation stimulants. This supports the assumption that starch could be used as additional substrate for biomass acidification. Moreover, as for the remaining stable silages, during storage of CM16%+S the major conversion of organic matter occurred in the first month. Indeed, conservation of VS for the starch silage was 94% after 30 days and 91% at the end of the storage.

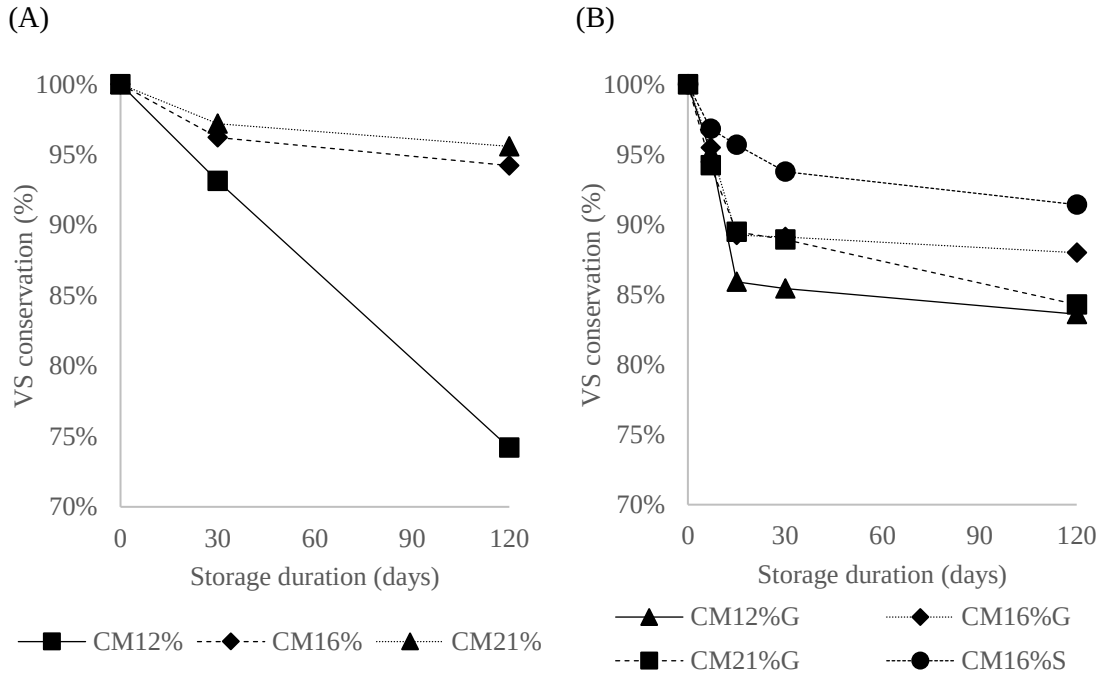

Figure 4-9 - Preservation of organic matter over storage duration. (A): effects of wheat straw addition; (B): co-ensiling conditions with glucose and starch.

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4.4.3.3. Effects of storage method on methane production during AD

4.4.3.3.1. BMP evolution

BMP values based on VS_{added} along ensiling duration are presented on Table 4-8. In the first place it is important to mention that BMP values for samples with 30 days of ensiling were quite lower than expected. This should be related to a reactivity issue of the inoculum used for this BMP batch. Therefore, this data was only used for qualitative considerations.

Regarding feedstocks, wheat straw addition decreased the BMP of fresh cattle manure from 257 $L_{STP}/kgVS_{added}$ to 232 $L_{STP}/kgVS_{added}$ for CM16% and 215 $L_{STP}/kgVS_{added}$ for CM21%. This evidences the low biodegradability of wheat straw in anaerobic digestion. In opposite, BMP of raw material was increased with the use of glucose: 319 $L_{STP}/kgVS_{added}$ for CM12%+G, 286 $L_{STP}/kgVS_{added}$ for CM16%+G and 271 $L_{STP}/kgVS_{added}$ for CM21%+G. This is in line with the theoretical methane yield of glucose (373 $L_{STP}/kgVS_{added}$) and the amounts of co-substrate used for the three experiments. Since pure starch also has an important BMP, 352±33 $L_{STP}/kgVS_{added}$ [106], the same trend was expected for the feedstock with starch addition. However, CM16%+S had a BMP before storage of 236 $L_{STP}/kgVS_{added}$, which was similar to the raw material without starch addition. Therefore, this indicates that starch was not fully degraded during the test with cattle manure. The reasons for this are still unclear, even if unequal BMP for commercial starch were already reported [106].

Anaerobic storage had a negative impact on BMP related to VS_{added} of single-handedly cattle manure. After 4 months, 16% of $BMP_{VSadded}$ was lost for CM12%. This indicates that there was a loss of global biochemical accessibility of biomass, which should be related to the damage of both structural carbohydrates and organic acids in the last 3 months of ensiling. The biomass conservation was improved by storing cattle manure with wheat straw. In fact, for both CM16% and CM21%, methane potential based on VS_{added} was stable after 4 months (within the accuracy of BMP tests), which also means that the conversion of cellulosic compounds did not have a significant impact on the accessibility of biomass.

In the contrary, BMP related to VS_{added} increased during ensiling with fermentation stimulants. On the one hand, this may be related to the accumulation of organic acids and alcohols over the storage period. As a matter of fact, some of these compounds have higher BMP by mass unit of VS than the substrates used for their production (since carbon is lost under the form of CO_2 during this process). This is the case of ethanol (BMP = 730 $L_{STP}/kgVS_{added}$) for assays with glucose addition and butyric acid (BMP = 636 $L_{STP}/kgVS_{added}$) for CM16%+S. Neither the production of lactic acid nor of acetic acid could stimulate this increase since they have the same methane potential of glucose. On the other hand, acid hydrolysis of structural carbohydrates may have led to the increase of accessibility of biomass over time, since these polymers are not fully biodegradable in mesophilic AD [96].

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Table 4-8 – BMP based on VS_{added}, its water-soluble fraction and energy conservation over storage period.

Condition	Duration (days)	BMP ($L_{STP}/kgVS_{added}$)	$BMP_{water\text{-}soluble}$ fraction (%$BMP_{crude\ material}$)	Conservation of original BMP (%)
CM12%	0	257 ± 6	17 ± 1.8	-
	30	237 ± 15	27 ± 1.9	86 ± 9
	120	217 ± 2.0	22 ± 0.7	63 ± 3
CM16%	0	232 ± 5	11 ± 0.6	-
	30	196 ± 4	18 ± 0.8	81 ± 4
	120	234 ± 4	21 ± 0.8	95 ± 4
CM21%	0	215 ± 4	8 ± 0.7	-
	30	186 ± 1.9	13 ± 4	84 ± 3
	120	228 ± 12	16 ± 1.3	101 ± 7
CM12%G	0	319 ± 9	54 ± 4	-
	7	328 ± 2.0	46 ± 1.4	98 ± 3
	15	347 ± 8	46 ± 3	94 ± 5
	30	306 ± 9	49 ± 2.2	82 ± 6
	120	341 ± 8	50 ± 3	89 ± 5
CM16%G	0	286 ± 3	42 ± 1.4	-
	7	298 ± 7	37 ± 2.2	100 ± 3
	15	339 ± 11	33 ± 3	106 ± 4
	30	265 ± 2	36 ± 1.7	83 ± 2
	120	312 ± 3	38 ± 1.5	96 ± 2
CM21%G	0	271 ± 10	37 ± 4	-
	7	294 ± 13	31 ± 3	102 ± 8
	15	318 ± 7	29 ± 1.9	105 ± 6
	30	244 ± 6	28 ± 2.4	80 ± 6
	120	307 ± 9	28 ± 1.6	95 ± 6
CM16%S	0	236 ± 5	8 ± 1.9	-
	7	264 ± 9	15 ± 0.8	108 ± 5
	15	275 ± 7	15 ± 1.7	112 ± 5
	30	210 ± 7	18 ± 1.1	83 ± 5
	120	254 ± 7	22 ± 1.8	98 ± 5

In order to better understand the main phenomena occurring during ensiling, and to evaluate the energy conservation of storage trials, weight losses were taken into account and BMP related to $VS_{original}$ was determined. The conservation of the initial methane potential is exposed on Table 4-8 and the evolution of $BMP_{VSoriginal}$ over storage period is presented in Figure 4-10.

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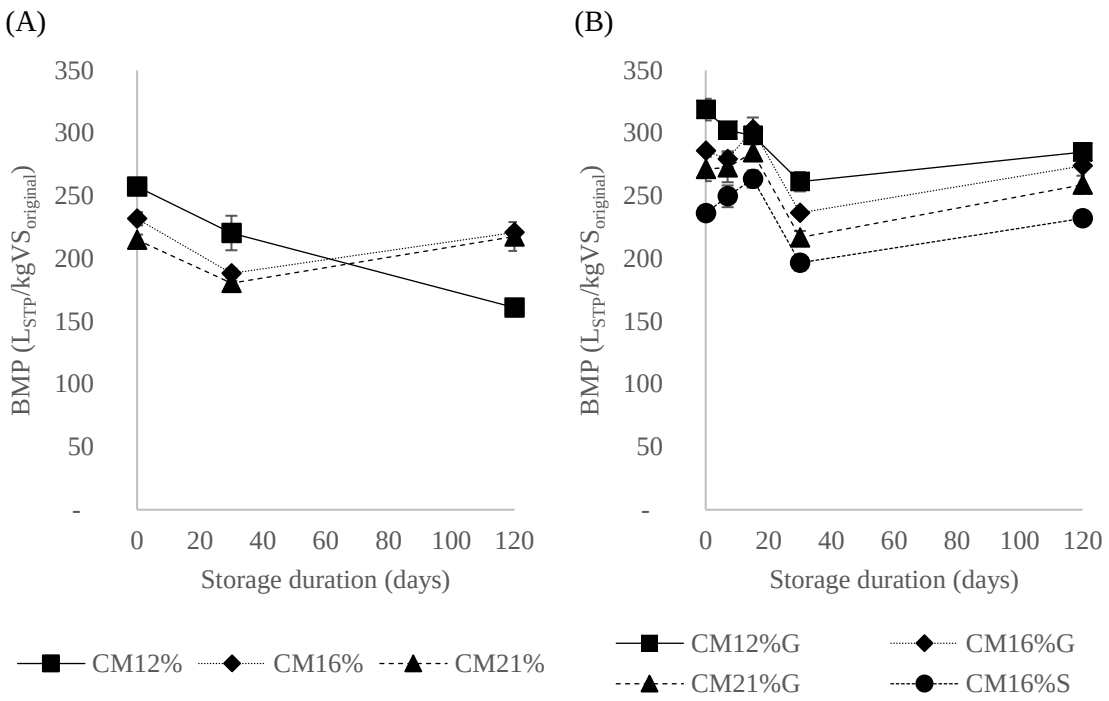

Figure 4-10 - BMP evolution of crude material over storage duration. (A): effects of wheat straw addition; (B): co-ensiling conditions with glucose and starch. Methane potential is based on $VS_{original}$, therefore considering storage losses.

Concerning CM12%, losses were quite more accentuated for BMP based on $VS_{original}$. At the end of the ensiling, around 37% of the initial methane potential was lost for this condition. Nevertheless, this represents an improvement as compared to the 46% of original BMP that was lost after 4 months of fresh cattle manure ensiling in our previous work [99]. In this latter study, degradation of structural carbohydrates and VFA was more advanced. The same trend should be expected for CM12% and so, heavier energy losses should occur for even longer storage periods.

Wheat straw addition was proven to be an efficient method to keep the energy potential of the cattle manure used in this work. At the end of the 4 months, negligible losses of $BMP_{VSoriginal}$ occurred for both CM16% and CM21%. This demonstrates that the wide formation of side-products (secondary fermentation and proteolysis) did not have a significant impact on energy preservation during storage.

Regarding co-ensiling assays with glucose, the increase of $BMP_{VSadded}$ was clearly counterbalanced by the storage losses. This means that bio-accessibility of feedstock was not improved enough through acid hydrolysis of cellulosic compounds. Therefore, and since biomass was stabilized for co-silages with glucose, minimal variation of the BMP based on $VS_{original}$ occurred during storage of these raw materials. After 4 months, original BMP was preserved at 89±5% for CM12%+G, 96±2% for CM16%+G and 95±5% for CM21%+G. Once more, this shows that clostridial fermentation in the last 3 months of CM21%+G condition had negligible impact on the methane potential of biomass.

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Starch addition to cattle manure led to a substantial improvement of original methane potential in the early days of ensiling. Indeed, after 15 days, initial BMP had been increased by 12%. Thus, the biochemical accessibility increased during this period. Since the degradation of structural carbohydrates did not enhance the methane potential for the remaining conditions, this result should be related to the conversion of starch during this period. However, this was counteracted by the degradation of structural organic matter in the long term. At the end of the storage, full preservation of original BMP (within the accuracy limits) occurred for CM16%+S.

4.4.3.3.2. BMP distribution in particulate and water-soluble phase

In the first 30 days of storage, the contribution of the water-soluble phase to the BMP increased for the conditions without addition of fermentation stimulants (Table 4-8, 4th column). This effect was more important for raw materials with higher water availability. However, in the 3 following months, BMP distribution evolved differently for biomasses without co-substrates and with only wheat straw addition. For CM12%, water-soluble fraction contributed to 27% of the total BMP after 30 days of ensiling and decreased to 22% at the end of the 4 months. This suggests that, during the last 90 days, the loss of water-soluble BMP (due to VFA degradation) was higher than the loss of particulate BMP. In contrast, for CM16% and CM21%, the contribution of the water-soluble fraction to the BMP kept improving until the end of ensiling. Despite of that, solubilization was less significant than in the first month of storage, due to the reduced fermentation rates after biomass stabilization.

Similarly, the BMP of feedstock with starch was solubilized over storage period, especially in the first week. Indeed, water-soluble fraction contributed to only 8% of total methane potential before storage and increased to 15% after 7 days and 22% at the end of ensiling. This growth was more important than for CM21%, which was the silage with the characteristics closer to those of CM16%+S (initial TS content and $\%BMP_{water\text{-}soluble}$). However, this also demonstrates that some starch remained in the particulate phase.

In contrast, the contribution of the water-soluble fraction to the BMP decreased during ensiling with glucose addition. This was basically observed in first 7 days of storage, during which there was a vast LAB fermentation of glucose. Therefore, this trend should be related to the yield of WSC that was used for bacterial growth.

4.4.3.3.3. Kinetics

Anaerobic digestion assays are also characterized by the 1st-order rate coefficient (k) of biomass degradation. This parameter is closely linked to the biochemical accessibility of biomass, hence the interest for biomass conservation purposes. Nevertheless, while testing long-term storage, different inoculum were used for the various series of BMP test. Contrary to methane potential values (Table

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4-8), modifications of inoculum properties had an impact on the rate of biomass degradation during BMP assays, even if our inoculum source was always the same (digested sludge from the same municipal WWTP). Therefore, in this work, *k* values were only compared and interpreted for samples with the same storage period, *i.e.*, BMP tests performed with the same inoculum. The evolution of BMP rate coefficient over storage duration is presented in Figure 4-11.

The addition of wheat straw to cattle manure proportionally decreased the kinetics of feedstock degradation. This confirms that wheat straw did not contribute to the increase of easily reachable substrate for LAB fermentation during ensiling. On the opposite, the use of either glucose or starch as co-substrate led to the increase of the *k* value before storage. Thus, these compounds were more rapidly biodegradable in AD than the average of the organic matter present in fresh cattle manure.

Furthermore, from 15 days of ensiling there was large difference between the rate coefficient of CM12%+G and the other conditions with addition of fermentation stimulants. This supports the idea that bacterial and enzymatic activity may have exponentially increased during ensiling of raw materials with particularly high moisture contents.

Contrarily to the samples with less than 30 days of preservation, the rate coefficient of CM12% was inferior the one of CM16% (with wheat straw) at the end of the 4 months. Therefore, the degradation of water-soluble VS for prolonged periods of CM12% ensiling led to an excessive loss not only of BMP but also of biochemical reactivity and subsequently of the rate of methane production.

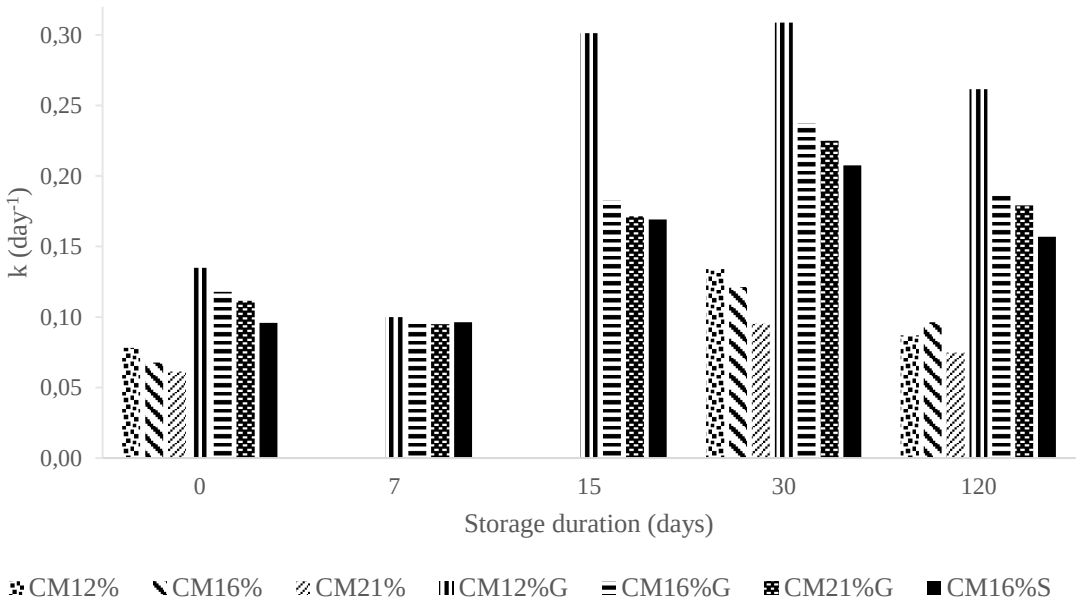

Figure 4-11 – Rate coefficient (k) of BMP assays over storage time. Standard deviations are not presented as differences among triplicates were negligible.

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4.4.4. Conclusions

Ensiling of single-handedly cattle manure was inefficient for methane potential conservation especially due to the high moisture content and to the absence of simple sugars in raw material. Around 37% of methane potential losses occurred after 4 months for this storage condition. The use of sugar-rich co-substrates enhanced ensiling performance. Wheat straw addition increased total solids content and stabilized biomass with a pH around 6.0. While using glucose as co-substrate, extensive heterofermentative LAB fermentation occurred and pH quickly drop to values below 4.0. Starch was used as substrate for fermentation but its hydrolysis limited biomass acidification. Despite the different modes of action, all three co-substrates led to minimal methane potential losses after 4 months of storage. Therefore, co-ensiling can be proposed as a valuable solution for energy conservation of cattle manure feedstocks in agricultural biogas plants.

4.4.5. Acknowledgements

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4.5. A retenir

La pratique la plus courante pour la gestion de fumier de bovin avant méthanisation est le stockage en tas à l'air libre. Cette condition de stockage est déconseillée car elle entraîne d'importantes émissions de gaz à effet de serre et des pertes du potentiel de production de méthane autour de 74% après 4 mois.

Ces pertes d'azote et de carbone peuvent être limitées par le stockage confiné du fumier de bovin, en particulier sur de courtes durées. Cependant, les propriétés chimiques inadaptées des fumiers de bovin frais limitent leur stockage sur de longues durées d'ensilage. Parmi ces caractéristiques, la faible teneur en matière sèche et l'absence de sucres facilement accessibles pour la fermentation sont les principaux facteurs responsables de l'inefficacité de la conservation. En conséquence, des pertes énergétiques entre 37% et 46% ont été observées après 4 mois d'ensilage de fumier de bovin frais.

Dans certains cas, l'ajout de paille de blé peut améliorer la performance du stockage confiné de fumier de bovin. Ceci a été observé pour les expériences du premier et du dernier article de ce chapitre. Dans ces études, la biomasse a été acidifiée jusqu'à un pH entre 5.5-6.0 pendant les premières semaines d'ensilage, ce qui a été suffisant pour inhiber l'activité méthanogène et stabiliser le fumier avec une teneur de matière sèche plus élevée. Ainsi, le fumier avec ajout de paille a-t-il conservé plus de 86% de son potentiel bio-méthanogène après 4 mois d'ensilage pour ces deux études. En revanche, pour le fumier utilisé dans les expériences décrites dans le deuxième article, l'ajout de paille n'a pas permis une acidification suffisante de la biomasse, la matière organique a été continûment dégradée au cours du stockage et les pertes énergétiques ont atteint 67% après 4 mois. Ces résultats contradictoires peuvent être expliqués par des différences dans les populations bactériennes, lesquelles varient pour le fumier selon, par exemple, l'alimentation du bétail ou la saison de l'année. En effet, les analyses de la composition du gaz produit pendant le stockage ont montré que le rapport CH_4/CO_2 dans les premiers jours d'ensilage était substantiellement supérieur pour le Fumier 2, ce qui met en évidence une activité méthanogène plus importante pour ce gisement. Bien que l'ajout de paille de blé ne puisse pas être généralisé comme étant un atout pour la conservation de la biomasse pendant le stockage, son utilisation diminue la teneur en humidité et joue un rôle important sur la tenue mécanique des silos, ainsi que sur la réduction des risques de production d'un effluent liquide.

L'utilisation d'autres co-substrats favorise la mise en place de conditions d'ensilage du fumier de bovin. Dans le cas de l'ajout d'acide formique (2 $\%_{MH}$), la conservation du potentiel énergétique a été d'environ 75% après 4 mois d'ensilage. Cet additif présente l'intérêt d'atteindre rapidement un pH inférieur à 4. Toutefois, l'ajout d'acide formique a seulement retardé l'activité bactérienne. Il est consommé pour des longues durées avec, pour conséquence, l'augmentation du pH au fil du temps. L'usage d'acide formique ne permet pas d'éviter la dégradation de la biomasse sur des périodes de stockage plus prolongées. En outre, il faut tenir compte de la difficulté de manipulation et des précautions supplémentaires à prendre avec ce type de composé au niveau agricole.

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L'ajout de sucres accessibles pour stimuler la fermentation a été une méthode efficace pour optimiser la conservation des fumiers de bovin pendant le stockage. En effet, le co-ensilage avec glucose a conduit à une acidification très efficace, ce qui a permis de conserver plus de 87% du potentiel en méthane initial et de supprimer les émissions gazeuses sur les 4 mois de stockage. Ces taux de préservation ont été encore plus élevés pour les concentrations de glucose (10 $\%_{MH}$) et les teneurs en matière sèche (15-25 $\%_{MH}$) les plus importantes. Malgré des résultats prometteurs, il est difficile de suggérer une teneur initiale de sucres disponibles commune à l'optimisation du stockage de tous les fumiers de bovin, étant donné que les mécanismes réactionnels d'acidification changeront selon les populations microbiennes présentes dans le gisement, notamment en ce qui concerne les bactéries homolactiques ou hétérolactiques. De plus, les essais de co-stockage de fumier avec de l'amidon (5 $\%_{MH}$) ont montré que des sucres plus complexes peuvent être utilisés en combinaison avec le fumier pour l'acidification de la biomasse, entraînant des pertes minimales d'énergie après 4 mois de co-stockage.

Tout bien considéré, le co-ensilage de fumier de bovin avec un co-substrat riche en sucres accessibles (glucose, amidon, etc.) et à teneur en matière sèche élevée semble être la méthode la plus pertinente pour la préservation du potentiel méthanogène de cette ressource avant méthanisation.

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5. SYNTHESE DES RESULTATS ET APPROCHE DE MODELISATION

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5.1. Synthèse des résultats

L'ensemble des expériences a montré que le stockage aérobie n'est pas une option viable en vue de l'utilisation des ressources en méthanisation. En revanche, des excellents taux de conservation énergétiques peuvent être obtenus si un stockage dans des conditions d'ensilage efficace est réalisé. La maitrise de l'ensilage est strictement liée à l'évolution du **pH** de la biomasse, étant donné que ce paramètre reflète les principaux changements biochimiques qui ont lieu au cours du conditionnement. Plus simplement, à partir de l'analyse de la Figure 5-1, il est possible d'observer qu'il existe une corrélation entre le pH à la fin des essais de stockage de longue durée et la conservation du **potentiel bio-méthanogène**. En effet, le PBM est largement conservé en ensilage pour des pH faibles et il y a de fortes pertes énergétiques lorsque l'acidité décroit.

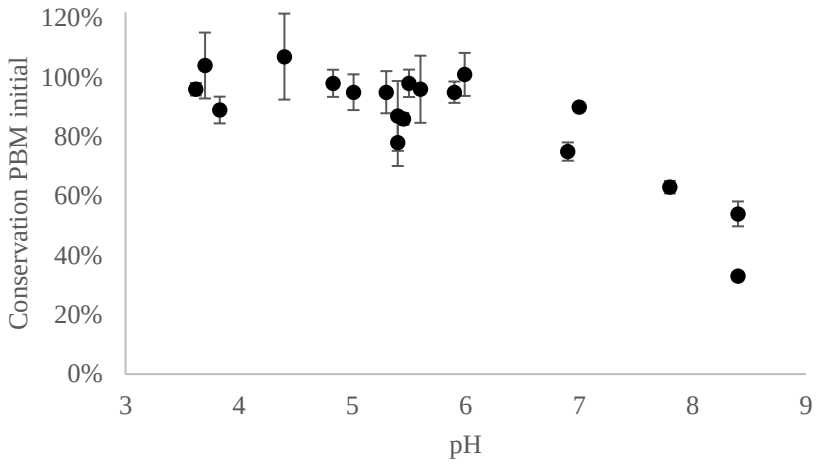

Figure 5-1– Conservation du PBM après stockage en fonction du pH final.

En conservant des conditions anaérobies dans le silo, l'évolution du pH de la biomasse dépendra principalement des propriétés de la matière première, en particulier:

- **Les sucres accessibles**: ce paramètre est probablement le plus important. Les sucres non-structurels sont consommés rapidement au début de l'ensilage par des **bactéries lactiques**, ce qui entraîne une acidification et une meilleure stabilité de la biomasse à long terme. Cette propriété représente la principale différence entre les deux matrices testées dans cette thèse. En effet, alors que les CIVEs sont rapidement acidifiées en raison de la forte présence de sucres accessibles (non seulement les sucres solubles), pour les fumiers de bovin frais l'acidification est trop lente en raison de l'absence de substrats organiques facilement biodégradables. Puisque cette stabilité à faible pH a un fort impact sur l'ensemble de l'activité microbienne et sur l'efficacité de l'ensilage, les pertes de matière organique et d'énergie sont largement plus faibles pour des CIVEs que pour du fumier de bovin frais. Cette hypothèse est soutenue par les taux élevés de conservation d'énergie obtenus pour le co-ensilage à longue durée de fumier de bovin avec des teneurs élevées de sucres accessibles.

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- **La matière sèche**: Cette propriété initiale des biomasses à stocker a un impact sur l'activité microbienne et, par conséquent, sur leur conservation. Comme on a pu le voir par exemple pour les essais avec CIVE 2, pour des faible teneurs de MS, l'acidification a été rapide mais pas assez importante pour éviter la consommation de l'acide lactique par **fermentation clostridiale**, ce qui a entraîné la remontée du pH. En outre, en utilisant une teneur en MS beaucoup plus élevée pour la CIVE 2, l'acidification s'est poursuivie, bien que plus lentement, malgré l'inhibition de l'activité clostridiale. Cela montre la plus grande sensibilité à la disponibilité en eau des populations clostridiales par rapport aux bactéries lactiques. Si le pH remonte, des populations sensibles à l'acidité auront une activité plus importante. Par exemple, une **fermentation d'acides aminés** extensive peut se produire. Ces réactions secondaires conduisent non seulement à la biodégradation de la matière organique, mais aussi à la formation d'autres sous-produits, y compris le H_2, qui sera émis dans l'atmosphère, entraînant des pertes d'énergie pendant le stockage. Toutefois, ces pertes seront limitées et mèneront seulement à la baisse du PBM si les mécanismes réactionnels de la méthanisation peuvent se développer. C'est le cas des certaines matrices à faible teneur en MS, avec des concentrations dominantes de méthanogènes et dont le pH est élevé, où la **méthanogenèse** de l'acétate peut se produire, conduisant à une perte massive de matière organique et d'énergie. Ces arguments peuvent expliquer les taux de conservation inférieurs pour des biomasses avec des faibles teneurs de MS (10-14%) pour la CIVE 2, mais aussi pour les Fumier 1 et Fumier 3 (pour un récapitulatif sur les paramètres testées en ensilage, voir : Tableau 2-1, page 34).

- **La microflore endogène**: La concentration initiale des micro-organismes endogènes intervenant dans les réactions mentionnées ci-dessus a une influence concrète sur le déroulement de l'ensilage. D'abord, une teneur importante en bactéries lactiques est fondamentale pour que l'acidification se passe correctement. Cela a été observé à la fois pour les deux CIVEs, mais aussi sur fumiers de bovin, étant donné que lors de la présence de substrats facilement biodégradables en grandes quantités, la production d'acide lactique a été rapide et abondante. Cependant, nous avons aussi constaté des différences significatives au sein de cette microflore endogène selon le gisement. D'une part, dans le cas de la CIVE 2 et du Fumier 2, les bactéries lactiques étaient majoritairement homo-fermentaires, se traduisant par une fermentation presque exclusive des sucres en acide lactique. D'autre part, pour le Fumier 3, il y avait une forte présence de bactéries hétéro-fermentaires, compte tenu de la production importante d'éthanol lors des co-ensilages avec du glucose. Etant donné que l'éthanol produit par des bactéries hétéro-fermentaires ne contribue pas à l'acidification de la biomasse, la quantité des sucres à consommer doit augmenter de façon à faire baisser le pH, ce qui peut avoir des conséquences sur la stabilité de l'ensilage. De plus, la concentration initiale d'autres populations bactériennes, telles que les méthanogènes, peut déterminer l'extension des pertes d'énergie dans les ensilages qui se stabilisent à pH plus élevé. Cela semble évident au regard des résultats obtenus avec fumier de bovin et ajout de paille de blé. En effet, dans la Figure 5-2, on peut observer que, même si pour les Fumier 1 et Fumier 3

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l'augmentation de la teneur en MS a été suffisante pour stabiliser la matière (pH = 5.5-6.0) et empêcher la méthanogenèse, le même phénomène n'a pas eu lieu pour le Fumier 2 (montée du pH à partir de 7 jours). Cela suggère que dans certains fumiers de bovin, la concentration des méthanogènes est déjà très élevée au début de stockage (mis en évidence par l'analyse du gaz produit dans les premiers jours d'ensilage); et que, sans un pH très acide, il est difficile d'empêcher que cette activité se développe.

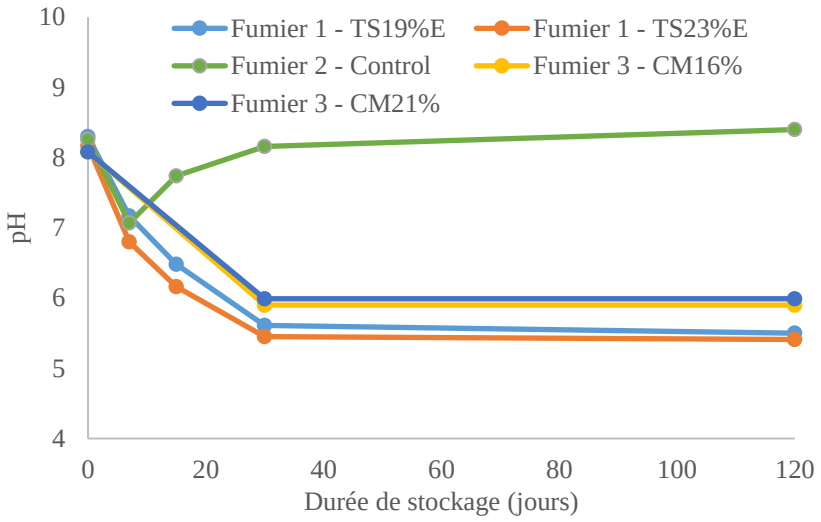

Figure 5-2 – Evolution du pH des co-ensilages de fumier avec paille de blé.

- **Le pH initial / pouvoir tampon**: Le pH du gisement est une indication de la basicité ou acidité des composés tampons présents dans la biomasse. Ces composés sont essentiels pour l'ensilage car ils déterminent la quantité d'acide lactique qui doit être produite pour baisser le pH et, par suite, influencent la quantité de sucres nécessaire pour la stabilité du stockage. Les résultats expérimentaux montrent à nouveau une différence pour cette propriété entre les ressources testées. En effet, pour les CIVEs le pH initial était de 5.8-7.2, ce qui indique une légère prédominance des composés donneurs de H^+. Au contraire, les fumiers de bovin étaient alcalins en raison de la présence d'azote ammoniacal (pH initial compris entre 7.7 et 8.3), ce qui a contribué à rendre son acidification plus difficile au cours du stockage dans des conditions d'ensilage.

En plus des paramètres listés ci-dessus, nous avons pu constater une solubilisation de la matière organique au cours du temps dans la plupart des essais réalisés. Cette solubilisation s'explique en partie par **l'hydrolyse des sucres structurels**, qui se produit lentement, mais normalement tout au long de l'ensilage. Par ailleurs, les résultats ont permis de mettre en évidence que cette hydrolyse peut entraîner des gains de bio-accessibilité et, par conséquent, une légère augmentation du PBM. Cependant, lors d'un ensilage instable avec développement d'une activité méthanogène, les produits de l'hydrolyse ne sont pas accumulés, ni sous la forme de sucres solubles, ni sous forme d'AGV. Ce mécanisme réactionnel mène à des pertes considérables de matière organique et de PBM. D'autre part, une **hydrolyse des composés azotés** a lieu au cours de l'ensilage. Cette hydrolyse augmente le risque de minéralisation de

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l'azote et des émissions de NH_3 pendant le stockage. Néanmoins, dans la majorité des essais d'ensilage, l'azote minéralisé reste sous forme de NH_4^+, en raison du faible pH.

En conclusion, les résultats montrent qu'il y a plusieurs tendances communes à l'ensemble des tests réalisés dans des conditions de stockage confiné, lesquels peuvent être expliqués en grande partie par les processus biochimiques et physico-chimiques résumés ci-dessus. Ces observations ont été les principales motivations pour l'élaboration d'un modèle qui pourrait expliquer les phénomènes majeurs qui ont lieu pendant l'ensilage, lequel est exposé ensuite.

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5.2. Approche de modélisation pour l'ensilage

5.2.1. Introduction

Le modèle présenté dans cette partie a été développé afin de décrire le processus d'ensilage, avec l'accent mis sur sa phase anaérobie. Pour cela, nous avons utilisé un jeu de réactions biologiques et d'équilibres chimiques qui permettent de représenter l'évolution temporelle des principaux composés au cours du stockage et, par conséquent, l'évolution du pH. Étant donné que l'évolution du pH est généralement corrélée avec la conservation énergétique de la biomasse, ce modèle doit permettre de prédire les meilleures conditions de stockage pour un gisement donné, en vue de son utilisation pour la production de biogaz, compte tenu de ses propriétés biochimiques. De plus, cet outil de simulation doit permettre une estimation de la perte de PBM à partir de la quantité de H_2 et CH_4 produite pendant le stockage.

La construction de ce modèle s'appuie sur d'autres modèles d'ensilage existants [73,74], mais avec une approche d'implémentation similaire à celle de l'ADM1 (*Anaerobic Digestion Model No.1*) [95], afin d'établir un lien mathématique entre le stockage de biomasse et la méthanisation.

Pour la calibration du modèle, des simulations ont été faites pour les ressources utilisées dans les expériences avec CIVE 2 et Fumier 2. Cela a permis d'assurer la validation du travail pour des matrices distinctes, avec plusieurs concentrations en sucres accessibles, matières sèches et populations bactériennes, ainsi que différents pH initiaux.

5.2.2. Unités, paramètres et variables

Les unités utilisées dans ce modèle sont décrites dans le Tableau 5-1. Pour la concentration des composés chimiques, l'unité choisie a été la DCO dégradable ($kgDCO.m^{-3}$) en raison de sa flexibilité dans l'étude des processus anaérobies, de son utilisation dans l'ADM1 et de son accessibilité expérimentale sur des échantillons solides et liquides. Pour les composés sans DCO, tels que le carbone inorganique (CO_2 et HCO_3^-) et l'azote inorganique (NH_4^+ et NH_3) la molarité a été utilisée comme unité.

Tableau 5-1 – Unités du modèle.

Mesure	Unités
Concentration	$kgDCO.m^{-3}$
Concentration de carbone/azote sans DCO	$kmol.m^{-3}$
Temps	j (jour)
Pression	bar
Volume	m^3
Température	K

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Les paramètres du modèle sont présentés dans le Tableau 5-2. Ceux-ci comprennent les coefficients stœchiométriques, les coefficients d'équilibre et constantes, ainsi que les paramètres cinétiques et vitesses.

Tableau 5-2 – Constantes et paramètres du modèle.

Symbole	Description	Unités
Coefficients stœchiométriques		
$f_{produit,substrat}$	Rendement du produit sur le substrat	$kgDCO.kgDCO^{-1}$
N_i	Teneur en azote du composé *i*	$kmol.kgDCO^{-1}$
Coefficients d'équilibre et constantes		
H_i	Constante de Henry pour le composé *i*	$kmol.m^{-3}.bar^{-1}$
$K_{a,acide}$	Constante de dissociation acido-basique	
pK_a	$-\log_{10}[K_a]$	
S_{TP}	Concentration du tampon initial	$kmol.m^{-3}$
Paramètres cinétiques et vitesses		
k_j	Constante cinétique de premier ordre pour le processus *j*	j^{-1}
$K_{S,j}$	Valeur de demi-saturation pour le processus *j*	$kgDCO.m^{-3}$
$I_{PH,j}$	Fonction d'inhibition pour le processus *j*	
$p_{M,j}$	pH de demi-activité pour le processus *j*	
q_j	Sensibilité de l'activité autour du p_M pour le processus *j*	
v_j	Vitesse cinétique du processus *j*	$kgDCO.m^{-3}.j^{-1}$
$Y_{b,i}$	Rendement de la biomasse *b* sur le substrat	$kgDCO.kgDCO^{-1}$
$\mu_{max,b,i}$	Taux de croissance maximal de Monod pour la biomasse *b*	j^{-1}

Les variables algébriques et d'état dynamique sont exposées dans le Tableau 5-3. Toutes ces variables ont été utilisées pour la modélisation de la phase anaérobie de l'ensilage à l'exception de la variable algébrique P_{IC}, laquelle a été introduite seulement dans les calculs de la respiration aérobie.

5.2.3. Processus biochimiques

5.2.3.1. Respiration aérobie

Lorsque le silo est fermé, la respiration aérobie de la biomasse se produit pendant plusieurs heures jusqu'à ce que l'oxygène piégé dans le dispositif soit totalement consommé. Cette respiration est décrite par la réaction suivante [12,74]:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \tag{5.1}$$

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Étant donné que la respiration entraîne une production et solubilisation de CO_2, le pH subira une légère baisse pendant cette phase. Notre approche a été de considérer une concentration initiale de CO_2 dans le ciel gazeux de 0.038%(V/V) et de 21%(V/V) après respiration. Ensuite le gaz dissout dans le milieu liquide a été calculé selon la loi de Henry :

$$S_{IC} = H_{IC} \times P_{IC} \tag{5.2}$$

Où la constante d'Henry pour le CO_2 est 0.035 kmol.m^{-3}.bar^{-1} [95] et P_{IC}, la pression partielle en CO_2, est égale à la pression totale du ciel gazeux (supposée égale à la P_{atm} = 1.013 bar) multiplié par la fraction volumique de CO_2 dans la phase gazeuse.

La quantité de sucres consommés au cours de la respiration aérobie a été considérée comme étant négligeable pour le modèle de la phase anaérobie. En effet, nous l'avons estimée à moins de 2% de la quantité de sucres accessibles initiale pour les deux biomasses testées. Ce calcul a été effectué avec l'hypothèse (raisonnable pour notre dispositif expérimental et pour un silo à l'échelle agricole) d'un rapport gaz/volume total de 0.4.

Tableau 5-3 – Variables algébriques et d'état dynamique.

Nom	Description	Unités
P_{IC}	Pression partielle du CO_2 gazeux	bar
S_{H+}	Ions H^+	kmol.m^{-3}
X_{CH}	Sucres difficilement accessibles	kgDCO.m^{-3}
S_{CH}	Sucres facilement accessibles	kgDCO.m^{-3}
S_{AL}	Acide Lactique	kgDCO.m^{-3}
S_{ET}	Ethanol	kgDCO.m^{-3}
X_{BL}	Bactéries lactiques	kgDCO.m^{-3}
S_{AB}	Acide butyrique	kgDCO.m^{-3}
S_{AC}	Acide acétique	kgDCO.m^{-3}
S_{H2}	Gaz hydrogène	kgDCO.m^{-3}
S_{IC}	Carbone inorganique	kmol.m^{-3}
X_{BC}	Bactéries clostridiales	kgDCO.m^{-3}
X_{PR}	Protéines	kgDCO.m^{-3}
S_{AA}	Acides aminés	kgDCO.m^{-3}
S_{IN}	Azote inorganique	kmol.m^{-3}
X_{BP}	Bactéries protéolytiques	kgDCO.m^{-3}
S_{CH4}	Gaz méthane	kgDCO.m^{-3}
X_{BM}	Bactéries méthanogènes	kgDCO.m^{-3}

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5.2.3.2. Phase anaérobie

La phase anaérobie de l'ensilage correspond à un nombre important de réactions qui se produisent soit séquentiellement soit en parallèle, en raison de la complexité de la flore microbienne endogène. L'approche présentée dans ce chapitre n'a pas pour objet de représenter tous les mécanismes réactionnels, mais plutôt de décrire le stockage confiné à partir des principaux phénomènes observables, en se basant sur deux modèles existants d'ensilage [73,74]. Le premier, conçu par Neal and Thornley [73], présente l'évolution du pH de l'ensilage à partir des réactions de fermentation lactique et clostridiale. Le deuxième a été développé par Pitt *et al*. [74] de façon à évaluer la qualité des ensilages à partir de son pH et comprend, dans la phase anaérobie du modèle, la réaction de fermentation lactique, mais aussi l'hydrolyse des hémicelluloses et la protéolyse. Au-delà de ces réactions, un mécanisme simplifié de méthanogenèse, crucial pour décrire des conditions d'ensilages dégradés, a été pris en compte dans notre approche. Finalement, le modèle peut être schématisé par la Figure 5-3, incluant les réactions suivantes :

1. Hydrolyse des sucres
2. Fermentation lactique
3. Fermentation clostridiale
4. Hydrolyse des protéines
5. Fermentation protéolytique
6. Méthanogenèse

Ces réactions peuvent être séparées en mécanismes extracellulaires et intracellulaires. Les réactions d'hydrolyse enzymatique/chimique (1 et 4) sont extracellulaires et sont présumées de premier ordre, ce qui est une simplification basée sur l'empirisme [95]. Leur cinétique est décrite selon l'équation ci-dessous :

$$v_j = k_j \times X_i \tag{5.3}$$

Les réactions de fermentation (2, 3 et 5) et de méthanogenèse (6) sont des transformations biologiques qui entraînent croissance et mort cellulaire. La mort cellulaire était présente dans l'approche originale, mais compte tenu de son impact négligeable sur les résultats, elle a été enlevée du modèle final pour une question de simplification. Ainsi, les cinétiques biologiques sont décrites par la consommation du substrat et croissance de la biomasse. Ces réactions biologiques sont présentées selon une cinétique de Monod basée sur la consommation du substrat :

$$v_j = \frac{X_{b,i} \times \mu_{max,b,i}}{Y_{b,i}} \times \frac{S_i}{K_{S,j} + S_i} \times I_{pH,j} \tag{5.4}$$

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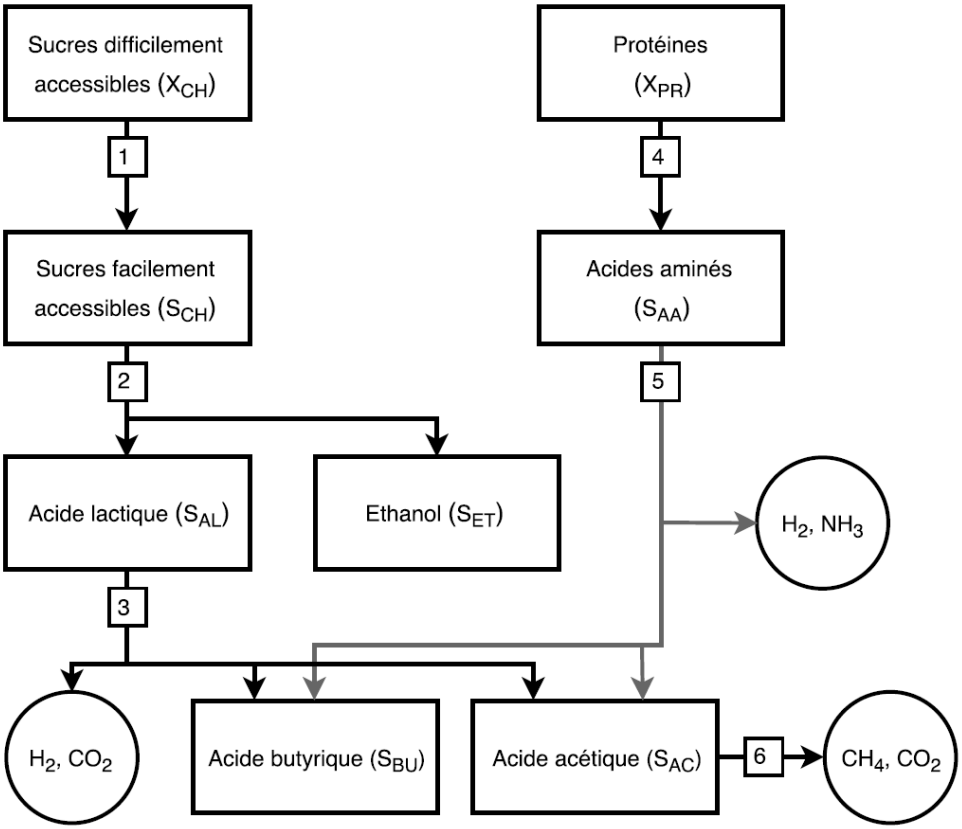

Figure 5-3 – Modèle de la phase anaérobie de l'ensilage. (1) Hydrolyse des sucres ; (2) Fermentation lactique ; (3) Fermentation clostridiale ; (4) Hydrolyse des protéines ; (5) Fermentation protéolytique ; (6) Méthanogenèse.

Dans cette équation, $X_{b,i}$ est la concentration des espèces qui consomment le substrat i, $\mu_{max,b,i}$ le taux de croissance maximum, $Y_{b,i}$ le rendement en biomasse, S_i la concentration en substrat, $K_{S,j}$ la constante de saturation associée au substrat. Le terme d'inhibition $I_{pH,j}$ traduit l'évolution de la vitesse en fonction du pH du milieu pour le processus j. La signification biologique de cette inhibition est liée au fait que la croissance de la biomasse et la consommation du substrat ne se produisent uniquement lorsque le pH de l'ensilage est supérieur à une valeur critique. Cette fonction est représentée par l'équation suivante [73] :

$$I_{pH,j} = \frac{(pH/p_{M,j})^{q,j}}{1 + (pH/p_{M,j})^{q,j}} \tag{5.5}$$

Dans l'équation ci-dessus, $p_{M,j}$ décrit le pH pour lequel l'activité est à moitié du maximum et q est la sensibilité de l'activité autour du p_M, comme illustré dans la Figure 5-4.

Les équations de vitesse pour les processus biochimiques de la phase anaérobie du modèle sont détaillées dans le Tableau 5-4 sous la forme d'une matrice de Petersen, qui présente un bilan DCO implicite. Dans ce tableau, la stœchiométrie des réactions est présentée sous la forme de rendement du produit sur le

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substrat ($f_{produit,substrat}$). De plus, les paires acide/base sont présentées comme la somme des concentrations des formes acide et base (par exemple $S_{IC} = S_{CO2} + S_{HCO3-}$). Etant donné que, ni le carbone inorganique, ni l'azote inorganique ne possèdent de la DCO, ces composés ont été implémentés avec une conversion en base molaire. Par exemple, dans le cas de la fermentation clostridiale, la conversion de carbone organique a été réalisée en sachant que la biodégradation de $1g_{DCO}$ de lactate produit 1/64 moles de carbone inorganique (sans compter le rendement bactérien). Pour l'azote inorganique, une conversion basée sur la teneur en azote des acides aminés (N_{AA}) a été appliquée.

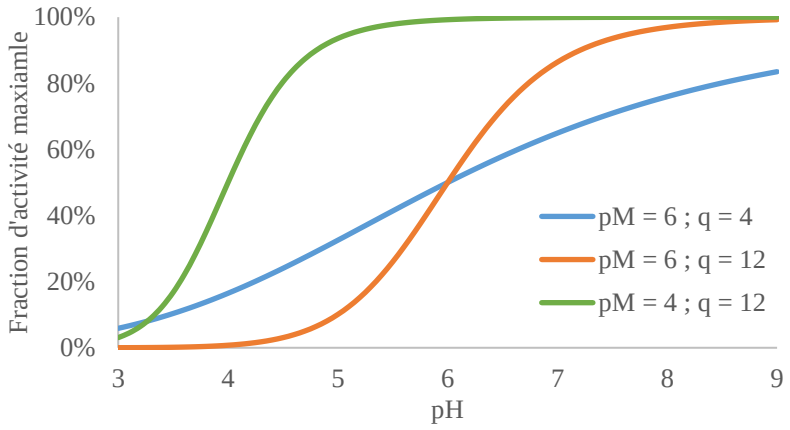

Figure 5-4 – Impact des différents paramètres de la fonction d'inhibition par rapport au pH sur l'activité microbienne maximale.

La matrice présentée dans le Tableau 5-4 comprend plusieurs approximations effectuées de façon à simplifier le système d'équations :

- La fraction des sucres facilement accessibles inclut non seulement des monosaccharides solubles, mais aussi une partie des composés particulaires non-structurels tels que l'amidon ou d'autres oligomères. Les réactions d'hydrolyse de ces derniers ne sont pas considérées comme étant un facteur limitant et, par la suite, sont écartées du modèle. Pour la fraction des sucres difficilement accessibles, nous considérons essentiellement les composés structurels, hémicelluloses et cellulose.
- L'acide acétique est utilisé comme molécule modèle d'autres AGV minoritaires formés pendant le stockage (acides propionique, valérique, caproïque et formique). Cela peut entraîner des différences par rapport aux sous-produits formés et au pH de l'ensilage.
- Chaque fermentation est représentée par une réaction générale, catalysée par une seule population bactérienne. Par exemple, dans le cas de la fermentation lactique, une réaction uniquement homo-fermentaire est utilisée pour la production à la fois d'acide lactique, mais aussi d'éthanol à partir des coefficients stœchiométriques. Cependant, étant donné que la production d'éthanol est théoriquement effectuée par des bactéries hétéro-fermentaires, cela introduit de l'erreur par rapport à la production d'autres sous-produits comme le CO_2. Aussi, pour la consommation de l'acide lactique, la même population clostridiale est utilisée pour la production non seulement d'acide

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butyrique, mais aussi d'acide acétique, de façon à considérer à la fois d'autres AGV minoritaires et une éventuelle réaction successive d'acétogenèse. Cette hypothèse sous-estime les pertes énergétiques dues à la production de H_2 et peut entraîner des écarts par rapport au pH obtenu.

- Des réactions de biodégradation de l'éthanol et de l'acide butyrique produits ne sont pas prises en compte, ce qui peut entraîner des erreurs pour des stockages très peu performants.
- L'azote inorganique consommé pour la croissance bactérienne (assimilation) est supposé négligeable et n'est pas pris en compte pour le bilan azote.
- Etant donné que les silos étaient régulièrement dégazés, l'équilibre gaz-liquide d'un système fermé n'était pas compatible avec les expériences. Cet équilibre n'a pas donc été pris en compte dans le modèle. Cependant, cela a pour conséquence la solubilisation totale des composés qui pourraient se volatiliser, comme le CO_2 dissous. Cette question est abordée en détail dans la partie 5.2.5. Implémentation.

5.2.4. Processus physico-chimiques

Les équilibres chimiques acide-base ont été utilisés dans ce modèle de façon à déterminer le pH de l'ensilage. Ces réactions constituent un jeu d'équations algébriques qui sont intégrées dans le modèle par un bilan de charges.

Pour que les phénomènes acide-base puissent être modélisés, comme des équations algébriques implicites, pour chaque réaction la somme des concentrations des paires acide-base a été considérée comme une variable d'état dynamique, comme illustré dans les équations suivantes :

$$S_{AL} - S_{AL-} - S_{HAL} = 0 \tag{5.6}$$

$$S_{ET} - S_{ET-} - S_{HET} = 0 \tag{5.7}$$

$$S_{AB} - S_{AB-} - S_{HAB} = 0 \tag{5.8}$$

$$S_{AC} - S_{AC-} - S_{HAC} = 0 \tag{5.9}$$

$$S_{IC} - S_{HCO3-} - S_{CO2} = 0 \tag{5.10}$$

$$S_{IN} - S_{NH3} - S_{NH4+} = 0 \tag{5.11}$$

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Tableau 5-4 – Vitesses et coefficients cinétiques pour les composés intervenant dans les réactions biochimiques.

Composante (i) ➔		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**Vitesses, v_j (kgDCO.m^{-3}j^{-1})**
(j) Processus 🡻		X_{CH}	S_{CH}	S_{AL}	S_{ET}	X_{BL}	S_{AB}	S_{AC}	S_{H2}	S_{IC}	X_{BC}	X_{PR}	S_{AA}	S_{IN}	X_{BP}	S_{CH4}	X_{BM}	
1	Hydrolyse des sucres	-1	1															$k_1 X_{CH}$
2	Fermentation lactique		-1	(1-Y_{BL}) x $f_{AL,CH}$	(1-Y_{BL}) x $f_{ET,CH}$	Y_{BL}												$\frac{X_{BL}\mu_{max,BL}}{Y_{BL}} \frac{S_{CH}}{K_{S2}+S_{CH}} \frac{(pH/p_{M2})^{q2}}{1+(pH/p_{M2})^{q2}}$
3	Fermentation clostridiale			-1			(1-Y_{BC}) x $f_{AB,AL}$	(1-Y_{BC}) x $f_{AC,AL}$	(1-Y_{BC}) x $f_{H2,AL}$	(1-Y_{BC}) / 96	Y_{BC}							$\frac{X_{BC}\mu_{max,BC}}{Y_{BC}} \frac{S_{AL}}{K_{S3}+S_{AL}} \frac{(pH/p_{M3})^{q3}}{1+(pH/p_{M3})^{q3}}$
4	Hydrolyse des protéines											-1	1					$k_4 X_{PR}$
5	Fermentation protéolytique						(1-Y_{BP}) x $f_{AB,AA}$	(1-Y_{BP}) x $f_{AC,AA}$	(1-Y_{BP}) x $f_{H2,AA}$				-1	N_{AA}	Y_{BP}			$\frac{X_{BP}\mu_{max,BP}}{Y_{BP}} \frac{S_{AA}}{K_{S5}+S_{AA}} \frac{(pH/p_{M5})^{q5}}{1+(pH/p_{M5})^{q5}}$
6	Méthanogenèse							-1		(1-Y_{BM}) / 64						(1-Y_{BM})	Y_{BM}	$\frac{X_{BM}\mu_{max,BM}}{Y_{BM}} \frac{S_{AC}}{K_{S6}+S_{AC}} \frac{(pH/p_{M6})^{q6}}{1+(pH/p_{M6})^{q6}}$
		Sucres difficilement accessibles (kgDCO.m^{-3})	Sucres facilement accessibles (kgDCO.m^{-3})	Acide lactique (kgDCO.m^{-3})	Ethanol (kgDCO.m^{-3})	Bactéries lactiques (kgDCO.m^{-3})	Acide butyrique (kgDCO.m^{-3})	Acide acétique (kgDCO.m^{-3})	Gaz hydrogène (kgDCO.m^{-3})	Carbone inorganique (kmol.m^{-3})	Bactéries clostridiales (kgDCO.m^{-3})	Protéines (kgDCO.m^{-3})	Acides aminés (kgDCO.m^{-3})	Azote inorganique (kmol.m^{-3})	Bactéries protéolytiques (kgDCO.m^{-3})	Gaz méthane (kgDCO.m^{-3})	Bactéries méthanogènes (kgDCO.m^{-3})	

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Où, par exemple dans l'équation 5.6, S_{AL} est la combinaison du paire acide-base, S_{HAL} est la forme associée et S_{AL-} est la forme dissociée. Ainsi, ces équations algébriques ont pu être formulées en tenant en compte les équilibres acide-base et les constantes d'équilibre (Tableau 5-5) respectives :

$$S_{AL-} - \frac{K_{a,AL} S_{AL}}{K_{a,AL} + S_{H+}} = 0 \tag{5.12}$$

$$S_{ET-} - \frac{K_{a,ET} S_{ET}}{K_{a,ET} + S_{H+}} = 0 \tag{5.13}$$

$$S_{AB-} - \frac{K_{a,AB} S_{AB}}{K_{a,AB} + S_{H+}} = 0 \tag{5.14}$$

$$S_{AC-} - \frac{K_{a,AC} S_{AC}}{K_{a,AC} + S_{H+}} = 0 \tag{5.15}$$

$$S_{HCO3-} - \frac{K_{a,CO2} S_{IC}}{K_{a,CO2} + S_{H+}} = 0 \tag{5.16}$$

$$S_{NH4+} - \frac{S_{H+} S_{IN}}{K_{a,NH4+} + S_{H+}} = 0 \tag{5.17}$$

L'équilibre ionique de l'eau est également décrit par la relation suivante :

$$S_{OH-} - \frac{K_{a,H_20}}{S_{H+}} = 0 \tag{5.18}$$

Tableau 5-5 – Constantes d'équilibre acide-base (298 K) [107].

Paire Acide / Base	**pK_a**	**K_a**
CO_2 / HCO_3^-	6.35	4.47×10^{-7}
ET / ET^-	15.5	3.16×10^{-16}
H_2O / (OH^- + H^+)	13.995	1.01×10^{-14}
HAB / AB^-	4.83	1.48×10^{-5}
HAC / AC^-	4.756	1.75×10^{-5}
HAL / AL^-	3.86	1.38×10^{-4}
NH_4^+ / NH_3	9.25	5.62×10^{-10}

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Ensuite, la concentration des ions H^+ et le pH sont calculés à partir d'un bilan des charges présentes dans l'ensilage :

$$\sum S_{C^+} - \sum S_{A^-} = 0 \tag{5.19}$$

Où $\sum S_{C^+}$ et $\sum S_{A^-}$ désignent les concentrations totales d'équivalents cationiques et anioniques, respectivement. La concentration d'équivalents pour chaque composé est égale à la valence de l'ion multipliée par sa concentration molaire. Pour que les variables exprimés en DCO soient intégrés, une conversion de sa teneur en grammes de DCO par charge est appliquée dans le dénominateur de l'équation.

Le bilan de charges avec tous les ions utilisés dans le modèle est représenté par l'équation suivante :

$$S_{TP+} + S_{NH4+} + S_{H+} - S_{HCO3-} - \frac{S_{AL-}}{96} - \frac{S_{AB-}}{160} - \frac{S_{AC-}}{64} - \frac{S_{ET-}}{96} - S_{OH-} - S_{TP-} = 0 \tag{5.20}$$

Dans l'équation ci-dessus S_{TP+} et S_{TP-} sont les concentrations en acide et base, respectivement, des composés tampon de la biomasse. Ces composés représentent des acides et bases présents dans la biomasse initiale qui n'ont pas de termes de consommation ou production et, par la suite, sont supposés constants tout au long du stockage. Ces termes peuvent aussi inclure d'autres acides organiques non mesurés ou des protéines et produits dérivés, en supposant qu'ils ne sont pas dégradés au cours de l'ensilage.

Pour le calcul des concentrations de composés basiques et acides du tampon, l'approche de Neal and Thornley [73] a été utilisée. Cette hypothèse considère que l'association et la dissociation sont des propriétés mutuellement exclusives du tampon, telle que la différence $S_{TP-} - S_{TP+}$ peut être décrite par l'équation ci-dessous :

$$S_{TP-} - S_{TP+} = \frac{S_{TP}\left(K_{a,TP}\, K_{b,TP} - {S_{H+}}^2\right)}{\left(S_{H+} + K_{a,TP}\right)\left(S_{H+} + K_{b,TP}\right) - S_{H+} K_{a,TP}} \tag{5.21}$$

Où S_{TP} désigne la concentration molaire initiale des composés tampon, $K_{a,TP}$ la constante de dissociation des composés acides du tampon et $K_{b,TP}$ la constante d'association des composés basiques du tampon.

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5.2.5. Implémentation

5.2.5.1. Modèle séquentiel

Afin de pouvoir utiliser le bilan de charges pour la résolution du modèle de la phase anaérobie, deux étapes préalables ont été effectuées, à savoir :

- L'estimation des propriétés des composés tampon ;
- La détermination du pH après la phase aérobie (respiration).

Selon l'équation 5.21, les propriétés du tampon sont définies par sa concentration initiale et par ses constantes de dissociation/association. Afin de réduire le nombre de variables à estimer, l'hypothèse de Neal and Thornley [73] de considérer $K_{a,TP} = K_{b,TP}$ a été effectuée. Ensuite, la différence $S_{TP-} - S_{TP+}$ a été calculée avec le bilan de charges et les valeurs initiales pour les variables algébriques et dynamiques du modèle. Cela a permis d'avoir plusieurs estimations de S_{TP} et $K_{a,TP}/K_{b,TP}$. Finalement, ces paramètres ont été raffinés en utilisant le bilan de charges pour simuler la descente de pH liée à la production d'acide lactique jusqu'au premier point expérimental en ensilage (7 jours). Cette approche peut aussi être considérée comme une mesure indirecte de la capacité tampon de l'ensilage, laquelle est souvent estimée comme étant la quantité de base nécessaire pour, par exemple, faire varier le pH de 4 à 6.

Pour la détermination du pH après respiration aérobie, le bilan de charges avec les propriétés du tampon précédemment estimées a été utilisé. Le seul changement effectué a été de considérer la montée de la teneur de CO_2 dans le ciel gazeux de 0.038%(V/V) (S_{IC} = 1.35 x 10^{-5} kmol.m^{-3}) initialement jusqu'à 21%(V/V) (S_{IC} = 7.45 x 10^{-3} kmol.m^{-3}) après respiration. La dernière concentration est calculée en considérant la conversion exclusive d'O_2 en CO_2 sous une pression de gaz constante.

Après ces calculs préliminaires, la phase anaérobie de l'ensilage a été modélisée sur MATLAB. Une implémentation « DAE » (*Differential and Algebraic Equation*) a été utilisée pour que les processus physico-chimiques puissent être intégrés algébriquement. Puisque l'ensilage est un procédé en mode discontinu (*batch)*, le modèle a été implémenté dans la configuration suivante :

- 16 équations différentielles pour représenter les variables d'état dynamique :

$$\frac{dX_{CH}}{dt} = -k_1 X_{CH} \tag{5.22}$$

$$\frac{dS_{CH}}{dt} = k_1 X_{CH} - \frac{X_{BL}\mu_{max,BL}}{Y_{BL}} \frac{S_{CH}}{K_{S2} + S_{CH}} \frac{(pH/p_{M2})^{q2}}{1 + (pH/p_{M2})^{q2}} \tag{5.23}$$

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$$\frac{dS_{AL}}{dt} = f_{AL,CH}(1 - Y_{BL})\frac{X_{BL}\mu_{max,BL}}{Y_{BL}}\frac{S_{CH}}{K_{S2} + S_{CH}}\frac{(pH/p_{M2})^{q2}}{1 + (pH/p_{M2})^{q2}} - \frac{X_{BC}\mu_{max,BC}}{Y_{BC}}\frac{S_{AL}}{K_{S3} + S_{AL}}\frac{(pH/p_{M3})^{q3}}{1 + (pH/p_{M3})^{q3}} \tag{5.24}$$

$$\frac{dS_{ET}}{dt} = f_{ET,CH}(1 - Y_{BL})\frac{X_{BL}\mu_{max,BL}}{Y_{BL}}\frac{S_{CH}}{K_{S2} + S_{CH}}\frac{(pH/p_{M2})^{q2}}{1 + (pH/p_{M2})^{q2}} \tag{5.25}$$

$$\frac{dX_{BL}}{dt} = X_{BL}\mu_{max,BL}\frac{S_{CH}}{K_{S2} + S_{CH}}\frac{(pH/p_{M2})^{q2}}{1 + (pH/p_{M2})^{q2}} \tag{5.26}$$

$$\frac{dS_{AB}}{dt} = f_{AB,AL}(1 - Y_{BC})\frac{X_{BC}\mu_{max,BC}}{Y_{BC}}\frac{S_{AL}}{K_{S3} + S_{AL}}\frac{(pH/p_{M3})^{q3}}{1 + (pH/p_{M3})^{q3}} + f_{AB,AA}(1 - Y_{BP})\frac{X_{BP}\mu_{max,BP}}{Y_{BP}}\frac{S_{AA}}{K_{S5} + S_{AA}}\frac{(pH/p_{M5})^{q5}}{1 + (pH/p_{M5})^{q5}} \tag{5.27}$$

$$\frac{dS_{AC}}{dt} = f_{AC,AL}(1 - Y_{BC})\frac{X_{BC}\mu_{max,BC}}{Y_{BC}}\frac{S_{AL}}{K_{S3} + S_{AL}}\frac{(pH/p_{M3})^{q3}}{1 + (pH/p_{M3})^{q3}} + f_{AC,AA}(1 - Y_{BP})\frac{X_{BP}\mu_{max,BP}}{Y_{BP}}\frac{S_{AA}}{K_{S5} + S_{AA}}\frac{(pH/p_{M5})^{q5}}{1 + (pH/p_{M5})^{q5}} - \frac{X_{BM}\mu_{max,BM}}{Y_{BM}}\frac{S_{AC}}{K_{S6} + S_{AC}}\frac{(pH/p_{M6})^{q6}}{1 + (pH/p_{M6})^{q6}} \tag{5.28}$$

$$\frac{dS_{H2}}{dt} = f_{H2,AL}(1 - Y_{BC})\frac{X_{BC}\mu_{max,BC}}{Y_{BC}}\frac{S_{AL}}{K_{S3} + S_{AL}}\frac{(pH/p_{M3})^{q3}}{1 + (pH/p_{M3})^{q3}} + f_{H2,AA}(1 - Y_{BP})\frac{X_{BP}\mu_{max,BP}}{Y_{BP}}\frac{S_{AA}}{K_{S5} + S_{AA}}\frac{(pH/p_{M5})^{q5}}{1 + (pH/p_{M5})^{q5}} \tag{5.29}$$

$$\frac{dS_{IC}}{dt} = \frac{(1 - Y_{BC})}{96}\frac{X_{BC}\mu_{max,BC}}{Y_{BC}}\frac{S_{AL}}{K_{S3} + S_{AL}}\frac{(pH/p_{M3})^{q3}}{1 + (pH/p_{M3})^{q3}} + \frac{(1 - Y_{BM})}{64}\frac{X_{BM}\mu_{max,BM}}{Y_{BM}}\frac{S_{AC}}{K_{S6} + S_{AC}}\frac{(pH/p_{M6})^{q6}}{1 + (pH/p_{M6})^{q6}} \tag{5.30}$$

$$\frac{dX_{BC}}{dt} = X_{BC}\mu_{max,BC}\frac{S_{AL}}{K_{S3} + S_{AL}}\frac{(pH/p_{M3})^{q3}}{1 + (pH/p_{M3})^{q3}} \tag{5.31}$$

$$\frac{dX_{PR}}{dt} = -k_4 X_{PR} \tag{5.32}$$

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$$\frac{dS_{AA}}{dt} = k_4 X_{PR} - \frac{X_{BP}\mu_{max,BP}}{Y_{BP}} \frac{S_{AA}}{K_{S5} + S_{AA}} \frac{(pH/p_{M5})^{q5}}{1 + (pH/p_{M5})^{q5}} \tag{5.33}$$

$$\frac{dS_{IN}}{dt} = N_{AA} \frac{X_{BP}\mu_{max,BP}}{Y_{BP}} \frac{S_{AA}}{K_{S5} + S_{AA}} \frac{(pH/p_{M5})^{q5}}{1 + (pH/p_{M5})^{q5}} \tag{5.34}$$

$$\frac{dX_{BP}}{dt} = X_{BP}\mu_{max,BP} \frac{S_{AA}}{K_{S5} + S_{AA}} \frac{(pH/p_{M5})^{q5}}{1 + (pH/p_{M5})^{q5}} \tag{5.35}$$

$$\frac{dS_{CH4}}{dt} = (1 - Y_{BM}) \frac{X_{BM}\mu_{max,BM}}{Y_{BM}} \frac{S_{AC}}{K_{S6} + S_{AC}} \frac{(pH/p_{M6})^{q6}}{1 + (pH/p_{M6})^{q6}} \tag{5.36}$$

$$\frac{dX_{BM}}{dt} = X_{BM}\mu_{max,BM} \frac{S_{AC}}{K_{S6} + S_{AC}} \frac{(pH/p_{M6})^{q6}}{1 + (pH/p_{M6})^{q6}} \tag{5.37}$$

- 1 équation algébrique qui rassemble tous les phénomènes physico-chimiques pour le calcul du pH :

$$\begin{aligned} S_{H+} + \frac{S_{H+}S_{IN}}{K_{a,NH4+} + S_{H+}} - \frac{K_{a,CO2}S_{IC}}{K_{a,CO2} + S_{H+}} - \frac{K_{a,AL}S_{AL}}{(K_{a,AL} + S_{H+}) \times 96} - \frac{K_{a,AB}S_{AB}}{(K_{a,AB} + S_{H+}) \times 160} \\ - \frac{K_{a,AC}S_{AC}}{(K_{a,AC} + S_{H+}) \times 64} - \frac{K_{a,ET}S_{ET}}{(K_{a,ET} + S_{H+}) \times 96} - \frac{K_{a,H_20}}{S_{H+}} \\ - \frac{S_{TP}(K_{a,TP}\,K_{b,TP} - {S_{H+}}^2)}{(S_{H+} + K_{a,TP})(S_{H+} + K_{b,TP}) - S_{H+}K_{a,TP}} = 0 \end{aligned} \tag{5.38}$$

Etant donné que ce modèle anaérobie ne prend pas en compte l'équilibre liquide-gaz, la résolution de l'équation 5.30 a l'inconvénient de considérer que tout le carbone inorganique produit reste solubilisé. Pour éviter des impacts importants de cette approximation sur le pH, nous avons supposé que la pression partielle de CO_2 dans la phase gazeuse ne peut pas dépasser 1 bar, ce qui correspond à un S_{IC} maximale de 3.50 x 10^{-2} kmol.m^{-3}. Cela a été intégré dans le modèle par la condition suivante :

$$\frac{dS_{IC}}{dt} = \begin{cases} Eq.5.30, & S_{IC} < 0.035\ kmol.m^{-3} \\ 0, & S_{IC} \geq 0.035\ kmol.m^{-3} \end{cases} \tag{5.39}$$

Même si cette hypothèse permet de résoudre la question de la quantité maximale de carbone inorganique dissous, sa vitesse d'accumulation dans la phase liquide sera supérieure dans le modèle à celle de la réalité. Néanmoins, cette différence de vitesses a été négligée, étant donné que le volume de ciel gazeux dans les silos est réduit et, par suite, la concentration de CO_2 dans le gaz monte rapidement dans premières semaines d'ensilage.

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5.2.5.2. Variables initiales et propriétés cinétiques : lien avec les données expérimentales

Afin de mettre le modèle anaérobie en place, les variables initiales ont été estimées par rapport aux mesures expérimentales issues de la caractérisation, de la façon suivante :

- X_{CH} *et* X_{PR} : La DCO de la phase particulaire a été mesurée, ainsi que sa fraction biodégradable (à partir des essais PBM). Cependant, ces analyses ne permettent pas de distinguer chaque fraction biodégradable de la DCO particulaire. Par suite, il a été supposé que toute la DCO de la phase particulaire correspondait à des protéines et sucres biodégradables. Il s'agit certainement d'une hypothèse erronée, mais qui n'a pas d'incidence pratique : au cours de l'ensilage, la fraction particulaire bio-accessible n'est jamais complètement métabolisée. Ainsi, X_{PR} a été estimé à partir de la mesure du $NTK_{particulaire}$ (en utilisant le paramètre N_{AA}) et le X_{CH} a été déterminé par la différence entre la $DCO_{particulaire}$ et le X_{PR}. Etant donné que, dans la plupart des essais d'ensilage il y a une solubilisation du particulaire facilement accessible au début du stockage, les X_{CH} et X_{PR} initiaux ont été déterminés par l'extrapolation de la tendance d'hydrolyse pour les points expérimentaux à partir de 7 jours d'ensilage. Ce calcul ne doit cependant pas être utilisé lorsqu'une partie de la DCO biodégradable est constituée de graisses, peu solubles dans l'eau : dans ces conditions, une détermination expérimentale des composés lipidiques doit être mise en œuvre. Néanmoins, les biomasses étudiées ici sont très pauvres en graisses.
- S_{CH} : La détermination de cette variable n'est pas possible expérimentalement dans sa totalité, en raison du fait que, pour ce paramètre, seuls les monosaccharides ont été analysés par CLHP. Pour cette raison, le S_{CH} initial a été estimé comme étant la quantité nécessaire de sucres pour la formation des AGV dans la première semaine d'ensilage. Pour tous les autres points, il a été présumé que seuls les sucres solubles analysés restaient dans la fraction S_{CH}.
- S_{AL}, S_{ET}, S_{AB}, S_{AC} : Calculés à partir des données obtenues en CLHP et chromatographie en phase gazeuse, après conversion en DCO. Il est important de souligner que S_{AC} comprend la DCO des acides acétique, propionique, valérique, caproïque et formique.
- S_{AA} : Ce paramètre ne peut pas être comparable de manière fiable aux résultats de l'azote organique soluble ($NTK_{soluble}$- NH_3-N), étant donné qu'il a été observé qu'une partie des acides aminés présents ne sont pas dégradables en conditions d'ensilage. Par conséquent, l'évolution de cette variable n'a pas été comparée aux résultats expérimentaux. Sa valeur initiale a été estimée comme étant la quantité nécessaire pour simuler les augmentations rapides de la concentration d'azote inorganique (S_{IN}) pendant l'ensilage, lesquels se produisent habituellement au cours des premières semaines.
- S_{IN} : Calculé à partir des analyses de l'ammonium (NH_3-N) en phase liquide.

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- S_{IC}, S_{H2} et S_{CH4} : Initialement, S_{IC} a été calculé à partir de la teneur en CO_2 gazeux après respiration, tandis que pour S_{H2} et S_{CH4} il a été assumé une concentration nulle. En revanche, les résultats de sortie du modèle n'ont pas été comparés aux résultats expérimentaux, compte tenu de l'insuffisance de mesures de composition de gaz effectuées.
- X_{BL}, X_{BC}, X_{BP}, X_{BM} : Aucune des mesures réalisées ne permet une détermination quantitative de la concentration des populations bactériennes dans la biomasse. Par conséquent, ces variables ont été estimées à partir de données bibliographiques. En effet, la valeur de 0.1 $kg_{biomasse}.m^{-3}$ estimée par Neal and Thornley [73] pour les bactéries lactiques a été utilisée pour toutes les populations bactériennes dans la matière fraîche de chaque matrice testée pour ce modèle. Cette valeur a été convertie en DCO en utilisant la formule empirique pour la biomasse $C_5H_7O_2N$ proposée par le modèle ADM1 [95] (M = 113 kg/kmol ; DCO = 160 kg/kmol).
- S_{H+} (pH): Pour le point de départ, le pH après respiration a été pris. Ensuite, les résultats du modèle ont été comparés au pH mesuré expérimentalement.

Concernant les paramètres stœchiométriques et cinétiques, lorsque cela était possible, des valeurs de la bibliographie [73,95] ont été prises et ensuite optimisées pendant les simulations. Cela a été le cas des rendements du produit sur le substrat ($f_{produit,substrat}$), des paramètres $p_{M,j}$ et q_j pour les fonctions d'inhibition par rapport au pH et des taux de croissance maximales de Monod ($\mu_{max,b}$). Pour la teneur en azote des protéines et acides aminés (N_{AA}), la valeur de 0.007 $kmol.kgDCO^{-1}$ suggérée par le modèle ADM1 [95] a été utilisé. De même, les rendements de la biomasse ont été estimés à partir des données de la littérature ([73] pour Y_{BL}, Y_{BC} et Y_{BP}; [95] pour Y_{BM}). En revanche, vu que les constantes cinétiques d'hydrolyse (k_j) en ensilage (conditions de conservation) ne peuvent pas être comparées à celles utilisées pour simuler la méthanisation (conditions de production de biogaz), leurs valeurs ont été estimées à partir de la vitesse de biodégradation des fractions particulaires X_{CH} et X_{PR} expérimentales au cours du stockage. Finalement, selon la littérature [73,108], les valeurs de demi-saturation pour les cinétiques de Monod sont très faibles et, par conséquent, ont des impacts négligeables dans les modèles. Dans le cas présent, une valeur de 0.1 $kgDCO.m^{-3}$ a été utilisée pour toutes les réactions où ce paramètre intervient, de façon à négliger l'effet de saturation mais, en même temps, empêchant mathématiquement que les fermentations continuent sans substrat présent.

5.2.6. Simulations

Les simulations ont été effectuées pour les variables d'entrée des expériences avec les ressources CIVE 2 et Fumier 2, afin de calibrer et optimiser les paramètres stœchiométriques et cinétiques du modèle. Cela comprend des biomasses avec différentes teneurs en matière sèche, sucres solubles/accessibles et pH initial, dont les propriétés sont résumées dans le Tableau 5-6. Etant donné que le modèle n'est pas conçu pour des quantités importantes d'acide formique, le co-ensilage du Fumier 2 avec acide formique

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n'a pas été testé. Les simulations ont été exécutées jusqu'aux durées finales des essais de stockage, à savoir 98 jours pour la CIVE 2 et 120 jours pour le Fumier 2.

Tableau 5-6 – Propriétés initiales des gisements utilisés pour les simulations, ainsi que les estimations de S_{CH}.

Gisement	Condition	pH	MS (%)	Sucres solubles (kgDCO.m^{-3})	S_{CH} (kgDCO.m^{-3})
CIVE 2	TS10%E	6.35	10.1	12.0	44.0
	TS14%E	6.17	14.1	18.3	48.0
	TS39%E	5.82	38.7	31.8	74.0
Fumier 2	Control	8.26	19.2	0	9.0
	Glucose 4%	7.91	22.3	45.2	76.2
	Glucose 10%	7.91	24.9	129.0	170.0

5.2.6.1. Fractions difficilement accessibles

Les résultats des simulations et estimations pour le particulaire difficilement accessible (X_{CH} et X_{PR}) sont présentés sur la Figure 5-5 pour la CIVE 2 et sur la Figure 5-6 pour le Fumier 2. La majorité des données estimées a suivi une lente dégradation de premier ordre pendant l'ensilage et par conséquent, le modèle a pu être correctement ajusté aux variables X_{CH} et X_{PR}. Néanmoins, pour certaines conditions – TS14%E (CIVE 2) et Glucose 4% (Fumier 2) – aucune hydrolyse du X_{CH} n'a été observée et des constantes cinétiques de valeur zéro ont été attribuées. Les approximations effectuées pour estimer la fraction des sucres difficilement accessibles doivent être à l'origine de cette non-détection de l'hydrolyse.

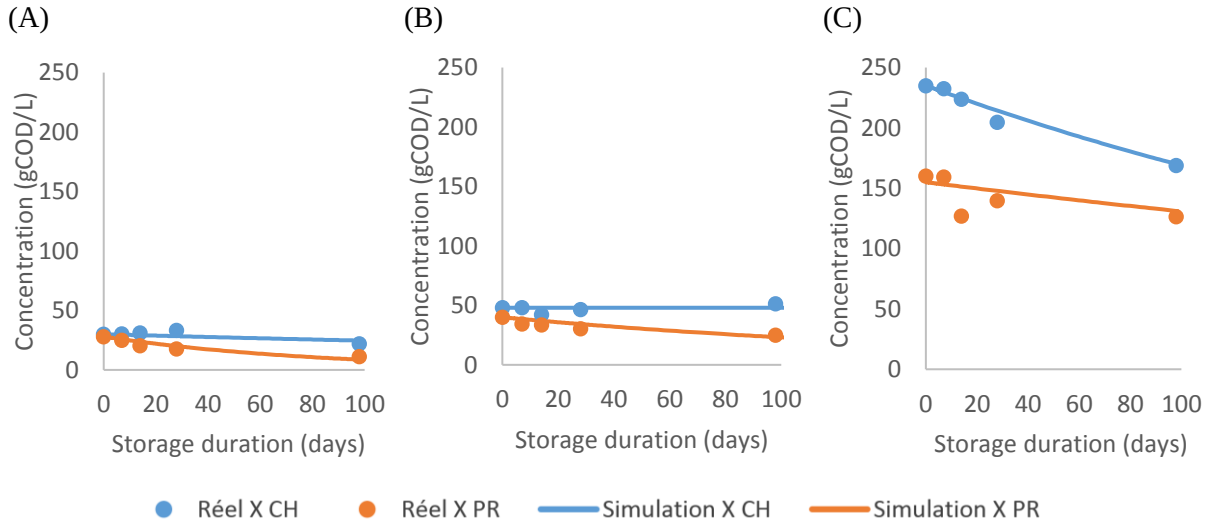

Figure 5-5 – Simulations et données expérimentales estimées pour l'évolution des fractions difficilement accessibles (X_{CH} et X_{PR}) de la CIVE 2. (A) TS10%E ; (B) TS14%E ; (C) TS39%E.

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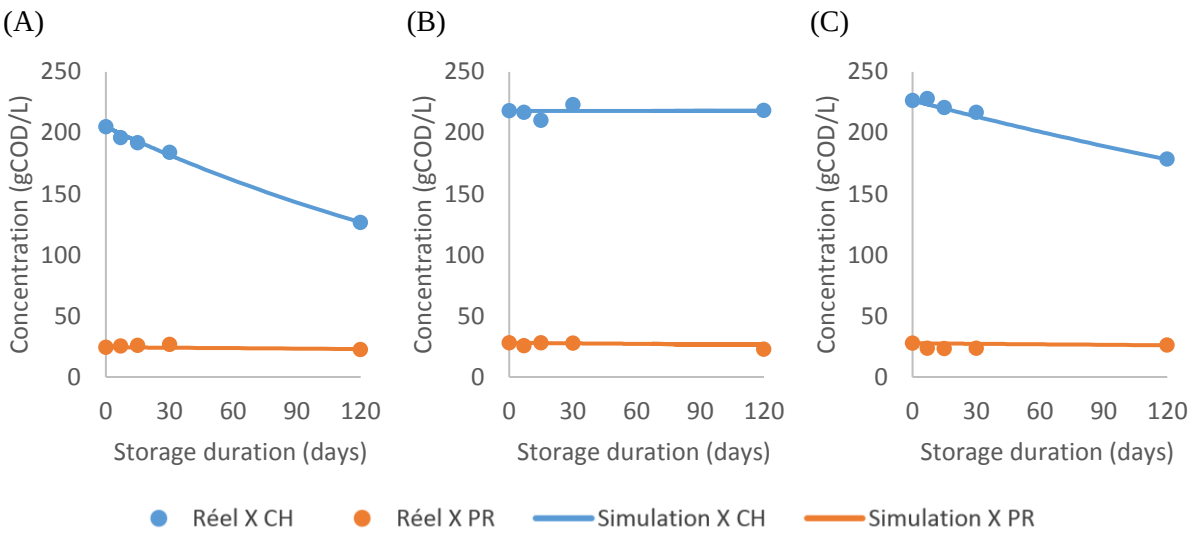

Figure 5-6 – Simulations et données expérimentales estimées pour l'évolution des fractions difficilement accessibles (X_{CH} et X_{PR}) du Fumier 2. (A) Control ; (B) Glucose 4% ; (C) Glucose 10%.

5.2.6.2. Fractions facilement accessibles

Les tendances observées expérimentalement pour la DCO facilement accessible (S_{CH}, S_{AL}, S_{AB}, S_{AC} et S_{ET}) pour des ensilages de CIVE 2 (Figure 5-7) e de Fumier 2 (Figure 5-8) sont, dans sa majorité, intégralement décrites par le modèle après l'optimisation des paramètres cinétiques et stœchiométriques.

Malgré tout, pour certaines variables il y a un écart entre l'évolution des prévisions du modèle et les estimations expérimentales. En effet, pour la simulation de la condition Glucose 10% des essais avec le Fumier 2, nous observons une accumulation des sucres facilement accessibles, en raison de l'hydrolyse de la fraction structurelle. Cependant, cette accumulation n'a pas été observée expérimentalement. Etant donné que le S_{CH} mesuré pendant l'ensilage comprend seulement les sucres solubles, cet écart indique que les sucres produits pendant l'hydrolyse resteront dans la partie particulaire accessible. Par ailleurs, l'éthanol produit pour les ensilages de CIVE 2 est dégradé dans le long terme, ce qui n'est pas comptabilisé dans les simulations, vu que cette réaction n'a pas été prise en compte dans la conception du modèle. Finalement, pour la condition d'ensilage la moins performante – essais Control avec le Fumier 2 – il a été observé expérimentalement une dégradation totale de composés organiques facilement biodégradables. C'est le cas par exemple pour l'acide butyrique, qui ne s'accumule pas au cours du stockage car il est rapidement dégradé. Néanmoins, les réactions d'acétogenèse n'ont pas été introduites dans le modèle et par suite, ce phénomène ne peut pas être décrit par les simulations.

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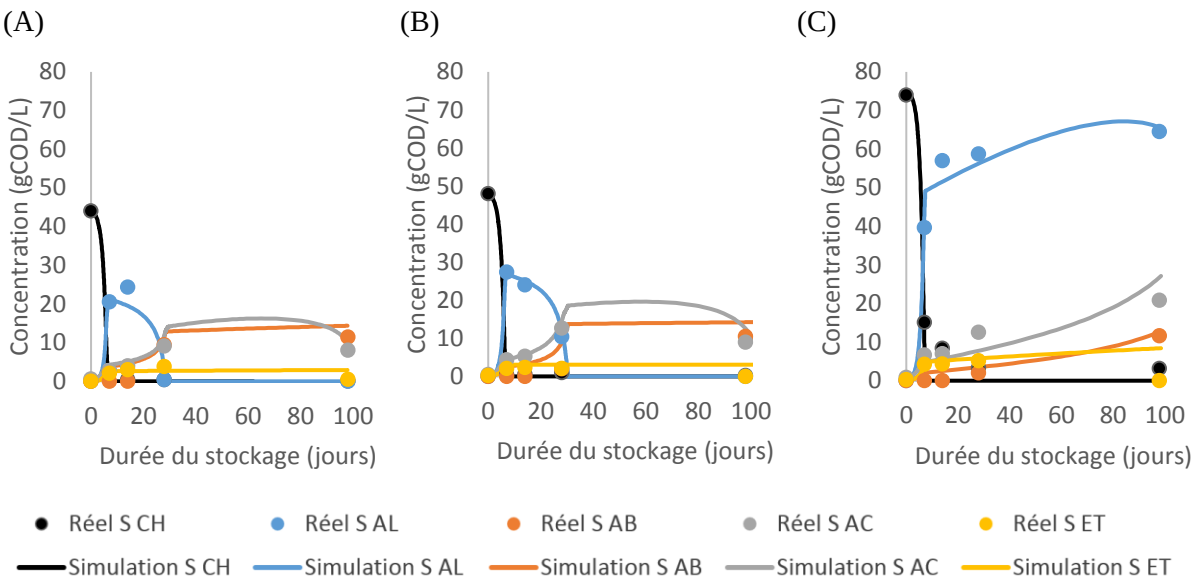

Figure 5-7 – Simulations et données expérimentales estimées pour l'évolution des fractions facilement accessibles (S_{CH}, S_{AL}, S_{ET}, S_{AB} et S_{AC}) de la CIVE 2. (A) TS10%E ; (B) TS14%E ; (C) TS39%E.

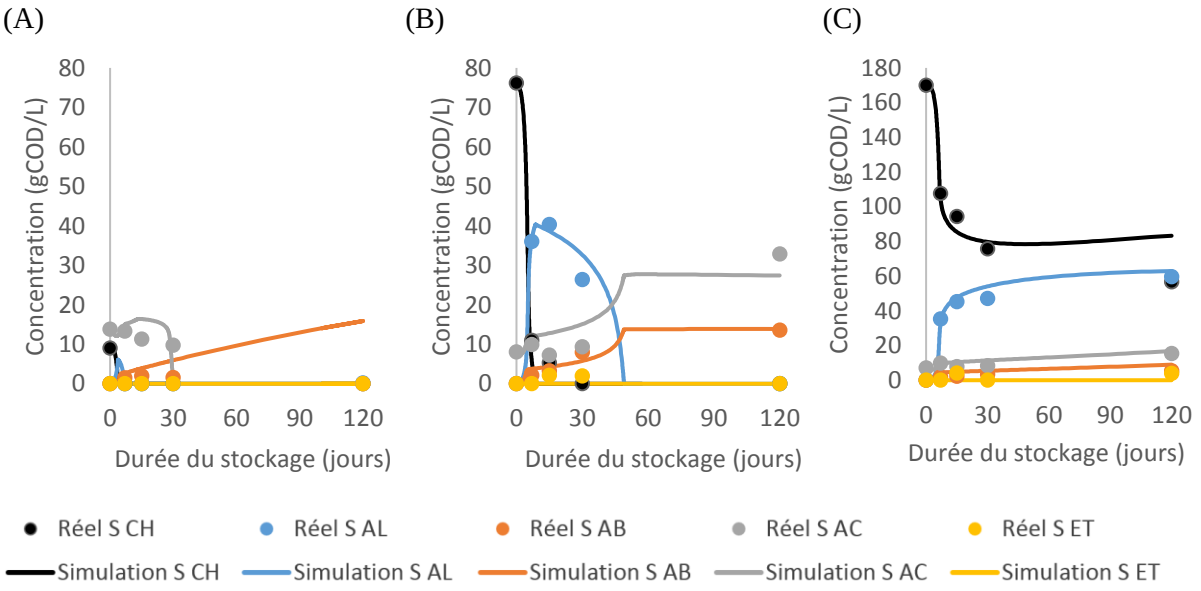

Figure 5-8 – Simulations et données expérimentales estimées pour l'évolution des fractions facilement accessibles (S_{CH}, S_{AL}, S_{ET}, S_{AB} et S_{AC}) du Fumier 2. (A) Control ; (B) Glucose 4% ; (C) Glucose 10% (présenté sur une échelle plus large).

5.2.6.3. Azote inorganique

Pour la majorité des essais ce modèle permet la représentation des variations d'azote inorganique pendant l'ensilage pour une teneur donnée d'acides aminés accessibles. D'une part, les résultats des simulations suggèrent qu'il y a une grande quantité de S_{AA} dans la CIVE 2, qui est dégradée en S_{IN} (Figure 5-9) lorsqu'il n'y a pas une forte acidité. D'autre part, pour le Fumier 2 (Figure 5-10), il y a une absence d'acides aminés accessibles et c'est la minéralisation lente des protéines qui conduit à la légère

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augmentation d'azote inorganique durant le stockage. De plus, pour l'essai Control avec le Fumier 2 il y a eu expérimentalement une chute de la teneur en azote inorganique entre 30 et 120 jours d'ensilage, alors que le S_{IN} du modèle a légèrement augmenté. Cette discordance peut être expliquée par un phénomène de volatilisation et perte d'une partie de l'azote inorganique (compte-tenu du pH élevé), lequel n'a pas été pris en compte dans le modèle. Enfin, la seule condition qui n'a pas pu être correctement modélisée pour l'azote inorganique a été la TS39%E avec CIVE 2, parce qu'il y a eu une énorme augmentation de la teneur en S_{IN} dans les premiers 7 jours, qui ne peut pas être seulement être due aux phénomènes intégrés dans le modèle.

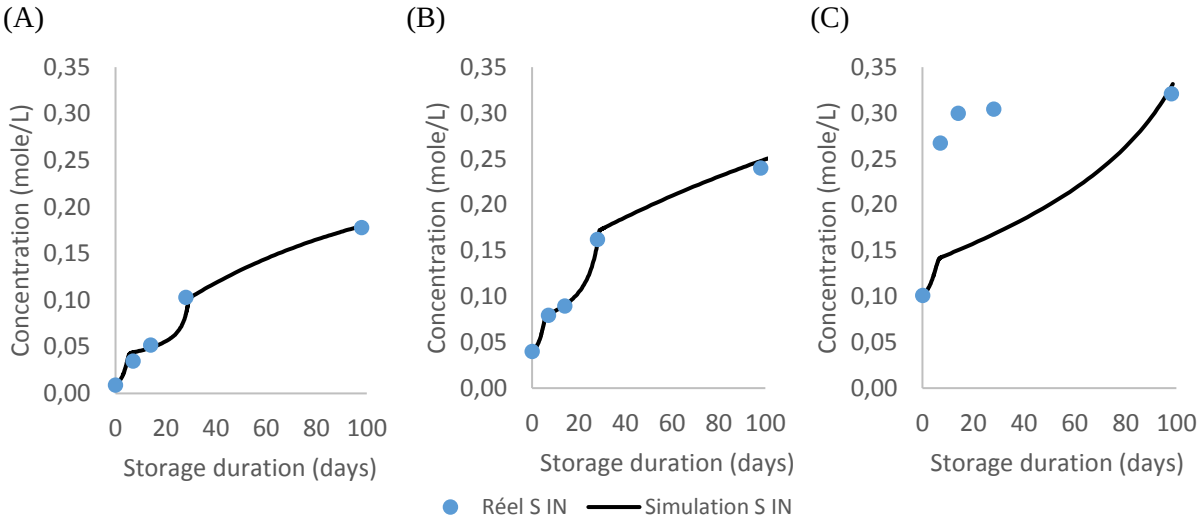

Figure 5-9 – Simulations et données expérimentales estimées pour l'évolution de l'azote inorganique (S_{IN},) de la CIVE 2. (A) TS10%E ; (B) TS14%E ; (C) TS39%E.

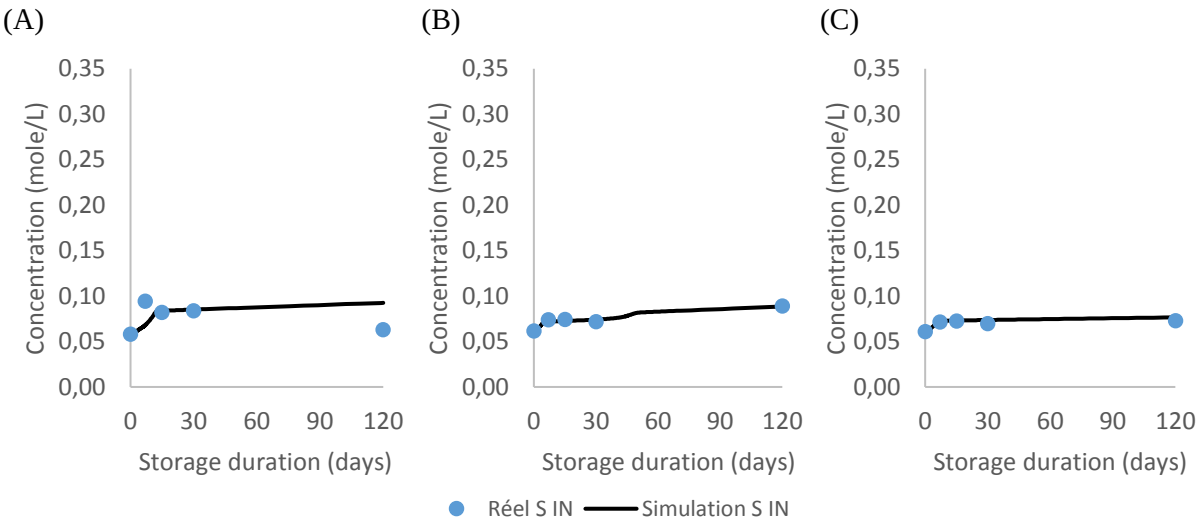

Figure 5-10 – Simulations et données expérimentales estimées pour l'évolution de l'azote inorganique (S_{IN},) du Fumier 2. (A) Control ; (B) Glucose 4% ; (C) Glucose 10%.

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5.2.6.4. pH

Le pH est l'un des paramètres les plus difficiles à modéliser, car de nombreuses espèces chimiques influencent sa valeur. Ces composés peuvent être dégradés au cours de l'ensilage et ont des constantes d'acidité variables. Malgré tout, la détermination de cette variable algébrique par le modèle a été un grand succès dans la plupart des cas testés (Figure 5-11 et 5 12), ce qui est dû en partie à l'utilisation du paramètre associé à la capacité tampon de la biomasse. Par ailleurs, l'efficacité de ces simulations a été strictement liée à la stabilité des ressources. En effet, pour les ensilages où la fermentation clostridiale ne s'est pas développée – TS39%E (CIVE 2) et Glucose 10% (Fumier 2) - l'évolution du pH est parfaitement décrite par le modèle. Inversement, pour les essais Control avec le Fumier 2 des différences significatives entre le pH mesuré et les simulations ont été observées, spécialement dans les premières semaines d'ensilage. Cet écart peut être expliqué par les difficultés à simuler l'évolution des AGV dans des conditions acétogènes et méthanogènes.

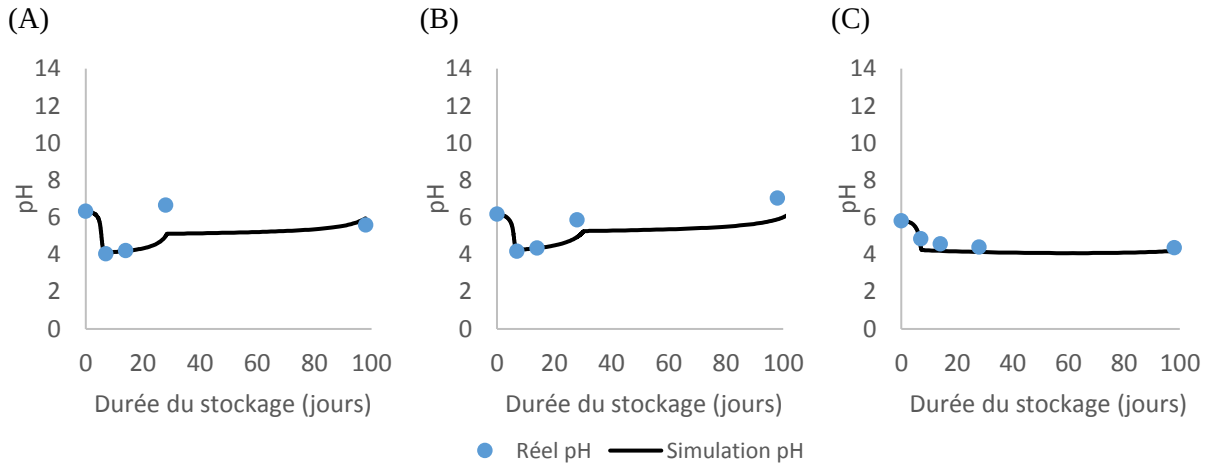

Figure 5-11 – Simulations et données expérimentales estimées pour l'évolution du pH de la CIVE 2. (A) TS10%E ; (B) TS14%E ; (C) TS39%E.

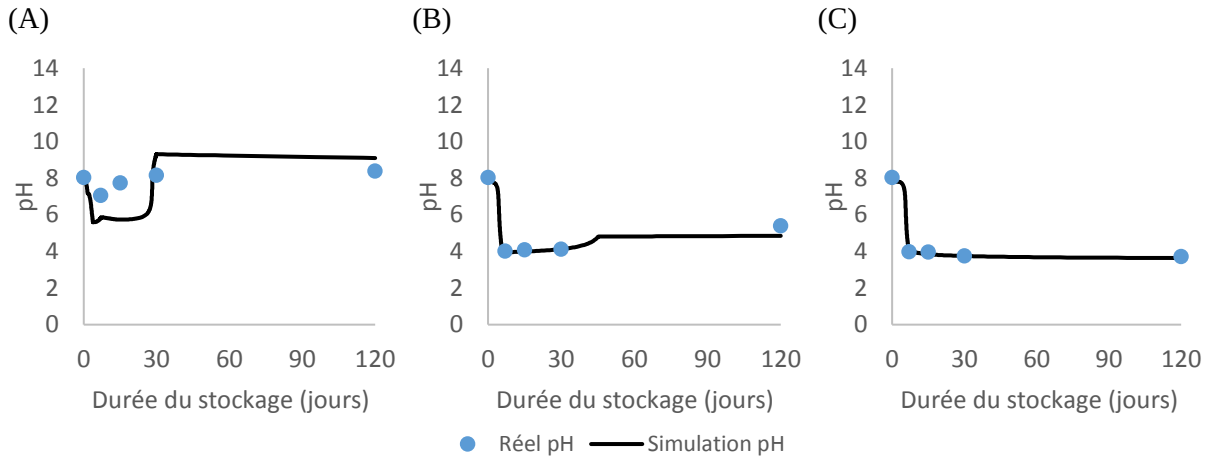

Figure 5-12 – Simulations et données expérimentales estimées pour l'évolution du pH du Fumier 2. (A) Control ; (B) Glucose 4% ; (C) Glucose 10%.

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5.2.6.5. Conservation du potentiel bio-méthanogène

La conservation du potentiel bio-méthanogène simulée (Figure 5-13 et 5 14) pour une durée d'ensilage définie ($\%PBM_{conservé,t}$) a été calculée à partir de la production de hydrogène et méthane, selon l'équation suivante :

$$\%PBM_{conservé,t} = 1 - \frac{S_{CH4,t} + S_{H2,t}}{DCO_{dégradable,i}} \tag{5.40}$$

Où $S_{CH4,t}$ et $S_{H2,t}$ désignent, respectivement, les productions cumulées de méthane et hydrogène pour une durée t (kgDCO.m^{-3}) et $DCO_{dégradable,i}$ représente la $DCO_{initiale}$ dégradable en conditions de digestion anaérobie, obtenue à partir des analyses de PBM et DCO.

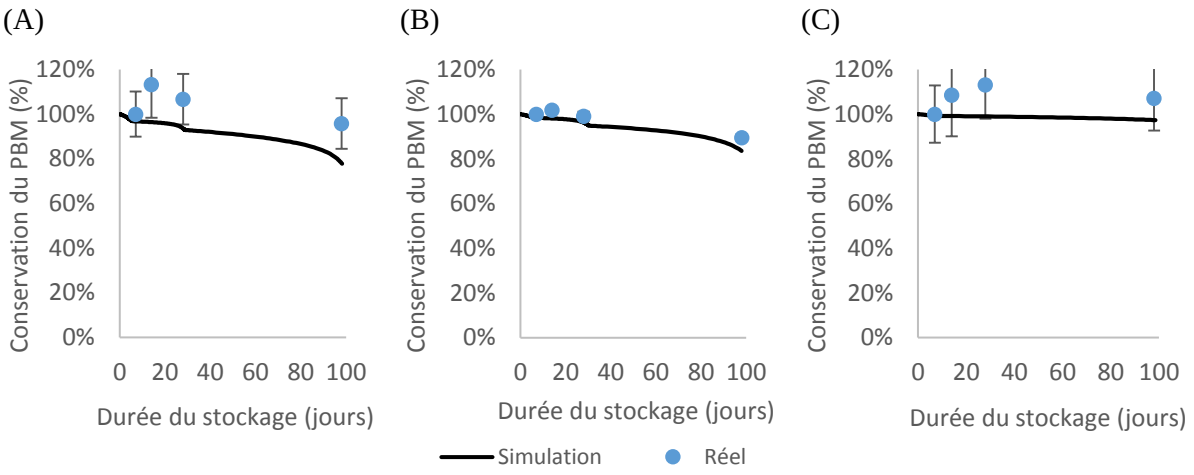

Figure 5-13 – Estimations avec les résultats des simulations et données expérimentales pour la conservation du potentiel bio-méthanogène de la CIVE 2. (A) TS10%E ; (B) TS14%E ; (C) TS39%E.

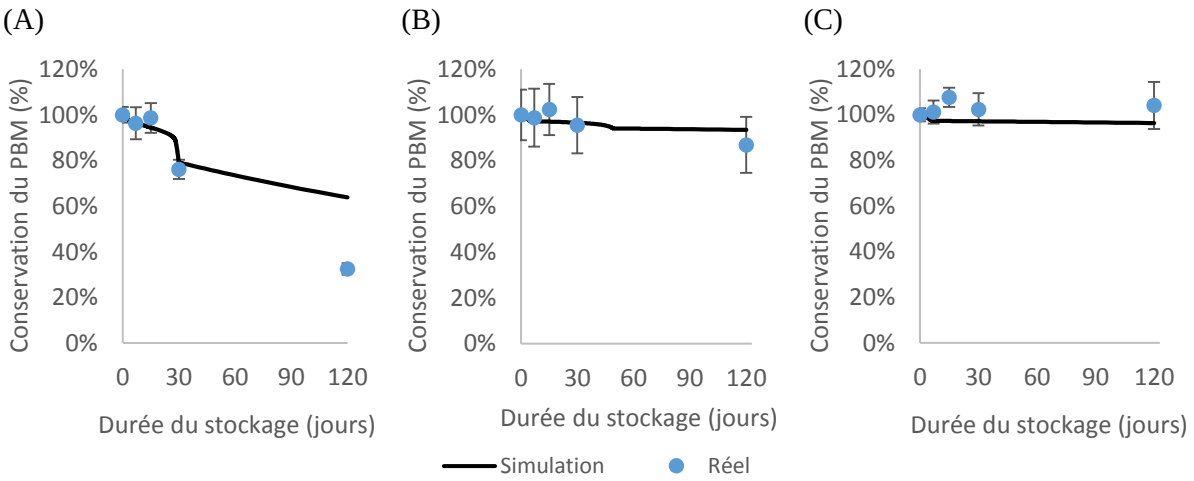

Figure 5-14 – Estimations avec les résultats des simulations et données expérimentales pour la conservation du potentiel bio-méthanogène du Fumier 2. (A) Control ; (B) Glucose 4% ; (C) Glucose 10%.

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Les simulations pour la conservation du PBM sont, dans la plupart des cas, qualitativement concordantes avec les résultats expérimentaux, permettant la détermination des meilleures conditions de stockage pour préserver le potentiel énergétique des ressources. Cependant, les résultats montrent que dans certains cas il est très difficile d'obtenir une estimation quantitative fiable du PBM conservé dans l'ensilage à partir seulement des pertes de DCO par production de H_2 et CH_4. Pour certaines conditions, cela est dû au fait qu'en réalité, pendant ensilage il peut y avoir des gains de bio-accessibilité grâce à l'hydrolyse des fractions difficilement accessibles, ce qui n'a pas été intégré dans le modèle. En outre, lorsque l'ensilage est très peu performant, à savoir pour la condition Control du Fumier 2, la préservation du PBM est surestimée par les simulations. Cela peut en partie être expliqué par l'omission des réactions de biodégradation de l'acide butyrique dans le modèle.

5.2.6.6. Paramètres suggérés et discussion

Comme le montrent les valeurs du Tableau 5-7, les résultats de la simulation ont été obtenus en faisant varier seulement un nombre limité de paramètres, notamment les constantes cinétiques et la stœchiométrie, ce qui illustre la robustesse du modèle.

Concernant les coefficients stœchiométriques, il a été observé que seulement une partie a variée selon le type de gisement utilisé. D'abord, pour les bactéries lactiques, les populations homo-fermentaires ont dominé au cours des ensilages, mais cela a été plus évident pour le Fumier 2, avec l'absence d'éthanol pendant son stockage. De plus, pendant la fermentation clostridiale, le rendement en acide butyrique a été plus faible pour les conditions avec le Fumier 2. Cela peut suggérer que l'activité des populations acétogènes est plus importante dans les fumiers de bovins. En revanche, pour la réaction de fermentation protéolytique, des rendements stœchiométriques identiques ont été utilisés pour les deux matières premières.

Les constantes cinétiques d'hydrolyse obtenues ont été très faibles, ce qui exprime le fait que l'ensilage est avant tout un procédé de conservation de la biomasse. Dans le cas des protéines, leur constante cinétique de dégradation a été supérieure pendant l'ensilage de CIVE 2 et pour des teneurs en MS plus faibles. Cela peut être lié non seulement à l'activité enzymatique dépendante de l'humidité du milieu, mais aussi à la dégradation d'enzymes protéolytiques au cours du préfanage de la biomasse. Cet effet par rapport à la teneur en matière sèche n'a pas été observé pour les essais avec Fumier 2 et la constante cinétique d'hydrolyse des protéines n'a pas variée pour les conditions testées. Néanmoins, il faut tenir compte du fait que pour les essais avec Fumier 2 la gamme de teneur en MS testée a été très restreinte. En ce qui concerne la constante cinétique d'hydrolyse des sucres structurels, les valeurs ont varié pour toutes les conditions et nous n'avons pas été en mesure d'établir une corrélation par rapport à la matrice testée, ou par rapport à la teneur en matière sèche et à la teneur en sucres accessibles. D'une part, cela peut être lié à des erreurs dans l'estimation de la fraction réelle des sucres difficilement accessibles. D'autre part, il est également plausible qu'il ne soit pas possible d'obtenir une tendance en utilisant une

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réaction simple de premier ordre, compte tenu de la variété des mécanismes anaérobies qui peuvent conduire à l'hydrolyse des sucres complexes. En effet, la conversion de ces polymères en conditions d'ensilage peut être, selon Dewar *et al*. [86] et McDonald *et al*. [12], catalysée soit par voie enzymatique, soit par voie chimique, mécanismes qui se produisent par exemple, à différents niveaux de pH.

Pour ce qui concerne les taux de croissance maximal de Monod, ils sont généralement différents dans les conditions testées. Pour les bactéries lactiques, le μ_{max} a légèrement diminué avec l'augmentation de la MS dans chacune des matières premières. Par exemple, dans les essais avec CIVE 2, l'augmentation de la teneur en matière sèche de 10% jusqu'à 39% a entraîné une diminution d'environ 10% du μ_{max}. Cette même augmentation de la teneur en MS a conduit à une réduction de presque 30% pour le taux de croissance maximal des bactéries clostridiales. Ces résultats montrent que la croissance de ces deux populations bactériennes dépend de la teneur en MS et que la sensibilité à la disponibilité de l'eau est beaucoup plus élevée pour les bactéries clostridiales, ce qui explique pourquoi il est préférable d'utiliser des concentrations en matière sèche plus importantes en ensilage pour atteindre une stabilité à faible pH. Pour le Fumier 2, cette sensibilité des bactéries clostridiales n'a pu être vérifiée que pour la variation de la matière sèche entre les essais Glucose 4% et Glucose 10%. Cela s'explique par le fait que pour les essais Control, la valeur précise de μ_{max} des bactéries clostridiales n'a pas pu être calculée, car la réaction a lieu avant le premier prélèvement. Par conséquent, des études expérimentales à des durées plus faibles que 7 jours devront être réalisées pour le fumier frais de façon à améliorer les connaissances sur les cinétiques de ces phénomènes. Tout comme pour les populations précédentes, lorsque les réactions de méthanogenèse se produisent au cours de l'ensilage, il a été possible d'identifier une augmentation du μ_{max} des bacteries méthanogènes pour des teneurs en MS plus faibles. En revanche, il n'a pas été possible d'établir une corrélation générale entre le μ_{max} des bactéries protéolytiques et les conditions testées en ensilage. En effet, pour la CIVE 2 il a eu une augmentation de l'activité protéolytique pour des concentrations en MS plus élevées, alors que pour le Fumier 2 aucune tendance n'a été observée parmi les teneurs en MS testées. Néanmoins, d'après la littérature [40,42,43], une baisse de l'activité bactérienne protéolytique est aussi attendue pour des plus faibles humidités de la biomasse. Cet écart par rapport à la bibliographie peut être dû au fait que la teneur réelle en acides aminés accessibles a été estimée à partir de la production d'azote inorganique durant l'ensilage, ayant certainement une erreur associée importante. Par conséquent, afin d'élaborer plus de conclusions sur l'activité protéolytique pendant l'ensilage, une détermination précise de la concentration de S_{AA}, ainsi que d'autres expériences de stockage à plusieurs teneurs en MS doivent être intégrées dans le modèle. Enfin, il est également important de noter qu'aucune comparaison entre les valeurs de μ_{max} de CIVE 2 et Fumier 2 n'a été effectuée, car ils dépendent de la quantité de biomasse bactérienne initiale, laquelle a été arbitrairement fixée pour chaque gisement à partir des données de la littérature.

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Tableau 5-7 – Valeurs suggérées pour les paramètres cinétiques et stœchiométriques du modèle selon la matrice testée.

Paramètre	Unité	Condition					
		CIVE 2			Fumier 2		
		TS10%E	TS14%E	TS39%E	Control	Glucose 4%	Glucose 10%
k_1	j^{-1}	0.002	0	0.0033	0.004	0	0.002
k_4		0.012	0.0055	0.0017	0.0005	0.0005	0.0005
$\mu_{max,BL}$	j^{-1}	0.72	0.69	0.65	>0.85[1]	0.85	0.71
$\mu_{max,BC}$		0.84	0.69	0.60	>0.65[1]	0.86	0.70
$\mu_{max,BP}$		0.56	0.58	0.58	0.23	0.29	0.23
$\mu_{max,BM}$		0.54	<0.46[2]	<0.46[2]	0.394	<0.22[2]	<0.22[2]
Y_{BL}	kgDCO. $kgDCO^{-1}$	0.265	0.265	0.265	0.265	0.265	0.265
Y_{BC}		0.265	0.265	0.265	0.265	0.265	0.265
Y_{BP}		0.265	0.265	0.265	0.265	0.265	0.265
Y_{BM}		0.055	0.055	0.055	0.055	0.055	0.055
$p_{M,2}$	-	4.3	4.3	4.3	4.3	4.3	4.3
$p_{M,3}$		5.5	5.5	5.5	5.5	5.5	5.5
$p_{M,5}$		5.5	5.5	5.5	5.5	5.5	5.5
$p_{M,6}$		7.0	7.0	7.0	7.0	7.0	7.0
q_2	-	35	35	35	35	35	35
q_3		12	12	12	12	12	12
q_5		12	12	12	12	12	12
q_6		12	12	12	12	12	12
$K_{S,2}$	kgDCO. m^{-3}	0.1	0.1	0.1	0.1	0.1	0.1
$K_{S,3}$		0.1	0.1	0.1	0.1	0.1	0.1
$K_{S,5}$		0.1	0.1	0.1	0.1	0.1	0.1
$K_{S,6}$		0.1	0.1	0.1	0.1	0.1	0.1
$f_{AL,CH}$	kgDCO. $kgDCO^{-1}$	0.92	0.92	0.92	1.00	1.00	1.00
$f_{ET,CH}$		0.08	0.08	0.08	0.00	0.00	0.00
$f_{AB,AL}$		0.54	0.54	0.54	0.33	0.33	0.33
$f_{AC,AL}$		0.29	0.29	0.29	0.50	0.50	0.50
$f_{H2,AL}$		0.17	0.17	0.17	0.17	0.17	0.17
$f_{AC,AA}$		0.88	0.88	0.88	0.88	0.88	0.88
$f_{AB,AA}$		0.06	0.06	0.06	0.06	0.06	0.06
$f_{H2,AA}$		0.06	0.06	0.06	0.06	0.06	0.06

[1,2]Les valeurs exactes n'ont pas pu être obtenues compte tenu des données expérimentales, car soit les réactions ont eu lieu avant 7 jours de stockage[1] ou n'ont pas été significatives jusqu'à la fin de la conservation[2].

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Parmi les autres paramètres pour lesquels des valeurs identiques ont été utilisées pour toutes les conditions de stockage, une discussion par rapport à ceux qui appartiennent à la fonction d'inhibition I_{pH} mérite d'être abordée. En effet, le modèle suggère que les bactéries lactiques ne sont pas inhibées sur presque toute la gamme de pH testée, en raison de leur faible pH de demi-inhibition ($p_{M,2}$ = 4.3) et de la forte sensibilité de leur activité autour de cette valeur (q_2 = 35). Cette sensibilité implique aussi que, pour des valeurs de pH inférieures à 4.3, l'activité lactique est très réduite, ce qui explique en partie l'arrêt de la consommation de sucres pour la condition Glucose 10% avec le Fumier 2. En ce qui concerne les autres réactions biologiques, les valeurs de pH de demi-inhibition utilisées ont été de 5.5 pour les fermentations clostridiale et protéolytique et de 7.0 pour la méthanogenèse. Étant donné que pour ces trois réactions la sensibilité autour de ces valeurs de pH est aussi élevée, cette fonction d'inhibition explique mathématiquement le délai de la croissance de ces populations bactériennes pour des ensilages proprement acidifiés.

Les résultats des simulations avec les paramètres exposés dans le Tableau 5.7 ont aussi montré que ce modèle peut décrire de manière très correcte des ensilages où la fermentation lactique prolifère et domine. Cependant, lorsque l'on avance sur la chaîne des réactions de fermentation (acidogenèse, méthanogenèse), les simulations sont moins efficaces. De fait, le modèle est trop simplifié au niveau de l'acétogenèse et méthanogenèse et, par conséquent, ces phénomènes ne peuvent pas être correctement décrits par les simulations. Cela signifie que, sous la forme actuelle, le modèle peut être utilisé pour exposer l'évolution de l'ensilage d'autres types de biomasses qui ont, au moins, une teneur en sucres accessibles élevées, tels que les cultures énergétiques, les fourrages, ou des déchets agro-industriels sucrés. Au contraire, pour des intrants très peu performants comme le fumier de bovin frais, ce modèle ne servira que pour indiquer que l'ensilage sera inefficace, qu'il y aura des pertes énergétiques élevées et qu'il faudra agir sur les caractéristiques initiales de ces biomasses pour améliorer la performance de conservation du pouvoir méthanogène. Afin d'obtenir des résultats plus quantitatifs dans le dernier cas, le modèle devra être complexifié au niveau des réactions qui ont lieu après la fermentation clostridiale.

En outre, le modèle permet une estimation qualitative de la meilleure condition pour préserver le potentiel énergétique de la biomasse. Néanmoins, quantitativement il y a encore des écarts entre certaines simulations et les résultats expérimentaux. D'une part, pour les stockages très peu performants, cela peut être encore une fois dû à la simplification du modèle dans les étapes finales de la méthanisation. D'autre part, pour certaines conditions il y a des phénomènes d'hydrolyse qui entraînent des gains de bio-accessibilité, lesquels ne sont pas pris en compte dans le modèle. Des travaux futurs concernant l'introduction d'un paramètre qui représente ces augmentations de PBM pourrait améliorer les estimations de conservation énergétique dans certains cas.

De plus, le modèle peut encore être amélioré par rapport à la détermination des paramètres cinétiques, afin de pouvoir utiliser une seule constante de vitesse par réaction. Pour cela, il est nécessaire d'inclure

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des phénomènes qui puissent expliquer les variations des paramètres dans le modèle actuel. Concernant les taux de croissance maximaux des bactéries lactiques, clostridiales et méthanogènes, l'utilisation d'une fonction d'inhibition par rapport à la teneur en matière sèche pourrait contribuer à l'harmonisation des cinétiques de chaque réaction. Pour cela, des essais d'ensilage à différentes teneurs de MS doivent être réalisées pour compléter le jeu des données expérimentales. Au contraire, pour les constantes cinétiques d'hydrolyse, aucune tendance n'a pu être identifiée en lien avec les propriétés initiales de la biomasse ou l'évolution de l'ensilage. Il a été précédemment suggéré que cela pourrait être dû au fait que les valeurs des constantes sont très faibles ou qu'il existe différentes voies d'hydrolyse, mais aussi parce qu'il existe un doute dans le calcul des fractions particulaires expérimentales. Ainsi, l'une des prochaines étapes pour l'optimisation du modèle devra passer par l'estimation exacte des fractions de DCO particulaire qui appartiennent aux protéines et sucres dégradables.

Finalement, le calcul précis d'autres données expérimentales comme le S_{AA}, S_{CH} et les concentrations des populations bactériennes doit être mis en perspective pour l'amélioration de la confiance sur les résultats du modèle. Cela peut comprendre, par exemple, le développement de nouveaux protocoles pour l'identification et quantification des composés faisant partie des sucres et des acides aminés facilement accessibles.

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5.3. A retenir

L'ensilage est la meilleure méthode pour conserver le potentiel bio-méthanogène de la biomasse et son succès est strictement lié à l'évolution du pH.

Nous avons développé un modèle mathématique pour expliquer les principaux phénomènes biochimiques qui ont lieu pendant l'ensilage et qui ont un impact significatif sur l'évolution du pH et de la conservation énergétique. Ce modèle s'est révélé suffisamment robuste pour décrire l'évolution des composés majeurs et du pH dans différentes conditions d'ensilage. Seuls très peu de paramètres ont nécessité d'être calés différemment, et il s'agit surtout de certains paramètres cinétiques, qui varient en fonction de la nature et des caractéristiques biochimiques du produit ensilé mais aussi de la teneur en eau. Ce modèle pourra donc être utilisé à l'avenir pour d'autres ressources, tels que les cultures énergétiques, les fourrages ou d'autres déchets riches en sucres accessibles.

Néanmoins, le modèle est encore légèrement limité par une description peu quantitative des ensilages inefficaces et de la conservation du potentiel bio-méthanogène. Des travaux futurs concernant l'inclusion d'autres réactions ou phénomènes inhibiteurs devront être entrepris. De plus, un travail plus poussé sur la caractérisation expérimentale des biomasses doit être réalisé. Il s'agit en particulier d'affiner la détermination expérimentale des composés particulaires et solubles (sucres et protéines notamment ici) afin de rendre le modèle d'ensilage plus complet, de sorte qu'il puisse couvrir d'autres types de ressources.

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6. CONCLUSIONS GENERALES ET PERSPECTIVES

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CONCLUSIONS GENERALES

L'un des enjeux majeurs dans l'optimisation de la filière de méthanisation concerne la gestion des gisements avant la production de biogaz. En effet, avec la nécessité d'un approvisionnement continu des installations de biogaz tout au long de l'année, certaines biomasses de production saisonnière doivent être stockées dans la plupart des cas pour des périodes prolongées, ce qui peut entraîner des pertes énergétiques conséquentes et des émissions de gaz à effet de serre.

Pour répondre à cette problématique, les travaux de cette thèse ont porté sur l'optimisation des pratiques de gestion des déchets agricoles en lien avec leur valorisation par méthanisation. Expérimentalement, cela a consisté dans l'étude des différentes **conditions de stockage**, en particulier le stockage **aérobie** ainsi que l'**ensilage**, dans l'optique de l'amélioration de la conservation du potentiel bio-méthanogène et de l'optimisation de la conversion de la matière organique lors de la méthanisation. Deux types de biomasses ont été étudiés : les **CIVEs** et les **fumiers de bovin**. Une approche de **modélisation** a été également mise en œuvre pour expliquer les mécanismes majeurs qui se produisent au cours du stockage confiné.

En raison du caractère très appliqué de ces travaux de thèse, les **conclusions** présentées ensuite sont à la fois **scientifiques**, mais aussi des **mesures pratiques** proposées afin d'apporter aux agriculteurs et exploitants des conseils techniques sur la gestion des ressources avant méthanisation.

STOCKAGE AEROBIE

Le stockage temporaire au contact de l'air est fréquemment utilisé par les agriculteurs pour des déchets agricoles comme le fumier de bovin. Cependant, l'ensemble des expériences a montré que **le stockage aérobie n'est pas une option viable** en vue de l'utilisation des ressources en méthanisation. Cela comprend la proscription des stockages en tas, en fosse ou tout autre type de modalité qui favorise le contact de la biomasse avec de l'air.

En effet, les matières organiques ont été largement dégradées pendant de longues périodes en présence d'oxygène et, pour cette modalité de stockage, la perte de potentiel de production de méthane des CIVEs a été supérieure à 80% après 3 mois. Pour le fumier de bovin, le stockage aérobie a entraîné d'importantes émissions de gaz à effet de serre et des pertes du potentiel de production de méthane autour de 74% après 4 mois.

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STOCKAGE CONFINE - ENSILAGE

L'e**nsilage** est le choix logique pour conserver la biomasse humide à long terme, car il peut **éviter** des **pertes d'énergie** et l'**émission de polluants** dans l'atmosphère si des conditions favorables sont présentes. Il s'agit d'un type de stockage qui repose sur la conservation anaérobie grâce à la stabilisation de la matière organique à pH acide, après fermentation lactique.

Les résultats expérimentaux ont montré que les performances de l'ensilage sont étroitement liées à l'évolution du pH, qui à son tour dépend fortement des caractéristiques biochimiques de la biomasse à stocker, notamment en ce qui concerne la teneur en **sucres accessibles** et **matière sèche**, mais aussi le type de **microflore endogène** et les **composés tampon** présents. En particulier, la teneur en sucres non-structurels (solubles ou polymères facilement accessibles) est cruciale pour le déroulement de l'ensilage, car ce sont ces composés qui vont être consommés rapidement au début de l'ensilage par des bactéries lactiques, conduisant à une acidification et une meilleure stabilité de la biomasse à long terme. Cette propriété représente la principale différence entre les deux matrices testées dans cette thèse (CIVEs et fumier) et c'est elle surtout qui explique les différents comportements observés en ensilage, lesquels sont présentés ensuite.

CIVES : *Ressource adaptée au procédé d'ensilage*

Le **stockage confiné des CIVEs** a limité les pertes de matière organique par l'acidification du gisement et la **conservation de l'énergie** de la biomasse après 3 mois a varié entre 78% et 96% en fonction des conditions testées. Les valeurs les plus élevées de potentiel bio-méthanogène après un stockage de longue durée ont été obtenues pour la matière fraiche ensilée immédiatement après la récolte.

Le **préfanage**, utilisé comme technique pour diminuer la teneur en eau des échantillons, n'a pas fourni un avantage significatif en termes de conservation d'énergie et **doit être évité lorsque les conditions météorologiques sont défavorables**. Dans de tels cas, il est préférable d'ensiler la biomasse rapidement après récolte. Néanmoins, si la teneur en MS est trop faible, une perte de lixiviat riche en carbone organique est probable lors du compactage du silo, dont la quantité augmentera au cours de l'ensilage en raison de la solubilisation de la biomasse. En conséquence, un dispositif de récupération de l'effluent liquide doit être prévu dans le silo, pour qu'il puisse être rapidement injecté dans le méthaniseur, afin d'éviter une fermentation ultérieure de la matière organique soluble et une perte d'énergie. En outre, il a été démontré que, même si une faible teneur en MS entraîne une certaine instabilité et une fermentation secondaire pendant l'ensilage, les pertes d'énergie seront limitées, voire contrebalancées par une augmentation des gains de bio-accessibilité au cours du stockage.

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FUMIER DE BOVIN : *Ressource inadaptée au procédé d'ensilage*

Les pertes d'azote et carbone peuvent être limitées par le stockage confiné du fumier de bovin, en particulier sur de courtes durées. Cependant, les propriétés chimiques inadaptées des fumiers de bovin frais limitent leur stockage sur de longues durées. Parmi les caractéristiques des fumiers, la faible teneur en matière sèche et l'absence de sucres facilement accessibles pour la fermentation sont les principaux facteurs responsables de l'inefficacité de la conservation. En conséquence, des pertes énergétiques entre 37% et 46% ont été observées après 4 mois d'ensilage de fumier de bovin frais.

Dans certains cas, **l'ajout de paille de blé peut améliorer la performance du stockage confiné de fumier de bovin**. Ceci a été observé pour les études où la biomasse a été acidifiée jusqu'à un pH entre 5.5-6.0 pendant les premières semaines d'ensilage, ce qui a été suffisant pour inhiber l'activité méthanogène et stabiliser le gisement avec une teneur de matière sèche plus élevée. Ainsi, le fumier avec ajout de paille a-t-il conservé plus de 86% de son potentiel bio-méthanogène après 4 mois d'ensilage. En revanche, pour du fumier de bovin avec une activité méthanogène élevée avant stockage, l'ajout de paille n'a pas permis une acidification suffisante de la biomasse, la matière organique a été continûment dégradée au cours du stockage et les pertes énergétiques ont atteint 67% après 4 mois. Les différences dans les populations bactériennes parmi les fumiers de bovin testés peuvent être expliquées par les changements de l'alimentation du bétail ou la saison de collecte. Bien que l'ajout de paille de blé ne puisse pas être généralisé comme étant un atout pour la conservation de la biomasse pendant le stockage, **son utilisation diminue la teneur en humidité** et permet une meilleure **tenue mécanique** des silos horizontaux, réduisant ainsi les risques de production d'un **effluent liquide** pendant l'ensilage des fumiers/lisiers de très faible teneur en MS (<10%).

L'utilisation d'autres co-substrats favorise la mise en place de conditions d'ensilage du fumier de bovin. En particulier, l'ajout de **sucres accessibles** pour stimuler la fermentation a été une **méthode efficace** pour optimiser la conservation des gisements. En effet, le co-ensilage avec glucose a permis de conserver plus de 87% du potentiel en méthane initial et de supprimer les émissions gazeuses pendant 4 mois de stockage. Ces taux de préservation ont été encore plus élevés pour les concentrations de glucose (10 $\%_{MH}$) et les teneurs en matière sèche (15-25 $\%_{MH}$) les plus importantes. Malgré des résultats prometteurs, il parait difficile de proposer une teneur initiale de sucres disponibles commune à l'optimisation du stockage de tous les fumiers de bovin, étant donné que les mécanismes réactionnels d'acidification changeront selon les populations microbiennes présentes dans le gisement, notamment en ce qui concerne les bactéries homolactiques ou hétérolactiques. De plus, les essais de co-stockage de fumier avec de l'amidon (5 $\%_{MH}$) ont montré que des sucres plus complexes peuvent être utilisés en combinaison avec le fumier pour l'acidification de la biomasse, entraînant des pertes minimales d'énergie après 4 mois de co-stockage.

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Tout bien considéré, le **co-ensilage de fumier de bovin avec un co-substrat riche en sucres accessibles** (glucose, amidon, etc.) et à teneur en matière sèche élevée semble être la méthode la plus pertinente pour la **préservation du potentiel bio-méthanogène** de cette ressource avant méthanisation.

MODELISATION : ***Outil d'aide à la décision pour des bonnes pratiques de stockage***

Nous avons développé un **modèle** mathématique pour expliquer les principaux phénomènes biochimiques qui ont lieu au cours de l'**ensilage** et qui ont un impact significatif sur **l'évolution du pH et de la conservation énergétique**. Six réactions biochimiques ont été utilisées dans cette approche : 1) hydrolyse des sucres, 2) fermentation lactique, 3) fermentation clostridiale, 4) hydrolyse des protéines, 5) fermentation protéolytique et 6) méthanogenèse.

Ce **modèle** s'est révélé **suffisamment robuste** pour décrire l'évolution des composés majeurs et du pH dans différentes conditions d'ensilage. Seuls très peu de paramètres ont nécessité d'être calés différemment, et il s'agit surtout de certains paramètres cinétiques, qui varient en fonction de la nature et des caractéristiques biochimiques du produit ensilé. Néanmoins, le modèle est encore limité par une description seulement qualitative des ensilages dits « peu performants » et de la conservation du potentiel bio-méthanogène. Cela signifie que, sous sa forme actuelle, le modèle peut être utilisé pour décrire l'évolution de l'ensilage d'autres types de biomasses qui ont, au moins, une teneur en sucres accessibles élevées, tels que les cultures énergétiques, les fourrages, ou des déchets agro-industriels sucrés. Au contraire, pour des intrants très peu performants comme le fumier de bovin frais, ce modèle ne servira que pour indiquer que l'ensilage sera inefficace, qu'il y aura des pertes énergétiques élevées et qu'il faudra agir sur les caractéristiques initiales de ces biomasses pour améliorer la performance de conservation du pouvoir méthanogène.

Somme toute, le **modèle** développé et calibré à partir des données expérimentales de la thèse pourra être utilisé pour **estimer les meilleures conditions d'ensilage à partir des caractéristiques du gisement** et, par conséquent, être un outil d'aide à la décision pour la gestion des ressources avant méthanisation.

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PERSPECTIVES

Du laboratoire à l'échelle réelle

Dans cette thèse nous avons conclu que le co-ensilage avec des substrats sucrés et une teneur élevée en MS semble être la meilleure solution pour la conservation à long terme du potentiel énergétique des déchets du type fumier de bovin. Cependant, ces expériences ont été seulement effectuées avec des molécules modèles telles que le glucose ou l'amidon. Ainsi, l'une des prochaines étapes de recherche sur les conditions d'optimisation du stockage devra comprendre l'étude de l'impact des co-substrats réels à utiliser au niveau de l'ensilage agricole de fumier de bovin, comme par exemple le maïs, la pulpe de betterave, la bagasse ou autres résidus de l'industrie sucrière. Aussi, des essais de co-ensilage avec des teneurs élevées de graisses seraient utiles pour comprendre les effets d'autres potentiels co-substrats sur la stabilisation des fumiers avant méthanisation.

De plus, nos essais de stockage ont été réalisés à une température constante de 25±2 °C. Néanmoins, la majorité des silos horizontaux agricoles est exposée au rayonnement solaire et est couverte avec des bâches noires, facilitant les échanges de chaleur et l'augmentation de température à l'intérieur du silo. En effet, lors d'un prélèvement effectué sur le site agricole de Béréziat pendant une journée de chaleur, nous avons mesuré une température de 44 °C dans la couche supérieure d'un silo réel. Des études futures devraient donc être menées pour déterminer les effets des températures élevées sur les choix des meilleures pratiques de stockage.

A l'échelle du laboratoire, le compactage des silos est difficilement abordable et pour cette raison nos expériences ont été effectuées à densité constante de 0.7 kg/L (valeur utilisée dans des silos agricoles). Cependant, selon la littérature, la densité du silo peut avoir une influence non seulement sur le PBM, mais aussi sur le coût de compactage et la production d'effluents liquides. Ainsi, des études à l'échelle réelle doivent être réalisées pour l'optimisation de ce paramètre en lien avec la valorisation de la biomasse par méthanisation.

Plus généralement, nos essais ont été réalisés dans un milieu considéré comme homogène du point de vue des différents paramètres physiques et biochimiques. Dans la réalité, des hétérogénéités se développent nécessairement lors du stockage, à la fois en raison des gradients de température déjà évoqués, mais également en raison de la nécessaire évolution spatiale du taux d'humidité. En effet, dans le cadre du projet SAM où cette thèse a été impliquée, des essais à l'échelle du terrain ont été réalisés par la société APESA. Ces tests ont montré des sérieuses difficultés en termes d'homogénéité de la biomasse dans le silo et des prélèvements effectués, ainsi que par rapport à la détermination des pertes massiques qui ont lieu pendant le stockage. Par conséquent, afin de valider les résultats obtenus en conditions de laboratoire, cette problématique doit être abordée à l'avenir et plus d'essais à l'échelle réelle doivent être conduits.

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Procédure expérimentale

La méthodologie développée et utilisée dans cette thèse a été extrêmement utile pour l'étude des différents compartiments biochimiques pendant le stockage. Toutefois, au cours de ces travaux, quelques limitations de la procédure expérimentale ont été identifiées. En particulier, le travail de modélisation a mis en évidence la nécessité d'amélioration de la quantification de certaines fractions et composés qui interviennent dans les principales réactions d'ensilage. Pour cette raison, un travail plus poussé sur la caractérisation expérimentale des propriétés des produits doit être réalisé dans les recherches futures. Il s'agit en particulier d'affiner la caractérisation chimique et la quantification des composés particulaires et solubles, comme les sucres et protéines. De plus, lorsque des substrats riches en graisses sont utilisés, une détermination expérimentale des composés lipidiques doit être mise en œuvre pour une distinction précise entre les différentes fractions biodégradables.

Modélisation

Même si le modèle développé est suffisamment robuste pour décrire les principaux phénomènes d'ensilage, il est encore possible de poursuivre son amélioration. En effet, des travaux futurs concernant l'inclusion d'autres réactions ou phénomènes (acidogenèse, méthanogenèse) devront être entrepris pour améliorer la description quantitative des ensilages inefficaces et de la conservation du PBM. De plus, l'introduction d'un paramètre qui représenterait les gains d'accessibilité dû à l'hydrolyse des polymères structurels devra être étudiée. Ensuite, le modèle peut progresser dans la détermination des paramètres cinétiques. Cela pourra passer par la réalisation des études cinétiques à différentes teneurs en matière sèche, de façon à pouvoir intégrer dans le modèle des fonctions d'inhibition dans les activités microbiennes par rapport à la MS du substrat. Finalement, un plan de développement du modèle plus ambitieux devra inclure l'impact des hétérogénéités spatiales (humidité, température, etc.) et les effets thermiques sur les cinétiques et la stabilisation de la biomasse.

Gestion de ressources avant méthanisation : et après le stockage ?

En conclusion, ces trois années de thèse ont apporté des résultats scientifiques et des mesures concrètes qui contribueront à l'optimisation de la filière de méthanisation dans le secteur de l'énergie et de l'environnement. En revanche, il existe encore de nombreuses pistes de recherche dans l'optimisation de la gestion de ressources avant méthanisation qui restent à explorer et qui n'ont pas été abordées. Par exemple, même si l'ajout de paille peut améliorer la performance du stockage des fumiers, son impact sur les besoins de prétraitements de la biomasse et sur le fonctionnement du digesteur anaérobie reste inconnu. Enfin, nous pouvons aussi citer les modalités de préparation mécanique et d'incorporation des ressources agricoles en lien avec leur valorisation par méthanisation, problématique qui sera aussi étudiée par le Laboratoire DEEP de l'INSA Lyon dans le projet PAM (Prétraitements Avant Méthanisation), financé par ADEME, que débutera dans le dernier trimestre de l'année 2017.

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ANNEXES

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A. Article supplémentaire: 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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A.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 [84] 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 [93] and should lead to substantial energy losses. These drawbacks can be mitigated if an efficient ensiling is carried out. According to Herrmann *et al.* [11], 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 [8,10,12,85]. 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

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optimization of biomass preservation before AD, which will have a major impact on the methane yield of agricultural biogas plants.

A.2. Materials and methods

A.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 A.1.

Table A.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

A.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

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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.

A.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 [21], the measures were corrected using the volatilization coefficients at 100 °C suggested by Porter and Murray [75]. pH was measured by a Consort C3020 device with a SP10B pH-electrode.

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

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equalized the sample ones. The BMP tests followed the recommendations provided by Holliger *et al.* [82].

A.3. Results and discussion

A.3.1. Effects of storage method

A.3.1.1. pH values

Lower pH values were obtained using ensiling as storage method, Figure A.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.

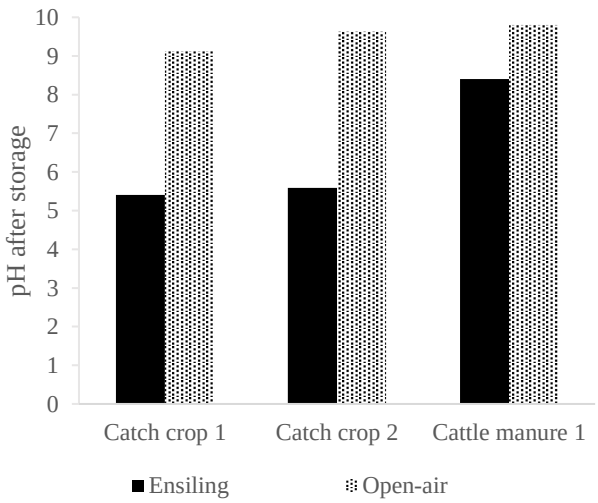

Figure A-1 – pH value after 3 months of storage for Catch crop 1 / Catch crop 2 and 4 months for Cattle manure 1.

However, pH values of silages were somewhat higher than those obtained by other authors for efficient lactate silages [11,14,16,22,23,25,30,40,62,63,87,100,101,109]. 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 [12]. 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

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mole of butyrate is produced from two moles of lactate [12]. Low TS content of catch crop (Table A.1) should have boosted secondary fermentation. Indeed, clostridia are known to be particularly sensitive to water availability and require wet conditions for active development [12]. According to Borreani et al. [40] 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.

A.3.1.2. Organic matter and energy conservation

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

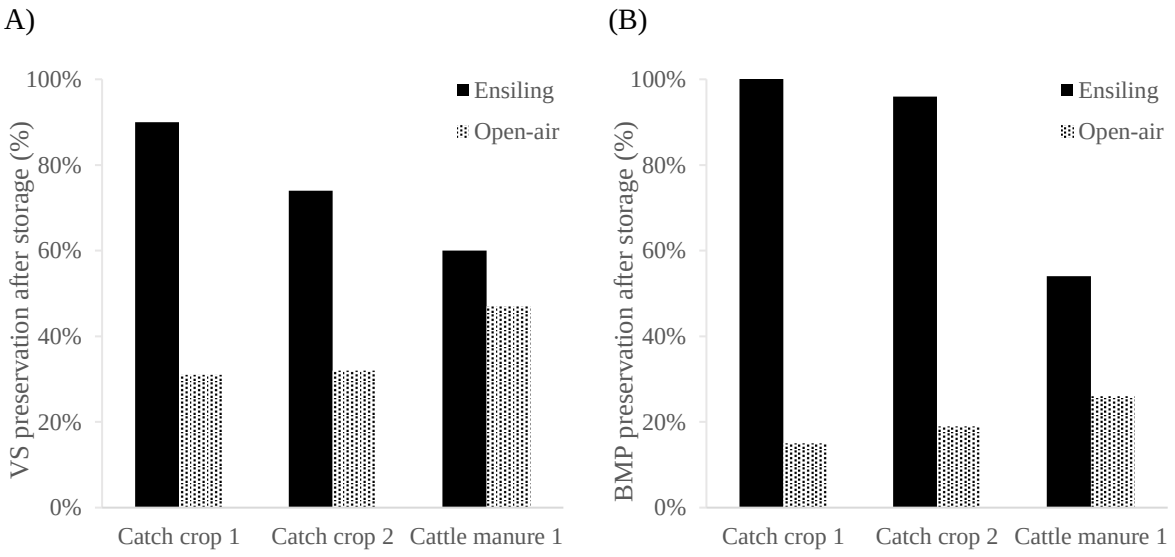

Figure A-2 - Organic matter (A) and BMP (B) losses after 3 months of storage for Catch crop 1 / Catch crop 2 and 4 months for Cattle manure 1. BMP losses are related to the differences between values on a $VS_{original}$ basis, therefore taking into account storage loss.

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

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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 [29,62,87], a partial degradation of (hemi-) cellulosic compounds occurs during ensiling. Since these polymers are not be fully biodegradable in mesophilic AD [96], 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 A.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.

A.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.

A.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 Figure A.3.

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.

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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.

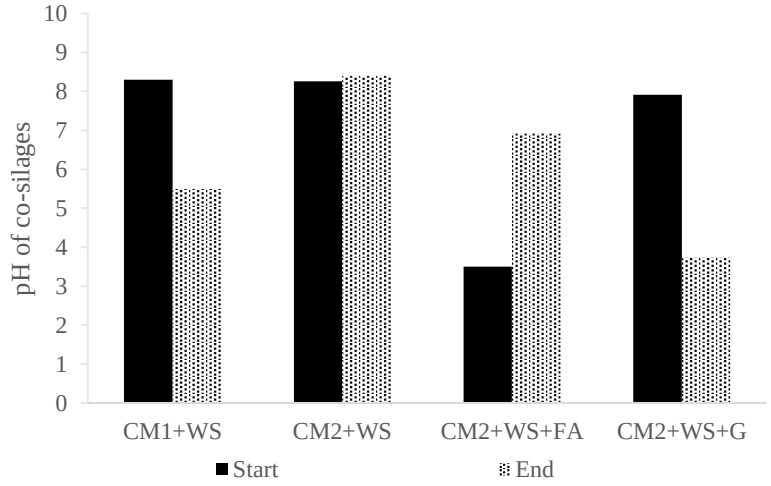

Figure A-3 - pH value before and after 4 months of co-ensiling assays. CM1+WS stands for Cattle manure 1 with wheat straw; CM2+WS for Cattle manure 2 with wheat straw; CM2+WS+FA for Cattle manure 2 with wheat straw and formic acid; CM2+WS+G for Cattle manure 2 with wheat straw and glucose.

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 [97], which according to Marañón *et al.* [98] 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.

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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.

A.3.2.2.Organic matter and energy conservation

Results presented in Figure A.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.

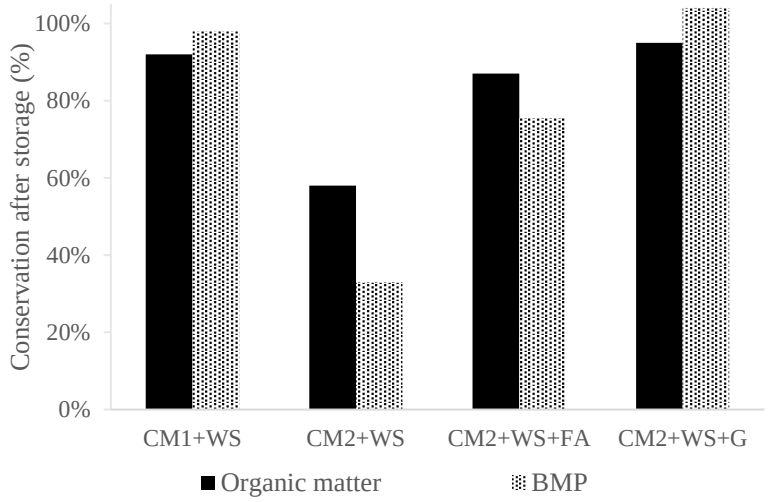

Figure A-4 - BMP and organic matter preservation of co-ensiled cattle manure after 4 months. CM1+WS stands for Cattle manure 1 with wheat straw; CM2+WS for Cattle manure 2 with wheat straw; CM2+WS+FA for Cattle manure 2 with wheat straw and formic acid; CM2+WS+G for Cattle manure 2 with wheat straw and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

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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.

A.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.

A.5. Acknowledgements

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B. Données supplémentaires

B.1. Optimizing agricultural wastes storage before anaerobic digestion: impact of ensiling on methane potential of lignocellulosic biomass

Tableau B-1 – Pertes de masse pendant stockage (B.1).

Condition	Durée du stockage (jours)	Pertes de masse (%MH)
TS18%E	0	-
	3	0.43
	15	0.84
	30	1.45
	90	1.59
	180	1.86
TS23%E	0	-
	3	0.66
	15	1.48
	30	1.56
	90	2.46
	180	3.71
TS18%A	0	-
	3	1.72
	15	6.27
	30	13.14
	90	41.54
	180	82.88

Tableau B-2 – Teneurs en matière sèche (MS) et matière volatile (MV) pendant stockage (B.1).

Condition	Durée du stockage (jours)	MS (%MH)	MV (%MH)
TS18%E	0	18.17	16.21
	3	17.82	15.85
	15	17.48	15.50
	30	16.96	14.97
	90	16.85	14.85
	180	16.62	14.62
TS23%E	0	23.42	20.98
	3	22.91	20.45
	15	22.27	19.80
	30	22.21	19.73
	90	21.49	18.99
	180	20.46	17.93
TS18%A	0	18.17	16.23
	3	15.88	14.19
	15	13.69	11.71
	30	12.46	10.42
	90	11.47	8.66
	180	30.21	20.31

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Tableau B-3 – Résultats de l'analyse élémentaire - K, P, S (%$MV_{original}$) (B.1).

Condition	Durée du stockage (jours)	K	P	S
TS18%E	0	0.53	1.66	0.08
	3	1.11	2.32	0.26
	15	0.97	2.25	0.29
	30	1.43	2.33	0.30
	90	1.53	2.39	0.28
	180	1.44	2.36	0.30
TS23%E	0	0.87	2.72	0.10
	3	0.97	2.53	0.22
	15	1.23	2.34	0.20
	30	1.29	1.98	0.21
	90	1.30	2.40	0.27
	180	1.25	2.41	0.28
TS18%A	0	0.53	1.66	0.08
	3	0.47	2.19	0.12
	15	0.76	1.91	0.06
	30	0.64	1.98	0.05
	90	0.11	2.06	0.03
	180	0.13	1.86	0.03

Tableau B-4 – Teneurs en sucres solubles (%$MV_{ajoutée}$) (B.1).

Condition	Durée du stockage (jours)	Cellobiose	Glucose	Eq. Xylose	Arabinose
TS18%E	0	0.00	0.08	0.02	0.02
	3	0.07	0.11	0.14	0.18
	15	0.02	0.14	0.08	0.15
	30	0.01	0.09	0.08	0.04
	90	0.01	0.05	0.08	0.02
	180	0.00	0.00	0.07	0.00
TS23%E	0	0.01	0.08	0.04	0.01
	3	0.02	0.08	0.08	0.07
	15	0.01	0.11	0.06	0.01
	30	0.06	0.02	0.01	0.02
	90	0.00	0.07	0.01	0.02
	180	0.00	0.00	0.00	0.00
TS18%A	0	0.00	0.08	0.02	0.02
	3	0.01	0.11	0.07	0.04
	15	0.00	0.11	0.01	0.01
	30	0.01	0.00	0.00	0.02
	90	0.02	0.03	0.01	0.02
	180	0.00	0.07	0.06	0.07

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Tableau B-5 – Teneurs en alcools (%$MV_{ajoutée}$) (B.1).

Condition	Durée du stockage (jours)	Méthanol	Ethanol	Butanol
TS18%E	0	0.00	0.00	0.00
	3	0.00	0.00	0.00
	15	0.00	0.00	0.00
	30	0.00	0.00	0.15
	90	0.00	0.00	0.03
	180	0.00	0.00	0.16
TS23%E	0	0.00	0.00	0.00
	3	0.00	0.00	0.00
	15	0.00	0.00	0.00
	30	0.00	0.00	0.10
	90	0.00	0.00	0.05
	180	0.00	0.00	0.12
TS18%A	0	0.00	0.00	0.00
	3	0.00	0.00	0.00
	15	0.00	0.00	0.00
	30	0.00	0.00	0.04
	90	0.00	0.00	0.00
	180	0.00	0.00	0.00

Tableau B-6 – Teneurs en acides gras volatils (%$MV_{ajoutée}$) (B.1).

Condition	Durée du stockage (jours)	Acide Lactique	Acide Formique	Acide Acétique	Acide Propionique	Acide Butyrique	Acide Valérique	Acide Caproïc
TS18%E	0	0.59	0.24	1.24	3.57	0.15	0.24	0.00
	3	4.71	0.02	3.90	1.98	0.55	1.71	0.00
	15	2.65	0.08	4.40	1.23	4.04	1.49	0.00
	30	0.08	0.01	6.11	1.07	4.55	1.37	2.67
	90	0.06	0.05	4.98	0.21	5.49	1.51	1.94
	180	0.00	0.00	5.75	0.18	6.35	1.61	2.75
TS23%E	0	0.13	0.33	0.79	1.24	0.16	0.25	0.00
	3	2.92	0.02	3.88	0.93	0.51	1.23	0.00
	15	0.74	0.03	4.92	1.46	3.87	0.67	0.25
	30	0.05	0.00	7.38	1.41	2.93	0.23	0.00
	90	0.04	0.04	8.39	1.84	3.34	1.21	0.00
	180	0.00	0.04	8.69	0.73	3.87	1.15	1.02
TS18%A	0	0.58	0.24	1.24	2.02	0.15	0.23	0.00
	3	1.20	0.03	2.39	0.29	0.26	0.65	0.00
	15	0.00	0.00	1.08	0.39	0.72	0.07	0.00
	30	0.02	0.00	2.46	0.02	0.89	1.83	0.00
	90	0.00	0.00	0.12	0.00	0.00	0.15	0.00
	180	0.00	0.11	0.00	3.57	0.00	0.00	0.00

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Tableau B-7 – Bilan DCO (B.1).

Condition	Durée du stockage (jours)	DCO_{brut} ($g/gMV_{original}$)	DCO soluble (%)	DCO particulaire (%)
TS18%E	0	1.279	7.79	92.21
	3	1.302	24.30	75.70
	15	1.445	26.05	73.95
	30	1.187	19.02	80.98
	90	1.381	30.37	69.63
	180	1.374	31.53	68.47
TS23%E	0	1.251	1.15	98.85
	3	1.286	16.41	83.59
	15	1.350	20.75	79.25
	30	1.284	22.46	77.54
	90	1.311	27.17	72.83
	180	1.065	23.64	76.36
TS18%A	0	1.279	7.78	92.22
	3	1.092	12.39	87.61
	15	0.902	8.05	91.95
	30	0.689	10.16	89.84
	90	0.397	6.87	93.13
	180	0.335	9.90	90.10

Tableau B-8 – Analyse des fibres ($\%MV_{particulaire}$) (B.1).

Condition	Durée du stockage (jours)	Hémicellulose	Cellulose	Lignine	Soluble
TS18%E	0	14.5	44.0	19.9	21.6
	3	18.4	48.6	13.6	19.4
	15	16.7	46.9	10.0	26.4
	30	16.3	50.6	10.7	22.4
	90	12.8	45.0	15.3	26.9
	180	16.1	50.6	15.7	17.7
TS23%E	0	18.3	44.1	18.8	18.8
	3	20.6	42.6	15.5	21.3
	15	18.6	43.1	14.6	23.7
	30	19.5	45.8	15.1	19.7
	90	13.2	43.4	17.3	26.1
	180	19.0	45.2	16.5	19.3
TS18%A	0	14.5	44.0	19.9	21.6
	3	19.1	43.2	19.7	18.0
	15	20.8	41.9	16.9	20.4
	30	18.0	43.4	18.3	20.3
	90	12.6	32.2	30.6	24.6
	180	13.6	29.3	34.9	22.2

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Tableau B-9 – Cinétiques des essais PBM (B.1).

Condition	Durée du stockage (jours)	k (jour^{-1})	V_{max} (L/kgMV$_{added}$)
TS18%E	0	0.13	182
	3	0.15	261
	15	0.15	278
	30	0.10	295
	90	0.19	286
	180	0.08	208
TS23%E	0	0.15	230
	3	0.12	260
	15	0.09	201
	30	0.13	226
	90	0.13	182
	180	0.14	246
TS18%A	0	0.06	273
	3	0.03	146
	15	0.00	330
	30	0.13	182
	90	0.15	261
	180	0.15	278

Tableau B-10 - Composition du gaz produit pendant stockage (%) (B.1).

Condition	Durée du stockage (jours)	H_2 (%)	O_2 (%)	N_2 (%)	CH_4 (%)	CO_2 (%)	H_2S (ppm)
TS18%E	0.5	2.0	0.5	69.6	0.0	27.9	0.0
	1.5	8.9	1.0	42.1	0.0	48.0	0.0
	3	6.1	2.9	26.7	0.0	64.3	20.3
	11	16.1	3.4	18.5	0.0	62.0	16.9
	14	17.6	1.7	5.2	0.0	75.5	43.8
	17	13.0	4.4	14.7	0.0	67.9	0.0
TS23%E	0.5	1.0	4.1	73.7	0.0	21.1	0.0
	1.5	10.8	3.6	43.4	0.0	42.3	0.0
	3	15.1	3.9	14.9	0.0	66.1	26.0
	11	13.1	0.8	3.5	0.3	82.2	85.7

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B.2. Optimizing storage of a catch crop before biogas production: Impact of ensiling and wilting under unsuitable weather conditions

Tableau B-11 – Pertes de masse pendant stockage (B.2).

Condition	Durée du stockage (jours)	Pertes de masse (%MH)
TS10%E	0	-
	7	0.45
	14	0.68
	28	1.76
	98	2.18
TS14%E	0	-
	7	0.45
	14	0.61
	28	1.20
	98	1.94
TS39%E	0	-
	7	0.67
	14	0.83
	28	0.98
	98	1.10
TS39%A	0	-
	7	7.52
	14	12.07
	28	19.27
	98	51.08

Tableau B-12 – Teneurs en matière sèche (MS) et matière volatile (MV) pendant stockage (B.2).

Condition	Durée du stockage (jours)	MS (%MH)	MV (%MH)
TS10%E	0	10.10	8.43
	7	9.70	8.02
	14	9.49	7.81
	28	8.49	6.79
	98	8.10	6.39
TS14%E	0	14.09	11.60
	7	13.70	11.20
	14	13.56	11.05
	28	13.04	10.52
	98	12.38	9.84
TS39%E	0	38.67	32.28
	7	38.25	31.82
	14	38.15	31.71
	28	38.06	31.61
	98	37.99	31.53
TS39%A	0	38.67	31.83
	7	33.68	25.91
	14	30.04	22.22
	28	28.71	20.37
	98	35.43	20.95

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Tableau B-13 – Résultats de l'analyse élémentaire - K, P, S (%$MV_{original}$) (B.2).

Condition	Durée du stockage (jours)	K	S	P
TS10%E	0	5.99	0.60	0.67
	7	2.75	0.39	0.31
	14	2.91	0.43	0.34
	28	2.96	0.18	0.36
	98	2.84	0.21	0.26
TS14%E	0	2.72	0.26	0.30
	7	3.16	0.44	0.35
	14	3.29	0.47	0.41
	28	3.49	0.33	0.40
	98	3.30	0.08	0.36
TS39%E	0	1.47	0.15	0.16
	7	3.50	0.47	0.42
	14	3.61	0.53	0.44
	28	3.69	0.46	0.42
	98	3.49	0.47	0.41
TS39%A	0	1.49	0.15	0.17
	7	3.94	0.04	0.49
	14	3.26	0.08	0.43
	28	3.31	0.13	0.40
	98	3.56	0.14	0.42

Tableau B-14 – Teneurs en sucres solubles (%$MV_{ajoutée}$) (B.2).

Condition	Durée du stockage (jours)	Cellobiose	Glucose	Eq. Xylose	Arabinose
TS10%E	0	0.13	5.72	6.06	0.06
	7	0.08	0.21	2.14	0.24
	14	0.00	0.09	0.00	0.30
	28	0.00	0.13	0.13	0.24
	98	0.00	0.00	0.09	0.00
TS14%E	0	0.02	5.78	6.79	0.11
	7	0.05	0.00	0.00	0.24
	14	0.04	0.00	0.05	0.29
	28	0.00	0.41	0.06	0.32
	98	0.00	0.04	0.10	0.05
TS39%E	0	0.02	2.27	3.37	0.00
	7	0.00	2.76	0.00	0.00
	14	0.00	1.33	0.00	0.21
	28	0.00	0.61	0.00	0.00
	98	0.00	0.32	0.00	0.27
TS39%A	0	0.02	2.31	3.42	0.00
	7	0.00	0.08	0.00	0.00
	14	0.00	0.08	0.07	0.08
	28	0.00	0.15	0.02	0.03
	98	0.00	0.03	0.00	0.00

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Tableau B-15 – Teneurs en alcools (%$MV_{ajoutée}$) (B.2).

Condition	Durée du stockage (jours)	Méthanol	Ethanol	Butanol
TS10%E	0	0.00	0.00	0.00
	7	0.00	1.10	0.00
	14	0.00	1.65	0.00
	28	0.77	2.44	0.00
	98	0.24	0.34	0.00
TS14%E	0	0.00	0.09	0.00
	7	0.00	0.77	0.00
	14	0.00	0.88	0.00
	28	0.66	0.82	0.00
	98	0.00	0.00	0.00
TS39%E	0	0.00	0.02	0.00
	7	0.00	0.40	0.00
	14	0.00	0.40	0.00
	28	0.36	0.49	0.00
	98	0.00	0.00	0.00
TS39%A	0	0.00	0.02	0.00
	7	0.00	0.03	0.00
	14	0.00	0.00	0.00
	28	0.00	0.00	0.00
	98	0.00	0.00	0.00

Tableau B-16 – Teneurs en acides gras volatils (%$MV_{ajoutée}$) (B.2).

Condition	Durée du stockage (jours)	Acide Lactique	Acide Formique	Acide Acétique	Acide Propionique	Acide Butyrique	Acide Valérique	Acide Caproïc
TS10%E	0	0.00	0.00	0.25	0.00	0.00	0.00	0.00
	7	21.64	0.06	2.36	0.20	0.00	0.00	0.00
	14	26.45	0.00	3.82	0.34	0.00	0.00	0.00
	28	0.53	0.51	5.60	1.41	6.96	1.37	0.84
	98	0.00	0.36	7.33	1.00	9.01	0.84	0.89
TS14%E	0	0.00	0.00	0.31	0.00	0.00	0.00	0.00
	7	19.81	0.00	2.66	0.25	0.00	0.00	0.00
	14	17.61	0.00	3.58	0.13	0.00	0.00	0.00
	28	8.09	0.11	7.85	0.70	0.92	0.00	0.00
	98	0.00	0.40	5.96	0.58	5.12	0.28	0.29
TS39%E	0	0.00	0.00	0.11	0.00	0.00	0.00	0.00
	7	7.22	0.00	1.13	0.06	0.00	0.00	0.00
	14	10.43	0.00	1.28	0.00	0.00	0.00	0.00
	28	10.79	0.02	1.80	0.00	0.22	0.00	0.00
	98	11.90	0.00	2.64	0.27	1.26	0.43	0.21
TS39%A	0	0.00	0.00	0.11	0.00	0.00	0.00	0.00
	7	1.55	0.11	1.54	0.00	0.16	0.09	0.00
	14	0.56	0.00	0.36	0.00	0.10	0.06	0.00
	28	0.36	0.08	0.00	0.00	0.23	0.00	0.00
	98	0.00	0.00	0.00	0.00	0.00	0.00	0.00

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Tableau B-17 – Bilan DCO (B.2).

Condition	Durée du stockage (jours)	DCO_{brut} ($g/gMV_{original}$)	DCO soluble (%)	DCO particulaire (%)
TS10%E	0	1.296	14.16	85.84
	7	1.117	47.27	52.73
	14	1.137	51.65	48.35
	28	1.145	52.59	47.41
	98	1.152	69.15	30.85
TS14%E	0	1.379	23.38	76.62
	7	1.122	45.51	54.49
	14	1.124	49.91	50.09
	28	1.077	47.08	52.92
	98	1.169	51.53	48.47
TS39%E	0	1.328	13.84	86.16
	7	1.216	38.78	61.22
	14	1.156	42.36	57.64
	28	1.156	43.40	56.60
	98	1.091	48.60	51.40
TS39%A	0	1.332	13.99	86.01
	7	1.001	25.00	75.00
	14	0.792	20.56	79.44
	28	0.687	23.50	76.50
	98	0.442	27.37	72.63

Tableau B-18 – Fractions du PBM (%) (B.2).

Condition	Durée du stockage (jours)	Phase soluble	Phase particulaire
TS10%E	0	14.8	85.2
	7	32.8	67.2
	14	44.9	55.1
	28	46.2	53.8
	98	46.3	53.7
TS14%E	0	20.9	79.1
	7	36.8	63.2
	14	47.3	52.7
	28	49.4	50.6
	98	46.2	53.8
TS39%E	0	12.4	87.6
	7	31.4	68.6
	14	46.3	53.7
	28	44.6	55.4
	98	39.3	60.7
TS39%A	0	12.3	87.7
	7	16.0	84.0
	14	19.5	80.5
	28	14.9	85.1
	98	21.6	78.4

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Tableau B-19 – Cinétiques des essais PBM (B.2).

Condition	Durée du stockage (jours)	k (jour^{-1})	V_{max} (L/kgMV$_{added}$)
TS10%E	0	0.21	203
	7	0.21	337
	14	0.14	367
	28	0.15	424
	98	0.18	428
TS14%E	0	0.32	176
	7	0.25	320
	14	0.15	354
	28	0.20	333
	98	0.24	333
TS39%E	0	0.25	363
	7	0.26	268
	14	0.18	291
	28	0.21	300
	98	0.22	297
TS39%A	0	0.25	363
	7	0.22	323
	14	0.20	284
	28	0.18	250
	98	0.18	143

Tableau B-20 - Composition du gaz produit pendant stockage (%) (B.2).

Condition	Durée du stockage (jours)	H_2 (%)	O_2 (%)	N_2 (%)	CH_4 (%)	CO_2 (%)	H_2S (ppm)
TS10%E	1	0.0	0.7	56.1	0.0	43.2	0.0
	3	0.0	1.7	30.5	0.0	67.8	0.0
	7	1.7	3.9	20.1	0.0	74.3	0.0
	24	2.3	11.5	28.3	0.0	58.0	0.0
	28	2.0	15.5	38.6	0.0	43.9	0.0
	59	1.6	13.3	49.2	0.0	36.0	0.0
	63	6.5	8.4	29.6	0.0	55.4	0.0
TS14%E	1	0.0	0.1	36.3	0.1	63.5	0.0
	4	0.0	4.1	29.7	0.0	66.2	0.0
	13	0.0	5.9	25.7	0.0	68.4	0.0
	25	0.6	13.2	47.4	0.0	38.7	0.0
	60	4.5	3.7	16.7	0.0	75.1	0.0
	67	5.6	11.3	34.2	0.0	48.9	0.0
TS39%E	3	0.0	2.0	25.7	0.0	72.2	0.0
	5	0.0	1.3	12.9	0.0	85.8	0.0

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B.3. Cattle manure for biogas production. Does ensiling and wheat straw addition enhance preservation of biomass and methane potential?

Tableau B-21 – Pertes de masse pendant stockage (B.3).

Condition	Durée du stockage (jours)	Pertes de masse (%MH)
TS13%E	0	-
	7	0.31
	15	0.58
	30	0.85
	120	4.05
TS19%E	0	-
	7	0.29
	15	0.51
	30	0.89
	120	1.22
TS22%E	0	-
	7	0.26
	15	0.43
	30	0.90
	120	1.30
TS13%A	0	-
	7	9.05
	15	22.05
	30	44.78
	120	89.20

Tableau B-22 – Teneurs en matière sèche (MS) et matière volatile (MV) pendant stockage (B.3).

Condition	Durée du stockage (jours)	MS (%MH)	MV (%MH)
TS13%E	0	12.80	10.23
	7	12.53	9.95
	15	12.30	9.71
	30	12.06	9.46
	120	9.12	6.44
TS19%E	0	18.78	15.86
	7	18.55	15.62
	15	18.37	15.43
	30	18.05	15.11
	120	17.78	14.82
TS22%E	0	22.44	17.93
	7	22.23	17.72
	15	22.11	17.58
	30	21.74	17.19
	120	21.42	16.85
TS13%A	0	12.80	10.23
	7	13.76	10.71
	15	15.09	11.69
	30	18.79	14.09
	120	69.76	44.92

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Tableau B-23 – Résultats de l'analyse élémentaire - K, P, S (%$MV_{original}$) (B.3).

Condition	Durée du stockage (jours)	K	S	P
TS13%E	0	3.73	0.11	0.22
	7	3.92	0.14	0.17
	15	3.96	0.38	0.17
	30	3.98	0.35	0.16
	120	4.27	0.06	0.05
TS19%E	0	2.63	0.07	0.15
	7	2.86	0.21	0.13
	15	2.89	0.46	0.12
	30	3.06	0.51	0.11
	120	3.07	0.48	0.11
TS22%E	0	2.33	0.07	0.14
	7	2.35	0.32	0.11
	15	2.35	0.34	0.11
	30	2.52	0.44	0.11
	120	2.93	0.49	0.12
TS13%A	0	3.73	0.11	0.22
	7	3.71	0.09	0.17
	15	3.71	0.11	0.14
	30	3.31	0.06	0.13
	120	3.05	0.11	0.23

Tableau B-24 – Teneurs en sucres solubles (%$MV_{ajoutée}$) (B.3).

Condition	Durée du stockage (jours)	Cellobiose	Glucose	Eq. Xylose	Arabinose
TS13%E	0	0.00	0.00	0.00	0.00
	7	0.00	0.00	0.00	0.00
	15	0.00	0.00	0.00	0.00
	30	0.00	0.00	0.00	0.00
	120	0.00	0.00	0.00	0.00
TS19%E	0	0.00	0.00	0.00	0.00
	7	0.00	0.00	0.00	0.00
	15	0.00	0.00	0.00	0.00
	30	0.00	0.05	0.00	0.00
	120	0.00	0.00	0.00	0.00
TS22%E	0	0.00	0.00	0.00	0.00
	7	0.00	0.00	0.00	0.00
	15	0.00	0.00	0.00	0.00
	30	0.03	0.05	0.00	0.00
	120	0.00	0.00	0.00	0.00
TS13%A	0	0.00	0.00	0.00	0.00
	7	0.00	0.00	0.00	0.00
	15	0.00	0.00	0.00	0.00
	30	0.00	0.03	0.00	0.00
	120	0.00	0.00	0.00	0.00

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Tableau B-25 – Teneurs en alcools (%$MV_{ajoutée}$) (B.3).

Condition	Durée du stockage (jours)	Méthanol	Ethanol	Butanol
TS13%E	0	0.00	0.00	0.00
	7	0.00	0.00	0.00
	15	0.00	0.00	0.00
	30	0.00	0.00	0.00
	120	0.00	0.00	0.00
TS19%E	0	0.00	0.00	0.13
	7	0.00	0.00	0.00
	15	0.00	0.00	0.00
	30	0.00	0.00	0.00
	120	0.00	0.00	0.00
TS22%E	0	0.00	0.00	0.13
	7	0.00	0.00	0.00
	15	0.00	0.00	0.00
	30	0.00	0.00	0.00
	120	0.00	0.00	0.00
TS13%A	0	0.00	0.00	0.00
	7	0.00	0.00	0.00
	15	0.00	0.00	0.00
	30	0.00	0.00	0.00
	120	0.00	0.00	0.00

Tableau B-26 – Teneurs en acides gras volatils (%$MV_{ajoutée}$) (B.3).

Condition	Durée du stockage (jours)	Acide Lactique	Acide Formique	Acide Acétique	Acide Propionique	Acide Butyrique	Acide Valérique	Acide Caproïc
TS13%E	0	0.00	0.25	1.27	0.23	0.33	0.00	0.00
	7	0.00	0.00	1.64	0.67	0.54	0.12	0.00
	15	0.10	0.00	2.80	1.34	1.33	0.48	0.00
	30	0.00	0.00	6.68	2.87	2.52	0.83	0.00
	120	0.00	0.00	1.08	0.60	0.00	0.00	0.00
TS19%E	0	0.00	0.27	1.08	0.15	0.18	0.00	0.00
	7	0.00	0.00	2.29	0.79	0.47	0.00	0.00
	15	0.05	0.00	2.55	0.98	1.11	0.13	0.00
	30	0.00	0.00	4.13	1.48	3.10	0.43	0.26
	120	0.00	0.00	7.40	1.78	4.40	1.19	0.89
TS22%E	0	0.00	0.23	1.06	0.13	0.13	0.08	0.00
	7	0.00	0.00	1.96	0.65	0.40	0.07	0.00
	15	0.03	0.00	2.15	0.78	0.85	0.15	0.00
	30	0.00	0.00	3.25	0.89	2.42	0.42	0.57
	120	0.00	0.00	6.35	1.34	4.93	1.46	1.46
TS13%A	0	0.00	0.28	1.27	0.23	0.33	0.00	0.00
	7	0.00	0.00	1.00	0.42	0.29	0.00	0.00
	15	0.00	0.00	0.94	0.46	0.41	0.18	0.00
	30	0.00	0.00	0.90	0.29	0.58	0.45	0.00
	120	0.00	0.00	0.00	0.00	0.00	0.00	0.00

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Tableau B-27 – Bilan DCO (B.3).

Condition	Durée du stockage (jours)	DCO_{brut} ($g/gMV_{original}$)	DCO soluble (%)	DCO particulaire (%)
TS13%E	0	1.477	18.65	81.35
	7	1.353	17.98	82.02
	15	1.359	21.72	78.28
	30	1.160	28.79	71.21
	120	0.727	12.14	87.86
TS19%E	0	1.390	13.97	86.03
	7	1.307	14.45	85.55
	15	1.229	17.94	82.06
	30	1.291	27.03	72.97
	120	1.153	26.42	73.58
TS22%E	0	1.274	11.37	88.63
	7	1.243	12.78	87.22
	15	1.243	14.26	85.74
	30	1.298	20.62	79.38
	120	1.148	27.04	72.96
TS13%A	0	1.477	18.64	81.36
	7	1.247	15.41	84.59
	15	1.217	15.15	84.85
	30	0.984	9.67	90.33
	120	0.639	8.48	91.52

Tableau B-28 – Analyse des fibres ($\%MV_{particulaire}$) (B.3).

Condition	Durée du stockage (jours)	Hémicellulose	Cellulose	Lignine	Soluble
TS13%E	0	39.6	32.7	7.5	20.2
	7	38.8	34.4	8.1	18.7
	15	36.8	34.6	8.1	20.6
	30	37.0	31.3	9.6	22.1
	120	34.4	34.8	12.5	18.2
TS19%E	0	33.2	41.5	7.1	18.2
	7	32.3	40.5	8.0	19.2
	15	31.6	40.9	6.4	21.0
	30	33.3	41.1	7.2	18.3
	120	34.5	38.8	7.3	19.4
TS22%E	0	33.0	44.8	7.6	14.6
	7	31.7	43.3	6.9	18.1
	15	32.8	43.3	6.5	17.3
	30	35.3	39.8	9.0	15.8
	120	32.5	38.0	11.0	18.5
TS13%A	0	39.6	32.7	7.5	20.2
	7	33.7	28.8	10.3	27.2
	15	33.8	35.7	7.4	23.1
	30	30.8	31.1	7.9	30.2
	120	30.5	23.6	13.1	32.7

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Tableau B-29 – Teneurs en NTK_{brut} et NH_3-N pendant stockage (%$MV_{original}$) (B.3).

Condition	Durée du stockage (jours)	NTK_{brut}	NH_3-N
TS13%E	0	4.03	1.30
	7	4.07	1.58
	15	4.09	1.98
	30	4.04	2.22
	120	3.55	2.15
TS19%E	0	2.72	0.75
	7	2.98	1.00
	15	3.05	1.19
	30	2.83	1.26
	120	2.66	1.38
TS22%E	0	2.29	0.62
	7	2.42	0.82
	15	2.45	0.87
	30	2.24	0.89
	120	2.37	1.14
TS13%A	0	4.03	1.30
	7	3.59	1.13
	15	3.18	1.04
	30	2.20	0.40
	120	1.67	0.06

Tableau B-30 - Composition du gaz produit pendant stockage (%) (B.3).

Condition	Durée du stockage (jours)	H_2 (%)	O_2 (%)	N_2 (%)	CH_4 (%)	CO_2 (%)	H_2S (ppm)
TS13%E	3	0.0	1.0	14.3	15.2	69.5	225
	7	0.0	0.5	3.3	21.3	74.8	741
	23	0.0	7.2	36.8	19.5	36.5	99
	49	0.0	1.9	15.8	48.3	33.9	970
	84	0.0	0.5	14.5	49.8	35.1	548
TS19%E	3	0.2	1.8	32.2	5.0	60.8	174
	7	0.1	1.7	17.6	12.7	67.9	281
TS22%E	3	0.4	1.5	29.3	2.6	66.1	258
	7	0.3	1.1	17.4	6.0	75.2	215

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B.4. Co-ensiling of cattle manure before biogas production: effects of fermentation stimulants and inhibitors on biomass and methane preservation

Tableau B-31 – Pertes de masse pendant stockage (B.4).

Condition	Durée du stockage (jours)	Pertes de masse (%$_{MH}$)
Control	0	-
	7	0.42
	15	0.91
	30	2.46
	120	6.97
Formic acid	0	-
	7	0.08
	15	0.11
	30	0.13
	120	2.40
Glucose 4%	0	-
	7	0.38
	15	0.50
	30	0.67
	120	1.78
Glucose 10%	0	-
	7	0.33
	15	0.53
	30	0.61
	120	1.04

Tableau B-32 – Teneurs en matière sèche (MS) et matière volatile (MV) pendant stockage (B.4).

Condition	Durée du stockage (jours)	MS (%$_{MH}$)	MV (%$_{MH}$)
Control	0	19.17	16.61
	7	18.83	16.26
	15	18.43	15.84
	30	17.13	14.50
	120	13.11	10.36
Formic acid	0	20.45	17.95
	7	20.39	17.89
	15	20.36	17.87
	30	20.34	17.84
	120	18.49	15.93
Glucose 4%	0	22.27	19.83
	7	21.97	19.52
	15	21.88	19.42
	30	21.74	19.28
	120	20.85	18.37
Glucose 10%	0	24.91	22.59
	7	24.66	22.33
	15	24.51	22.18
	30	24.45	22.11
	120	24.12	21.77

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Tableau B-33 – Résultats de l'analyse élémentaire - K, P, S (%$MV_{original}$) (B.4).

Condition	Durée du stockage (jours)	K	S	P
Control	0	1.87	0.06	0.06
	7	2.00	0.20	0.03
	15	1.94	0.07	0.02
	30	2.04	0.04	0.02
	120	1.86	0.03	0.02
Formic acid	0	1.95	0.35	0.04
	7	1.97	0.36	0.04
	15	1.97	0.36	0.05
	30	1.93	0.35	0.04
	120	1.73	0.09	0.02
Glucose 4%	0	1.50	0.08	0.05
	7	1.67	0.30	0.03
	15	1.69	0.31	0.03
	30	1.75	0.32	0.03
	120	1.53	0.24	0.03
Glucose 10%	0	1.26	0.07	0.04
	7	1.39	0.25	0.03
	15	1.37	0.26	0.03
	30	1.37	0.26	0.03
	120	1.37	0.26	0.03

Tableau B-34 – Teneurs en sucres solubles (%$MV_{ajoutée}$) (B.4).

Condition	Durée du stockage (jours)	Cellobiose	Glucose	Eq. Xylose	Arabinose
Control	0	0.00	0.00	0.00	0.00
	7	0.00	0.00	0.00	0.00
	15	0.00	0.00	0.00	0.00
	30	0.00	0.00	0.00	0.00
	120	0.00	0.00	0.00	0.00
Formic acid	0	0.00	0.00	0.00	0.00
	7	0.00	0.00	0.00	0.00
	15	0.00	0.00	0.00	0.00
	30	0.00	0.04	0.00	0.00
	120	0.00	0.00	0.00	0.00
Glucose 4%	0	0.00	16.62	0.00	0.00
	7	0.00	4.11	0.00	0.00
	15	0.00	1.98	0.00	0.00
	30	0.00	0.00	0.00	0.00
	120	0.00	0.00	0.00	0.00
Glucose 10%	0	0.00	40.20	0.00	0.00
	7	0.00	34.04	0.00	0.00
	15	0.00	30.08	0.00	0.00
	30	0.74	23.44	0.00	0.00
	120	0.80	17.68	0.00	0.00

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Tableau B-35 – Teneurs en alcools (%$MV_{ajoutée}$) (B.4).

Condition	Durée du stockage (jours)	Méthanol	Ethanol	Butanol
Control	0	0.00	0.00	0.00
	7	0.00	0.00	0.00
	15	0.00	0.00	0.00
	30	0.00	0.00	0.00
	120	0.00	0.00	0.00
Formic acid	0	0.00	0.00	0.00
	7	0.00	0.00	0.00
	15	0.00	0.00	0.00
	30	0.00	0.00	0.00
	120	0.00	0.00	0.00
Glucose 4%	0	0.00	0.00	0.00
	7	0.00	0.00	0.00
	15	0.00	0.42	0.00
	30	0.00	0.39	0.00
	120	0.00	0.00	0.00
Glucose 10%	0	0.00	0.00	0.00
	7	0.00	0.00	0.00
	15	0.00	0.68	0.00
	30	0.00	0.00	0.00
	120	0.00	0.67	0.00

Tableau B-36 – Teneurs en acides gras volatils (%$MV_{ajoutée}$) (B.4).

Condition	Durée du stockage (jours)	Acide Lactique	Acide Formique	Acide Acétique	Acide Propionique	Acide Butyrique	Acide Valérique	Acide Caproïc
Control	0	0.00	0.00	3.70	1.85	0.00	0.00	0.00
	7	0.00	0.00	3.41	1.44	0.42	0.42	0.00
	15	0.00	0.00	2.38	1.75	0.57	0.31	0.00
	30	0.00	0.00	0.23	3.53	0.49	0.00	0.00
	120	0.18	0.00	0.00	0.00	0.00	0.00	0.00
Formic acid	0	0.00	8.23	1.48	0.28	0.00	0.00	0.00
	7	0.40	8.80	1.19	0.28	0.13	0.13	0.00
	15	0.47	8.60	1.20	0.28	0.00	0.00	0.00
	30	0.57	9.80	1.10	0.26	0.12	0.00	0.00
	120	0.13	0.00	5.59	1.08	1.52	0.49	0.29
Glucose 4%	0	0.00	0.00	1.84	0.80	0.00	0.00	0.00
	7	13.49	0.00	2.03	0.57	0.47	0.47	0.00
	15	15.21	0.00	1.96	0.55	0.72	0.00	0.00
	30	10.02	0.00	2.12	0.60	1.78	0.00	0.28
	120	0.00	0.00	2.16	0.42	3.22	0.66	4.47
Glucose 10%	0	0.00	0.00	1.30	0.65	0.00	0.00	0.00
	7	11.17	0.00	1.76	0.47	0.35	0.35	0.00
	15	14.46	0.00	1.84	0.47	0.43	0.00	0.00
	30	15.09	0.00	2.02	0.45	0.63	0.00	0.00
	120	19.50	0.00	3.95	0.75	1.04	0.00	0.00

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Tableau B-37 – Analyse des fibres (%$MV_{particulaire}$) (B.4).

Condition	Durée du stockage (jours)	Hémicellulose	Cellulose	Lignine	Soluble
Control	0	35.0	41.3	9.5	14.2
	7	34.2	40.9	9.7	15.3
	15	34.2	37.7	10.2	17.9
	30	33.3	41.3	12.0	13.4
	120	27.4	35.2	17.4	20.0
Formic acid	0	37.5	37.6	8.5	16.4
	7	36.3	37.5	8.0	18.2
	15	35.4	38.4	8.2	17.9
	30	35.2	39.7	7.5	17.6
	120	32.4	41.3	10.8	15.6
Glucose 4%	0	34.3	38.8	13.2	13.8
	7	36.1	37.0	8.2	18.7
	15	33.3	35.2	6.8	24.7
	30	34.4	36.8	7.2	21.7
	120	34.8	40.2	8.3	16.8
Glucose 10%	0	32.3	35.3	6.8	25.6
	7	31.2	33.2	6.8	28.8
	15	29.1	33.5	6.1	31.3
	30	30.2	33.2	6.0	30.6
	120	29.5	34.7	6.6	29.2

Tableau B-38 – Fractions du PBM (%) (B.4).

Condition	Durée du stockage (jours)	Phase soluble	Phase particulaire
Control	0	15	85
	7	16	84
	15	17	83
	30	16	84
	120	8	92
Formic acid	0	10	90
	7	ND	ND
	15	ND	ND
	30	14	86
	120	24	76
Glucose 4%	0	31	69
	7	28	72
	15	30	70
	30	28	72
	120	38	62
Glucose 10%	0	20	80
	7	55	45
	15	53	47
	30	50	50
	120	53	47

ND : non déterminé car les données des PBM de la phase soluble n'étaient pas exploitables.

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Tableau B-39 – Cinétiques des essais PBM (B.4).

Condition	Durée du stockage (jours)	k (jour^{-1})	V_{max} (L/kgMV$_{added}$)
Control	0	0.07	216
	7	0.05	219
	15	0.05	237
	30	0.04	214
	120	ND	ND
Formic acid	0	0.07	237
	7	0.06	190
	15	0.05	211
	30	0.06	185
	120	0.03	276
Glucose 4%	0	0.11	207
	7	0.08	227
	15	0.07	241
	30	0.09	227
	120	0.07	258
Glucose 10%	0	0.17	243
	7	0.10	267
	15	0.10	286
	30	0.15	272
	120	0.08	314

ND : non déterminé car la production de méthane n'était pas du premier ordre.

Tableau B-40 - Composition du gaz produit pendant stockage (%) (B.4).

Condition	Durée du stockage (jours)	H_2 (%)	O_2 (%)	N_2 (%)	CH_4 (%)	CO_2 (%)	H_2S (ppm)
Control	1	0.0	0.7	50.8	10.0	38.5	265
	10	0.0	1.2	5.3	35.6	58.0	349
	14	0.0	0.5	1.8	47.6	50.1	134
Formic acid	1	0.1	11.2	66.7	0.3	21.6	0
Glucose 4%	1	0.5	0.9	28.9	6.9	62.7	394
	10	10.8	1.7	11.0	2.6	74.0	165
Glucose 10%	1	0.3	1.0	37.7	4.3	56.6	89
	10	1.4	2.1	38.3	0.5	57.8	23

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B.5. Effects of storage conditions, total solids content and silage additives on fermentation profiles and methane preservation of cattle manure before anaerobic digestion

Tableau B-41 – Pertes de masse pendant stockage (B.5).

Condition	Durée du stockage (jours)	Pertes de masse (%$_{MH}$)
CM12%	0	-
	30	0.65
	120	2.45
CM16%	0	-
	30	0.54
	120	0.82
CM21%	0	-
	30	0.52
	120	0.82
CM12%G	0	-
	7	0.4
	15	1.3
	30	1.3
	120	1.5
CM16%G	0	-
	7	0.6
	15	1.5
	30	1.5
	120	1.7
CM21%G	0	-
	7	1.05
	15	1.92
	30	2.01
	120	2.86
CM16%S	0	-
	7	0.58
	15	0.80
	30	1.15
	120	1.58

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Tableau B-42 – Teneurs en matière sèche (MS) et matière volatile (MV) pendant stockage (B.5).

Condition	Durée du stockage (jours)	MS (%MH)	MV (%MH)
CM12%	0	11.6	9.50
	30	11.0	8.91
	120	9.3	7.22
CM16%	0	16.3	14.16
	30	15.9	13.70
	120	15.6	13.45
CM21%	0	20.9	18.56
	30	20.5	18.14
	120	20.2	17.89
CM12%G	0	10.3	9.04
	7	9.9	8.63
	15	9.1	7.86
	30	9.1	7.82
	120	8.9	7.67
CM16%G	0	15.3	14.00
	7	14.8	13.45
	15	14.0	12.69
	30	14.0	12.67
	120	13.9	12.53
CM21%G	0	19.8	18.17
	7	18.9	17.30
	15	18.2	16.57
	30	18.1	16.49
	120	17.4	15.76
CM16%S	0	20.4	18.39
	7	19.9	17.91
	15	19.8	17.74
	30	19.5	17.44
	120	19.1	17.08

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Tableau B-43 – Résultats de l'analyse élémentaire - K, P, S (%$MV_{original}$) (B.5).

Condition	Durée du stockage (jours)	K	S	P
CM12%	0	2.50	0.10	0.08
	30	2.81	0.35	0.04
	120	2.76	0.05	0.03
CM16%	0	1.57	0.07	0.05
	30	1.73	0.30	0.02
	120	1.77	0.31	0.02
CM21%	0	1.06	0.07	0.04
	30	1.22	0.26	0.02
	120	1.25	0.27	0.02
CM12%G	0	1.38	0.12	0.05
	7	1.43	0.31	0.04
	15	1.65	0.34	0.04
	30	1.60	0.35	0.04
	120	1.57	0.36	0.04
CM16%G	0	0.92	0.09	0.03
	7	1.05	0.22	0.03
	15	1.16	0.25	0.03
	30	1.14	0.26	0.03
	120	1.11	0.27	0.03
CM21%G	0	0.73	0.07	0.03
	7	0.86	0.18	0.02
	15	0.93	0.21	0.03
	30	0.89	0.22	0.03
	120	0.86	0.21	0.03
CM16%S	0	1.09	0.07	0.04
	7	1.23	0.27	0.03
	15	1.30	0.30	0.03
	30	1.30	0.32	0.03
	120	1.33	0.32	0.03

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Tableau B-44 – Teneurs en sucres solubles (%$MV_{ajoutée}$) (B.5).

Condition	Durée du stockage (jours)	Cellobiose	Glucose	Eq. Xylose	Arabinose
CM12%	0	0.00	0.00	0.00	0.00
	30	0.00	0.11	0.00	0.00
	120	0.00	0.20	0.00	0.00
CM16%	0	0.00	0.00	0.00	0.00
	30	0.00	0.04	0.00	0.00
	120	0.00	0.06	0.00	0.00
CM21%	0	0.00	0.00	0.00	0.00
	30	0.00	0.03	0.00	0.00
	120	0.00	0.08	0.00	0.00
CM12%G	0	0.00	52.60	0.00	0.00
	7	0.10	25.50	0.00	0.00
	15	0.00	0.00	0.00	0.00
	30	0.00	0.13	0.00	0.00
	120	0.00	0.08	0.08	0.00
CM16%G	0	0.00	40.54	0.00	0.00
	7	0.10	9.40	0.00	0.00
	15	0.00	0.73	0.00	0.00
	30	0.00	0.10	0.00	0.00
	120	0.00	0.11	0.00	0.00
CM21%G	0	0.00	35.60	0.00	0.00
	7	0.09	9.12	0.00	0.00
	15	0.00	0.00	0.00	0.00
	30	0.00	0.07	0.00	0.00
	120	0.00	0.00	0.00	0.00
CM16%S	0	0.00	0.00	0.00	0.00
	7	0.00	0.00	0.00	0.00
	15	0.00	0.00	0.00	0.00
	30	0.00	0.07	0.00	0.00
	120	0.00	0.10	0.00	0.00

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Tableau B-45 – Teneurs en alcools (%$MV_{ajoutée}$) (B.5).

Condition	Durée du stockage (jours)	Méthanol	Ethanol	Butanol
CM12%	0	0.00	0.00	0.00
	30	0.00	0.00	0.00
	120	0.00	0.00	0.00
CM16%	0	0.00	0.00	0.00
	30	0.00	0.00	0.00
	120	0.00	0.00	0.00
CM21%	0	0.00	0.00	0.00
	30	0.00	0.00	0.00
	120	0.00	0.00	0.00
CM12%G	0	0.00	0.00	0.00
	7	0.00	4.90	0.00
	15	0.00	13.93	0.00
	30	0.00	16.60	0.00
	120	0.00	16.61	0.00
CM16%G	0	0.00	0.00	0.00
	7	0.00	4.61	0.00
	15	0.00	10.00	0.00
	30	0.00	11.41	0.00
	120	0.00	11.85	0.00
CM21%G	0	0.00	0.00	0.00
	7	0.00	5.80	0.00
	15	0.00	9.50	0.00
	30	0.00	10.17	0.00
	120	0.00	10.29	0.00
CM16%S	0	0.00	0.00	0.00
	7	0.00	0.30	0.00
	15	0.00	0.31	0.00
	30	0.00	0.00	0.00
	120	0.00	0.41	0.12

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Tableau B-46 – Teneurs en acides gras volatils (%$MV_{ajoutée}$) (B.5).

Condition	Durée du stockage (jours)	Acide Lactique	Acide Formique	Acide Acétique	Acide Propionique	Acide Butyrique	Acide Valérique	Acide Caproïc
CM12%	0	0.00	0.48	3.58	0.65	0.24	0.00	0.00
	30	0.00	0.00	9.94	2.74	1.46	0.00	0.00
	120	0.00	0.00	4.55	3.30	0.49	0.00	0.00
CM16%	0	0.00	0.31	1.87	0.28	0.00	0.00	0.00
	30	0.00	0.00	6.26	1.75	1.01	0.00	0.00
	120	0.00	0.00	7.07	1.81	1.74	0.75	0.29
CM21%	0	0.00	0.22	1.40	0.22	0.00	0.00	0.00
	30	0.00	0.00	4.61	1.06	0.98	0.00	0.00
	120	0.00	0.00	5.58	1.33	1.48	0.59	0.34
CM12%G	0	0.08	0.00	2.39	0.46	0.00	0.00	0.00
	7	17.07	0.00	2.99	0.66	0.00	0.00	0.00
	15	23.67	0.00	3.29	0.57	0.31	0.00	0.00
	30	24.18	0.00	3.83	0.00	0.00	0.00	0.00
	120	17.43	0.00	6.87	1.15	0.39	0.00	0.00
CM16%G	0	0.04	0.00	1.77	0.00	0.00	0.00	0.00
	7	13.93	0.00	2.13	0.43	0.00	0.00	0.00
	15	17.52	0.00	2.18	0.37	0.18	0.00	0.00
	30	18.36	0.00	2.47	0.00	0.00	0.00	0.00
	120	16.37	0.00	3.26	0.53	0.00	0.00	0.00
CM21%G	0	0.04	0.00	1.54	0.00	0.00	0.00	0.00
	7	12.91	0.00	1.67	0.30	0.00	0.00	0.00
	15	13.18	0.00	1.68	0.27	0.59	0.00	0.00
	30	13.00	0.00	0.00	0.00	0.00	0.00	0.00
	120	0.00	0.00	2.03	2.05	5.76	0.38	0.97
CM16%S	0	0.00	0.23	1.62	0.28	0.00	0.00	0.00
	7	1.31	0.21	3.83	0.73	2.11	0.00	0.00
	15	0.04	0.07	3.69	0.83	3.00	0.00	0.28
	30	0.00	0.00	4.93	0.89	4.64	0.00	1.19
	120	0.00	0.00	5.53	1.11	5.04	0.47	1.74

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Tableau B-47 – Analyse des fibres (%$MV_{particulaire}$) (B.5).

Condition	Durée du stockage (jours)	Hémicellulose	Cellulose	Lignine	Soluble
CM12%	0	34.1	30.9	10.2	24.9
	30	31.9	28.3	13.1	26.8
	120	28.8	27.5	13.7	29.9
CM16%	0	34.8	41.3	8.3	15.5
	30	32.2	33.2	12.0	22.6
	120	33.4	30.0	13.6	23.0
CM21%	0	38.6	46.1	9.0	6.2
	30	35.5	40.6	9.7	14.2
	120	34.6	34.5	12.1	18.8
CM12%G	0	29.6	29.1	7.6	33.6
	7	24.5	23.8	6.3	45.4
	15	28.1	28.2	6.5	37.1
	30	27.1	28.4	6.7	37.9
	120	27.7	27.5	8.8	36.0
CM16%G	0	31.6	37.7	7.6	23.1
	7	26.9	31.2	6.2	35.7
	15	28.8	33.7	7.1	30.4
	30	30.0	33.0	7.5	29.5
	120	29.7	27.5	12.2	30.6
CM21%G	0	34.4	40.5	7.6	23.1
	7	30.5	34.5	7.0	28.0
	15	32.7	34.3	9.6	23.5
	30	34.0	38.0	7.8	20.2
	120	35.2	33.9	11.6	19.3
CM16%S	0	30.6	35.8	10.1	23.5
	7	28.0	28.0	8.9	35.1
	15	29.0	28.9	9.8	32.2
	30	32.2	30.9	7.3	29.5
	120	33.9	32.0	6.7	27.3

Tableau B-48 - Composition du gaz produit pendant stockage (%) (B.5).

Condition	Durée du stockage (jours)	H_2 (%)	O_2 (%)	N_2 (%)	CH_4 (%)	CO_2 (%)	H_2S (ppm)
CM12%G	8	0.0	0.6	5.9	0.2	93.3	399
CM16%G	8	0.0	0.8	5.8	0.0	93.3	123
CM21%G	8	0.8	0.6	1.8	0.1	96.6	35
	27	22.4	1.6	7.1	0.0	69.0	10
	34	22.1	1.8	9.1	0.1	66.9	13
	44	17.3	1.7	13.7	0.0	67.3	14
CM16%S	2	22.5	3.2	6.7	3.3	64.4	13
	8	18.1	0.0	5.9	1.9	74.2	101

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B.6. Screening d'additifs: résultats majeurs

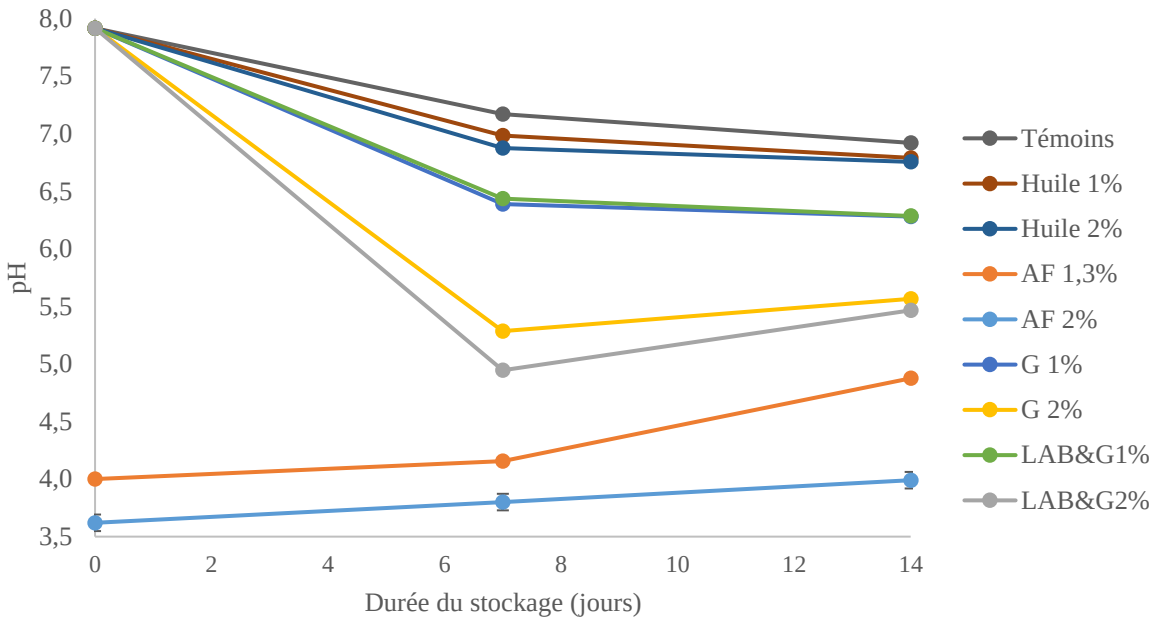

Figure B-1 – Impact de l'ajout de co-substrats à faibles concentrations sur l'évolution du pH du fumier de bovin frais jusqu'à 14 jours d'ensilage. Témoins : fumier de bovin frais ; Huile : Huile de colza ; AF : Acide formique ; G : Glucose ; LAB&G : bactéries lactiques et glucose. Concentrations d'additifs en g/g_{MH}.

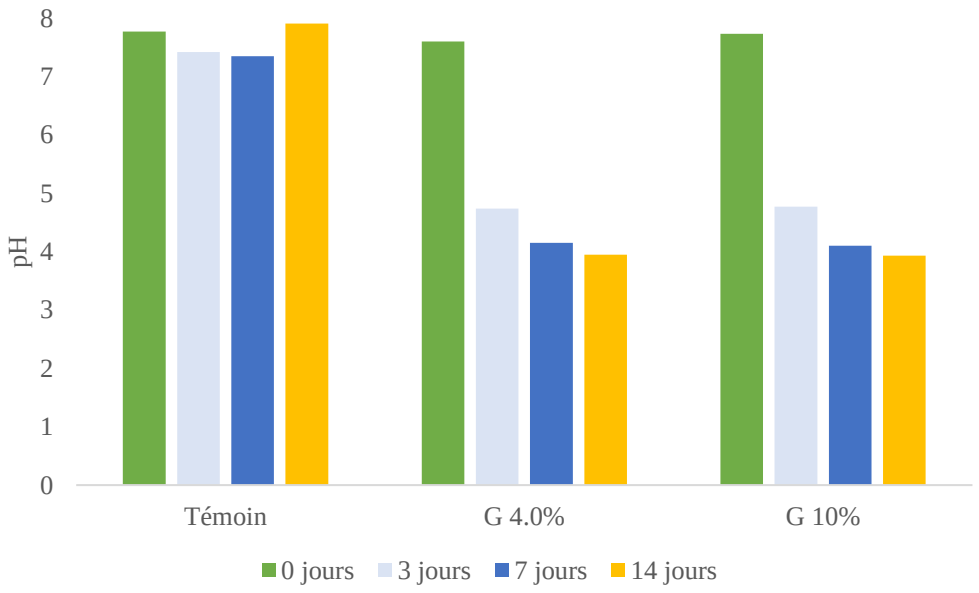

Figure B-2 - Impact de l'ajout des concentrations importantes de glucose sur l'évolution du pH du fumier de bovin frais jusqu'à 14 jours d'ensilage. G : glucose. Concentrations d'additifs en g/g_{MH}.

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THESE DE L'UNIVERSITE DE LYON OPEREE AU SEIN DE L'INSA LYON

NOM : TEIXEIRA FRANCO DATE de SOUTENANCE : 12 Octobre 2017

Prénoms : Rúben

TITRE : Optimisation des pratiques de gestion des déchets agricoles en lien avec leur valorisation par méthanisation

NATURE : Doctorat Numéro d'ordre : 2017LYSEI092

Ecole doctorale : Ecole Doctorale de Chimie de Lyon

Spécialité : Environnement

RESUME :

A l'instar d'autres pays européens, la France se mobilise actuellement pour la valorisation énergétique de ses déchets agricoles par méthanisation. Néanmoins, la production d'une partie de ces ressources n'est pas constante toute au long de l'année. En conséquence, afin de permettre l'approvisionnement continu des installations de biogaz, elles doivent être conservées parfois pendant de longues périodes (plusieurs mois).

Cette thèse porte sur l'optimisation des pratiques de gestion des ressources agricoles avant méthanisation. Différentes conditions de stockage ont été étudiées, en particulier le stockage aérobie ainsi que l'ensilage, dans l'optique de l'amélioration de la conservation du potentiel bio-méthanogène et de l'optimisation de la conversion de la matière organique lors de la méthanisation.

Grâce à une méthode expérimentale originale, la caractérisation multiphasique des déchets à différents stades de leur gestion a été mise en œuvre, à la fois sur les compartiments biochimiques et sur la biodisponibilité du carbone pour la méthanisation. Dans une première partie, la préparation et le stockage de cultures intermédiaires à vocation énergétique ont été étudiés au laboratoire. Les résultats obtenus ont permis de démontrer l'avantage majeur de l'ensilage plutôt que le stockage à l'air libre pour la conservation du pouvoir méthanogène. Deuxièmement, des essais de stockage de longue durée ont été réalisés pour des fumiers de bovin. Ces expériences ont permis de mettre en évidence la mauvaise conservation des fumiers et l'intérêt de l'utilisation de co-substrats pour améliorer la performance du stockage en conditions d'ensilage. Afin d'expliquer les mécanismes majeurs qui se produisent au cours de l'ensilage, une approche de modélisation a été utilisée pour synthétiser et interpréter les résultats. Ces travaux de recherche ont permis d'identifier les bonnes pratiques de gestion pour les intrants de méthanisation agricole afin d'optimiser au mieux la conservation des ressources.

MOTS-CLÉS : Biomasse, Déchets agricoles, Cultures à vocation énergétique, Fumier de bovin, Stockage, Ensilage, Méthanisation, Potnetiel bio-méthanogène, Modélisation

Laboratoire (s) de recherche : Laboratoire DEEP (Déchets Eaux Environnement Pollutions)

Directeur de thèse: Rémy BAYARD et Pierre BUFFIERE

Président de jury :

Composition du jury :
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