Optimization of the process parameters controlling dry anaerobic digestion of spent animal bedding in leach-bed reactors

Silvio Riggio

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Which is the optimum process temperature?

3°

Is spent animal bedding adapted to be treated in LBR? Which is the performance of the process?

1°

Tank
Leachate
°C
Process temperature

LBR

VFA VFA VFA

Leachate circuit

Solid substrate

Biogas circuit

Digestate

Spent animal bedding

How can VFAs be managed?

4°

What specific role do the leachate and digestate play?

2°

MÉTHANISATION PAR VOIE SÈCHE DISCONTINUE DES FUMIERS : OPTIMISATION DES PARAMÈTRES OPÉRATIONNELS DU PROCÉDÉ

OPTIMIZATION OF THE PROCESS PARAMETERS CONTROLLING DRY ANAEROBIC DIGESTION OF SPENT ANIMAL BEDDING IN LEACH-BED REACTORS

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Optimization of the process parameters controlling dry anaerobic digestion of spent animal bedding in leach-bed reactors

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Abstract

Anaerobic Digestion (AD) is a process which allows the treatment of organic waste and the production of renewable energy. In particular, dry AD allows the treatment of solid organic substrates, offering several possibilities to the enhancement of agricultural waste such as spent livestock bedding (a mixture of straw, faeces and urine). Among the available biotechnologies in AD, leach-bed reactor (LBRs) is a promising but yet poorly known process both at scientific and industrial level.

In order to develop this process, several issues have been studied: (i) the bio-physico-chemical characterization of spent animal bedding and its digestion potential in LBRs; (ii) the optimization of the start-up and the operating temperature of the digesters; (iii) the co-digestion of spent animal bedding with an easily-degradable substrate and the issues connected to the management of the volatile fatty acids (VFAs) produced.

The results showed that spent animal bedding is a slowly-degradable substrate which needs a long digestion time. However, it is a substrate suitable to be treated through AD displaying high degradation and methane production rates when processed in LBRs. This substrate is, therefore, a valuable organic resource in the agricultural context.

Spent animal bedding was shown to contain an active methanogenic population able to start the process efficiently, both in thermophilic and mesophilic temperature, without requiring a specific external inoculation. An economic study at industrial scale proved that this peculiarity can be used to diminish the investment costs and then promote the development of this process. Moreover, thermophilic temperature was proved to be less advantageous over mesophilic condition. In fact, despite the very close methane yield reached in both temperature range, the different biogas production rates in thermophilic conditions would lead to a reduction of the final electric energy production in this condition. Mesophilic temperature was then shown to be the best operating condition for this process.

Finally, the role played by the leachate recirculation in the mobilization of the VFAs accumulating in the solid bulk was highlighted in the case of a reactor co-digesting slowly- (spent livestock bedding) and easily-degradable substrates. A strategy was even proposed to efficiently face such a problem by optimizing both the VFA extraction and consumption with the objectives of increasing the overall process efficiency.

In the end, this work allowed to optimize some important parameters for the correct management of the LBRs. This technology was proved to be efficient in the treatment of spent livestock bedding, both as a sole substrate or in co-digestion with an easily-degradable substrate. This research study demonstrates that LBRs is an adapted process for the agricultural context and this technology can easily answer to the full scale issues usually encountered. This work represents a significant advance towards the comprehension and development of LBRs to treat agricultural waste and, more generally, to the development of renewable energies based on biomass.

Sintesi

La digestione anaerobica (DA) é un processo che permette, al contempo, il trattamento di rifiuti organici e la produzione di energia rinnovabile sotto forma di biogas. La DA a secco, in particolare, permette il trattamento di substrati solidi, offrendo svariate possibilità per il trattamento di rifiuti di origine agricola come lo sterco, un rifiuto zootecnico composto da una miscela di paglia, feci e urine. Tra le biotecnologie disponibili in digestione anerobica, i "leach-bed reactors" (LBRs) costituiscono una valida opzione, tuttavia, poco conosciuta e poco sviluppata sia a livello scientifico che industriale.

Ai fini di ottimizzare questo processo, diverse problematiche sono state affrontate: (i) la caratterizzazione bio-fisico-chimica dello sterco e del suo potenziale energetico in LBRs; (ii) l'ottimizzazione dell'inoculo dei reattori e della temperatura di digestione; (iii) la codigestione dello sterco con un substrato facilmente biodegradabile e le problematiche connesse alla gestione degli acidi grassi volatili cosi prodotti.

I risultati mostrano che lo sterco é un substrato lentamente biodegradabile che necessita di tempi di digestione lunghi. Tuttavia é un residuo agricolo adatto ad essere valorizzato attraverso la DA e le rese di degradazione e produzione di metano raggiunte in LBRs sono significativamente alte. Questo substrato é di conseguenza una risorsa organica preziosa nel contesto agricolo.

E stato dimostrato che lo sterco contiene una popolazione di batteri metanogeni attiva capace, sia in regime mesofilico che termofilico, di iniziare il processo di digestione anaerobica efficacemente, senza l'aggiunta di un'inoculo esterno specifico. Un'analisi economica ha dimostrato che questa caratteristica può essere sfruttata per diminuire l'investimento iniziale di un progetto a scala industriale, favorendone, quindi, lo sviluppo sul mercato. Per di più, i risultati mostrano che il regime di termofilia per la digestione dello sterco in LBR non comporta vantaggi sulla produzione finale di metano e che, al contrario, diminuisce le rese di produzione di energia elettrica. Il regime mesofilico, quindi, si è dimostrato il più adatto a questo processo.

Infine, il ruolo giocato dalla percolazione del lisciviato sulla mobilizzazione degli acidi grassi volatili, accumulatisi nella frazione solida, é stato messo in luce nello studio di un reattore di codigestione, mescolando una frazione lentamente biodegradabile (lo sterco) ed una facilmente biodegradabile. Una strategia é stata sviluppata per affrontare sia il problema dell'estrazione che quello del consumo di questi metaboliti nell'obiettivo di migliorare le rese globali del processo.

In conclusione, questo lavoro ha permesso di ottimizzare alcuni parametri fondamentali per la gestione di un LBR. Questo tecnologia si è dimostrata efficace nel trattamento dello sterco, sia in monodigestione che in codigestione con un substrato facilmente biodegradabile. Questa ricerca mostra che l'utilizzo dei LBR é appropriato al contesto agricolo e che la modificazione dei parametri di controllo permette a questo processo di rispondere efficacemente alle problematiche affrontate sul terreno. Questo lavoro rappresenta un significativo avanzamento scientifico verso la comprensione e lo sviluppo dei LBR per il trattamento di residui agricoli e più globalmente, delle energie rinnovabili a biomassa.

Résumé

La Digestion Anaérobie (DA), ou méthanisation, est un procédé qui permet le traitement de déchets organiques et la production d'énergie renouvelable sous forme de biogaz. La DA par voie sèche permet en particulier la valorisation de substrats solides, offrant plusieurs possibilités aux traitements de résidus d'origine agricole tels les fumiers, des substrats constitués d'un mélange de paille, fèces et urine accumulés dans les litières des étables. Parmi les technologies disponibles en méthanisation, les « leach-bed reactors » (LBRs), constituent une option valide mais toutefois peu connue et peu développée soit au niveau scientifique qu'industriel.

Dans le but d'optimiser ce procédé, plusieurs problématiques ont été affrontées : (i) la caractérisation bio-physico-chimique du fumier et du potentiel énergétique exprimé dans un LBR; (ii) l'optimisation de l'inoculation des réacteurs et de la température de digestion ; (iii) la co-digestion du fumier avec un substrat facilement biodégradable et la problématique reliées à la gestion des acides gras volatiles (AGVs) ainsi produits.

Les résultats montrent que le fumier est un substrat lentement biodégradable qui nécessite un long temps de digestion. Cependant, il s'agit d'un déchet agricole adapté à la valorisation par méthanisation et dont les rendements de dégradation et de production de méthane en LBRs sont intéressants industriellement. Ce substrat est par conséquent une ressource organique précieuse dans le contexte agricole.

Il a été montré que le fumier bovin contient une population méthanogène active capable de démarrer un procédé de digestion anaérobie efficacement sans l'ajout d'un inoculum externe spécifique, autant en mode mésophile que thermophile. Une analyse économique a démontré que cette propriété peut être exploitée afin de diminuer les coûts d'investissement initiaux d'un projet à l'échelle industrielle, en favorisant de cette manière le développement de la filière. De plus, les résultats montrent que pour la digestion du fumier en LBRs le mode thermophile ne comporte aucun intérêt par rapport à la production finale de méthane (qui est similaire pour les deux régimes) et que, au contraire, la valorisation par cogénération du méthane produit en thermophile diminue le

rendement de production électrique surtout à cause d'une production de méthane très importante en début de digestion. Le régime mésophile parait donc être le mode de fonctionnement le plus adapté dans ce contexte.

Enfin, le rôle joué par la percolation du lixiviat sur la mobilisation des AGV accumulés dans la fraction solide a été mis en lumière dans un réacteur de co-digestion traitant une fraction de lentement biodégradable (le fumier) et une fraction facilement biodégradable. Une stratégie a été développée afin d'étudier le problème de l'extraction et de la consommation des AGV dans le but d'améliorer le rendement global du procédé.

Pour conclure, ce travail a permis d'optimiser certains paramètres fondamentaux dans la gestion d'un LBR. Cette technologie s'est révélée efficace dans le traitement du fumier, autant en mono-digestion qu'en co-digestion avec un substrat facilement biodégradable. Ces recherches montrent que l'utilisation des LBR est appropriée au contexte agricole et que la modification des paramètres de contrôle permet à ce procédé de répondre efficacement aux problématiques du terrain. Ce travail représente une avancée significative vers la compréhension et le développement des LBRs pour le traitement des résidus agricole et, plus globalement, des énergies renouvelables mobilisant des biomasses agricoles.

Samenvatting

Vergisting is een proces voor de behandeling van organisch afval en de productie van duurzame energie. Vooral, droge vergisting het behandelen van vaste organische substraten toestaat, die diverse mogelijkheden bieden om de valorisatie van landbouwafval zoals verbruikte stalstrooisel (een mengsel van stro, feces en urine). Een van de beschikbare biotechnologie in vergisting is Leach-bed reactor (LBRs), maar nog weinig bekend proces, zowel op wetenschappelijk als op industrieel niveau.

Om dit proces te ontwikkelen, zijn verschillende kwesties onderzocht: (i) de bio-fysisch-chemische karakterisering van verbruikte stalstrooisel en de potentieel vergisting in LBRS; (ii) het optimaliseren van het opstarten en de bedrijfstemperatuur van de kookketels; (iii) de co-vergisting van verbruikte stalstrooisel met een makkelijk afbreekbaar substraat en de kwesties in verband met de geproduceerd vluchtige vetzuren.

De resultaten toonden dat verbruikte stalstrooisel is een langzaam afbreekbaar substraat dat een lange vergisting tijd nodig heeft. Het is echter een geschikt substraat te behandelen door vergisting tonen hoge afbraak en methaan productie snelheden bij verwerking in LBRs. Dit substraat is dan een waardevolle biologische bron in landbouwcontext.

Aangetoond is dat verbruikte stalstrooisel actief methanogene populatie om het proces efficiënt te starten bevatten, zowel bij mesofiele en thermofiele temperatuur, zonder specifieke externe inoculatie. Een economische studie op industriële schaal bewezen dat deze eigenaardigheid kan worden gebruikt om de investeringskosten te verminderen en het bevorderen van de ontwikkeling van dit proces. Bovendien werd aangetoond dat thermofiele temperatuur minder gunstig dan mesofiele temperatuur. Ondanks de zeer nauwe methaanopbrengst bereikt in beide temperatuurgebied verschillende biogasproductie prijzen thermofiele omstandigheden zou leiden tot een vermindering van de uiteindelijke productie elektrische energie in deze toestand. Mesofiele temperatuur werd vervolgens naar de beste bedrijfsomstandigheden voor dit proces.

Tenslotte, de rol van het percolaat recirculatie bij het mobiliseren van de vluchtige vetzuren die is geaccumuleerd in de vaste massa werd benadrukt in de reactor voor langzaam co-vergisting (verbruikte stalstrooisel) en makkelijk afbreekbaar substraat. Een strategie werd voorgesteld om het probleem te overwinnen door het optimaliseren van zowel de vluchtige vetzuren winning en gebruik voor de efficiëntie van het totale proces te verhogen.

Uiteindelijk, in deze studie belangrijke parameters voor het juiste beheer van de LBRs werden geoptimaliseerd. Deze technologie werd bewezen efficiënt in de behandeling van verbruikt stalstrooisel te zijn, zowel als enige substraat of in co-vergisting met een gemakkelijk afbreekbaar substraat. Dit onderzoek toont aan dat LBRs is een aangepast proces voor de agrarische context en deze technologie kan eenvoudig antwoord op de volledige schaal problemen die meestal voorkomen. Dit werk is een belangrijke stap naar een beter begrip en ontwikkeling van LBRs aan agrarisch afval te behandelen en aan de ontwikkeling van hernieuwbare energie uit de biomassa.

Abbreviations

AD	Anaerobic digestion	RE	Renewable energy
BMP	Biomethane potential	RM	Raw mass
CAPEX	Capital expenditure	S/X	Substrate VS/Inoculum VS
CH_4	Methane	SB_cow	Spent cow bedding (SB_cow_g, SB_cow_h and SB_cow_m)
CHP	Combined heat and power	SB_cow_g	Spent bedding from cows fed with round bale grass silage (as roughage)
CO_2	Carbon dioxide	SB_cow_h	Spent bedding from cows fed with hay (as roughage)
COD	Chemical oxygen demand	SB_cow_m	Spent bedding from cows fed with maize silage (as roughage)
CODs	Soluble chemical oxygen demand	SB_goat	Spent goat bedding
CODt	Total chemical oxygen demand	SB_horse	Spent horse bedding
CSTR	Continuous stirred tank reactor	SB_sheep	Spent sheep bedding
FAN	Free ammonia nitrogen	TAN	Total ammonia nitrogen
H_2	Hydrogen	TKN	Total Kjeldahl nitrogen
IRR	Internal rate of return	TS	Total solids
LBR	Leach-bed reactor	TVFA	Total volatile fatty acid
OM	Organic matter	VFA	Volatile fatty acid
PP	Payback period	VS	Volatile solids

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CHAPTER 1

Literature review

The objectives of this chapter are to describe the context and the basic knowledge developed in the frame of this PhD work. First of all, the social and economic context, mainly focusing on French case, is defined. Then, the history and basic knowledge of the anaerobic digestion process and the main process operating parameters are described. Given the extension of the scientific information on this biological process, the author focused only on the aspects playing an important role in the understanding of this PhD manuscript. Then, the focus is set on the technological features of the anaerobic digestion (AD) process studied: leach-bed reactors (LBR). Finally, based on previously reported knowledge, the cornerstones of this PhD work are presented including the structure of the thesis manuscript. It is reminded that the literature review in this chapter aims at introducing the issues of this work and, for this reason, it should be regarded as complementary to the more specific literature overview provided in the introduction section of the research chapters (2, 3, 4 and 5).

1.1 Introduction

1.1.1 Generalities

Population will turn from the actual 6.9 billion to 9 billion within 40 years. Population growth is then the major challenges of 21st century and with it all problems connected to food, water, shelter and energy supply. Energy harnessing defines the lives of all organisms and superorganisms as humans. Energy is one of the aspects that will challenge the most human development in future years (Fox, 2011). Human evolution, until now, was based mainly on fossil fuels (coal, charcoal, oil, etc.) exploitation. Their use increased exponentially during the industrial revolution through the invention of the steam engine and the possibility to accomplish an amount of work unexpected before, indirectly leading towards the technical development that we know today (Smil, 2004). However, the use of fossil fuel is connected to long biogeochemical carbon cycle. The rapid liberation of huge and slowly stored amount of carbon is seriously affecting our planet leading to the well-known climate change (Prentice et al., 2001). This, as well as, the depletion of fossil fuel

sources in future years is pushing human kind to look into new and more sustainable energy sources. Nowadays, technologies for engineering the new world exist and just need to be optimized (Fox, 2011) and one should contribute actively to reach sustainable development objectives. Among the existing green biotechnologies, AD allows to produce a clean fuel (based on a closed carbon cycle with respect to atmospheric carbon dioxide) but at the same time offers a valid solution to the disposal of different kind of organic waste, to the reduction odour nuisance and of pathogens (Chynoweth et al., 2000). AD occupies then an important place among existing biotechnologies. However, still a lot of problems are connected to its use and implementation, and research is called to actively solve them.

1.1.2 Methane historical discovery

We have suggestions of the use of biogas for heating bath water in Assyria already back in the 10th century B.C. (Bond and Templeton, 2011). However, the first observations of biogas leading to our current use dates back to 1776, when father Carlo Campi observed "piccole fiammelle" (small flames) on the surface of a lake in San Colombano al Lambro (Milan) and asks Alessandro Volta (1745-1827), a young Italian physicist, to study this phenomenon. One year later, in "Lettere sull'aria infiammabile native delle paludi (Milano 1777)" A. Volta described for the first time a new gas that he supposed coming from organic matter degradation and that he addresses to with these exact words: "*Quest'aria arde assai lentamente con una bella vampa azzurrina...*" (this air burn quite slowly with a beautiful blue flame) (Fondazione Alessandro Volta, 2016). Methane and its combustion properties were discovered! Many scientists were interested by that discovery and his work was translated in several languages. A. Volta corresponded with eminent scientists like A. L. Lavoisier (1743 – 1794) in France. Indeed, this latter obtained evidence that Volta's gas was "hydrogenium carbonatrum". Only later, in 1892, this gas was named officially "methane" by the international Congress on Chemical Nomenclature (James G. Ferry, 1993). In 1920, the understanding of this process was sufficient to stimulate the development of anaerobic digesters

(Beline et al., 2013). However, many more years were necessary to exactly identify the biological pathways and the microorganism intervening in this process. To these days new important discoveries on this process are made suggesting that we are far from the complete understanding of this complex natural process and, more in general, of the carbon cycle in such specific environment (Vanwonterghem et al., 2016).

Real applications based on the harnessing of AD gas did not wait long after methane was discovered and developed along with research. Small devices such as lamps were already tested by A. Volta himself. Nonetheless, large-scale applications were sparingly reported in New Zealand, in the United Kingdom and India to fuel street lamps in 1890s. In 1921, in China, a digester of 8 m^3 was first commercialized while, in Germany, in 1920 a sewage treatment plant was built and, in 1950, an agricultural one. Energy production through AD gained momentum during the first oil crisis in 1970s (Bond and Templeton, 2011) since price of oil raised dramatically. At that period, many countries promoted the use of alternative energy source such as biogas. China, for example, promoted the installation of household anaerobic digesters which led this country to become the biggest rural biogas user in the world at that time (Ni and Nyns, 1996).

Nowadays AD is considered as no way around biotechnology. It is used both for organic matter (OM) stabilization and/or energy production both in developed and developing countries for household and industrial purposes. Biogas production through AD is a green energy source, and then an important and promising part of the world energy mix, but also a very important process for managing efficiently organic waste of different kinds (Beline et al., 2013). However, it is important to notice a clear temporal difference in the development of AD processes: the first ones were in fact developed to treat liquid waste streams while the processes treating the solid ones developed later. The reasons of such a delay were linked to the strong interactions between the process parameters (Forster-Carneiro et al., 2008), the complexity of the substrate treated, the dynamic evolution of the microbial biomass and also the substrate properties (e.g. porosity, volumetric mass density, etc.) modification during digestion (Shi et al., 2013). The need of a dynamic and global approach,

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mixing biological, physical and chemical knowledges affected consistently its development. Despite of that, recent studies show that a similar level of industrial development between these two process categories has been reached, in Europe at least (Baere et al., 2012).

1.1.3 Social and economic context

European Union Renewable Energy Directive requires to fulfil at least 20% of its total energy consumption with renewable energies by 2020 (European Commission, 2016a). More recently, European commission agreed on a new target of at least 27% of final energy consumption as a whole by 2030 (European Commission, 2016b). In France, at a national level ,these objectives were translated by national laws Grenelle 1 and Grenelle 2 in 23% of renewable energy share by 2020 and were further enhanced to 32% by the "Loi sur la transition énergétique" by 2030 (Assemblée Nationale, 2015).

It is estimated that, in France, by 2030, the globality of the ressources that can be mobilized would represent a potential energy of about 56 TWh per year if treated in AD plants. In 2014 the share of energy from biogas represented only the 2.2% of the total energy consumption, much lower compared to the ones represented by wood for energy (39.0%) or hydraulic energy (23.6%) but at a similar level than wind energy (6.6%) or photovoltaic energy (2.3%) (Ministère de l'Ecologie du Développement durable et de l'Energie, 2015). Nonetheless, these latter (wind and photovoltaic) are intermittent energy sources since highly dependent on meteorological conditions and daylight whereas this is not the case for AD. Thus, energy from AD, and more broadly from biomass, presents an enormous advantage in this sense since its use can be controlled and a demand-driven is possible (Hahn et al., 2014). Moreover, AD is not dependent of geophysical position (impacting wind and solar power) and it is adapted to every context.

The national French interest in developing AD sector is reflected by governmental incentives which permit to promote the implementation of industrial sites on its territory. In only these last 3 years two main modifications were done to the original statute fixing the feed-in-tariffs for

electrical energy produced through AD (Decree of 19 May, 2011). The first modification included specific incentives for treatment of husbandry waste (Decree of 30 July, 2013) and the second increased the incentives for electricity production but removed the ones for heat production (Française, 2015).

The development of decentralized AD facilities like farm-scale processes, consisting in the creation of local and numerous sources of energy in the territory, is one important axis supported by the French government. The objective of constructing 1000 anaerobic digesters by the end of 2020 and 1500 by the end of 2030 proves this commitment. However, nowadays, France still has relatively few farm-scale AD plants: less than 210 in 2014. This number is very low when compared to Germany which is the European leader with about 8,000 agricultural plants in 2015 ("IEA Bioenergy," 2015). As a consequence, a real need in developing this sector is highlighted.

But France is not the only country interested in the development of decentralized energy production as in farm-scale anaerobic digester. Indeed, this interest is shared by several other countries as numerous recent scientific publications reported: Karellas et al. (2010), in Greece; Klavon et al. (2013), in United States; Wilkinson, (2011), in Australia; Tranter et al. (2011), in England. Wilkinson, (2011) clearly states that decentralized energy production through AD have to face several problems related to specific context, i.e. policy, substrates availability and technology development. Each country must then face this issue specifically and no general solutions seem possible. Each country must be able to support in the best way the development of this sector within its territory by promoting the use of the technology that better suits to the kind of substrate produced. The French study from Buffière et al. (2009) already highlighted the lack of information about dry AD and the consequent difficulty in its implementation at national scale. Thus, research in this field is particularly important in order to support industrial development and increase the AD share for reaching the forecasted renewable energy targets in France.

1.1.4   Agricultural substrates: spent livestock bedding

The analysis of the resources available on a given territory is a very important step in the development of an adapted technology suitable to its treatment.

In France, agricultural waste is estimated to be 90% of the resource that can be mobilized for energy production through AD by 2030. That would mean an annual energy production of 51 TWh. In this part, 41% is estimated coming from animal dejections which represents about 95.5 10^6 tons of raw matter (ADEME, 2013) per year. This amount is quite constant in time because of the stability of the amount of animals grown, but a slight decrease in future years could occur because of the tendency in growing animal outside the stables.

One of the main characteristic of manure is its total solids (TS) content which can vary from few percentage points, and called then slurry, to really high percentage like 30% or 40% (ADEME, 2013) which normally characterizes manure from goats, sheep and horses. Slurry is normally found in stable where animal dejections are collected by frequently scraping the soil stable, while dry manure is produced by regular addition of a bedding material in the stabling area offering higher comfort to animals. Bedding material, most of the case straw in France, mixed with faeces and urine, accumulates in the litter for several days or weeks before being cleaned out. Such a waste is referred to as spent animal bedding (Tait et al., 2009), and it is the main substrate investigated in this PhD work. In France, stabling practices producing spent animal bedding are very common: 75% of the animal dejections, considering all animals, are estimated to be solid (while the rest are under a liquid form) (ADEME, 2013). Cattles represent the biggest share of husbandry in France with 7.8 million of animals in 2011 (FranceAgriMer, 2013). A recent study estimated that 54% of the total amount of cattle manure is solid with a minimum TS content of 18% (Degueurce et al., 2016a). Spent cow bedding is then by far the most common in France. However, even if the number of cattle overtakes those of other kinds of livestock: 5.1 million of sheep, 1.3 million of goats and 0.5 million of horses in 2011 (Agreste, 2015; FranceAgriMer, 2013), the totality of the waste

produced by the husbandry of these latter is dry (FranceAgriMer, 2013) and then interesting as a resource. In conclusion, spent animal bedding represents an important resource in the French context and its use as a second generation biomass seems important for the development of a decentralized energy production sector in this country.

1.2 Anaerobic digestion

1.2.1 Introduction

AD is the natural process driving the biological conversion of organic carbon into CO_2 and CH_4, in anaerobic condition and in redox values ranging between -300 and -500 mV (Chynoweth, 1996). AD is a marvellous selective natural process because it allows reducing a large varieties of different organic substrates into one very useful molecule (i.e. methane) (Ferreira and Trierweiler, 2009). In nature, this process has been observed in wetlands, rice fields but also in the intestines of several animals (Chynoweth, 1996).

Methane is a colourless and odourless hydrocarbon, combustible with an oxygen to methane ratio of 2 and with a lower heating value of 50.01 MJ kg^{-1} (802.32 kJ mol^{-1} or 35.80 MJ Nm^{-3}). Methane is also the most reduced form of carbon and the one having the highest energy potential. In fact, Gibbs free energy yield through AD is two or three times higher compared to other bioenergy process like bioethanol and biodiesel (Kleerebezem et al., 2015). Methane is a very interesting molecule since easily exploitable in engine and transported in existing infrastructures like gas pipelines. Its production is much easier than other fuels like methanol or hydrogen whose commercial development is reduced due also to their most difficult production (Chynoweth et al., 2000). Energy balance compared to hydrogen states that even if H_2 has effectively a higher lower heating value, only a small fraction of H_2 can be produced by OM degradation compared to CH_4. Compared to ethanol, methane does not need pure culture or any substrate pre-treatment, resulting then in higher process efficiencies (Chynoweth et al., 2000). Finally, stabilization of the substrate and its possible use as organic fertilizer is an additional characteristic of AD in comparison with

other biotechnologies. Thus, not only the improved energy balance but also the waste treatment and the post-treatment use are real advantages of the anaerobic digestion (Mata-Alvarez et al., 2000).

1.2.2 Anaerobic digestion in four steps

Four distinct chronological steps are involved in the biological transformation of OM into methane and carbon dioxide: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Figure 1). These four stages are rapidly described based on these authors description mainly: Amani et al. (2010), Demirel and Scherer (2008) and Moletta (2008).
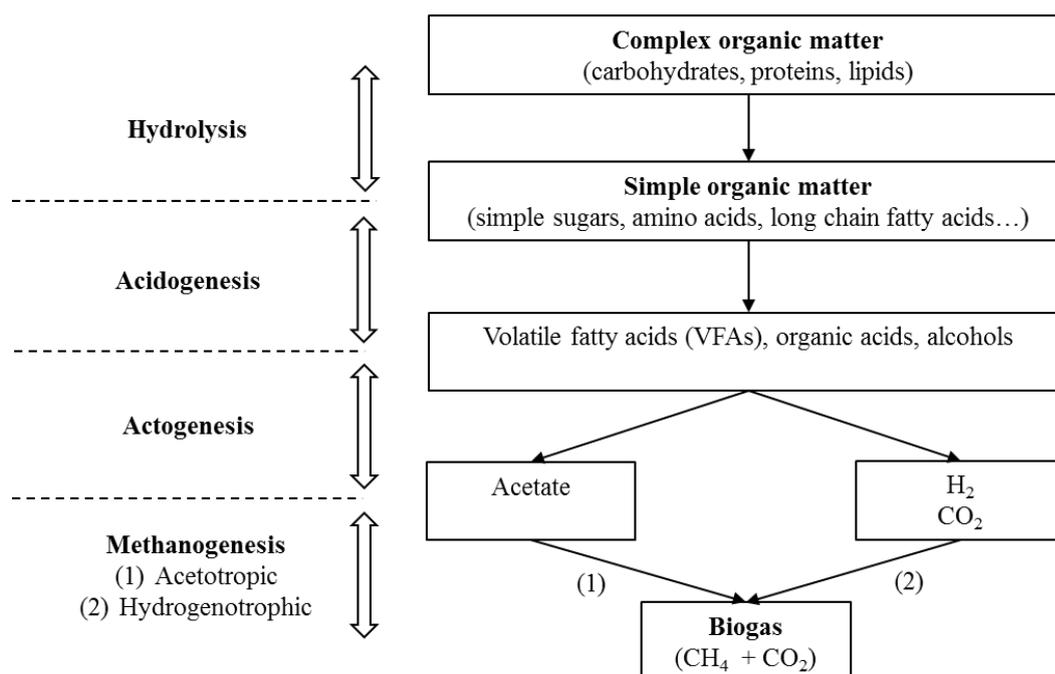
Figure 1: Biological phases of the anaerobic digestion process (adapted from Demirel and Scherer (2008))

Hydrolysis

Hydrolysis is the stage in which complex OM, composed by carbohydrates (cellulose, hemicellulose, starch, etc.), proteins and lipids is converted in soluble and simpler monomers like sugars, amino-acids and fatty acids. The bacteria involved in this steps are called hydrolytic bacteria, a very heterogeneous group of microorganisms which can be both strictly or facultative anaerobic. Hydrolysis is normally catalysed by extra cellular enzymes (e.g. protease or cellulase)

which fasten the breaking of the complex molecules. The rate of this step strictly depends on substrate structure and it can become the AD rate limiting one when a complex substrate, e.g. a lignocellulosic material (such as agricultural waste), is digested. In fact, the presence of recalcitrant compounds like lignin or crystallized cellulose into these substrates hampers the proper hydrolysis of the OM. This issue is often solved by undergoing through a pre-treatment step (biological, chemical or physical) of the substrate (Yang et al., 2015).

Acidogenesis

Acidogenesis consists in the conversion of the hydrolysed compounds into volatile fatty acids (VFAs): acetic, propionic, (iso-) butyric and (iso-) valeric acids; more complex carboxylic acids (e.g. caproic, lactic acid), alcohols (ethanol, methanol) and also H_2 and CO_2. One of the more striking properties of acidogenic bacteria is their rapid growth. Their doubling time is estimated at 30 min (Kothari et al., 2014), which is much higher than the ones of the other steps. This disequilibrium is one of the main causes of failure in anaerobic processes.

Acetogenesis

Acetogens allow converting all VFAs and alcohols into acetate, formate, H_2, and CO_2. Their growth rate is slightly higher than that of methanogens, and estimated ranging between 1.5 to 4 days (Kothari et al., 2014). Acetogens are divided in two groups, the OHPA or Obligate hydrogen producing acetogens, which produce acetate and H_2 from acidogenesis waste, and homoacetogens producing acetate mainly through H_2 and CO_2. OHPA reactions are endergonic ($\Delta G > 0$) in standard condition and only the constant consumption of the H_2 (by methanogens or homoacetogens bacteria for example) permits it to continue.

Methanogenesis

The last step of the AD, methanogenesis is driven by strictly anaerobic bacteria which belong to the Archaea domain. Methanogens doubling time is estimated to range between 2 and 4 days

(Kothari et al., 2014). Two main processes of methane formation are known, a first one driven by hydrogenotrophic methanogens and a second one by aceticlastic methanogens. The first one, producing about 30% of the methane in a reactor, consists in the reduction of formate or CO_2 with H_2 to produce CH_4. These reactions are thermodynamically favoured ($\Delta G < 0$) only when H_2 partial pressure is above 10^{-6} Pa. The second pathway, producing about 70% of the methane, consists in the conversion of acetate into CH_4 and CO_2.

1.2.3   Parameters influencing Anaerobic Digestion

Anaerobic process is a very complex process on which chemical, physical and biological parameters have a big influence. Knowing their effects and relationships inside an anaerobic reactor is important in order to control such process. The most important parameters affecting AD process, and more in particular AD of solid substrate, are discussed hereafter.

pH and alkalinity

pH, or the concentration of hydrogen ions in the liquid phase, is a paramount parameter in AD processes. The sensitivity to this parameter is mainly due to the coexistence of four different steps in AD in a single vessel (except in specific process designs), each one having its own optimum. Hydrolysis and acetogenesis have their optimum respectively at 5.5 and 6.5 (Kothari et al., 2014) while neutral pH is optimum for acetogenesis (Xu et al., 2015) and for methanogenesis (Kothari et al., 2014). Different optimum ranges for the AD analysed as a global process were reported in literature but it can be stated that they are commonly found between pH 6.5 and 8.5 (Bernet and Buffière, 2008). Even if pH affects directly the micro-organisms activity, it also plays a major role in VFA and ammonia inhibitions mainly by changing their speciation and making them more available to bacteria (see paragraphs 1.2.4). Given the importance of pH for correct microorganism activity and in reason of the higher sensibility of methanogens to this parameter, pH is often

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controlled at industrial level by addition of external compounds like sodium bicarbonate which moves pH towards neutral values (Amani et al., 2010).

The pH stability in a given system depends on the alkalinity, or the buffering capacity of the reactive medium. The pH variation when acidic or basic compounds are added to the system is then lower when the alkalinity is significant. The main weak acid/base couples which participate to the overall buffering capacity of a digester are carbonate, ammonium, phosphate, VFAs and sulfide (Sun et al., 2016). However, the main contribution to the alkalinity in anaerobic digester is given by carbonates (H_2CO_3/HCO_3^-, pKa ~ 6.4) which are found at really high concentration in anaerobic digester. A second important source of alkalinity, especially when animal dejections which are rich in nitrogen compounds are used, is ammonia. High amounts of ammonia in the system cause an increase of the pH towards alkaline values (NH_3/NH_4^+, pKa = 9.25) and could cause failure of the system.

Temperature

Temperature is generally known to affect each biological process by increasing its kinetics following the Vant'hoff law. Its impact is so strong that the differences in kinetics between acidogens and methanogens have often been solved by carrying out this two steps at different temperatures in order to slow down the first and speed-up the second (Amani et al., 2010).

In nature methane is formed over a wide range of temperature form 0°C (ice fields) to 97 °C (hot springs) (Zeeman et al., 1988). However, three optimal temperature ranges were identified in AD: psychrophilic (10°C to 20°C), mesophilic (20°C to 45 °C) and thermophilic (50 °C to 65 °C) (Kothari et al., 2014). Indeed, each optimum corresponds to the highest activity of different specific populations.

Psychrophilic conditions are poorly studied until now and applications are often restrained to specific zones with cold climate. Because of their really slow kinetics, psychrophilic reactors often need to be inoculated massively (Lettinga et al., 2001; Massé and Saady, 2015). The most employed

processes are run under mesophilic conditions (Kothari et al., 2014) since they allows to have high degradation kinetics and their energy requirements are easily filled (e.g. through the heat recovered by biogas burning in cogeneration engines). Thermophilic temperatures present, on the other side, interesting advantages in relation to kinetics and consequent reduced digestion time for several substrates (Hegde and Pullammanappallil, 2007; Labatut et al., 2014; Pohl et al., 2012). Moreover, this allows to reduce significantly the amount of pathogens in the digestate (Gómez et al., 2011; Jha et al., 2013; Kim et al., 2002), which can be important in particular contexts where inlet substrates are biologically contaminated. However, some drawbacks are connected to thermophilic processes. The higher kinetics can cause a rapid hydrolysis and acidogenesis leading to a VFA accumulation in the system with possible failure. Thermophilic processes are found to be more sensitive to environmental changings because of the restricted microorganisms populations involved compared to the mesophilic ones (Moset et al., 2015). Moreover, sensitivity to lipid-rich substrates were also recorded since thermophilic microorganism are inhibited by high concentration of long chain fatty acids (Palatsi et al., 2009). Nitrogen inhibition is also often reported in literature in relation to thermophilic conditions. Thermophilic treatment can then be problematic when treating nitrogen-rich substrates like animal dejections. In fact, at this temperature the nitrogen equilibrium between NH_4^+/NH_3 is pushed toward NH_3 which is considered to be the most toxic form for microorganisms since permeable for cell (see paragraph 1.2.4). However, (Yenigün and Demirel, 2013) reported also two different studies showing that the ammonia inhibition thresholds are higher in thermophilic conditions compared to mesophilic ones, reducing by far this issues.

Inoculum

Inoculation refers to the amount of active biomass brought to a system at its start-up in relation to the amount of fresh matter treated. Given the repeated start-up in a batch system, inoculation is particularly important for discontinuous process rather than for continuous ones in which inoculation is carried out only once. The inoculation is expressed in literature in very

different way taking into account the VS, the TS or the raw weight of the substrate and the inoculum used (Yang et al., 2015). As an example: Motte et al. (2013) used the substrate-to-inoculum S/I on a volatile solids basis (substrate VS/ inoculum VS); Yap et al. (2016) used the inoculum-to-substrate ratio I/S on a VS basis too; Kusch et al. (2012) and Kusch et al. (2008) used, instead, the I/(I+S) expressed on a TS basis (inoculum TS/(inoculum + substrate) TS).

The amount of inoculum brought to a system has a big impact on the process kinetics since it accelerates the consumption of intermediate compounds and then the overall AD degradation kinetics (Motte et al., 2013). Addition of high amount of inoculum permits to reduce the digestion period but inoculum occupy a significant volume in the reactor reducing in that way the volumetric productivity (Yang et al., 2015) of the system. The look for the optimum conditions between kinetics and efficiency is then a real issue in each digester.

For optimal digestion performance, the amount of inoculum should be adapted to the kind of substrate treated. In fact, easily-degradable substrates, tending to produce rapidly VFAs would need higher amount of inoculum to counteract rapid reactor acidification. As an example, reactor loaded with easily-degradable substrates like maize silage showed risk of acidification with a S/I (VS basis) of 0.5 (Kusch et al., 2012) while a higher S/I (VS basis) between 10 and 4.4 was considered optimal to digest spent horse bedding in similar batch reactors (Kusch et al., 2008). In this sense, Liew et al. (2012) found an optimum S/I (VS basis) of 2 for several lignocellulosic waste like corn stover, wheat straw, leaves and yard waste.

Inoculum can influence the digestion also through its chemical properties like C/N ratio (section 1.2.3), alkalinity, nutrients, etc. (Yang et al., 2015). However, a very important role is played by the adaptation of the bacteria consortium to a specific substrate composition and/or degradation conditions like temperature and nitrogen content as the following example show. The use of three different liquid inocula and three different S/I in the dry digestion of corn stover showed that to each inoculum used corresponded a different optimum S/I (VS basis), as well as a different methane yields (at the optimum of each condition) (Xu et al., 2013). Guendouz et al.

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(2010) found that a non-adapted inoculum can affect methane production kinetics when treating municipal solid waste and three consecutive batches were necessary before reaching stable methane yield. Zeeman et al. (1988) found that inoculum can adapt to temperature variations but that changing from mesophilic to physchrophilic condition is slower than changing from mesophilic to thermophilic temperatures. Van Velsen (1979) reported the absence of lag phase in the methane production when varying the ammonia concentration in the digester (from 0.6 to 3 g L^{-1} of ammonia) where an inoculum previously adapted to high ammonia concentration (2.4 g L^{-1} of ammonia) was used; this was not the case when a non-adapted inoculum (adapted to 0.8 g L^{-1} of ammonia) was used.

To sum-up inoculation is a paramount step of the AD. The good amount and the best inoculum in respect to the substrate must be found in order to optimize a batch process.

Substrate physical properties

Physical properties like particle size, porosity and hydraulic conductivity have a significant impact on the digestion process of solid waste. Biological hydrolysis of solid substrates occurs via a surface biofilm degradation mechanism (Vandevivere et al., 2003) which makes particle size playing an important role in AD. Liotta et al. (2014) showed that by increasing the particle size of carrots, an easily-degradable substrate, methane production kinetics decreased but not the final methane yield. A surface dependent disintegration kinetic was successfully used to model this process (Esposito et al., 2011). On the other side, Motte et al. (2013) showed that by milling (and then decreasing particle size) straw, a lignocellulosic substrate, not only the degradation kinetic increased but also the methane yield, since this particle size reduction increased the accessible surface area to biological degradation by breaking recalcitrant compounds like lignin (Yang et al., 2015).

Among physical solid properties, porosity (empty volume / total volume) and hydraulic conductivity, indirectly related to particle size (Murray, 1995) and then to mass transfer, were

shown to play an important role in percolation process as LBRs treating solid waste. In those cases, methane yield was shown to increase by enhancing the porosity of a solid substrate by addition of inert material: pistachios shell to digestate manure (Myint and Nirmalakhandan, 2009) and gravel to municipal solid waste (Valencia et al., 2009). During digestion, the decrease of the porosity in a solid bulk and, as a consequence of its hydraulic conductivity, can cause problems of water channelling in percolating systems. This was shown to hamper the optimal substrate degradation (André et al., 2015). Physical properties of the substrates and their evolution during digestion play then a very important role in the degradation of solid substrates.

Substrate composition: C/N

Carbon-to-nitrogen ratio, C/N, is an important parameter since it reflects the substrate balance in respect to the microorganism growth, and then the efficiency of the process. Carbon quantifies the energy source in the substrate whereas nitrogen its nutritive capacity. C/N is strictly linked to the effect of nitrogen on the AD performance, indeed, limitation in nitrogen leads towards limited bacterial growth and poorer performance while too high concentration leads to inhibition (see paragraph 1.2.4). Even if the optimum C/N value varies in relation to the substrate (Kothari et al., 2014), this is commonly set between 20 and 30 (Forster-Carneiro et al., 2008; Hills, 1979; Jha et al., 2011; Xu et al., 2014). Such a big range is often due to the fact that often the fractions of carbon and nitrogen considered are not only the available ones but the total amounts present in the reactor. Optimal C/N was also shown to vary in relation to operating temperature mainly because of a variable need in both sources and a different inhibition threshold connected to nitrogen speciation: C/N ratio of 27 and 31 were respectively found as optimum in mesophilic and thermophilic condition for reactors digesting dairy manure, chicken manure and straw (Wang et al., 2014).

Co-digestion is a very useful way to modify the C/N of a fed mixture. As an example, agricultural waste (e.g. straw or cereal residues) are often poor in nitrogen and have a C/N higher than 50 (Kothari et al., 2014). These wastes can then be co-digested with animal manures which are

known to contain high amount of nitrogen (see paragraph 1.2.4). Their co-digestion has been widely described in literature and a lot of examples has been reported by Mata-Alvarez et al. (2014).

Total solids

The amount of water is a parameter particularly important in AD process working with high TS content. Water deficiency highly limits microorganism activity and mobility, coproducts dilution, diffusion and transfer of soluble compounds (Abbassi-Guendouz et al., 2012; Pommier et al., 2007; Yang et al., 2015). Because of that, lower methane productions were reported by Abbassi-Guendouz et al. (2012) and Le Hyaric et al. (2011) when increasing TS in the digesters. In dry AD, 30% TS was found to be a critical threshold above which performance drops significantly (Abbassi-Guendouz et al., 2012). High TS content in the digester was found to cause higher accumulation of intermediate compounds, like VFAs (Motte et al., 2013), causing, indirectly, a process inhibition (see paragraph 1.2.4).

Finally, the low total solid content in dry AD process modifies the microorganism growth mode and, as a consequence, their morphology. The high TS content, often linked to lack of free water, hampers the development of free microorganisms and favours the development of biofilms on the substrate (Vandevivere et al., 2003). In dry system the substrate would play both the role of energy source and support for biomass which seem an advantageous strategy since the use of organic material as support material for bacterial growth increases methane production significantly (Ward et al., 2008).

1.2.4   Inhibition

VFA

All along the AD process operation, intermediate compounds are contemporarily produced and consumed. However, when their production and consumption rate differs, intermediates accumulate and inhibitory concentrations can be reached. The intermediates accumulating the most

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in AD process are VFAs. The most observed VFAs are acetic, propionic and butyric acids (Karthikeyan and Visvanathan, 2012a): indeed, acetic acid is the main precursor of methane and all carbonate compounds (except CO_2) pass from this form before being transformed into methane; propionic and butyric acids consumption requires specific thermodynamic conditions ($\Delta G > 0$ in standard conditions) to be consumed, in particular propionic acid (Amani et al., 2010), making their consumption more difficult.

VFA accumulation directly impacts the pH with a tendency to decrease its value (pKa close to 4.7). The pH drop has, as previously mentioned, a strong impact on all biochemical equilibrium in the system (see paragraph 1.2.3). At low pH, VFAs are mainly found under a unionized form which make them more toxic for cells since they can easily pass through their membrane (Amani et al., 2010). VFA inhibition is then strictly linked to pH (and then to alkalinity which could hamper pH drop) more then to their real amount even if some authors report inhibitory level of 0.9 g L^{-1} of propionic acid (Wang et al., 2009), 2 g L^{-1} for acetic acid and 8 g L^{-1} for the total acid concentration (Karthikeyan and Visvanathan, 2012a). In fact, Gourdon and Vermande (1987) reported that no inhibition to propionic acid up to a concentration of 6 g L^{-1} at a pH of 7.4 digesting pig manure, and that propionic acid should simply be considered as a warning more than as a disturbance of the process. The same conclusions for butyric and isobutyric acids were found by digesting manure in continuous-stirred tank reactor (CSTR) at thermophilic temperature (Ahring et al., 1995). VFA toxicity should be looked with caution and analysed in every specific context in relation to the pH and the alkalinity. For this reason several indirect parameters like acetic acid-to-propionic acid ratio or VFAs-to-alkalinity ratio are often used to control possible critic states in anaerobic digesters (Karthikeyan and Visvanathan, 2012a).

Nitrogen

Proteins, urea and nucleic acids are the main source of nitrogen in anaerobic digesters (Yenigün and Demirel, 2013). More specifically, in manure the ammonia is derived mainly from

the urea component contained in urines (Whitehead et al., 1989). Cow urine, as an example, contains from 6.8 to 21.6 g N L^{-1} (Bristow et al., 1992). During AD, the hydrolysis and solubilisation of proteins, urea and nucleic acids leads to mineralization of nitrogen and its accumulation in the systems since nitrogen in not directly ejected from the system as CH_4 and CO_2; this makes the digestate a great source of nitrogen and of other fertilizer compounds. (Vaneeckhaute et al., 2013). Yabu et al. (2011) and Yenigün and Demirel (2013) reported, for example, a mineralization ranging between 60% to 80% of total organic nitrogen present in substrates. This range can evidently change from process to process in relation to degradability, retention time, process design, etc..

Even if nitrogen is necessary for normal bacterial activity, high concentrations of nitrogen compounds have been reported as inhibitory of AD, especially for methanogens (Kayhanian, 1999). According to Rajagopal et al. (2013), concentrations between 50 and 200 mg L^{-1} are beneficial to AD. However inhibiting level are very disparate in literature, varying from 1.4 to 14 g L$^-$1 (Chen et al., 2008). This is due to the difference in the system and the substrate used, the inoculum adaptation, process temperature and pH.

Inorganic nitrogen is present in anaerobic digestors in two forms: NH_3 (called free ammoniacal nitrogen, FAN) and NH_4^+. Two mechanisms are hypothesised to inhibit AD, (i) NH_4^+ inhibiting the enzymes synthesing methane directly and (ii) passive diffusion of NH_3 in the cell causing proton imbalance and/or potassium deficiency (Kayhanian, 1999). This latter is recognized as the most important inhibitory mechanism. The FAN is related to the total ammoniacal nitrogen, TAN, by a relation varying in relation to temperature and pH (Karthikeyan and Visvanathan, 2012a). By increasing temperature, the equilibrium pushes towards NH_3 which is the reason why thermophilic conditions are often associated to ammonia inhibition. However, according to Yenigün and Demirel (2013), higher NH_3 concentration in thermophilic condition are also accompanied by higher threshold for inhibition. In addition, several authors showed also that TAN inhibition

diminished in presence of other ions as K^+, Ca^{2+}, Mg^{2+} (Chen et al., 2008), but details about these interactions are still poor.

Metal ions - Potassium and Sodium

Potassium is not often directly addressed as inhibitory; however, its high concentration in spent animal bedding makes it a valuable element to consider. Indeed, macro-minerals such as potassium and sodium are essential in animal nutrition to ensure proper functioning of their metabolism, 177 g K d^{-1} cow^{-1} and 66.5 g Na d^{-1} cow^{-1} for lactating dairy cattle (Pettygrove and Heinrich, 2009).

Given the animal diet it is normal to find high concentration of K^+ and Na^+ in the animal excrements. Fang et al. (2011) and Pettygrove and Heinrich (2009) reported a concentration of 4.4 g K^+ L^{-1} (0.57% of the total dry weight) and 1.1 g Na^+ L^{-1} in cattle manure. Cations, such as potassium and sodium, are required for the effective functioning of AD systems. However, at high concentrations, it may cause inhibition of AD process (Karthikeyan and Visvanathan, 2012b). The K^+ and Na^+ inhibitory effect is rarely mentioned in literature. Low concentrations of potassium (< 400 mg L^{-1}) have demonstrated an enhancement of the AD in mesophilic and thermophilic condition (Chen et al., 2008). Concerning potassium and sodium, inhibition of AD process has been reported from 6 g K^+ L^{-1} to 28 g K^+ L^{-1} (Chen and Cheng, 2007; Fang et al., 2011; Jard et al., 2012; Kugelman and McCarty, 1965) treating sludge, swine waste, algae and desugared molasses and from 3 to 11 g Na^+ L^{-1} (Jard et al., 2012).

Other inhibitions

A high number of elements are necessary for correct bacterial growth. At too low concentrations a deficiency can occur, while at too high concentrations an inhibition could take place. In both cases the reactor performance will be compromised (Bernet and Buffière, 2008).

Cobalt, iron, nickel, sulfide, selenium, tungsten, molybdenum, barium, magnesium, and sodium are the micronutrients required in relatively small quantities by some microorganisms (Amani et al., 2010) and their presence should be ensured in the digesters. Beside them, many other compounds were found to affect the AD process: light metals (e.g. Na^+, K^+, Ca^{2+}, Mg^{2+}, etc.), heavy metals (e.g. Zn^{2+}, Cd^{2+}, Hg^{2+}, etc.), long chain fatty acids (LCFA). All these possible inhibitions will not be discussed in this manuscript since not directly linked to the topic. The readers should refer to the work of Amani et al. (2010), Rajagopal et al. (2013) and Chen et al. (2008) for further details.

1.3   Anaerobic digestion technologies – Leach-Bed Reactors (LBRs)

1.3.1   Technology classification

In order to well understand the functioning of LBRs, a short overview on AD technologies is necessary to put LBRs in relation with all the other existing processes.

The adaptation to the context and to the variety of organic substrates led to the development of several anaerobic digestion reactor designs, and new reactor designs are still proposed. In this sense, animals represent a real source of inspiration since their digestive systems offer a very large variety of examples for designing new and more efficient AD plants (Godon et al., 2013). Three main process characteristics will be highlighted here to finally define LBRs: the *total solids*, the *operation mode* and the *complexity* of the system.

Total solid content is a cornerstone of AD process, separating processes able to treat solid waste streams from the ones treating liquid waste streams. Two main categories are identified: wet process, working at a TS < 15% and dry process working at a TS > 15% (Kothari et al., 2014). As mentioned previously, dry process evolution at industrial scale is far more recent (1980s') compared to wet technologies; this is also reflected by the scientific research in this field (see paragraph

1.1.1). However their rapid development at industrial scale in the last years (Baere et al., 2012) is due to their interesting features in comparison to wet processes Table 1.

Table 1: Strengths and weaknesses of dry processes based on Jha et al. (2011), Karthikeyan and Visvanathan (2012a) and Kothari et al. (2014)

	Strengths	Weaknesses
Technical	- Small volume - Low consumption of water - Low production of digestate - Easy handling of digestate (solid) - No need in post-treatment (e.g. dewatering, phase separation, etc.) - Less energy requirement	- Incomplete mixing
Biological	- Less sensitive to acidification - Differentiation between methanogenic and acidogenic zones (Martin, 2001)	- Complex heterogeneous structure, composition and size of the medium - Inhibition problem due to high load and accumulation of VFAs - Poor start-up performances - Long retention time needed - Possible inhibition due to high TS
Economical	- Reduced costs	

The reduced use of water allows having a smaller working volume to build and to warm up, reducing significantly the investment costs. Moreover, this contributes to the production of a solid digestate which does not need any further post-treatment to be handled and spread onto soils. Consequent simpler design and reduced costs for substrate transport are further positive characteristics of dry processes (Karthikeyan and Visvanathan, 2012a). Nonetheless some drawbacks are connected to them and in particular to the biological aspects. In dry processes OM in often loaded without any pre-treatment (e.g. particle size reduction) which makes of it a heterogeneous matrix more difficult to digest than aqueous streams or small particle-size substrates. This diminishes the conversion performance and generally increases digestion time. However,

biodegradability is not affected if proper conditions (like not too high TS) in the digesters are maintained (Jha et al., 2011). Finally, high organic load could result in reactor acidification and generally in a higher inoculation. However, the heterogeneity of the matrix can also represent a positive aspect of dry systems because of the theoretical separation of methanogenic and acidic zones inside of the reactor which make it more robust in biological terms (Martin, 2001).

The operational mode refers to the way the reactor is fed. Two main categories are defined: continuous and discontinuous (or batch) reactors. In the first configuration, new substrate is continuously added to the reactor and the same volume is taken out. In relation to the frequency of feeding the definition of semi-continuous reactors is sometimes used. On the other hand, in batch process the substrate is loaded in the digester which is then completely emptied after completion of the digestion (Vandevivere et al., 2003). In dry discontinuous reactors, no moving parts are present, which, besides reducing investment and maintenance costs, allows accepting undesirable like pebbles or ropes which are naturally present in the inlet OM in rural areas. However, they suffer of reduced mixing and not complete substrate degradation. Problems of clogging and percolation are often reported while discontinuous loading impact biogas production and start-ups which have to be repeated several times a year and which make the operator face risks of explosion. In spite of these drawbacks (further detailed in the next paragraph), poorer degradation efficiency are often balanced by lower investment and energy requirement which makes them suitable for farm-scale implementation (Weiland, 2010).

System complexity refers to physical separation of the several biological steps of AD (see paragraph 1.2.2). Two main categories are defined in literature: one-stage when all the different AD steps take place in the same reactor and two-stage when hydrolysis/acidogenesis and acetogenesis/methanogenesis are separated and take place in two different reactors (Kothari et al., 2014). The choice between these two configurations is often done based on the type of substrate used and, more in particular on its aptitude to undergo acidification. Examples of single or two-stage process will be given using, as an example, the LBR in the next paragraph.

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1.3.2 Leach-bed reactors (LBRs)

Process description

LBRs are dry and discontinuous digesters which can be used in several configurations (complexity) because of their aptitude to be coupled. LBRs consist in the separation of a solid static phase, which is the solid dry substrate, and a liquid mobile phase called leachate. The simplest LBR configuration is the single-stage, called also one-stage or batch (Figure 2). This latter consists in the use of only one reactor where all the four steps of AD take place simultaneously (see paragraph 1.2.2). Such a configuration needs the rector to be well inoculated from the beginning in order to not fall into acidification because of the high organic load (see paragraph 1.2.3). However, even if the use of a single-stage system is common at a lab-scale, the configuration coupling several reactors is often preferred at industrial-scale: sequential LBRs configuration. This latter was first developed in the USA and known as SEBAC (Sequential Batch Anaerobic Composting) (Chynoweth et al., 1991) (Figure 2). This consists in the use of three reactors in sequence staggered in time: new, mature and old reactor (which refers to the digestion time). In this configuration the freshly loaded reactor (new) is connected through the leachate to the old one with the aim of injecting a stabilized leachate, transporting alkalinity and inoculum, on the top of the new reactor and sending solubilised flushed compounds (mainly VFAs) from the new to the old reactor in order to consume them rapidly and do not inhibit the new reactor start-up. Once stabilized, the new reactor can be run as a single-stage and later it could help a new reactor to start (closing the cycle). Sequential LBRs have the advantage to overtake the initial acidification because of the synergy created between new and old reactors in consuming VFAs. This configuration permits to treat very easily-degradable substrate like municipal solid waste (Chynoweth et al., 1992) or market waste (Tubtong and Towprayoon, 2010).

Finally, LBRs were used also as simply acidification reactors aiming at producing VFAs that were extracted and sent in a methanogenic reactor, often an UASB (Upflow Anaerobic Sludge

Blanket), and then called hybrid batch-UASB system (Figure 2). This configuration, completely separating acidogenesis and methanogenesis, is a classic example of a two-stage system, first proposed by Ghosh (1985) in order to optimise separately the acidogenesis and methanogenesis phase (whose optimum condition are known to be different (see paragraph 1.2.3), reaching in that way better performance. This configuration was successfully developed by Shin and Han (2000) and called MUSTAC (Multi-Step Anaerobic Composting). MUSTAC consisted in several acidic LBRs and only one UASB for VFAs conversion into methane with the double aim of increasing the total methane production and smoothing overall methane fluctuations. Acidogenic reactors were characterized by a short digestion time (Browne et al., 2013; Nizami et al., 2010) (often coupled with a post-treatment like composting to further stabilize the substrate) and also by the recovery of certain amounts of hydrogen (Han and Shin, 2004). The production of hythane, a mixture of hydrogen and methane, the first issued by the dark fermentation in acidogenic reactor and the second by the anaerobic digestion in methanogenic reactor, is then possible in such a configuration (Liu et al., 2013). However, in spite of the interest of this process, hythane production is beyond the scope of this manuscript and is not described any further.
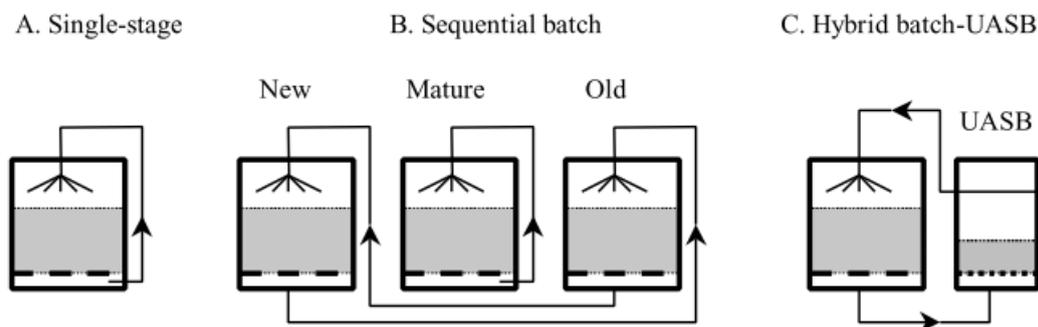
Figure 2: Existing LBR configurations (Vandevivere et al., 2003)

Recirculation in leach-bed reactor

A particular aspect of LBRs concerns the leachate recirculation. Indeed, this seems particularly important in dry static reactor where substrate is not mixed as LBRs. The effects of

leachate recirculation on the digestion process are associated to various complementary mechanisms: humidification, transfer and inoculation (Komilis et al., 1999).

The first effect played by leachate recirculation is the humidification of the solid bulk phase. It is reminded that high TS content inside the reactor decreases microorganism activity and, as a consequence the reactor performance (see paragraph 1.2.3). A not efficient aspersion of the leachate on the solid bulk top and problem of channelling, compaction and unidirectional flow are often the main cause of a delayed reach of optimal humidity inside the reactor (Uke and Stentiford, 2013). At industrial scale, the use of electrical tomography in LBRs stressed the difficulty in reaching optimal humidification of the substrate and the need in optimizing injection technologies (Degueurce et al., 2016b). To face this problem, flooding the substrate all along the digestion was tested by Kusch et al. (2012) while Benbelkacem et al. (2010) tested 12 hours flooding and drain. Both of them reported a positive impact on reactor performance compared to discontinuous recirculation: the first higher kinetics but similar methane production after 60 days and the second lower kinetics but higher methane yield after 600 days of operation (mathematical simulation between 200 and 600 days). In addition, flooding 30% of the bed was found to give better methane yield than no flooding by André et al. (2016b). However, in spite of the positive results obtained, flooding questions the design of the processes at industrial scale (e.g. garages are not designed to support such liquid pressure inside) but remains an option that needs to be further investigated to discover all its potentiality and limits.

Discontinuous recirculation is considered a good mean to improve biodegradation. In process simulating landfill degradation of municipal solid waste in bioreactors (long digestion time and small recirculated volumes) the increase of the leachate rates is found a good mean to reduce digestion time (Benbelkacem et al., 2010; Chugh and Clarke, 1998; Šan and Onay, 2001; Sponza and Ağdağ, 2004). However, compared to flooding, discontinuous recirculation resulted in lower kinetics (Kusch et al., 2012). Continuous recirculation, on the other side, was found with no positive effect compared to discontinuous recirculation in a well inoculated reactor treating maize silage and

it was found even to favour acidification in not well inoculated reactor (Kusch et al., 2012). This shows the importance of the inoculation and the substrate treated on the role of recirculation.

The second role played by the leachate recirculation is the transport of nutrients and intermediate compounds through the bed (Browne et al., 2013; Jha et al., 2011; Šan and Onay, 2001). This seems particularly important in a system as a LBR where no mixing is done. The role of transport was elucidated by testing the performance of a LBR loaded with different layer of substrate and inoculum (Veeken and Hamelers, 2000). The VFA accumulation in fresh substrate layers (leading to inhibition) when no recirculation was applied and the good performance obtained (no VFAs accumulation) when this latter was effectuated proved the importance of leachate recirculation in mobilizing intermediates in LBRs. Moreover, not only the compounds but also microorganisms were proved to be transported by leachate recirculation and to colonized the solid bed in the sense of recirculation treating food waste (Xu et al., 2014). It is easy to understand that the role of transfer acquires a greater importance in coupled systems where inoculum and nutrients can be exchanged (i.e., as in SEBAC process). The system complexity plays then a very important role on the choice of the best recirculation strategy.

If the qualitative roles of recirculation are relatively well known, their actual setting (e.g. volume recirculated and frequency of recirculation) are difficult to generalize because of the inter-relation with other parameters like substrate degradability, inoculation and, digester and process design. This means that specific assessment of these parameters in LBRs treating a slowly-degradable substrate as spent animal bedding are necessary.

1.3.3 Industrial LBR-based processes

Generalities

Mainly based on the process developed in France by 1937 by Ducellier and Isman to treat spent animal bedding with vegetable waste in underground silos (Molnar and Bartha, 1988), several

process using the principle of LBR have been industrialized. The most known in scientific literature are Biocel and SEBAC.

Based on the PhD thesis of Ten Brummeler, (1993) and his several publications (Ten Brummeler and Koster, 1990; Ten Brummeler et al., 1992, 1986), an industrial site using Biocel technology was constructed by Orgaworld in 1997 in Lelystad, The Netherlands. This site operated 14 digesters of 720 m^3 (480 m^3 working volume) working in parallel with a batch duration of 21 days at mesophilic temperature and treating 50,000 t of bio-waste (organic fraction of municipal solid waste (MSW) per year (Ten Brummeler, 2000).

On the other side, based on the work of Chugh et al. (1999), Chynoweth et al. (1991) and O'Keefe et al. (1993), an industrial SEBAC process was implemented in Florida (USA) by the company Sigarca (USA, 1995) for the treatment of different kind of bio-waste like municipal solid waste or animal waste.

However, beside these two examples, many other companies developed or are developing their own technologies in Europe: Bekon, Bioferm, Smartferm, Kompoferm, etc and finally Naskeo Environnement. All based on the same principles, some specificity concerning the garage tightness, door opening system, building material (concrete garages or metal boxes) and specific leachate control differentiate them.

Naskeo/Methajade process : LBR configuration of this PhD thesis

This LBR-based process commercialized by Naskeo/Methajade was firstly developed by the French company Methajade for the treatment of farm waste. In 2014 Naskeo Env. acquired this company and improved this process. Nowadays, Naskeo Env. has 15 full scale plant operating on this dry discontinuous process in France (Naskeo, 2015). In the following paragraph, a general garage-system based on the Naskeo/Methajade design is described. The main problematic connected to this process will be clearly highlighted in order to better understand the issues treated in the next chapter of this manuscript.

Digesters and tank

Two main components compose a LBR process, the digesters and the tank. In the first, the solid substrate is loaded while in the second the leachate is stored (Figure 3). Digesters and tanks are connected through a leachate circuit. Leachate is sprinkled on the top of the solid substrate, let percolate through it, collected at the bottom and pumped back to the tank. The tank plays then a role of dilution and buffer volume between the digesters which are then not directly connected. Normally four digesters work in parallel staggered in time with batch duration variable between 40 and 60 days in mesophilic conditions (e.g. with a batch duration of 60 days and four digesters, each 15 days a batch is emptied and loaded again). In this way a more stable biogas production is ensured. It is interesting noting that in this system solid and liquid fractions are separated and that, theoretically, new solid and liquid containers (digesters or tanks) can easily be added if necessary.

Loading and emptying operations

The reactors are consecutively (e.g. every 15 days in the previous example) emptied and loaded with new fresh matter which is previously mixed with digested substrate (digestate) from an old digester to ensure inoculation and a rapid start-up. The leachate is never replaced and its level is completed with fresh water if needed. The digester is closed and let run for its entire batch duration. During substrate digestion the solid is moistened with leachate according to specific strategies (different for any process). Once the batch duration is over, the reactor is opened, emptied and reloaded and the digestion cycle continues.

Biogas conversion and energy production

Biogas produced in each digester or tank (20% of the biogas was estimated to be produced in the leachate storage by Kusch (2008) in an industrial site) is collected and converted. Three conversion modes exist nowadays: direct injection into the gas grid, direct combustion into biomass

boiler for heat recovery, and in a combined heat and power (CHP) unit to produce heat and electricity, better known as cogeneration. The number of French AD plants using these three different biogas conversion modalities is reported in SINOE (2003-2016): 213 cogeneration, 140 biomass boiler and 9 biogas injection.

The most common practice in Naskeo sites, and thus the one considered in this manuscript, is the cogeneration: a part of the heat is used to fulfil internal process needs (like keeping digester temperature) and another is used in other external activities (like wood drying, building heating system, etc.) (no more compulsory since 2016); on the other hand, electricity is sold and the prices fixed by national decrees (see paragraph 1.1.3).

A CHP unit is a very important part of the process. A CHP unit is a combustion engine characterized by a specific combustion nominal power, or maximum combustion power, and a minimum combustion power (often close to the half of its nominal power), below which the engine shuts down, In normal conditions the CHP unit works within this power range continuously. Indeed, repeated shuts down reduce significantly its lifetime. An important parameter associated to the CHP units is the electrical power efficiency. This latter is at its highest level when the engine works at its nominal power whereas it decreases at lower powers. This makes the functioning at nominal power the most interesting since electrical production is the major source of income for an AD plant. The last limitation of a CHP is the methane content in the biogas. Variable from model to model, this threshold is often 45-50%, which means that a biogas with a lower content cannot be burned in a CHP. These specific characteristics of a cogeneration engine play a very important role in the feasibility of a process.
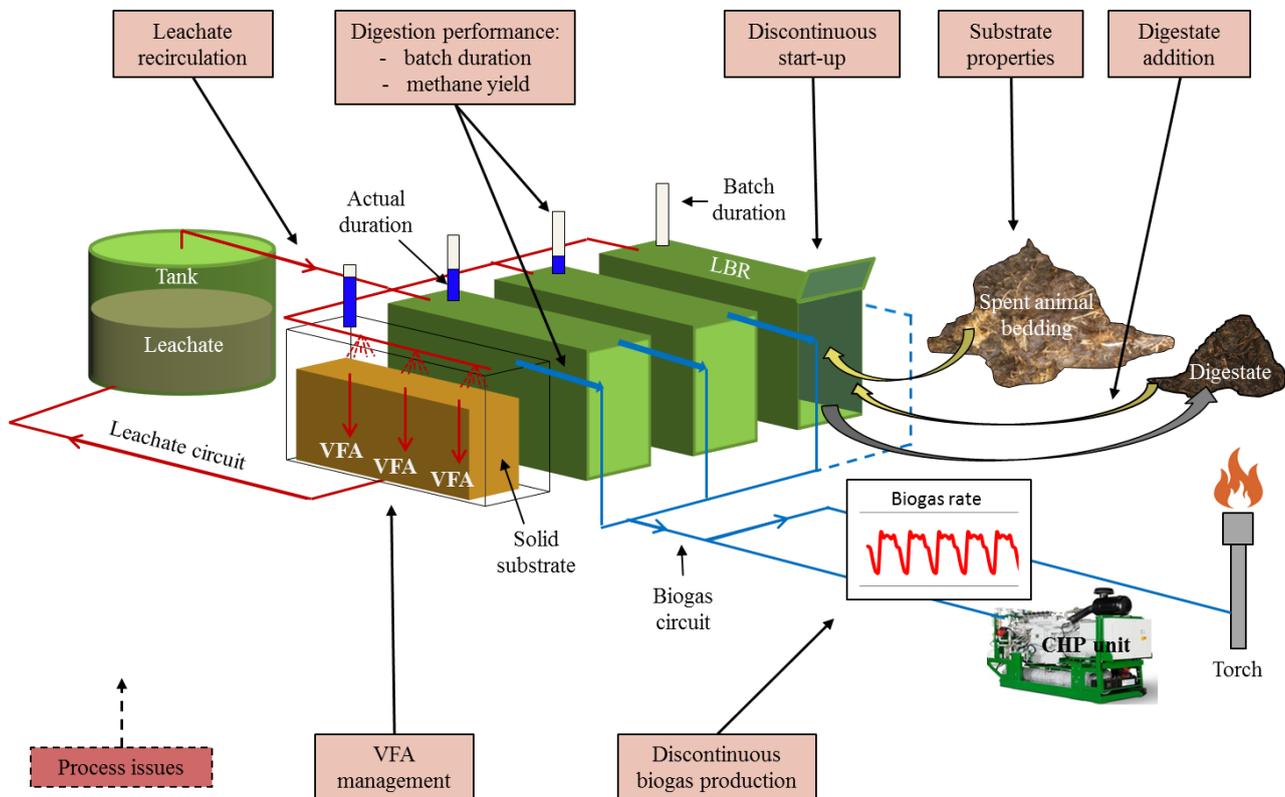
Figure 3: Operating principle of the Naskeo/Methajade sequential LBRs process

1.3.3.2 Process issues

The sequential LBR process described in this manuscript present several issues that affect its economic viability and development. These issues have been highlighted in Figure 3 (pink bullet points) and then discussed in the next sub-sections.

Substrate properties

In general, the biological, chemical and physical characteristics of the substrate used in a process play a key role on the process itself since they determine its range of action, i.e. limits. Therefore, studying the characteristics of different types of spent animal bedding, their adaptability to LBRs process, the problem encountered during their digestion as well as the energy efficiency recovered from their treatment, are all key basic pieces of information. If a lot of data is available in literature about manure (animal faeces), very few studies analysed properties of spent animal

bedding and even less on its digestion in LBRs. The acquisition of this knowledge was found to be an important step in order to successively improve the process efficiency.

Increasing system efficiency

The efficiency of an anaerobic digestion process is based on the amount of methane produced in a certain time slot and in relation to the energy potentially recoverable from the substrate itself. At the same time, engineering problems connected to the reactor size of the must be considered. Therefore, the increase of the methane production in a shorter time and a smaller digester volume is the most important objective to increase the project feasibility. The increase of the degradation rate of the substrate can be achieved through the modification of two main parameters: the inoculum quantity and quality as well as the temperature (see paragraph 1.2.3). These strategies were tested experimentally and analysed from an economic and technical angles, which often represent the real limits of the process.

VFA management and leachate recirculation

Dry AD systems and in particular LBRs are characterized by a high organic load which can lead to an acidification. Strategies like massive inoculation can be used to counteract this phenomenon but the economic impact of such approach would be significant since very little fresh matter would be loaded at every batch. Sequential LBRs based process offers the possibility to manage these VFAs through the synergic relations created by coupling several LBRs with each other (see paragraph 1.3.2). The management of VFAs through leachate recirculation is then fundamental to control the process digestion. This latter represents, in fact, the only controlling tool that the operator can use to drive the process in terms of substrate degradation and biogas production during digestion. For this reason leachate recirculation management is particularly important.

Discontinuous process and biogas exploitation

A sequential LBR system is a discontinuous process where reactors are staggered in times. Even if this operation strategy is convenient for farm management, this represents a real drawback of the process (see paragraph 1.3.2). In this sense start-up phase needs to be perfectly managed in order to avoid serious economic loss. For each start-up phase, a reactor needs a certain time slot in order to reach a stable biogas production rate and an optimum gas quality in order to be used. The consequent discontinuous biogas production is thus a major issue in order to produce electricity in a CHP unit and the process should be operated in order to work within CHP unit technical limits. Discontinuous biogas production is a major issue in sequential LBR site and a biogas combustion power higher or lower than the working CHP range will inevitably result in a biogas loss. The same will occur when the methane content in biogas is below the minimum acceptable threshold (often between 45% and 50%), like when a new batch is started and methane content in the head space is still low.

1.4   Thesis objectives and structure

Based on the issues highlighted above four main general objectives were identified. Each of them is treated in a separate chapter as a scientific article (published or submitted): chapters 2, 3, 4 and 5. Chapters 1 and 6 are deal with the introduction and the conclusion of the present PhD thesis, respectively.

Chapter 1 provides background information regarding anaerobic digestion, LBR process and the structure of the PhD thesis

Chapter 2: focus on spent animal bedding properties and aptitude in being digested in a LBR. Several types of spent animal bedding from different animals, all susceptible to be treated in a LBR process, were characterized and their digestion performance determined. In this chapter the evolution of the main parameters (i.e. biogas volume produced, methane content in the biogas, pH,

VFAs concentration, etc.) during the digestion in a LBR were presented as well as the degradation efficiency in this kind of process and the drawbacks connected to it (Figure 4).
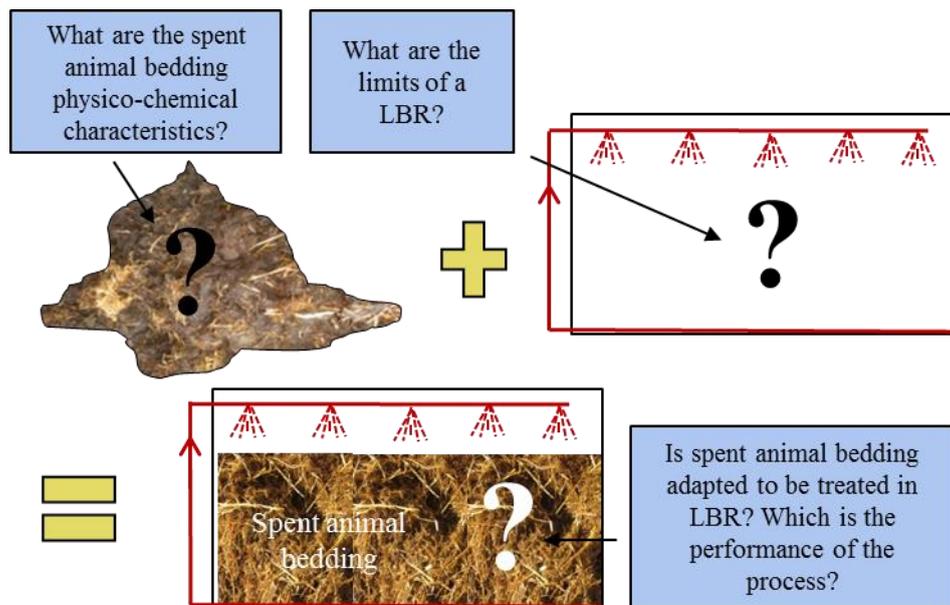
Figure 4 : Graphical abstract of the first issue faced in this PhD thesis: physico-chemical characterization of several types of spent animal bedding, description of the problematics connected to a LBR process and biogas performance reached

Chapter 3 discusses the possibility to improve the industrial site profitability through the optimization of the reactor inoculation at the start-up phase. Difference in the role played by liquid inoculum and solid one were highlighted and the best start-up strategy proposed. The economic interest in adding solid digestate is evaluated in economic terms (Figure 5).
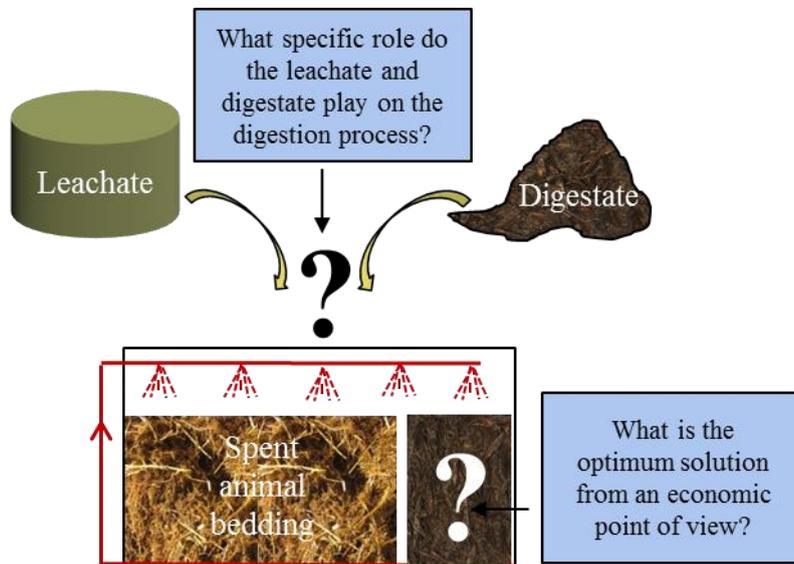
Figure 5 : Graphical abstract of the second issue faced in this PhD thesis : the role played by both leachate and digestate on the digestion process and the economic comparison of these two strategies

Chapter 4 reasons the possibility to operate the bioreactor in thermophilic conditions with the aim to diminish batch incubation and highlights the problem linked to the biogas exploitation in this condition (Figure 6).
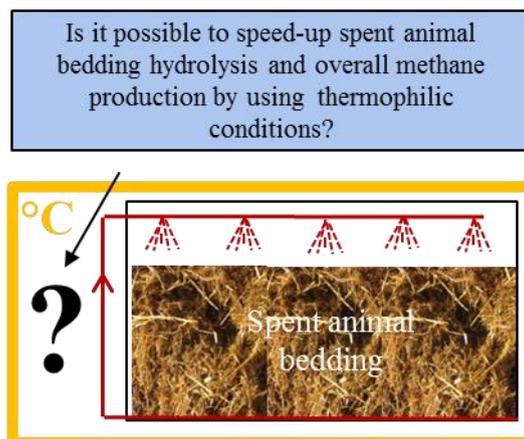
Figure 6 : Graphical abstract of the third issue faced in this PhD thesis: the advantages and drawback of treating spent animal bedding in thermophilic conditions

Chapter 5 proposes a strategy to control the digestion process through a leachate management in the case of co-digestion of a slowly- and easily-degradable mixture. This chapter gives insights to the operator regarding the importance of leachate management during the acidification phase which is likely to occur seasonally in farm-scale site (Figure 7).
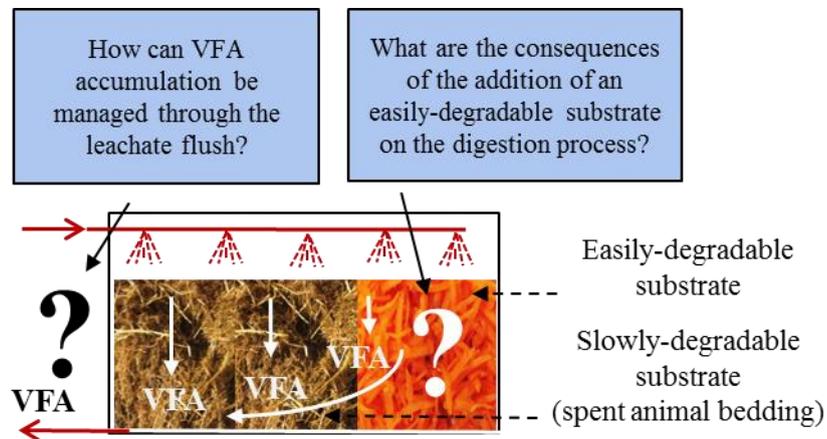
Figure 7 : Graphical abstract of the fourth issue faced in this PhD thesis: the extent of inhibition when adding a significant fraction of an easily-degradable substrate and the use of the leachate flush to manage volatile fatty acid accumulation

Chapter 6 highlights the major findings and the potential impact of the research at industrial scale, and provides future recommendations for LBR management.

Finally, Figure 8 allows to better visualise the PhD structure in chapters as well as the substrate and the system used in each of them. Initially spent animal bedding digestion is analysed and optimized in a single-stage process while, later, the case of co-digestion of easily and slowly-degradable matter is studied in an LBR simulating a sequential system.

Chapter 1	• General overview of the context • Basic knowledge on anaerobic digestion process • Leach-bed reactor description and PhD issues presentation	
Chapter 2	• Spent animal bedding characterization • Digestion performance in LBR	**Substrate** Mono-digestion: Spent animal bedding **Process** Single-stage LBRs
Chapter 3	• Leachate and digestate role during start-up • Economic analysis of different start-up strategies	
Chapter 4	• Comparison of thermopilic and mesophilic temperatures	
Chapter 5	• VFAs management and leachate flush strategy	**Substrate** Co-digestion: easily- and slowly-degradable substrates **Process** Sequential LBRs
Chapter 6	• Conclusions and perspectives	

Figure 8: Structure of the PhD manuscript

CHAPTER 2

Study of the spent animal bedding digestion in single-stage LBR

The objective of this chapter is to assess the influence of the physico-chemical properties of the substrate (spent livestock bedding) on the LBR process performance. Several types of spent livestock bedding were characterized in order to determine their properties (e.g. VS/TS, BMP, fibre content, etc.), variability and finally adaptability to the anaerobic digestion. In this chapter, the main issues connected to the operation of LBR and more especially the treatment of spent animal bedding are discussed: biogas loss at the start-up, batch duration, overall methane production and accumulation of intermediate metabolites. This chapter developed the knowledge necessary to propose some new operating strategies to optimize the treatment of spent animal bedding in leach-bed reactors.
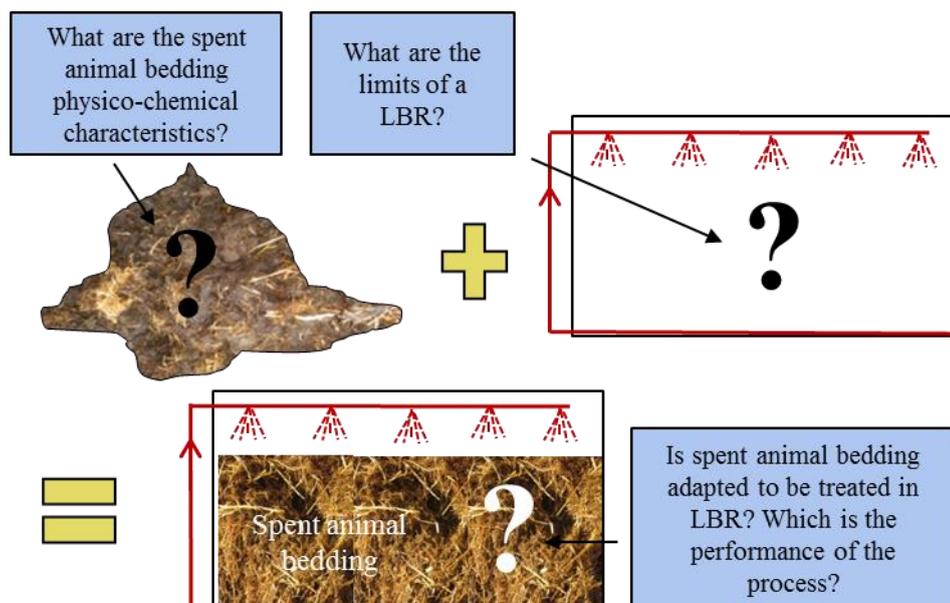
Figure 9 : Graphical abstract of the first issue faced in this PhD thesis: physico-chemical characterization of several types of spent animal bedding, description of the problematics connected to a LBR process and biogas performance reached

2.1   Mesophilic anaerobic digestion of several types of spent livestock bedding in a batch leach-bed reactor: substrate characterization and process performance

2.1.1   Introduction

Dry anaerobic digestion processes have spread widely in the past few years compared to the wet ones due to their advantages in accepting substrates with total solids (TS) higher than 20%

(Karthikeyan and Visvanathan, 2012a). In countries like France, agriculture and livestock rearing are well developed and the use of primary products (e.g. cereals) as feed for anaerobic digestion (AD) plants is forbidden by national legislation (Assemblée Nationale, 2014). In such a context, manure occupies a dominant position among rural waste. Indeed, the French national audit FranceAgrimer (2012) reported an annual production of about 90 million tonnes of solid manure and 180 million tonnes of slurry.

Thanks to its particular characteristics mainly related to a high nitrogen content and alkalinity, manure have been used in AD for a long time, especially in co-digestion with other substrates. This is also linked to the very tight legislation on manure management because of problems like greenhouse gas emission, nitrogen contamination of groundwater (Smith and Frost, 2000) and nuisance odours (Wilkie, 2000). Manure can vary greatly in relation to its TS, which mainly depends on the farm housing practices. In deep-litter housing systems, bedding is used to absorb excrements and urine, thus creating a solid waste rather than a liquid one (*i.e.* slurry). Such solid waste is a soiled bedding that accumulates in the stables and which is referred to as spent bedding (Tait et al., 2009) or spent straw if the latter is used for bedding material. In France, where deep-litter housing practices are widely used, 53.8% of the produced spent cow bedding has a TS higher than 18% (Degueurce et al., 2016a) which can in turn support the development of AD processes adapted to their treatment.

One of the AD processes gaining a foothold in the rural context is the Leach-Bed Reactor (LBR) operated in batch mode. In this dry AD process, the solid substrate is loaded into the reactor, while a liquid phase, usually stored in a separate container, is sprinkled over the solid bulk, percolates through the waste bed and finally returns to its storage tank. So far, only few authors investigated the digestion of spent animal bedding as sole substrate in LBR (Table 2), with the first example reported 30 years ago by Hall and Hawkes (1985). In literature, the most common spent bedding treated in LBRs mainly originated from cow stables and only a few from horse ones. It is

worth noting that very little evidence on the use of spent sheep bedding was reported (Blanco et al., 2010), while nothing was ever reported regarding goat bedding.

Batch LBRs offer several complementarities to animal husbandry and in particular when deep-litter housing practices are implemented. Firstly, being a discontinuous process, it is perfectly suited to the cyclic cleaning of stables, thus allowing the reduction of storage time as well as problems arising from it, such as odour nuisances , soil contamination and the loss of volatile solids (VS) by oxidation (Cui et al., 2011). Secondly, the process accepts undesirables like pebbles or ropes, which are commonly found in farm waste (Møller et al., 2004) and would create operating problems in conventional continuous stirred-tank reactors (CSTRs). Thirdly, thanks to the presence of bedding material, the substrate is characterized by good porosity, which is essential for adequate percolation (Myint and Nirmalakhandan, 2009). Furthermore, the robust and simple LBR design, free of any moving parts, reduces costs related to electro-mechanical spare parts investment and maintenance, making it highly suitable for rural context (Karthikeyan and Visvanathan, 2012a). On the other hand, certain problems may originate with this process: an incomplete degradation of the substrate due to bad percolation and compaction (André et al., 2015), an unstable biogas production due to its discontinuous loadings, as well as a difficulty to exploit all the biogas produced due to the low methane content during the first days.

Considering that, in France, spent bedding from animal stables represents the highest fraction of the feed mixture in LBRs at industrial scale, it is important to better understand its characteristics, diversity and putative differences during its digestion for industrial-scale applications. In contrast to manure and straw separately, the properties of spent bedding are scarcely described in the literature. Moreover, spent bedding has to be considered as a substrate on its own since animal mechanical action on the litter and biological degradation during litter accumulation can modify the original properties of straw and animal excrements (Tait et al., 2009).

Table 2 : Comparison of literature results: performance of leach-bed reactor (LBR) treating spent livestock bedding or a synthetic mixture of livestock faeces and an external bulking agent

Substrate	Reactor	Temperature	Batch duration	Methane yield	References
[1] spent cow straw bedding	LBR	mesophilic (30°C)	40 days 70 days	166 L kg^{-1} VS 215 L kg^{-1} VS	(Hall and Hawkes, 1985)
[1] spent cow straw bedding	LBR	mesophilic (35°C)	30 days	114.5 NL kg^{-1} VS	(Degueurce et al., 2016c)
[1] spent cow straw bedding	LBR	mesophilic (37°C)	32 days	-	(André et al., 2015)
[1] spent cow straw bedding	LBR	-	-	-	(Shewani et al., 2015)
[1] spent cow straw bedding	LBR	mesophilic (35°C)	30 days	114.5 NL kg^{-1} VS	(Degueurce et al., 2016c)
[1] spent pig/swine straw bedding	LBR	mesophilic (37°C)	50 days	not provided clearly	(Yap et al., 2016)
[1] spent horse straw bedding	LBR	mesophilic (35°C)	45 days	170 NL kg^{-1} VS	(Kusch et al., 2008)
[2] cow manure (faeces only) + straw	LBR sequence	psychrophilic (20°C)	84 days (21 days per batch)	147 NL kg^{-1} VS	(Massé and Saady, 2015)
[2] sheep manure + straw	LBR	mesophilic (35°C)	94 days	184 NL kg^{-1} VS	(Blanco et al., 2010)
[2] raw manure slurry + pistachios half-shell	LBR	psychrophilic (22 °C)	-	-	(Myint and Nirmalakhanda, 2009)
[1] spent horse softwood-pellet bedding	LBR	mesophilic (34-36°C)	57 days	44.8 L kg^{-1} VS [*]	(Wartell et al., 2012)
solid phase of raw dairy manure slurry	LBR	thermophilic (50°C)	60 days	214 - 227 L kg^{-1} VS	(Rico et al., 2015)
[2] cow manure + wood powder/chips	LBR	psychrophilic (20-24°C)	-	not provided clearly	(Demirer and Chen, 2008)

[1] Spent bedding: a mixture of excrements and bedding material (straw) directly sampled from the stables
[2] Synthetic mixture of excrements and bedding/bulking agent
[*] No adequate conditions for the process

Characterization of spent bedding and variability among different types is crucial to understand the properties of these substrates before loading them into an anaerobic digester. Additionally, it is

worth mentioning that the few studies investigating the digestion of spent bedding in LBR operated in different conditions (e.g. inoculation, leachate recirculation, etc.), thus hampering a clear and direct comparison of the substrates and their performances in LBR.

For these reasons, the present work aims at characterizing spent animal bedding of different origins and assessing their biological degradation in a batch LBR. The main anaerobic digestion performances are described and compared, and the operating parameters that need to be carefully taken into account when treating these organic substrates in LBRs are analysed in detail.

2.1.2   Materials and Methods

Substrates and inoculum collection

Substrates were collected from deep-litter stables where the bedding was mainly wheat straw. All of them were collected in winter to avoid any variability due to seasonality. Substrates were chosen to be representative of the types of spent bedding that can be found in practice, in relation to both animal species and diet. Six different types of spent bedding were collected, each from one stable: sheep (SB_sheep), goat (SB_goat), horse (SB_horse), and three different cow substrates (SB_cow). Since spent cow bedding is by far the most common in France (FranceAgrimer, 2012), a supplementary division of SB_cow into three subcategories was made, based upon the roughage used in animal diet: round bale grass silage (SB_cow_g), clamp maize silage (SB_cow_m) and hay (SB_cow_h)

Table 3). This sub-division was also supported by the fact that the type of roughage is indirectly related to different breeds of livestock and therefore to the stabling objectives (*i.e.* milk, meat or cheese production) and it has been shown to influence faeces properties like methane potential (Møller et al., 2014). All substrates were collected during stable cleaning out in order to be representative of their real properties before a possible AD treatment. Six samples of almost 20 kilos were collected from different parts of the litter and then mixed following the protocol

described by Gy (1998). The cleaning operation and the sampling protocol ensured the collection of a sample which was representative of the entire site. The substrates were kept frozen at -20°C in plastic bags and one day before utilization they were thawed at ambient temperature.

Liquid inoculum was obtained from an UASB (Upflow Anaerobic Sludge Blanket) reactor treating sugar industry wastewater in the south of France. Before inoculation, the sludge granules were first broken up in glass reactors by continuous magnetic mixing for 3 to 5 days and were then kept in anaerobic conditions at a concentration of about 10 g VS L^{-1} for almost two weeks, until they reached their endogenic production level of about 1.33 NmL CH_4 g^{-1} VS d^{-1}.

Experimental apparatus

The reactors used were 7 L jacketed glass containers of 14.5 cm diameter and 43 cm high (Figure 10).
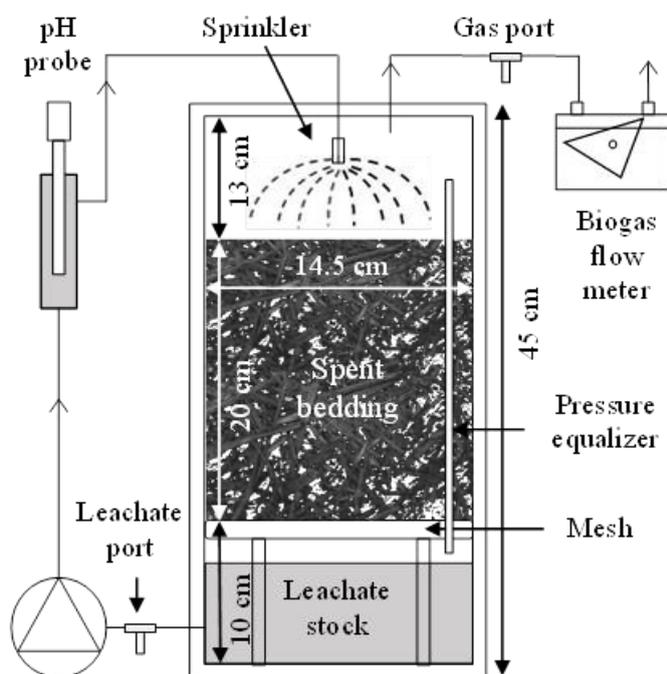
Figure 10 : Experimental leach-bed reactor

The solid and the liquid fractions were separated by a mesh (1 mm holes) placed at 10 cm from the bottom of the reactor, which allowed the leachate to be stored in the volume below. A tube

connecting the headspace and the volume under the mesh was added in order to equalize the pressure in the event of the bulk becoming too compact. A peristaltic pump enabled the leachate to recirculate from the bottom to the top. One small glass flask (140 mL) was fitted into the liquid circuit, in order to measure the pH using a fixed pH probe. A biogas circuit connected the top of the reactor to a volumetric biogas counter. Two ports, one on the liquid and the other on the gas circuit, facilitated the collection of samples for analysis.

Experimental set-up

All the kinds of spent bedding were tested in duplicate in two successive series of 6 reactors. In the first, spent bedding from sheep, goat and horse was analysed separately while in the second, the three different types of cow bedding were assessed.

To better compare the results, all batches were run following the same experimental protocol. The spent bedding load was about 410 g TS (between 1.2 and 1.6 kg of fresh substrate), whereas the total amount of water in the system was set by fixing the system TS (fresh substrate, water and sludge) between 12% and 13%. Since each type of spent bedding had a similar water-holding capacity the amount of free water for recirculation was similar in all reactors, thus ensuring a complete recirculation of the leachate each time. The systems were all inoculated at a substrate-to-inoculum ratio S/X (VS substrate/VS inoculum) of 10, in order to ensure the presence of methanogens and a good start-up of the process. However, inoculum was not considered to participate to the methane production in the reactor because of its rather small concentration. Before each loading, spent bedding and the inoculum were first mixed together. Then, the inoculated substrate was manually placed inside the reactor and compacted with a weight of 8 kg, in order to simulate the compaction taking place in industrial site due to the height of the substrate pile. In a final step, the chosen amount of water was added. Once filled, the solid phase was saturated by continuous recirculation of the leachate for about 15 min. Thereafter, the liquid recirculation was run automatically 10 times a day for 1 minute at a flow rate of about 1.6 L min^{-1}. Such a high total

amount of water recirculated per day (about 40 L kg^{-1} TS d^{-1}) was fixed in order to provide homogenous sprinkling over the bulk and to bring the liquid phase into equilibrium with the solid phase, thus accessing the bulk conditions through leachate analysis. The digestion was stopped after 60 days which is a common digestion period for a mesophilic process digesting slowly-degradable substrate as spent animal bedding.

Sampling and analysis

Characterization of the initial substrate was carried out in triplicate and mean values were estimated with their standard deviation. The TS content was measured after drying the fresh substrate at 105 °C for 24 h, followed by 3 h of calcination at 550 °C for determination of VS. All the other analysis were carried out on substrates previously freeze-dried and milled to 1 mm. Total phosphorus was measured with the BIOTECK ELX800 microplate reader after a complete calcination in an acidic environment where sulphuric and nitro-perchloric acids were added. NTK was measured by the Kjeldahl method after 3h of calcination and successive titration with a BUCHI 370-K apparatus. Total Organic Carbon (TOC) was estimated by a carbon analyser (TOC-V CHS/CSN, Shimadzu Corporation) performing combustion of the sample at 680°C with a catalyser (cobalt/platinum) and pure oxygen. A Van Soest fractionation (Van Soest, 1963), able to separate the soluble fraction, the cellulose, the hemicellulose and the lignin, was carried out using a Fibrebag system (Gerhardt). Finally, a BMP test was performed in accordance with the protocol described by Motte et al. (2014). Further information about the NTK, COT and Van Soest fractionation protocols used are also given by Motte et al. (2014). During the digestion process, leachate was sampled after a recirculation cycle and all the analysis carried out in simplicate. At each sampling a volume between 10 and 20 mL was taken by the mean of the port present in the liquid circuit. The sampling frequency was adapted to the parameters evolution in order to precisely follow the start-up period: 4 times per week the first 3 weeks and then twice per week until the end. . Total alkalinity was measured on the raw leachate at a pH of 4.30, as recommended by Hill and Jenkins (1989). Soluble

chemical oxygen demand (CODs) and volatile fatty acids (VFAs) were measured on a leachate previously filtered at 0.45 μm. 0-1.500 mg L^{-1} spectroquant kits were used to analyse COD, VARIAN I-MET-0084 gas chromatograph with helium as the gas vector was used to measure VFAs and Mettler Toledo InPro 4260i probes connected to a Mettler Toledo pH M300 operational manual transmitter were used to measure pH. Ion concentrations (i.e. Cl^-, NO_2^-, NO_3^-, PO_4^{3-}, SO_4^{2-}, Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+}) in the leachate were measured only at the end of the digestion using the ICS-3000 ion chromatography system after filtering the raw leachate at 0.2 μm. Analyses on biogas were done daily except during the week-end. Biogas volumetric production was measured with Ritter flow meters (Milligascounter MGC-1 V3) and its composition was analysed with a micro-GCPRO CP-4900 using helium as the gas vector.

In the graphs, the mean values between the duplicates were reported without error bars for an easier presentation of the results. The only exception concerned the biogas data of SB_cow_g. In fact, due to the malfunctioning of one of the biogas volumetric counters in the duplicate set-up, the results of only one reactor were used. On average, the error made between duplicates on each analysis (calculated as |x1 – x2| / M(x1, x2) with x1 and x2 the measures and M the mean value of the measures) was: 26% on the daily biogas volume produced, 16% on the TVFA and total alkalinity, and 2.3% and 0.4% on the CH_4 content and pH, respectively.

Statistical analysis

An analysis of variance, ANOVA, was carried out using R commander (Fox, 2005) in order to evaluate the statistical significance of the results obtained from characterization (TS, VS, BMP and, carbon, nitrogen, phosphorous and fibre content) and from final methane yields. Kinetic parameters of methane production were calculated with R Grofit (Kahm et al., 2010). The Gompertz equation was used to fit the experimental results.

$$A(t) = A_{max} \cdot \exp \{- \exp [((\mu_{max} \cdot \exp(1))/A_{max}) \cdot (\lambda - t) + 1)]\}$$

Where A is the cumulative methane production (NmL CH$_4$ g^{-1} VS), t is the batch duration (day), λ is the lag phase time (day), μ_{max} is the maximum methane production rate (NmL CH$_4$ g^{-1} VS d^{-1}). Finally, linear correlations estimated with the Pearson correlation coefficients were searched among all the parameters. All these results are in reported in supplementary material (section 7)

2.1.3   Results and discussion

2.1.3.1   Substrate characterization

Physico-chemical characteristics

All the types of spent bedding were visually heterogeneous as a consequence of their high straw content. All of them were well compacted and in an ongoing composting process, as evidenced by the warm temperature inside the bulk (not measured). This was not the case for the SB_horse which accumulated for only a few days, which is a very short time compared to the other substrates (

Table 3). Indeed, the frequency of litter change, or the time between two stable cleanings, can vary significantly on account of sanitary requirements and farm practices. In line with this parameter, SB_horse represents an exception since horses are mostly reared for leisure and sport rather than for meat production and a much greater attention is paid to their comfort. As reported by Møller et al. (2004) and Cui et al. (2011), the frequency of litter change also plays an important role in the degradation process. In fact, composting of the most degradable fraction can start easily in good biological conditions such as in a litter, even when the limited aeration and the increasing compaction diminish this process.

Another parameter that could highly affect the spent bedding properties is the amount of straw per animal (expressed in livestock unit, LU, (Eurostat, 2013)) per day. As for the frequency of litter change, the amount of straw depends on the animal and the farming practices and SP_horse

represents once again an exception for its high value. By mixing the straw to the animal faeces, these latter are diluted and properties like TS, VS, C/N, fibre composition and BMP are modified. Hence, spent bedding characteristics are often found in between the ones of raw faeces and straw.

The ANOVA, performed on TS, VS, carbon, nitrogen, phosphorous and fibre content, proved that all substrates were statistically different ($p\text{-value} < 0.05$).TS is a very important parameter for AD process design (Møller et al., 2004) because it impacts the loading charge and the methane production per volume of digester. TS of SB_cow was found, on average, lower than SB_horse and SB_sheep (

Table 3). TS values similar to the ones measured in this study were reported in literature: 26.3% (Hall and Hawkes, 1985) and 22.2% (Degueurce et al., 2016c) for spent cow bedding and 38.0% (Kusch et al., 2008) for spent horse bedding. TS of straw is generally higher than 89% (Cui et al., 2011; Wartell et al., 2012; Motte et al., 2013; Massé and Saady, 2015) while TS of cow faeces was estimated between 9.8 and 15.6% (Møller et al., 2004). Higher values were reported for horse faeces, ranging between 20% and 42% (Wartell et al., 2012), as well as for sheep droppings, equal to 49% (Blanco et al., 2010). Even if goat faeces are similar to sheep ones, the effective lower value reported in

Table 3 must be due to a lower amount of straw compared to SB_sheep.

A very narrow range of volatile solids (VS) contents, between 82.3% and 88.9% of TS, was found for the studied spent bedding materials. This is likely due to the similar VS content for bedding and faeces (Cui et al., 2011; Massé and Saady, 2015; Møller et al., 2004; Motte et al., 2013; Wartell et al., 2012).These values show the presence of a good fraction of degradable matter in the spent bedding and support the interest in using it for energy recovery.

Animal excrements and urine in particular are known to be rich in nitrogen compounds and a positive linear correlation ($R^2 = 0.95$) was found between nitrogen content and soluble fraction of the substrate. Values of 30.30 g N kg^{-1} TS and 27.95 g N kg^{-1} TS have been reported by Demirer

and Chen (2008) and Møller et al.(2004), respectively. Similar total nitrogen concentrations were measured in all the types of spent bedding, ranging between 17.6 and 23.7 g N kg^{-1} TS (

Table 3). Once again, SB_horse was an exception with a TS equal to 11.8 g N kg^{-1} TS:, probably due to the high amount of straw. The mixture of animal excrements and straw allows to decrease the total amount of nitrogen, which can be inhibitory for AD at high concentration, and to increase the C/N ratio to optimum values for AD (i.e. 20-30) (Karthikeyan and Visvanathan, 2012). C/N value of 164 for straw is reported by Motte et al. (2014), while a value under 10 for cow faeces was found by Hills (1979). The C/N ratios of the substrates used in this study ranged from 20 and 28, except for SB_horse whose C/N ratio was 42. A similar value (39.4) for spent horse bedding was reported by Cui et al. (2011), while Degueurce et al. (2016b) reported a value of 17 for spent cow bedding.

Phosphorous content in the spent bedding was found to vary significantly (p-value < 0.05) in relation to the animal. On average, SB_cow were found to have a lower content than the others while the highest phosphorous concentration was measured for SB_goat, 8.4 g P kg^{-1} TS.

Given the poor percentage of soluble and lignin fraction in straw, 10.2% and 7.4% (on a VS basis) respectively (Motte et al., 2013), high content of these two fractions in spent bedding is mainly due to faeces. Generally, higher values of cellulose than hemicellulose were found in all the samples (

Table 3) as reported also by Demirer and Chen (2008) and Massé and Saady (2015). This is probably related to the high percentage of straw in all the substrates (Liew et al., 2012; Motte et al., 2013). The higher fraction of cellulose in SB_horse and SB_cow_m (44.2% and 38.5% respectively) compared to other substrates can be related to the frequency of litter change, which corresponded to 3 days and 3 weeks old, respectively. Given its high content and degradation rate in lignocellulosic substrate (Liew et al., 2012) cellulose can rapidly be degraded when good conditions of moisture and bacterial concentration are reached, as in the case of litter. This supports the view

according to which a reduced time in the litter and/or in storage before AD could represent an advantageous strategy in order to increase energy production.

Table 3 : Characterization of six types of spent animal bedding

Substrate		SB_sheep	SB_goat	SB_horse	SB_cow_h	SB_cow_g	SB_cow_m
Breed		-	-	-	Simmenthal	Blonde d'Aquitaine	Prim'Holstein
Roughage feed		-	-	-	Hay	Baled grass silage	Clamp maize silage
[a]Amount of straw [1]	[b]kg LU^{-1} d^{-1}	9.8	4.5	11.3 (Wartell et al., 2012)[*]	6.5	>3	8-10
Freq. litter change		2-3 months	2-3 months	2-3 days	2 months	2-3 months	3 weeks
[c]TS	%	37.0 ± 1.2	29.8 ± 0.8	34.3 ± 0.4	23.7 ± 0.1	25.6 ± 0.8	28.8 ± 0.3
[c]VS/TS	%	88.9 ± 4.5	84.4 ± 2.7	86.8 ± 1.2	83.7 ± 0.3	82.3 ± 3.8	84.0 ± 1.7
[c]C	g C kg^{-1} TS	427.4 ± 2.7	438.1 ± 2.0	498.5 ± 5.5	505.6 ± 5.9	525.1 ± 2.4	498.6 ± 2.3
[c]N	g N kg^{-1} TS	19.1 ± 0.2	21.7 ± 1.2	11.8 ± 0.7	17.6 ± 0.8	23.7 ± 0.7	17.7 ± 0.5
[c]P	g P kg^{-1} TS	6.8 ± 0.6	8.4 ± 0.0	5.6 ± 0.4	4.1 ± 0.3	3.7 ± 0.4	4.6 ± 0.8
C/N/P	-	62 / 2 / 1	51 / 2 / 1	88 / 2 / 1	121 / 4 / 1	141 / 6 / 1	109 / 3 / 1
C/N	-	22 / 1	20 / 1	42 / 1	28 / 1	22 / 1	28 / 1
[c]Soluble	[d]% VS	31.8 ± 1.2	36.7 ± 1.3	19.0 ± 0.3	25.9 ± 0.3	38.1 ± 1.5	25.9 ± 2.2
[c]Hemicellulose	[d]% VS	23.8 ± 0.1	20.6 ± 0.8	28.5 ± 0.4	28.4 ± 1.5	21.2 ± 1.0	21.2 ± 1.0
[c]Cellulose	[d]% VS	30.7 ± 1.0	34.7 ± 1.7	44.2 ± 1.1	33.0 ± 2.5	28.4 ± 0.8	38.5 ± 2.0
[c]Lignin	[d]% VS	13.7 ± 2.0	8.0 ± 0.6	8.3 ± 1.0	12.8 ± 1.0	12.3 ± 1.0	12.0 ± 1.5

[a] This information has not been measured directly but provided by the farmers as average value on their farm
[b] LU: livestock unit. Milk cows = 1LU, goat and sheep = 0.100 LU and horse =0.800 LU up to the European legislation
[c] Mean value ± standard deviation of the triplicates
[d] On a volatile solid basis
[*] The exact value is not known by the farmer. However it was visually higher than the one of the other substrates

Methane potential

BMP represents the amount of CH_4 that can be produced by anaerobic digestion per mass of substrate usually expressed on a VS basis. Even if the BMPs measured were significantly different (p-value < 0.05), their range of variation was quite narrow: 192- 239 NmL CH_4 g^{-1} VS with the lowest values belonging to SP_cow_g and highest to SB_horse (Table 4). Few BMPs of spent livestock bedding are reported in literature: 277 NmL CH_4 g^{-1} VS for horses (Kusch et al., 2008), 173.5 NmL CH_4 g^{-1} VS for cows (Degueurce et al., 2016c) and 195-218 NmL CH_4 g^{-1} VS for pigs/swine (Yap et al., 2016). The use of straw as bedding material was proved valuable from a bioenergy perspective especially when compared with other bedding materials like softwood chips or pellets, mainly because straw contributes significantly to methane production (Wartell et al., 2012) Several BMPs for wheat straw were also reported in literature with values ranging from 54 to 245 NmL CH_4 g^{-1} VS (Li et al., 2013; Liew et al., 2012; Møller et al., 2004; Motte et al., 2014; Wartell et al., 2012). High variability in straw BMPs in relation to the crop used is also reported by Wu et al. (2010). The BMP of faeces has also been reported to vary significantly. Values ranging from100 to 316 NmL CH_4 g^{-1} VS were reported for cow (Labatut et al., 2011; Møller et al., 2014, 2004) and for horse (Wartell et al., 2012) faeces. Finally BMP values of 204, 155 and 159 mL CH_4 g^{-1} VS for solid manure from dairy cows, horse and goat (no details about the bedding are given) were recently published by Kafle and Chen (2016). All these values show the BMP variability of faeces among different animals and within the same animal category. Thus, the addition of straw to animal faeces can either increase or decrease the final BMP in relation to the animal and the straw used. Nevertheless, the addition of straw to faeces has a great impact on the volume of methane produced per raw mass (RM) since the global TS increases. Thus, even if spent bedding has a poor biodegradability compared to other substrates like fruit and vegetable waste or kitchen waste (342 NmL CH_4 g^{-1} VS and 541 NmL CH_4 g^{-1} VS, Li et al., 2013), its high TS concentration and high VS per gram of raw matter (RM) makes it an interesting substrate for industrial purposes. In fact,

expressed in NmL CH$_4$ g^{-1} RM, BMP values of spent bedding (Table 4) are found between fruit and vegetable waste (11 mL CH$_4$ g^{-1} RM) and kitchen waste (123 mL CH$_4$ g^{-1} RM). Therefore, spent bedding has to be considered a valid substrate for dry AD and its physical properties make it suitable for percolation system in LBRs.

Table 4 : BMP test results and methane yields measured in leach-bed reactors

		SB_sheep	SB_goat	SB_horse	SB_cow_h	SB_cow_g	SB_cow_m
[a] BMP	NmL CH$_4$ g^{-1} VS	204 ± 8	227 ± 8	239 ± 7	216 ± 6	192 ± 3	228 ± 2
	NmL CH$_4$ g^{-1} RM	65 ± 3	57 ± 2	71± 3	43 ± 2	40 ± 1	55 ± 1
[b] Methane yield in LBR (60 days)	NmL CH$_4$ g^{-1} VS	201 ± 31	196 ± 33	206 ± 32	185 ± 4	168[*]	204 ± 20
	NmL CH$_4$ g^{-1} RM	64 ± 10	49 ± 8	61 ± 10	37 ± 2	35[*]	49 ± 5
LBR/BMP ratio		(99 ± 16)%	(86 ± 15)%	(87 ± 14)%	(85 ± 3)%	88%[*]	(89 ± 9)%

[a] Mean value ± standard deviation
[b] Mean value ± range (maximum/minimum)
[*] Value from only one reactor of the pair

2.1.3.2   Process analysis

pH and alkalinity

For all the types of spent bedding considered, the pH ranged between 6.6 and 7.6 during the digestion process (Figure 11a). In this way, all the reactors were run in optimal conditions since AD is known to perform efficiently in a pH range of 6.8-7.6 (Jha et al., 2011). The decrease in pH observed during the first days of the digestion process is common in batch systems and it is due to the accumulation of VFAs (discussed later). Depending on the kind of spent bedding, the pH in this study started to increase after 3 to 10 days, reaching stable values between 7.0 and 7.5.
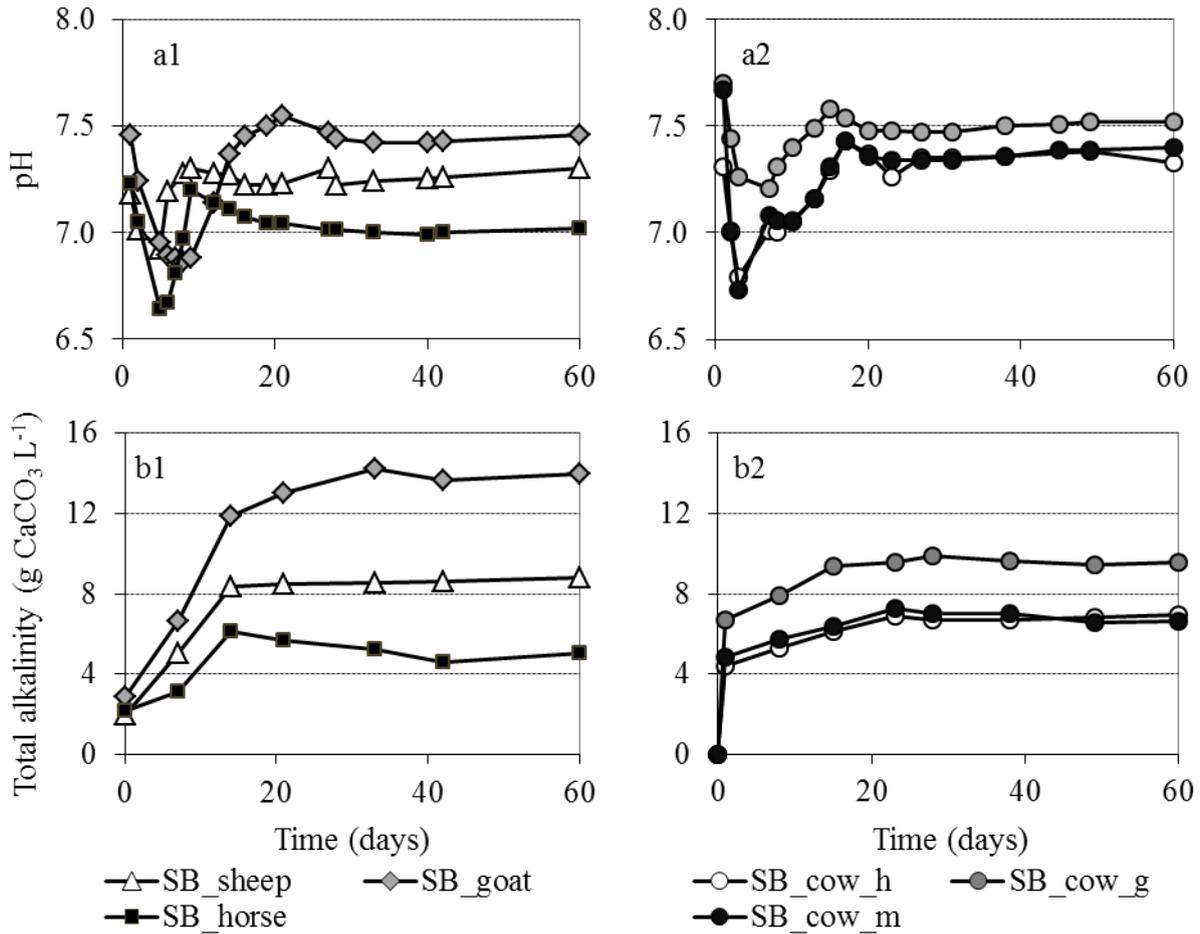
Figure 11 : Parameters monitored in the leachate during the digestion process in a LBR of six different spent animal bedding: (a1 - a2) pH and (b1 - b2) total alkalinity in g $CaCO_3\ L^{-1}$

pH is closely linked to alkalinity and therefore to several acid/base couples present in the system. Here, the most important and common ones were VFAs, with an average pKa of about 4.8, the couple CO_2/HCO_3^- with a pKa of 6.25 and, finally, NH_3/NH_4^+ which raises pH toward alkaline values due to a pKa of 9.25. The good stability of the batch reactors studied in this work was linked to the presence of high amounts of NH_3/NH_4^+ and bicarbonates which helped to keep the pH at levels suitable for methanogenesis. During roughly the first 15 days, the alkalinity increased (Figure 11b) due to a changing balance between VFA accumulation, which tends to acidify a system, and the hydrolysis of molecules containing nitrogen and the carbonate compounds which tends to increase the pH. After this phase, given that all the VFAs were consumed and the nitrogen and carbonate compounds reached a steady concentration, the alkalinity remained constant throughout

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the digestion period ranging from 5.0 g $CaCO_3$ L^{-1} (SB_horse) to 9.5 g $CaCO_3$ L^{-1} (SB_cow_g). Reactors loaded with SB_goat showed a more specific behaviour: a stable pH was reached later, most likely due to a higher VFA accumulation, and the final value of about 14 g $CaCO_3$ L^{-1} was consistently higher compared to the other substrates. Similar values for alkalinity, close to 10 g $CaCO_3$ L^{-1}, were also recorded by Massé and Saady (2015) when treating a synthetic mixture of cow faeces and straw. The high alkalinity measured in all the systems treating spent bedding makes it, independently of its origin, a very good substrate for co-digestion as it stabilizes the pH.

VFAs

VFAs are the main metabolic intermediates of anaerobic digestion and their accumulation depends on both their rates of production and consumption. The imbalance between VFA production and consumption, processes which are carried out by different microbial populations, is one of the main challenges in batch digestion in a single stage using highly-concentred substrates.

As observed by other authors using LBRs (Ten Brummeler, 2000), an accumulation of VFAs (Figure 12 a1-a2) was observed during the first days, indicating an effective imbalance between hydrolysis/acidogenesis and acetogenesis/methanogenesis. All the various types of spent bedding behaved similarly and the total VFA (TVFA) peaks corresponded to the minimal pH values. TVFA peaks were reached between day 3 and day 10 and then VFA concentrations decreased at different rates. VFA concentrations below 1 g COD L^{-1} were reached at different times starting from day 12. 10 days more were needed by SB_goat, for whom a particularly high TVFA peak of 14 g COD L^{-1} was recorded. It is worth noting that, for SB_goat, such an accumulation of VFAs did not affect the overall reactor performance. Higher TFVA concentrations, reaching 20 g L^{-1} or more with no inhibition, have been observed by other authors treating more degradable substrates like vegetables and municipal solid waste (Aymerich et al., 2013; Kusch et al., 2012; Shi et al., 2013; Ten Brummeler, 2000) In anaerobic digesters working at high solid concentrations such as LBR,
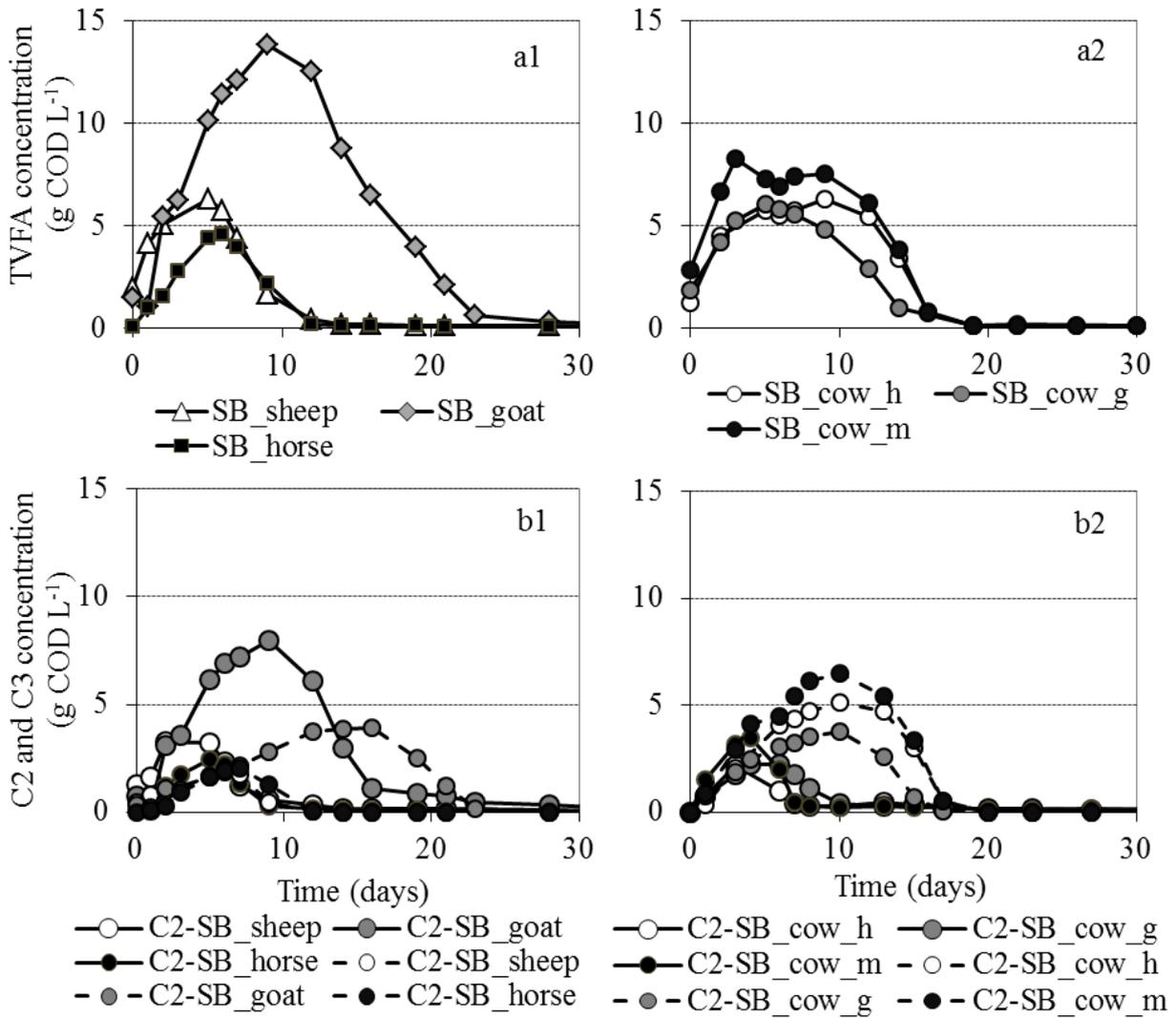
Figure 12 : Volatile fatty acids measured in the leachate during the digestion process in a LBR of six different spent animal bedding: (a1 - a2) total volatile fatty acids concentration in g COD L^{-1}, (b1 - b2) acetic (C2) and propionic (C3) acid concentrations in g COD L^{-1}

concentrations of this magnitude are common. For this reason, high levels of alkalinity, like those reported in this work with spent bedding, are essential to prevent reactor acidification.

The predominant acids produced in all the systems (Figure 12 b1-b2) were acetic and propionic acids (about 80% of the TVFA in terms of COD), which corresponds also to the data reported by Rico et al. (2015) and Yap et al. (2016). This seems to be specifically related to spent bedding since other authors, using the same system but treating organic fraction of municipal solid waste (Ten

Brummeler, 2000), maize silage (Kusch et al., 2012) or canning plant waste (Aymerich et al., 2013), observed an additional high concentration of butyric acid.

For all the types of spent bedding treated, a delay in the degradation rate of propionic acid, compared to that of the others, was observed. Propionic acid consumption only started when all other VFAs had been degraded. In fact, the specific thermodynamic conditions necessary for the consumption of propionic acid to take place have been found difficult to reach, mainly in relation to H_2 partial pressure in the liquid phase (Aymerich et al., 2013).

Figure 12 b1-b2 clearly illustrates the profiles of acetate (C2) and propionate (C3) concentrations during batch operations. While, for SB_horse and SB_sheep, the acetic and propionic acids were consumed almost simultaneously, for the other types of spent bedding there was a gap of about 5 days. On the other hand, no clear relation was found in the magnitude of the concentration peaks of the acetic and propionic acids since the first were found to be higher with SB_sheep, SB_goat and SB_horse but lower for all the types of SB_cow.

Methane production

Variations in the amount and composition of the biogas produced give important information about system behaviour, possible inhibitions and overall kinetics. Methane is, in fact, the final product of a long sequence of reactions as well as a sought-after and valuable molecule. Figure 13 a1- a2 shows the biogas production rate during the experiments. Three main groups were identified based on the lag phase, which was estimated by fitting the experimental data with the Gompertz equation (data not shown). SB_sheep and SB_horse had an average lag-phase of 1.1 days, SB_cow of 4.2 days and SB_goat of 6.4 days. However, the methane production rate peaks among these groups were not found statistically different. The reasons of such a different initial methanogenic activity were not clear. No significant correlations were found between the lag-phase and the substrate properties highlighted through characterization. Problems related to the original

methanogenic population present in the substrate, specific inhibition or nutrients deficiency could be one of the causes.
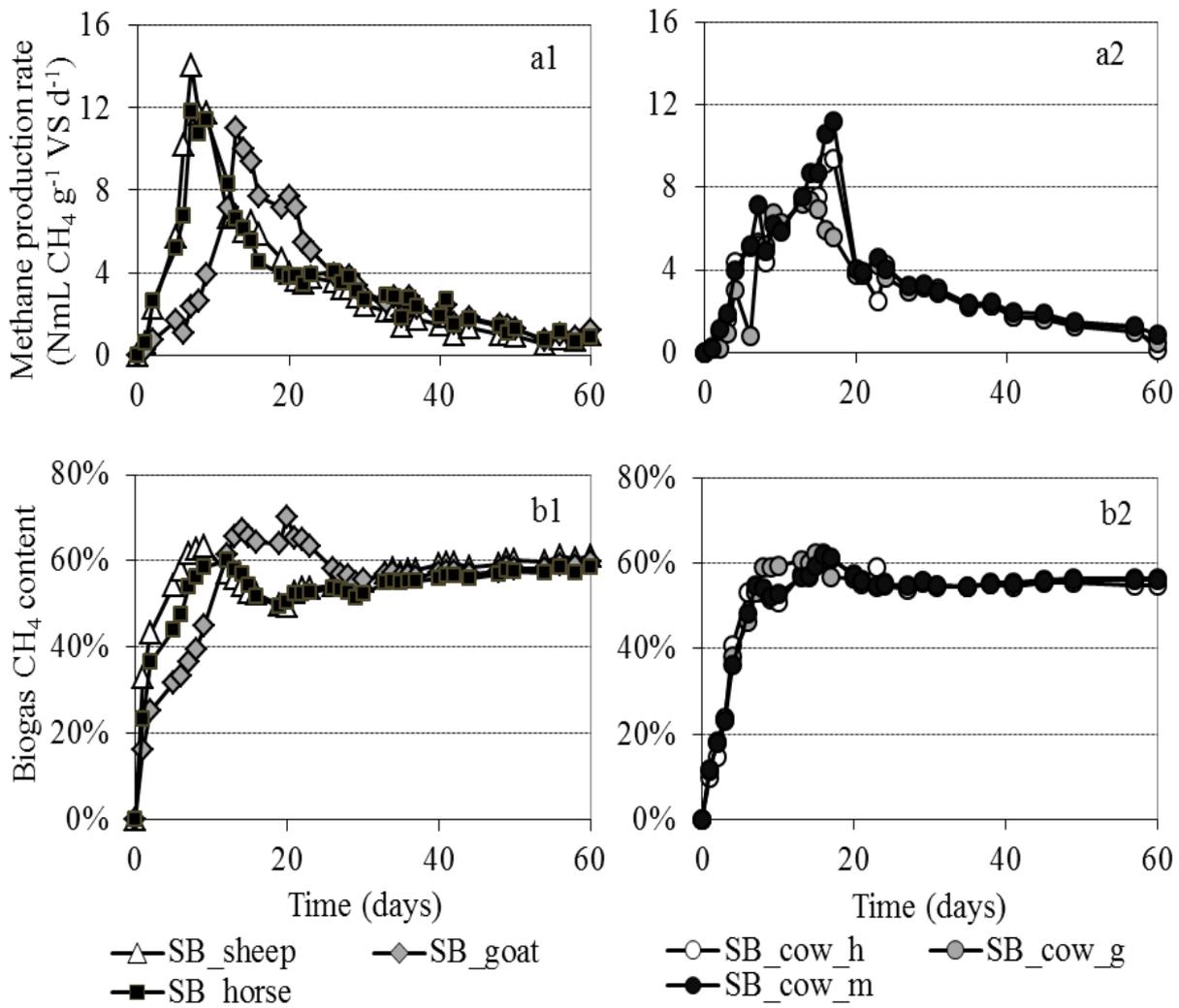
Figure 13 : (a1 - a2) methane production rate in NmL CH_4 g^{-1} VS d^{-1} and (b1 - b2) methane content in biogas during the digestion process of six different spent animal bedding in a LBR

As observed for several lignocellulosic substrates (Liew et al., 2012), a biphasic biogas production was found for all types of spent bedding. The biggest differences between substrates were observed during the first part of the digestion, when more easily-degradable fractions of the substrates were degraded and the highest methane production rates were measured. On the other hand, less degradable fractions played a role in maintaining a low but fairly constant production of

methane which was very similar for all the substrates, as showed by the methane production rates after 25 days of operation.

Finally, the presence of one or two peaks in the methane production rate was related to the rate of VFAs consumption. Therefore, the bigger the gap in degradation between the acids produced the more likely would there be two biogas peaks (as for SB_cow_m).

The methane content in the biogas showed a similar trend for all substrates except SB_goat. Methane content increased rapidly during the first 10 days, reaching stable values comprised between 55% and 60% (Figure 13), whereas the increase in methane content was slower for SB_goat, indicating some difficulty in the establishment of methanogens in this last system, as the high lag phase showed.

For all the substrates, methane yield expressed in terms of VS ranged between 168 and 206 NmL CH_4 g^{-1} VS (Figure 14 a1–a2). Since the inoculum used was estimated to produce less than 4% of the total methane in one batch reactor, all the methane measured was considered to be produced only by spent bedding. The errors in the measured biogas volume did not allow differentiating the studied substrates based on this parameter (p-value = 0.82). However, similar methane yields in LBR were reported by treating spent bedding from cow, horse and sheep stables in literature (Table 2). Further comparison in batch system for spent straw was reported by Cui et al. (2011), 150 NmL CH_4 g^{-1} VS after 30 days. Other comparisons are difficult to make because few authors have used spent bedding as a sole substrate. It is important noting that, after 60 days of digestion in LBR, methane yield was 89 (± 11)% (error calculated as a standard error of laboratory, SEL) of the of the BMP (Table 4) and VS degradation of the substrate (48 ± 3)%.This proved that an LBR process is efficient in treating spent livestock bedding.
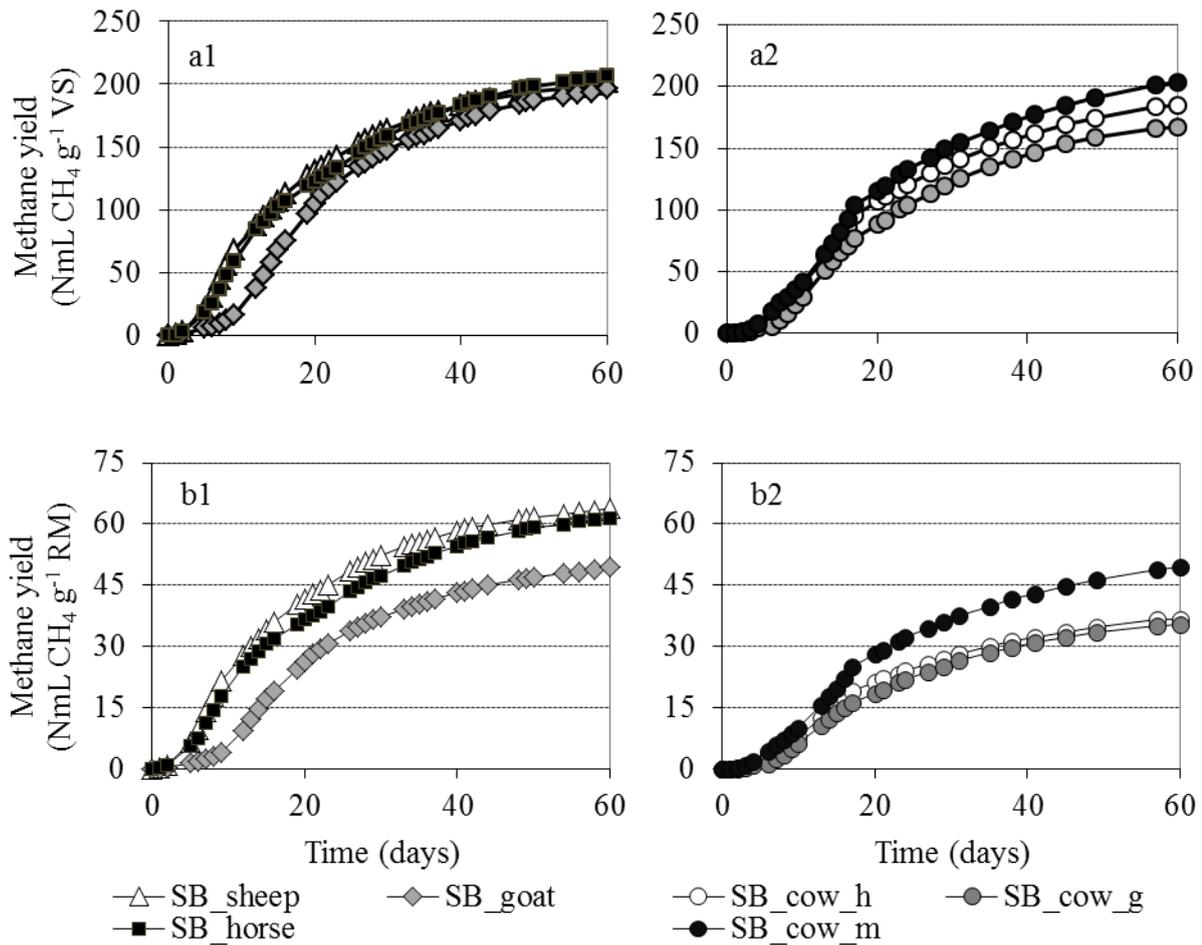
Figure 14 : (a1 - a2) methane yield in NmL CH_4 g^{-1} VS, (b1 - b2) methane yield in NmL CH_4 g^{-1} RM (raw mass) during the digestion process of six different spent animal bedding in LBRs

Methane yield on a raw mass basis gives information of interest for industrial applications. As a function of this parameter, 3 groups can be distinguished (p-value = 0.065) in Figure 14 b1-b2: 64 and 61 NmL CH_4 g^{-1} RM for SB_sheep and SB_horse, 49 NmL CH_4 g^{-1} RM for SB_goat and SB_cow_m, and 37 and 35 NmL CH_4 g^{-1} RM for SB_cow_h and SB_cow_g. Since the VS/TS ratio were similar for all six types of spent bedding, the methane yield expressed on a raw mass basis is linked to the production level expressed on a VS basis by the TS content. This indicates simply that the drier the substrate, the higher the organic load in a given digester can be.

Percolation and compounds accumulation

For all kinds of spent bedding studied, percolation of the leachate through the bulk was possible thanks to the physical structure provided by the straw. No addition of extra bulking material, as performed by Demirer and Chen (2008) with wood chips and Myint and Nirmalakhandan (2009) with pistachios shell, was necessary to ensure a proper leachate percolation. By the end of the process, however, a loss of bulk volume, compaction of the waste bed and the consequent diminished potential for percolation were observed, suggesting a loss of rigidity of the straw fibres (visual observation). André et al. (2015) showed that during the digestion process, the permeability of the spent bedding decreases consistently and spent bedding becomes an almost impermeable substrate after 30 days of digestion. Nevertheless, at least in the first stages of the process, the amount of straw (per animal and per day), is a very important parameter insofar as straw gives spent bedding a porous structure facilitating percolation. When this latter is not optimal, problems may arise even in the early stages of the process, hampering the proper degradation of the substrate.

In the perspective of the reuse of the liquid fraction from previous batches to start up new batches, the accumulation of non-degraded compounds can become a possible cause of failure if the initial dilution is not adequate. After 60 days, Total Kjeldahl Nitrogen (TKN) and potassium were found to be the compounds of major interest. A final concentration in the liquid phase ranging from 1.7 to 4.0 g L^{-1} for K^+ and from 0.6 to 2.7 g L^{-1} for TKN was recorded during this experiment. Potassium was found below the threshold considered strongly inhibitory 2.5 to 4.5 g L^{-1} (Karthikeyan and Visvanathan, 2012a). Acclimation to K^+ concentrations up to 6 g L^{-1} have been reported while for higher values the inhibition on methanogenic activity was persistent (Chen and Cheng, 2007).

The nitrogen inhibition is known to depend on several factors mainly connected to the inoculum and the acclimation of bacteria. In the literature, a 50% reduction in methanogen activity has been associated with a wide range of total ammonia nitrogen (TAN), going from 1.7 to 14 g N

L^{-1} (Chen et al., 2008). Furthermore, the importance of the speciation of nitrogen compounds has often been pointed out because any toxicity has often been ascribed to NH$_3$ (Rajagopal et al., 2013). Necessary for bacterial growth at concentration below 200 mg L^{-1} (Chen et al., 2008; Karthikeyan and Visvanathan, 2012a), at high concentrations, NH$_3$ may inhibit growth due to its capacity to penetrate into the microbial cell. It is interesting to notice that an antagonistic effect on TAN inhibition has been related to minerals in the phosphorite ore (K$^+$, Ca^{2+}, Mg^{2+}). Hence, the presence of high amounts of K$^+$ can play a positive role in reducing the ammonia inhibition (Chen et al., 2008), an important consideration for anaerobic digesters treating spent bedding which is naturally rich in both compounds. Based on that, the really high TKN concentration in the leachate of SB_goat (2.7 g L^{-1}coupled with the low concentration in K$^+$ measured (1.7 g L^{-1}) could explain the delay in the start of methane production for this substrate. In any case, the accumulation of N in the system should be viewed with reserve and the threshold for actual inhibition needs to be evaluated in each specific case.

Finally, it is worth mentioning the presence in the leachate of a slowly-degradable COD fraction whose tendency is to accumulate in the system. Between 5 and 10 g L^{-1} of CODs were measured in the leachate after 60 days, however that represents a negligible fraction of the methane potential left over. High COD concentration in the leachate at the end of the process was recorded also by Yap et al. (2016).

Water balance

Spent animal bedding from deep litter stable was shown to have a high TS, ranging between 24% and 37%. However, after 60 days of digestion digestate showed a similar TS content, ranging between 13% and 15% (Table 5). A water mass balance was carried out on all the batches in order to determine the amount of water necessary to run a LBR. The error made was less than 5% probably due to the water vapour contained in the biogas which was not taken into account. Considering the amount of water added at the start-up and collected in the end it was possible to

compute the amount of water actually absorbed by the solid matrix. This latter was found between 1.2 and 1.9 kg_{water} kg^{-1} TS_{added}, or between 0.3 and 0.7 kg_{water} kg^{-1} RM_{added}.

Table 5: Analysis of the water mass balance on the LBRs. The results of both duplicates for each substrate studied are reported

	TS spent bedding	TS digestate	Mass Balance (water loss)	Water absorbed by the substrate in a cycle	
	%	%	%	kg_{water} kg^{-1} TS_{added}	kg_{water} kg^{-1} RM_{added}
SB_sheep1	37%	15%	3%	1.9	0.7
SB_sheep2	37%	15%	3%	1.9	0.7
SB_goat1	30%	14%	4%	1.4	0.4
SB_goat2	30%	15%	3%	1.2	0.4
SB_horse1	34%	14%	3%	1.6	0.6
SB_horse2	34%	14%	4%	1.7	0.6
SB_cow_h1	24%	14%	4%	1.4	0.3
SB_cow_h2	24%	14%	4%	1.3	0.3
SB_cow_g1	26%	14%	5%	1.4	0.4
SB_cow_g2	26%	14%	2%	1.2	0.3
SB_cow_m1	29%	14%	5%	1.8	0.5
SB_cow_m2	29%	13%	4%	1.8	0.5

The amount of water absorbed at the end of a cycle by each substrate was found to be directly linked to its initial TS, as shown in Figure 15. This result is important since it proves that to run LBR, a considerable amount of water is needed. For every kg of spent bedding an amount of fresh water ranging from 0.3 L and 0.7 L must be added, less than in a wet process but far from being negligible. Furthermore this amount can be easily calculated by simply knowing the substrate initial TS.
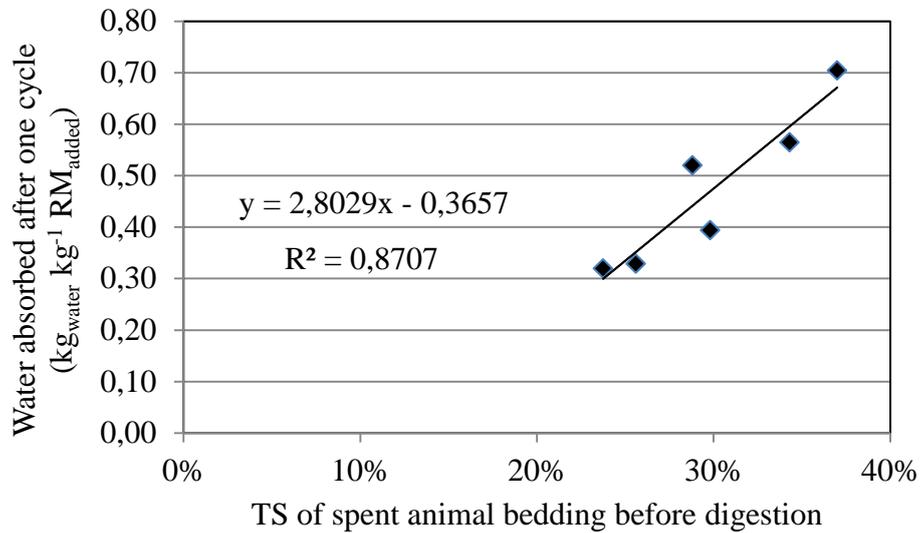
Figure 15 : Amount of water absorbed after one cycle by the bulk in relation with its total solids (TS) before digestion

2.1.3.3 Digester design

In order to optimize the economic performance of batch systems such as LBRs, two specific stages, linked to the discontinuous loadings, require a particular attention: the start-up phase and the final phase. For the start-up phase, the two important parameters are the time needed to reach the minimum percentage of methane in the biogas allowing it to be burned in a CHP unit and, the percentage of methane not exploited during this period. In the final phase the most important is the batch duration, when low methane production needs to deal with economic issues.

During the start-up phase methane content in a digester headspace increases until reaching a steady level. Nowadays, CHP units with different thresholds for minimum CH_4 content in biogas, ranging from 30% to 50%, are available on the market. However, in this work, data comparison was done for biogas compositions between 20% and 40% only because, at industrial scale, the biogas injected into the CHP unit is actually a mixture of the biogas coming from several batches (operated in parallel but at different stages: multi-stage process), and hence, biogas from a new batch can be accepted even if its CH_4 content is lower than the minimum needed by the CHP unit installed on site. It is clear from Table 6a that the higher the methane content necessary for combustion in CHP

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unit, the higher the time needed to reach the desired methane content in the headspace of the digester and the higher the amount of methane not exploited. Taking the example of a biogas exploitable at a methane content of 30%, the duration needed to reach this value varied from 2.3 days (SB_sheep) to 6.0 days (SB_goat) while the methane not exploited for all the types of spent bedding varied from 1.7% to 4.2%. In this example, it is worth noting that the SB_goat needed a higher number of days to reach a usable methane content of 30% in the headspace but it was not the substrate producing the most not exploitable methane in the end (SB_horse in the present case). Thus, the optimization of the start-up phase in relation to the substrate is an important issue for LBRs in order to improve the methanogenic kinetics, enabling the time to reach the desired CH_4 content in the biogas and the quantity of unavailable methane to be reduced to a minimum. Furthermore, it is important to notice that the CH_4 content in the biogas at the outlet depends, among other parameters, on the headspace volume which dilutes the biogas produced during the start-up period. In the present work, the ratio between the volume of the headspace and the volume occupied by the organic substrate equalled 1, which is higher than the ratio actually found on site. For this reason, the above-mentioned values are overestimated compared to full-scale digesters.

By drawing the attention to the final phase of the digestion process, the batch duration is the parameter to take into account in the design of a discontinuous reactor. Mesophilic LBRs are normally unloaded and reloaded every 60 days. Taking the total specific methane production on day 60 as a reference, it is possible to evaluate the methane production performance of each type of spent bedding in respect to the time and to calculate in that way the percentage of methane not exploited when reducing the batch duration. Table 6b shows, for example, that by reducing the batch digestion to 55 days or 50 days, the not exploited methane ranged, respectively, from 1.9% to 2.7% and from 3.4% to 6.4% in relation to the substrate. However, the consequent decrease in the incomes could be balanced by a smaller digester volume (if the amount of spent bedding to treat per year is fixed) for example. The search for the optimum economic balance is then necessary to set this parameter.

Table 6 : Optimization of start-up and batch duration

a - the time (days) needed to reach the specific CH_4 content in the headspace of the digester and the percentage of methane not exploited (compared to the total production after 60 days)

b - the percentage of CH_4 not exploited (compared to the total production after 60 days) when diminishing the batch duration

a	Analysis of the start-up					
	Time					
CH_4 content	SB_sheep	SB_goat	SB_horse	SB_cow_h	SB_cow_g	SB_cow_m
20%	1.5 d	3.4 d	1.9 d	3.1 d	3.4 d	3.1 d
25%	1.8 d	4.4 d	2.5 d	3.3 d	3.7 d	3.6 d
30%	2.3 d	6.0 d	3.2 d	3.6 d	4.1 d	4.2 d
35%	2.9 d	7.0 d	3.9 d	3.9 d	4.9 d	5.3 d
40%	3.5 d	8.3 d	4.6 d	4.4 d	5.7 d	6.1 d
	Percentage of CH_4 not exploited					
CH_4 content	SB_sheep	SB_goat	SB_horse	SB_cow_h	SB_cow_g	SB_cow_m
20%	0.5%	1.3%	0.8%	1.2%	1.0%	1.3%
25%	0.7%	2.0%	2.3%	1.8%	1.4%	2.1%
30%	1.7%	2.9%	4.2%	2.4%	1.9%	3.3%
35%	3.4%	4.1%	6.2%	3.0%	3.0%	6.0%
40%	5.2%	6.0%	8.2%	4.4%	4.1%	8.1%

b	Analysis of the batch duration					
	Percentage of CH_4 not exploited					
Days	SB_sheep	SB_goat	SB_horse	SB_cow_h	SB_cow_g	SB_cow_m
40	8.9%	12.6%	11.3%	12.9%	15.2%	13.6%
45	5.7%	8.0%	7.0%	8.4%	10.9%	9.2%
50	3.4%	4.4%	3.8%	4.7%	6.4%	5.7%
55	2.0%	2.5%	1.9%	1.5%	2.7%	2.5%
60	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

2.1.4   Influence of leachate recirculation

In order to study the effect of the leachate recirculation on the digestion of the spent bedding, a further experiment was carried out. Three stainless-steel reactors of 100 L were used following the same principle than the glass reactor previously described. The details regarding this experiment are provided in section 2.1.2. Reactors were loaded with 16 kg of spent cow bedding and 7.5 kg of sludge and 3 kg of water. A very close substrate-to-inoculum ratio than the one previously used was set: S/X of 11. The same loading protocol was applied: the reactors were loaded, the liquid fraction let recirculate for 10-15 minutes and, in the end, the reactors were closed and the digestion started; the experiment was stopped after 60 days. The amount of leachate recirculated per day was set at 10 L kg^{-1} TS d^{-1} and 1 L kg^{-1} TS d^{-1} for R1 and R2, respectively (leachate recirculation frequency was of 5 and 0.5 times per day, respectively). R3 setting was similar to R1 for the first 8 days, until the maximum concentration of VFAs was measured, and then switched to be as R2. This was done in order to study the effect of reducing the amount of leachate recirculated per day once methanogenesis was established. Figure 16 shows that methane production rates presented a similar evolution than the other substrate previously treated (Figure 13); the peak was reached after about 10 days from the start and, after that, the methane production decreased slowly until reaching 1 NmL CH_4 g^{-1} VS d^{-1}. Beside a slight difference in the peak value of methane production rates (about 9 NmL CH_4 g^{-1} VS d^{-1} for R1 and 8 NmL CH_4 g^{-1} VS d^{-1} for R2 and R3), the methane production rates were very similar all along the digestion whatever the recycling strategies. No difference was observed by reducing by 10 the amount of leachate recirculated, both by setting this parameter from the beginning of by modifying it during the digestion process, at least in the recirculation range covered.
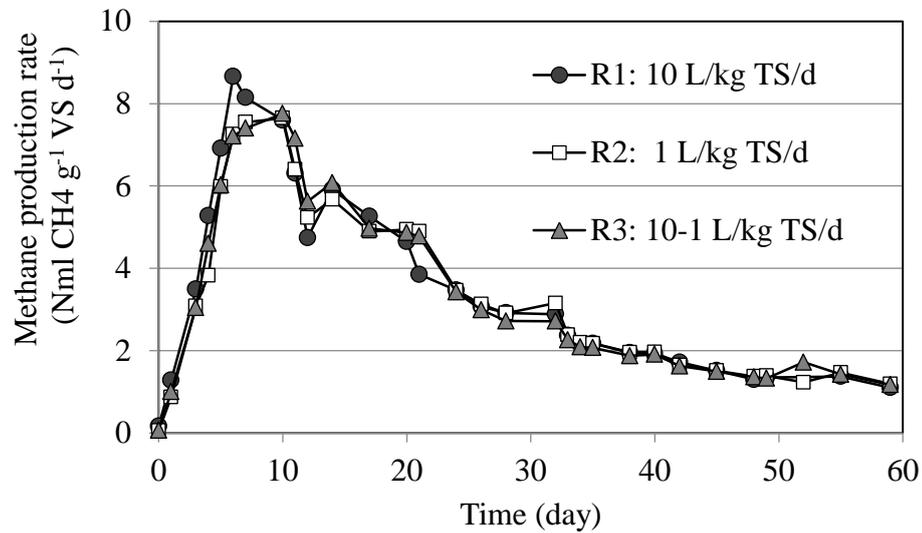
Figure 16 : Methane production rates of three LBRs with different internal recirculation conditions

The influence of the elapsed time between two recirculations (frequency), the volume recirculated, the initial solid-to-liquid ratio (TS of the system in this manuscript) on the methane production kinetics were also studied by (Degueurce et al., 2016d). They concluded that the frequency has the highest impact while the other two factors had a minor one. They stated also that different settings of these parameters could lead to similar effects in the studied ranges: frequency from 1 d^{-1} to 0.16 d^{-1} (i.e. from once per day to once every 6 days), a recirculated volume between 0.8 and 3.5 L kg^{-1} TS and a TS% between 10 and 11% (as in our case study).

In contradiction with the study of Degueurce et al. (2016c), we have found here that the periodicity and the volume recirculated had no influence on the process (excluding the coincidence of having found the same performance with two different settings). This was related to different initial conditions: in the present study, the reactors were first recirculated for 10-15 minute at the start-up in order to be close to saturation whereas this procedure was not adopted by Degueurce et al. (2016c) who poured only a small initial volume of leachate on the top of the reactor. Initial TS inside the bulk were then different at the start-up and this could have affected the entire process. In this sense, leachate recirculation strategy can influence methane production kinetics by controlling the TS content in the bulk. In fact, AD is known to be affected by this parameter (see

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paragraph1.2.3). This is a significant result which means that recirculation of the leachate does not affect methane production when substrate water saturation is reached at the start-up.

On the other hand, Figure 17 illustrates the VFA concentration in the leachate. Even if the values measured were quite low (i.e., less than 2 g L^{-1}), some effects of the recirculation on this parameter can be observed. A higher concentration of VFAs in the leachate were found when recirculating the most, as in R1 and R3, compared to R2. This was related to the higher wash out of these compounds during the initial stage of acidification. On the other hand, a minor VFA concentration was measured in R1 compared to R3 starting from day 8, after the modification of the recirculation strategy in R3. This suggests that recirculation affects the VFAs mobility and transfer between solid and liquid phase. This phenomenon, that in this experiment was barely observable, would finally play an important role in systems accumulating a lot of VFAs like when co-digesting easily-degradable substrates (see section 5.2).
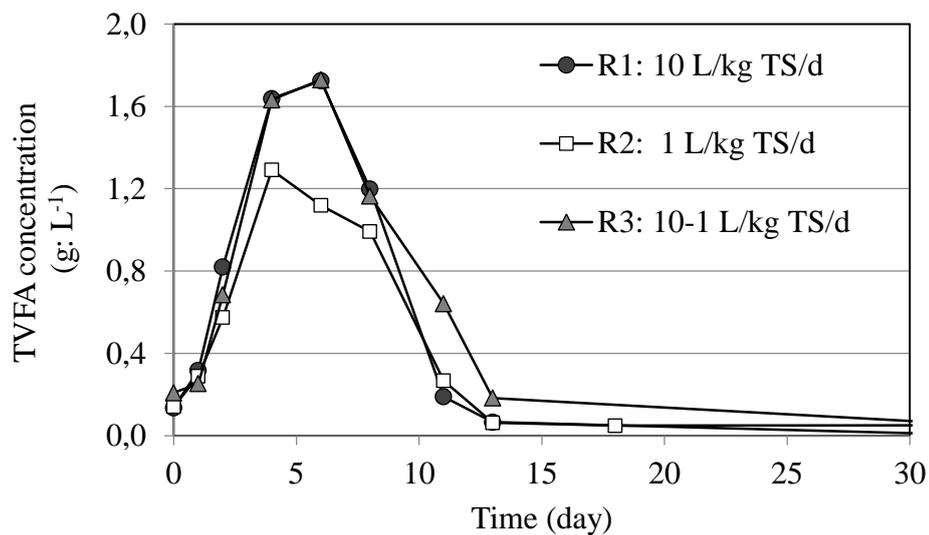
Figure 17 : Volatile fatty acids concentration in three LBRs with different internal leachate recirculation conditions

2.1.5 Conclusions

Several types of spent bedding were collected from deep-litter stables, and their physico-chemical properties were compared. Spent horse bedding differed from the other substrates because of a greater amount of straw added to the litter and a more frequent litter change. However, all the substrates showed similar VS/TS content, C/N (except that of horse) and BMP. In addition, LBR was shown to be an adapted process for the treatment of spent animal bedding since an average of 89 ($\pm$ 11)% of the BMP was reached after 60 days of operation. Spent goat bedding and spent sheep bedding behaved similarly to the other substrates even if this first showed a difficult start-up leading to an acidification phase. Therefore, spent animal bedding is a promising substrate for farm-scale LBR plant and its digestion as sole substrate is feasible. Moreover, the overall process stability suggests that a co-digestion with more easily-degradable substrates could be done. Long term accumulation of nitrogen and potassium in the leachate was identified as the main concern with this kind of substrate. Batch duration and start-up strategies appeared as two possible levers for enhancing performance at industrial scale. Finally, the recirculation appeared to affect the VFA concentration in the leachate; this phenomenon is supposed to play a significant role when digesting an easily-degradable mixture.

CHAPTER 3

Study of the start-up phase

This chapter focused on one specific phase of the digestion process in a LBR: the start-up. The role played by the leachate and the digestate on the digestion performance (i.e. methane production and process stability) were studied in detail. In addition, given the big impact of the start-up phase at industrial scale an economic analysis aiming at the comparison of these to possible inoculation was effectuated. In the end, the best strategy for inoculating a LBR treating spent animal bedding was proposed based on experimental and economic results.
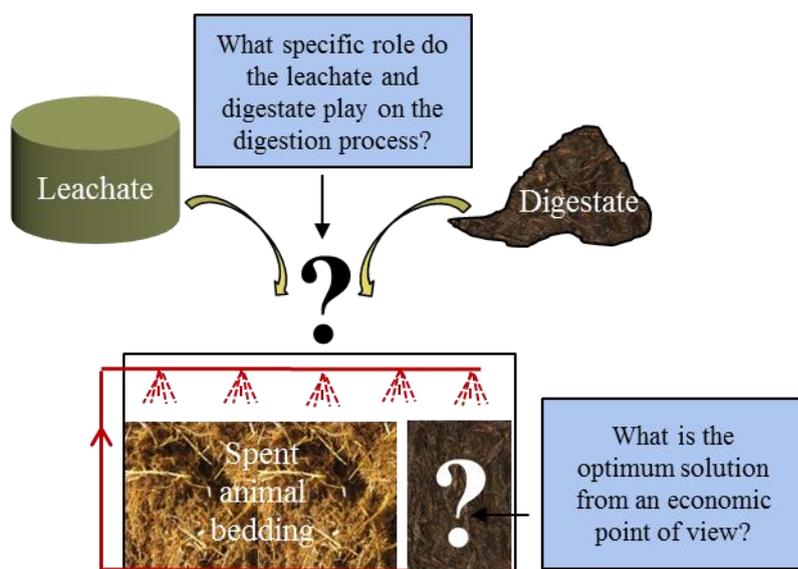
Figure 18 : Graphical abstract of the second issue faced in this PhD thesis : the role played by both leachate and digestate on the digestion process and the economic comparison of these two strategies

3.1 Start-up strategies for leach-bed reactors treating spent cow bedding: impact on process and economic performance

3.1.1 <u>Introduction</u>

Anaerobic Digestion (AD) is a widespread biotechnology converting different kinds of organic residue into biogas, a mixture of carbon dioxide and methane. Among the existing dry AD processes, leach-bed reactors (LBRs) were proved to efficiently treat spent animal bedding from cow (André et al., 2016b; Degueurce et al., 2016d; Riggio et al., 2017a), horse (Kusch et al., 2008; Riggio et al., 2017a), pig (Yap et al., 2016), goat and sheep (Riggio et al., 2017a) stable. At farm scale, several reactors are operated in batch mode and run in parallel in order to obtain a fairly stable biogas production. The digesters are regularly emptied and reloaded at different periods with the help of a wheel loader (Kusch et al., 2011). Two separated phases, the liquid and the solid, co-exist throughout the process, and both are usually involved in the start-up phase of a LBR.

A batch anaerobic digestion process needs the implementation of an appropriate start-up phase in order to create the best conditions for the establishment of the biological activity and thus generate as much biogas as possible within a limited time slot. Microbial inoculation is particularly important for discontinuous processes since it has to be repeated at every digester loading event. For example, at an industrial site using four LBRs in parallel with a batch duration of 50 days, about 30 reactor loadings per year are carried out. Hence, the assessment and optimization of the start-up strategy is critical.

With this aim in mind, the liquid fraction and/or the solid fraction obtained from a previous LBR can be incorporated into the new substrate in order to facilitate the start-up of the biological reactions. The liquid fraction, named leachate, is generally used to humidify the solid waste in order to start the AD process which could be hampered if the total solids concentration is too high (Abbassi-Guendouz et al., 2012; Bollon et al., 2011). In LBRs, the addition of the leachate alone has been shown to improve the start-up phase of the anaerobic digestion process thanks to the

microbial inoculation as well as the nutrients and alkalinity addition as previously described by Pognani et al. (2015) treating organic fraction of municipal solid waste (OFMSW) and, Kusch (2008) and Degueurce et al., (2016c) using spent cow and horse bedding, respectively. The importance of the adaptability of the inoculum to the substrate and to the process in terms of nutrients (e.g. nitrogen) and trace elements concentration was studied by Xu et al. (2013) on corn stover digestion and Facchin et al. (2013) on food waste treatment; those results higlighted the complexity of the inoculation step and the importance to well assess this parameter in order to reach optimal digestion performance. In addition, Degueurce et al., (2016c) supported the idea that leachate has poor inoculation properties by demonstrating that the bacterial consortia present in the solid and liquid phases are different, with no significant microbial population intermixing. Then the role of leachate still remains an open question.

The solid fraction, called digestate (digested solid material from a previous treatment cycle), is generally mixed with the fresh substrate in order to enhance the overall process kinetics as well as to avoid process failures due to the accumulation of inhibitory intermediate compounds (Di Maria et al., 2012; Kusch et al., 2008; Motte et al., 2013; Riggio et al., 2017b; Saady and Massé, 2015). However, the volumetric methane productivity (methane production per digester volume) may be affected since the solid digestate occupies a significant space in the digester working volume (Yang et al., 2015). Increasing the volumetric methane productivity is indeed one of the major challenges in the anaerobic digestion of solid substrates (Yang et al., 2015). Furthermore, in this context, the addition of an external solid source of inoculum raises questions because manure is known to contain methanogens insofar as AD is a process taking place in the digestive system of all ruminants (Solli et al., 2014; Sun et al., 2015).

Even though the role of leachate and digestate as sources of microbial inoculum in LBRs has been studied and reported separately in the literature, a direct and parallel comparison of their influence on the start-up phase of a LBR treating spent cow bedding has not yet been described, nor

has their respective economic impact. In the scientific literature, only an economic analysis using a LBR process was found for the treatment of the OFMSW in a landfill context (Chynoweth et al., 1992). On the other side, the use of dry animal manure such as spent cow bedding, was shown to be an interesting organic waste material for significantly increasing the economic performance of biogas plants (Asam et al., 2011) because of its dry content and subsequent high methane potential. The objectives of this study are then: 1) to identify the impact of adding leachate or solid digestate for starting up LBR on the degradation kinetics of spent cow bedding and methane production yield; 2) to carry out an economic simulation based on these experimental data in order to highlight the economic issues related to the two start-up strategies; and 3) to propose the best inoculation strategy when treating spent cow bedding in LBRs. Both experimental investigation and economic analysis of a LBR process for the treatment of spent cow bedding represent a novelty in the scientific literature and an important support for industrial development.

3.1.2   Materials and Methods

Substrates and inoculum

Spent cow bedding, corresponding to a mixture of wheat straw and faeces, was collected in October 2014 in a cow dairy farm located in South-West France. This farm used a deep-litter housing system and grew 30 Blonde d'Aquitaine cows whose roughage feeding was composed by 50 % hay and 50 % grass silage. During the cleaning-out of the litter, after accumulation for about 1 month in the stable, six samples of about 10 kg each were sampled from different parts of the litter and were then mixed together manually following the protocol described by Gy (1998) to ensure homogeneity. This overall sample was stored in plastic bags of 8 kg each and kept at ambient temperature for one week before use in order to maintain its natural biological activity. Spent bedding from only one bag was used to load all the reactors.

The solid digestate and leachate used for inoculation were collected from a previous experiment using the same experimental system and treating spent cow bedding similar to the one used in the present study. Table 7 reports the main physico-chemical characteristics of the spent cow bedding, the digestate and the leachate used. The methane production rate of this digester, run for more than 60 days, was about 0.06 NL CH_4 kg^{-1} VS h^{-1} (VS, volatile solids). This value was low enough to consider negligible the methane production originating from digestate in the new batch. The leachate contained 13.0 g L^{-1} of total chemical oxygen demand (CODt) and 4.0 g L^{-1} of soluble chemical oxygen demand (CODs), no trace of volatile fatty acids (VFAs) and, 1.0 g L^{-1} and 2.0 g L^{-1} of NH_4^+ and K^+, respectively.

Table 7 : Characterization of the solid and liquid substrates: total solids (TS), volatile solids (VS), VS to TS ratio and biomethane potential (BMP)

	TS (wet basis)	VS (wet basis)	VS/TS	BMP
	%	%	%	NL CH_4 kg^{-1} VS
Spent cow bedding	29.8 ± 1.6	23.7 ± 0.2	79.5	205.7 ± 15.4
Digestate	13.7 ± 0.3	10.4 ± 0.3	75.6	-
Leachate	1.03 ± 0.01	0.38 ± 0.02	36.8	-

The results are mean values of the triplicates ± standard deviation

Experimental apparatus

The 6 reactors used were identical: 7 L jacketed glass containers and 14.5 cm in diameter (Figure 19). In each reactor, the solid and liquid fractions were kept apart by a sieve (1 mm holes) placed at 10 cm up from the bottom of the reactor, which enabled the leachate to be retained in the lower volume at the bottom. A tube connecting the headspace and the volume under the mesh was added in order to equalize the pressure in the event of the bulk becoming too compact. A peristaltic pump enabled the leachate to recirculate from the bottom to the top. One small glass flask of 140 mL was fitted into the liquid circuit in order to measure pH using a fixed pH probe. Two ports, one

in the liquid and one in the gas circuits, facilitated the collection of samples for analysis. The reactors were maintained in mesophilic conditions (37°C) using a thermo-regulated water bath.
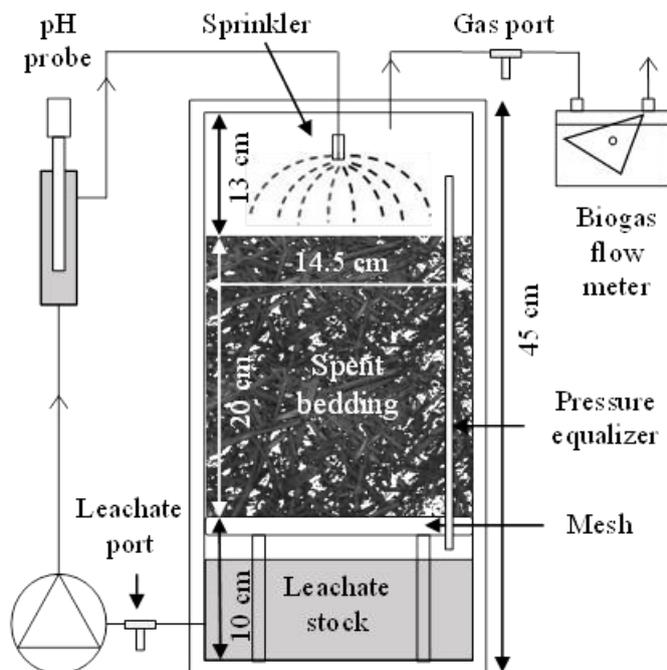
Figure 19: Experimental leach-bed reactor (LBR)

Experimental setting

Three conditions were tested in duplicate to evaluate the different start-up strategies for a LBR packed with spent bedding (Table 8): the addition of a liquid fraction (leachate, L), a solid fraction (digestate, D), and tap water only (blank, B).

For condition D, the substrate-to-inoculum ratio on a volatile solids (VS) basis was 6.8 while on a total solids (TS) basis, expressed as TS digestate / (TS digestate + TS substrate), it reached 13.5 %. Kusch et al. (2008) reported an optimum between 10 % and 20 % (on a TS basis) when treating spent horse bedding. This inoculation ratio is also representative of the one implemented at farm-scale. The leachate used for the condition L, on the other hand, was set using a leachate completed with tap water in order to reach the total amount of liquid desired: the total solid concentration (liquid and solid phase) of all the reactors was set between 10 % and 11 %. In this

case, the substrate-to-inoculum ratio was 45.3 on a VS basis and 4.2 % on a TS basis. This indicated that the VS brought via the inoculation were less when using the leachate compared to the digestate. For the reactors B, only tap water was used to moisten the spent bedding and no further inoculation was added to that already present in the spent cow bedding.

Table 8 : Experimental loading of the three tested conditions: blank (B), condition D and condition L

		Spent cow bedding	Digestate	Leachate	Water
		kg	kg	L	L
Blank (B)	B1	1.30	-	-	2.28
	B2	1.32	-	-	2.28
Condition D	D1	0.97	0.33	-	2.10
	D2	0.97	0.33	-	2.10
Condition L	L1	1.30	–	1.69	0.59
	L2	1.30	-	1.70	0.58

The mixture (spent bedding and digestate, when applicable) was compacted with a weight of 8 kg in order to ensure the same compaction in all the reactors. Finally, the liquid fraction (composed of leachate and/or water) was poured over the solid bed and left to percolate down. A continuous recirculation for 15 min at a flow-rate of about 1.6 L min^{-1} allowed to humidify homogeneously the bulk phase (Abbassi-Guendouz et al., 2012). Thereafter, the recirculation was ensured automatically 10 times per day for 1 minute at a flow-rate of about 1.6 L min^{-1}. This high total amount of water recirculated daily, set at 40 L kg^{-1} TS d^{-1} as done by Riggio et al. (2017a) in a similar system, served to ensure the liquid phase remained in equilibrium with the solid phase, thus permitting the assessment of the bulk conditions through analysis of the leachate.

Analysis

Characterization of the initial raw spent cow bedding was carried out. The TS concentration was measured after drying at 105 °C (378 K) for 24 h, followed by 3 h of calcination at 550 °C (823 K) for determination of VS. Finally, the spent bedding was freeze-dried and crushed to 1 mm particle size prior to BioMethane Potential (BMP) tests. These tests were carried out in batch of 500 mL inoculated with anaerobic sludge from a sugar treatment plant in accordance with the procedure described by Riggio et al. (2017a). The analyses were performed in triplicate and the average values with standard deviation were reported.

During the digestion process only the liquid phase and gas were analysed. The analysis frequency was adapted to the parameters evolution in order to precisely follow the start-up period: 3-4 times per week at the beginning until once per week in the end. The analyses of the liquid phase were done after a recirculation cycle. A volume between 10 and 20 mL was sampled by the mean of the port present in the liquid circuit. Total alkalinity and pH were measured on the raw leachate. Alkalinity was measured at a pH of 4.30, as advised by Hill and Jenkins (1989) and, the pH with Mettler Toledo InPro 4260i probes connected to a Mettler Toledo pH M300 operational manual transmitter. Volatile fatty acids (VFAs) were measured on a leachate previously filtered at 0.45 μm using the VARIAN I-MET-0084 gas chromatograph with a 15 m × 0.53 mm column (Alltech FFAP EC^{TM} 1000) and a flame ionization detector (FID) using helium as the gas vector. Biogas volume was measured with a Ritter flow meter (Milligascounter MGC-1 V3) and its composition was analysed with a micro-GCPRO CP-4900 using helium as the gas vector. The biogas volumes were expressed in standard condition: 273.15 K and 101,325 Pa. The average values of the pairs and their variation ranges (minimum and maximum values) are presented.

Economic assessment

The experimental data were processed together with the economic information delivered by the French company Naskeo Environnement. For industrial confidentiality reasons, all the exact

details used cannot be disclosed and the results obtained have been arbitrarily normalized with respect to the values obtained at 11.5 kt y^{-1} (kilometric ton per year).

The biogas plant considered in the simulations was a farm-scale (100 kW_{el} to 300 kW_{el}) AD plant using several LBRs to treat spent cow bedding. The site design comprised a minimum of four digesters operated in parallel with a certain interval between each reactor start-up (e.g. 4 digesters, batch duration of 60 days and 15 days of interval) and a tank collecting the leachate. The considered incomes were those from the selling of heat and electricity produced through a Combined Heat and Power (CHP) unit. The feed-in tariffs used were those in accordance with actual French legislation (Decree of 19 May, 2011, Decree of 30 July, 2013). The selling and purchase of spent bedding and digestate were not considered.

Along with the capital expenditure (CAPEX), two additional economic parameters were selected based on Karellas et al., (2010) to evaluate the performance of the two start-up strategies: the Payback Period (PP) and the Internal Rate of Return (IRR). The PP, expressed in years, is the duration required to recover the cost of an investment. Its equation is:

$$PP = C/I \qquad \text{(Eq. 1)}$$

with C, cost of project (investment - grants) and I, annual cash inflows (annual revenue - operating costs). The IRR is defined as the value of the Discount Rate necessary to the Net Present Value function (NPV) (the sum of the present values of all the benefits and cost cash flows) to equal zero, which means that the present value of the investment funds equals the present net revenues from an operation. Its equation is:

$$\text{NPV} = \sum_{n=0}^{N} \frac{C_n}{(1+r)^n} - C_o = 0 \qquad \text{(Eq. 2)}$$

with C_n: net cash inflow during the period n; C_o: total initial investment costs; n: number of time periods (N= 15 years); r: discount rate. The higher the IRR, the more profitable the project.

To sum up, PP has the advantage of being explicit but it does not take into account important factors like the value of money over time (money at the present time is worth more than the same

amount in the future due to its potential earning capacity) and the risk of an investment; IRR is a more precise but complicated parameter that takes these latter into account, permitting a comparison of projects of different size. More information about the hypotheses and the economic inputs are reported in the supplementary material (section 7.2).

3.1.3   Results and discussion

3.1.3.1   *Process performance*

Biogas production

The analysis of the cumulative volume of methane produced and the methane yield provides a comprehensive evaluation of process performance for the three different operating conditions (Figure 20 a - b). The error between duplicate was really low indicating the precision of measures in the three conditions and the reproducibility of the reactor set-up. The blank, where only tap water was added, showed a cumulative volume of methane produced that was 9.0 % lower compared to the batches where leachate was added (condition L). However, after 60 days, the blank reached a methane yield of 169.0 NL CH_4 kg^{-1} VS on average, which represents 82 % of the BMP of spent cow bedding (205.7 NL CH_4 kg^{-1} VS). Very similar methane yields were reported by Riggio et al. (2017a) when treating spent cow bedding for the same batch duration. These results suggest that the AD process in batch mode treating spent cow bedding with only the addition of water can start up reasonnably well and also the microbial inoculation provided by the solid manure itself is sufficient. Similar results were found by Kusch et al. (2008) with spent horse bedding and water.

The cumulative volume of methane produced in conditions L and D (addition of leachate and digestate, respectively) were linked to the difference in the amount of fresh matter loaded (Table 8). In fact, after 60 days of operation, condition D produced 13.5 % less volume of methane than condition L (Table 9). Nevertheless, the methane yield, expressed as the ratio of the total volume of

methane produced during a batch to the quantity of VS added as spent bedding at the beginning of the batch, was 15.8 % higher for condition D.
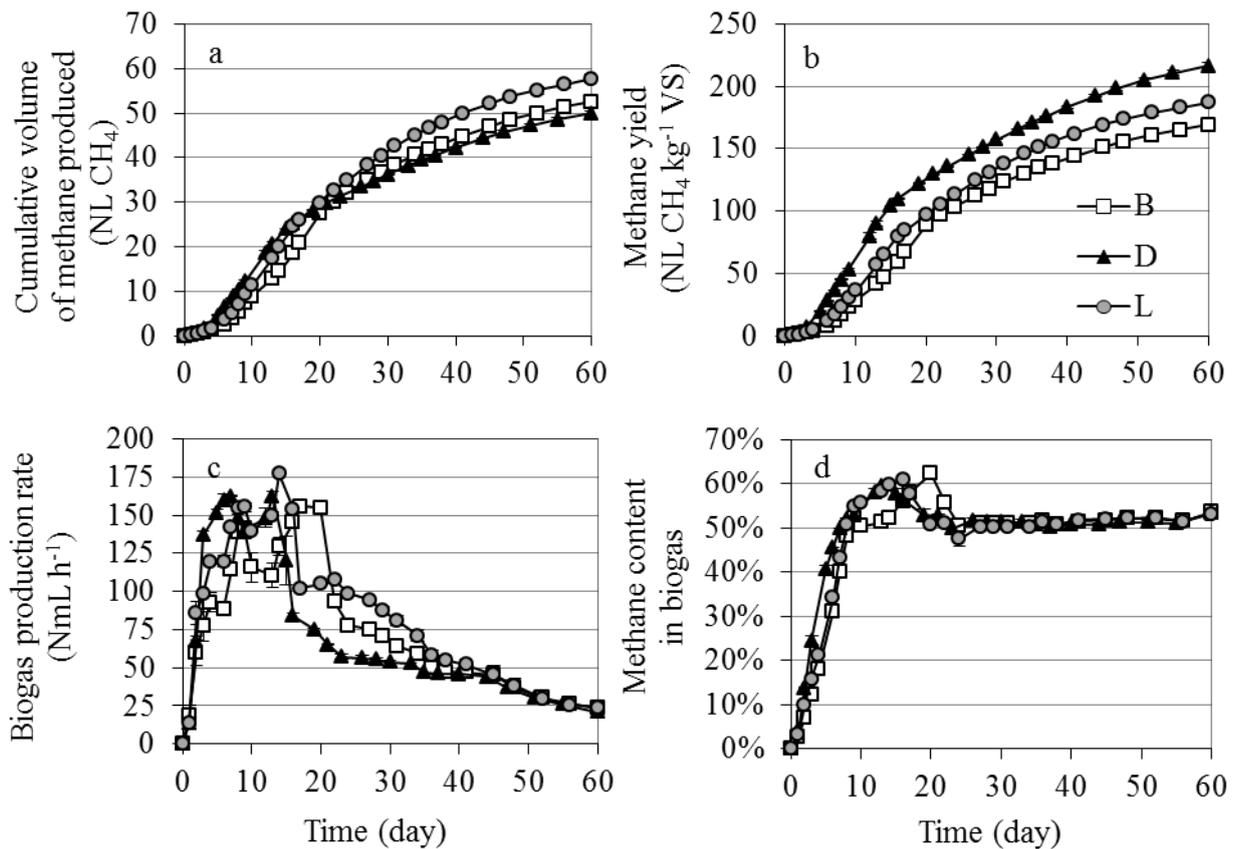
Figure 20 : Comparison of the biogas parameters analysed all along the digestion process of three different experimental loadings: spent cow bedding and water (B), spent cow bedding and leachate (L) and spent cow bedding and digestate (D). (a) cumulative methane produced, (b) methane yield, (c) biogas production rate and (d) methane content in biogas produced; (b) methane yield; (c) biogas production rate; (d) methane concentration in biogas

The contribution of digestate amendment to the methane produced was estimated to be lower than 6.0 % of the total methane produced (by considering a constant methane production rate of 0.06 NL CH_4 kg^{-1} VS h^{-1}). Hence, the part of methane produced from digestate degradation cannot fully explain the higher methane yield which was certainly related to faster overall degradation kinetics. Higher kinetics of substrate degradation after inoculation with digestate were also recorded by Di Maria et al. (2012) and Kusch et al. (2008). These results can be explained by the possible greater

amount of specific microorganisms brought into the system through the addition of solid digestate and/or by the microbial consortia present in the digestate which seem better adapted to solid substrate degradation. The observation that the microbial consortia in the leachate and solid phase differ, with little exchange between them (Degueurce et al., 2016c), could support this explanation.

The biogas production rates in Figure 20c indicate that faster kinetics were obtained with condition D, followed by condition L and then B. Different production rates were observed up to about 40 days; thereafter, for all the conditions, very close values were recorded until the end. The methane concentration in biogas displayed in Figure 20d was found slightly better when adding digestate, as also reported by Di Maria et al. (2012). However, the very tiny difference observed between the use of leachate or water during the first 10 days proved the very limited inoculation capability provided by the leachate in comparison to water. A more significant delay was observed for condition B after 10 days, attesting of the positive effect played by the leachate or the digestate.

In conclusion, the addition of digestate led to increased methane production kinetics and permitted a higher methane yield at 60 days while the addition of leachate led to higher volumetric methane production.

Table 9 : Average methane performance in the three tested conditions after 60 days of digestion

	Cumulative volume of methane	Methane yield
	NL CH_4	NL CH_4 kg^{-1} VS
Blank (B)	52.5 ± 0.8	169.0 ± 1.5
Condition D	49.9 ± 0.6	216.5 ± 2.4
Condition L	57.7 ± 1.0	187.0 ± 3.1
Comparison of conditions D and L	-13.5%*	15.8%*

The results are mean values of the duplicate ± range of variation (minimum and maximum value)
*(D-L)/L

pH, VFAs and alkalinity

In the three conditions tested, the process was stable overall and no significant inhibition was detected. The lowest pH values were observed for the blank condition (Figure 21a). However, the pH remained above 6.5, even during the first days during which the highest VFA peak was monitored (Figure 21b). After the acidification phase, the pH stabilised at values ranging between 7.0 and 7.4 for all three conditions. A constant difference between condition L and the other two is explained by the higher alkalinity of the leachate (Figure 21c) mainly because of the presence of NH_4^+ in the solution. Indeed, alkalinity in L was about 2 g $CaCO_3$ L^{-1} and 3 g $CaCO_3$ L^{-1} higher than in B and in D, respectively. Hence, leachate can contribute significantly to a greater stability of the process.

The concentration of total volatile fatty acids (TVFA) in conditions D and L (Figure 21b) followed a similar trend: a peak was reached after 7 days of operation (8 and 5 g COD L^{-1}, respectively), and, after 15 days, no more VFAs were recorded. The lower TVFA concentration in condition D in comparison to L was mainly related to a lower load of spent bedding (Table 8). The difference between conditions L and B was due to slower propionic acid consumption in the latter (Figure 21d), meaning that the acetogenesis kinetics were slower in the blank. A faster degradation of propionic acid could be linked to a higher alkalinity and/or pH rather than to the inoculation, since this leachate inoculation was poorer compared to that of the digestate. A faster consumption of propionic acid observed in condition L when compared to condition D, indicates that the leachate played a more important role than the digestate in propionic acid consumption.
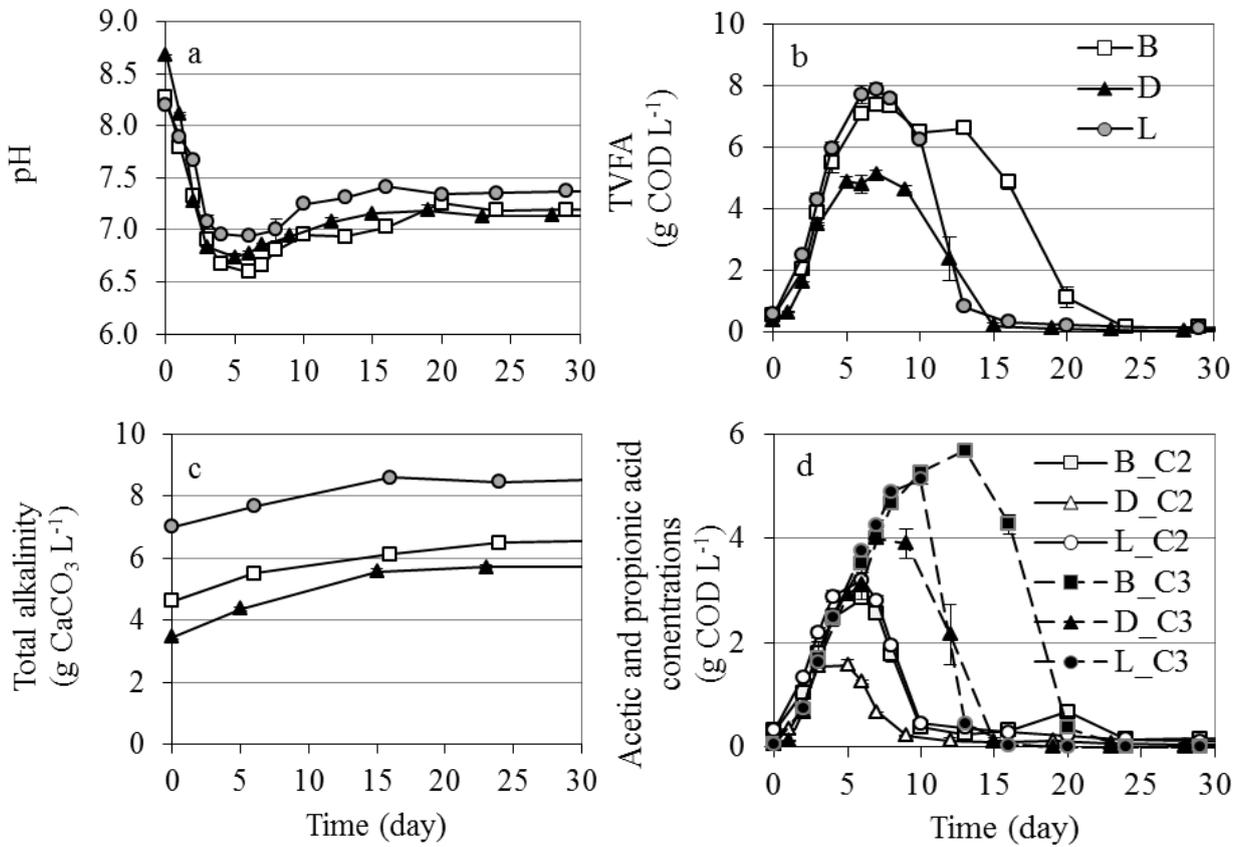
Figure 21 : Comparison of the leachate parameters analysed all along the digestion process of three different experimental loadings: spent cow bedding and water (B), spent cow bedding and leachate (L) and spent cow bedding and digestate (D). (a) pH; (b) total volatile fatty acid concentration; (c) total alkalinity; (d) acetic (C2) and propionic (C3) acid concentrations

3.1.3.2   Economic analysis

A qualitative economic analysis was carried out in order to compare the two inoculation strategies: conditions D and L. Real values could not be provided for confidentiality reasons.

The evolution of the Payback Period (PP), the Internal Rate of Return (IRR) and the Capital Expenditure (CAPEX) were analysed in relation to the annual tonnage processed (capacity) and the batch duration. Further results about the sensitivity analysis of four important external process parameters (grants accorded to the project, the fraction of heat sold, the preparation time for loading and emptying the digesters and the haulage distance for substrate collection) were provided in the supplementary materials (section 7.3).

Capacity (annual tonnage processed)

The CHP power chosen (100 kW$_{el}$ to 300 kW$_{el}$) and the experimental methane yields determined for each strategy (condition D or L) set the lower and upper capacity limits for the simulation: 5.0 kt y^{-1} and 15.0 kt y^{-1}. Figure 22 a - b shows that the PP decreased and the IRR increased with the capacity of the plant. These trends are common in AD processes (Walla and Schneeberger, 2008) and show an improved economic margin for bigger plants due to economies of scale. Lantz (2012) agreed on the importance of the scale of biogas production at farm-scale plants. In our conditions, a comparison was considered economically interesting only for capacities higher than 8.0 kt y^{-1} because for lower quantities a rapid increase of the PP was observed along with a decrease in the IRR (Figure 22 a - b). To further support this choice, it is interesting to note that below a capacity of 8.0 kt y^{-1} the IRR was too low, turning negative at about 6.0 kt y^{-1} (an IRR of approximatively 10 % is generally required in farm-scale projects (Wresta et al., 2015)).

When increasing the annual capacity, the discontinuous variation of the feed-in tariffs, the number of digesters and the CHP power installed (i.e. five consecutive drops in the feed-in tariffs, a gradual increase from 4 to 9 digesters, 25 kW$_{el}$ step-by-step increase in the CHP power from 100 kW$_{el}$ to 300 kW$_{el}$) led to irregularities in the evolution of the IRR and PP (e.g. for 8.0 kt y^{-1} and 9.5 kt y^{-1}). However, these latter did not affect the overall economic analysis.

In the selected capacity range, condition L performed, overall, slightly better than condition D: condition L's IRR and PP values better than, or similar to, those of D. That means that the 15.8 % higher methane yield in condition D counterbalanced the 34 % higher digester working volume needed in this condition but it did not allow overtaking condition L from an economic point of view. In spite of these results, the difference found in the PP and IRR were not judged significant to prefer one strategy over the other, and this for the entire range of capacity analysed.
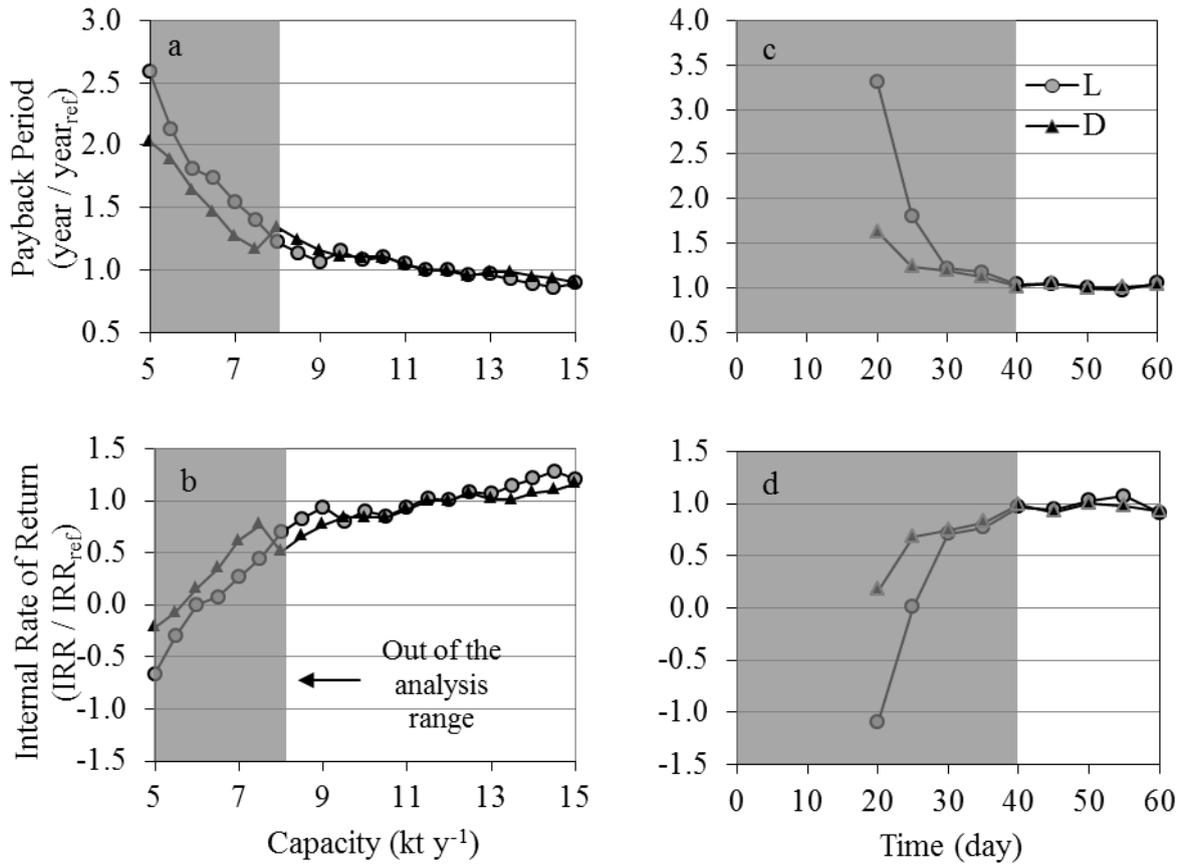
Figure 22 : Results of the economic simulation performed on an industrial site using a sequential LBR process considering a digester number varying from 4 to 9 and an electric cogeneration power ranging from 100 to 300 kW_{el}. Evolution of the (a) Payback Period and Internal (b) Rate of Return in relation to the annual capacity. Evolution of the (c) Payback Period and (d) Internal Rate of Return in relation to the batch duration

Batch duration

Given the more rapid kinetics observed when using digestate instead of leachate as inoculum, batch duration represents an important parameter requiring close analysis. Figure 22 c-d represent the variation over time of the PP and IRR, respectively, for an average capacity of 11.5 kt y^{-1}.

Similarly as for the batch duration, it is important to note that below 30 days the process is not economically viable because of the low IRR, and that a more stable evolution (especially of the IRR) is obtained after 40 days. That supports the choice of a batch duration not below this threshold at industrial scale. Moreover, batch durations below 40 days would mean lower degradation and treatment of the organic substrate (environmentally not advisable) and an under-conversion of its

energy potential (about 15 % of methane lost at day 40 compared to day 60). Therefore, the impact of batch duration on the economic assessment was studied between 40 days and 60 days.

Both the PP and the IRR in the selected range of analysis were found very close regarding the conditions tested (Figure 22 c-d) and, as for the capacity, batch duration was not found a discriminant parameter allowing the choice between the two strategies.

For capacities different (varying from 8.0 and 15.0 kt y^{-1}) from the one studied (11.5 kt y^{-1}) similar results were observed (not shown). However, it is interesting to note that for smaller capacities, the batch duration was found to impact significantly the economic performance whereas for bigger no particular effect resulted from its modification. As a consequence, batch duration of 60 days should be preferred for small capacities while the choice of batch duration would depend on a specific objective for bigger sites (i.e. to treat the maximum amount of substrate or to increase its degradation).

Capital expenditure

CAPEX is a very important parameter to consider from an economic point of view because it quantifies the capital needed to practically undertake a new project. Figure 23 shows the CAPEX evolution (normalized) in condition D and L, and the additional investment of condition D over condition L. CAPEX normally increases in relation to the capacity (Figure 23a) and the batch duration (Figure 23b) in both conditions. However, these evolutions follow significantly different trends. When considering these growths in the range of interest (8.0 – 11.5 kt y^{-1} for the capacity and 40 – 60 days for the batch duration) the additional investment in condition D is estimated to be 18% higher, on average, compared to L. This represents a significant percentage, especially for small project whose feasibility is often difficult to reach.
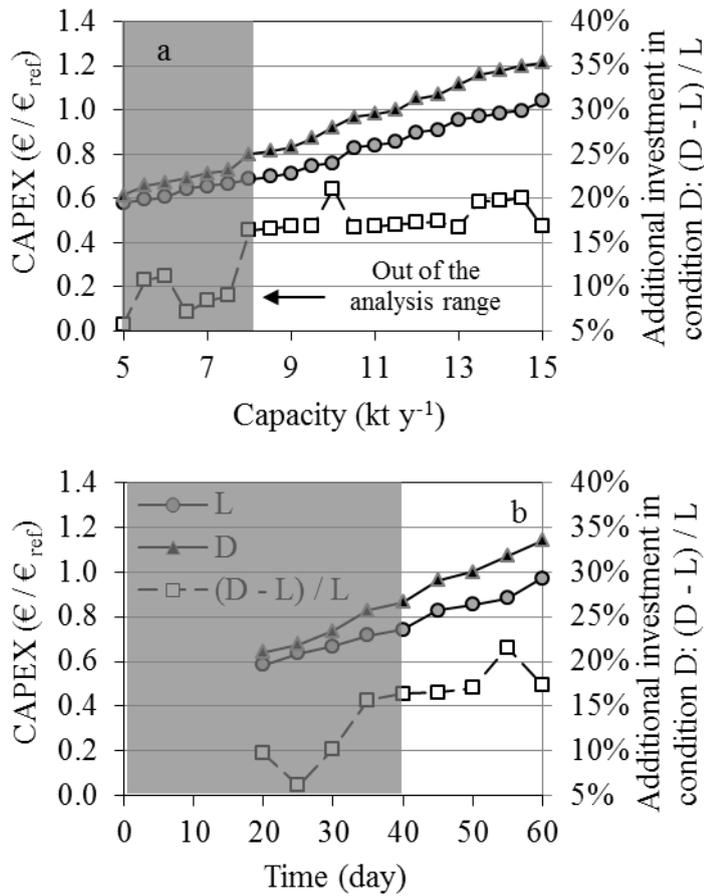
Figure 23 : Results of the economic simulation performed on an industrial site using a sequential LBR process considering a digester number varying from 4 to 9 and an electric cogeneration power ranging from 100 to 300 kW_{el}. Evolution of the CAPEX in relation to (a) the annual capacity and (b) the batch duration

This result underlines that the addition of digestate affects significantly the initial investment by the mean of the increase of the digesters volume. In fact, engineering construction represents a big share of the CAPEX. In view of the above, condition D has a significant disadvantage compared to condition L and this latter is then the best choice from an economic point of view.

3.1.4   Conclusion

Two start-up strategies for LBR treating spent cow bedding were tested: the addition of leachate or solid digestate. Both were found to impact positively the overall performance of the process, the first improving the process stability (pH and higher alkalinity) and the second enhancing the substrate degradation kinetics. The economic analysis showed no significant

differences in the payback period and internal rate of return connected to these two start-up strategies. However, despite these results, the bigger working volume (34 % in this case study) required when adding solid digestate affected considerably the initial investment: 18 % higher compared to the use of leachate. Therefore, leachate addition, associated to a smaller working volume and lower initial costs, seems acceptable from the economic point of view when treating a substrate already rich in methanogens like spent cow bedding. These economic results promote further development of farm-scale AD process and the increase of decentralized energy production.

CHAPTER 4

Comparison of the mesophilic and thermophilic anaerobic digestion of spent cow bedding in leach-bed reactors

In this chapter, the effect of operating the LBR in thermophilic condition as a tool to diminish batch duration and consequently improve the overall performance of the process was investigated. In fact, in chapter 2, the long incubation time necessary to reach a significant BMP when treating spent animal bedding had already been identified as one of the levers for possible process optimization. Furthermore, the problematic connected to the exploitation of the biogas in a Combined Heat and Power (CHP) unit were analysed as well as their impact on the plant performance.
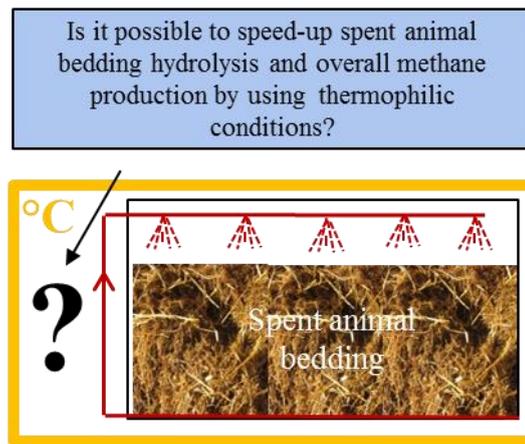
Figure 24 : Graphical abstract of the third issue faced in this PhD thesis: the advantages and drawback of treating spent animal bedding in thermophilic conditions

4.1 Comparison of thermophilic and mesophilic temperatures on the anaerobic digestion of spent cow bedding in leach-bed reactors

4.1.1 Introduction

Anaerobic digestion (AD) is a very widespread biological process aiming at the treatment of organic waste and the production of green energy. Spent animal bedding, a mixture of animal faeces and a bedding material both accumulating in the stall, has been proved to be efficiently treated in discontinuous dry anaerobic digesters such as leach-bed reactors (LBRs) (Riggio et al., 2017a). Spent animal bedding constitutes a slowly-degradable substrate mainly because of its high content in lignocellulosic material (Buffiere et al., 2006); for this reason digestion times ranging from 40 to 60 days are generally used in discontinuous LBRs at industrial scale. In order to improve the economic performance of the entire process, there is a real interest in increasing the substrate degradation kinetics and then reduce the overall digestion time. The use of thermophilic conditions in LBRs treating spent cow bedding could represent an effective solution to enhance the process performance. Indeed, by comparing the influence of mesophilic and thermophilic temperatures, several authors agreed on the faster degradation kinetics when using thermophilic conditions to treat different substrates: spent horse bedding (Böske et al., 2015), the organic fraction of municipal solid waste (Fernández-Rodríguez et al., 2013), spent cow bedding (Gómez et al., 2011), wood chips (Hegde and Pullammanappallil, 2007) and cow dung (Jha et al., 2013).

In the literature, few data comparing mesophilic and thermophilic treatment are available on spent animal bedding consisting of faeces and straw. Böske et al. (2015) used a continuous upflow anaerobic solid-state (UASS) reactor to treat spent horse bedding whereas Gómez et al. (2011) used a dry unmixed batch system to digeste spent cow bedding. In thermophilic conditions, the first authors observed higher kinetics and methane yield than at a mesophilic temperature while the second reported higher kinetics and a lower methane yield. In addition to the discrepancy in their results, these latter hardly seem applicable to a different system such as a LBR.

The use of LBRs in thermophilic condition is not very common in scientific literature: Koppar and Pullammanappallil (2013) used it to treat citrus peel waste, Liang et al. (2014) to treat smooth cordgrass and Rico et al. (2015) to treat raw dairy manure (among other types). Moreover, no direct comparison has been made between LBRs run in mesophilic and thermophilic temperatures and nor has research involved two specific challenges connected with this discontinuous process: the start-up conditions (i.e. the inoculation) and the discontinuous biogas production. The first, repeated at every digester loading, affects the methane production rates if not well managed, while the second causes problems for combustion in a Combined Heat and Power (CHP) unit. More particularly, this latter issue is very important when dealing with LBR plants in rural areas since a limited number of reactors (the main cause of a fluctuating biogas production) is often chosen to make this process economically feasible.

The efficiency of thermophilic treatment of spent cow bedding in LBRs remains an open question. Therefore, the aim of this work was to investigate if the operation of thermophilic mode when treating spent cow bedding in LBRs could be an effective measure for reducing the digestion time and increasing methane yield. In order to reach this objective, specific problems related to inoculation and the challenge connected to the combustion of biogas in a CHP unit were analyzed in detail.

4.1.2 Material and Methods

Experimental set-up

The three leach-bed reactors used in the present study were made of stainless-steel and the internal diameter and total height were 40 cm and 80 cm, respectively, for a total volume of about 100 L. A mesh (3 mm holes) placed at 20 cm from the bottom separated the solid and the leachate volumes: 75 L and 25 L, respectively. Each reactor was connected to a dedicated thermo-regulated water bath to maintain the temperature. A centrifugal pump (Rover Pompe BE-M 20) was used to sprinkle the

leachate stored at the bottom of the reactor to the top of the bulk. A valve in the leachate circuit allowed taking leachate samples while a port on the biogas circuit allowed gas sampling for analysis. The operation principle of the present LBRs is further detailed by Riggio et al. (2017a).

Substrate collection and experimental conditions

Three conditions were tested: two reactors were inoculated with a mixture of digestate and leachate and were operated under mesophilic (37°C) or thermophilic (55°C) conditions (reactors named M_inoc and T_inoc, respectively); a third reactor was started up in thermophilic conditions but without specific inoculation (T_no inoc). Two successive runs were carried out in order to establish the process's stability and repeatability. The spent cow bedding used in each run was collected at the same farm during two different stable cleanings. The substrates sampled were stored for 2-3 days in plastic bags at ambient temperature before being used. The solid digestates and leachates used in run 1 were sampled from two previous batches adapted to thermophilic and mesophilic conditions for over three months (with two consecutive loadings). For run 2, the digestates and leachates collected at the end of run 1 were used instead. Total solids (TS), volatile solids (VS) and biomethane potential (BMP) tests (on raw matter) of digestate and spent cow bedding used in both run are reported in Table 10a. The protocols used have been described by Riggio et al. (2017a).

The operating conditions for each run are described in Table 10b. In each reactor, about 1.5 kg of total solids (TS) of spent bedding was added. For the reactors inoculated, solid digestate was mixed to the spent bedding to reach a digestate TS/(substrate TS + digestate TS) of 13%. The leachate was diluted before being added to the reactor in order to keep a $N-NH_4^+$ concentration in the leachate below 0.9 g L^{-1} at the start-up and to avoid any risk of nitrogen inhibition (Angelidaki and Ahring, 1993). The total amount of leachate to be added was chosen to keep the initial TS of the mix (manure + digestate + leachate) at 11.5%, close to the one reported by Riggio et al. (2017a) in similar systems. Before starting the digestion process, leachate was recirculated continuously for

10 min in order to achieve water saturation of the waste bed. Finally, the reactors were closed and

the internal recirculation of the leachate was scheduled twice a day for a total volume of 1 L kg^{-1} TS

d^{-1}.

Table 10 : (a) Total solids (TS), volatile solids (VS), ratio of VS to TS (VS/TS) and the BioMethane potential (BMP) of the substrates loaded in the reactors in run 1 and run 2; (b) loading set-up of run1 and run 2

a		Digestate T	Digestate M	Spent cow bedding
Run 1				
TS	kg TS kg^{-1} RM	$(14.9 \pm 0.5)\%$	$(15.5 \pm 0.0)\%$	$(25.5 \pm 0.1)\%$
VS	kg VS kg^{-1} RM	$(11.3 \pm 0.4)\%$	$(11.6 \pm 0.0)\%$	$(22.8 \pm 0.1)\%$
VS/TS	%	76.2%	75.0%	89.4%
BMP	NmL CH_4 g^{-1} VS	-	-	282 ± 25
Run 2				
TS	kg TS kg^{-1} RM	$(14.3 \pm 0.6)\%$	$(15.7 \pm 2.6)\%$	$(28.1 \pm 1.9)\%$
VS	kg VS kg^{-1} RM	$(11.1 \pm 0.3)\%$	$(12.7 \pm 1.9)\%$	$(23.7 \pm 2.2)\%$
VS/TS	%	78.1%	81.0%	84.5%
BMP	NmL CH_4 g^{-1} VS	-	-	-

Average values of the triplicate and standard deviation

b		Spent cow bedding	Digestate	Leachate	Water
		kg	kg	L	L
Run 1	T_inoc	5.35	1.51	4.0	4.0
	T_no inoc	5.35	-	4.0	4.0
	M_inoc	5.35	1.36	4.0	4.0
Run 2	T_inoc	5.90	1.56	3.0	4.4
	T_no inoc	5.90	-	3.0	4.4
	M_inoc	5.90	1.60	5.0	2.4

During digestion, volatile fatty acids (VFAs), pH, alkalinity and biogas volume and its composition were monitored. The frequency of analysis and the protocols used have been described in section 2.1.2.

Hypothesis for electrical production

Electrical production in a CHP unit from biogas produced in mesophilic and thermophilic conditions was compared using data from run 1. The simulation considered the following hypothesis: treatment of 9,400 tons/year of spent cow bedding and 4 LBRs working in parallel, staggered over time with a batch duration of 44 days: every 11 days a LBR was emptied and reloaded. 4 LBRs were chosen as a representative number of digesters in this kind of farm plant. In fact, more digesters would affect the economic feasibility of the project. 44 days were chosen not only because it is a realistic digestion time for this kind of substrate but also because the methane yield of the two conditions tested were the same after this time slot, cancelling the influence due to this factor on the comparison. Finally, a staggering time of 11 days between digesters start-ups was indirectly set after the previous choices: 4 digesters and 44 days of batch duration.

Biogas storage was not considered. A CHP unit with an electrical nominal power (P_{nom}) of 250 kW_{el} (Schnell, 2016) was chosen, based on the average annual energy production of the site (considering the annual amount of substrate and the duration time evoked above). A minimum methane content of 45% was set for injection into the CHP unit and an electrical efficiency (η_{el}) of 45.5% was considered at P_{nom} on manufacturer recommendation. The electrical efficiency was considered to vary linearly (Bianchi et al., 2014) between electrical nominal power (max_η_{el} = 45.5%) and the electrical minimal power P_{min} (min_η_{el} = 41.0%) with the equation min_η_{el} = max_η_{el} × 0.9. It is important to note that the electrical minimal power P_{min} (50% of P_{nom}) corresponds to the power under which the CHP shuts down.

4.1.3   Results and Discussion

4.1.3.1   Effect of inoculation in thermophilic conditions

Figure 25a permits a comparison to be made between the specific methane production (SMP) rates in thermophilic conditions with and without the addition of solid digestate as inoculum. SMP rates between the two conditions were different: for the first 12 days in run 1 and 24 days in run 2. Moreover, in both runs a lower SMP rate was measured during the first 5 days when the LBR was not inoculated with solid digestate. This result indicates that an inoculation with solid digestate mostly influenced the start-up phase of the process leading to higher SMP peaks. Figure 25b shows that the VFAs which accumulated during this period were rapidly consumed after 5 days in both conditions and runs.

Small differences were observed when adding solid inoculum, while different VFA concentration peaks between runs were due to the use of different samples of spent cow bedding. All along the digestion process, the pH remained in a suitable range for an optimized anaerobic digestion (i.e., 7.6 – 8.2).

In addition to the results of Chachkhiani et al. (2004) showing that thermophilic microbial community in cattle manure is present even if at a subdominant level compared to mesophilic communities, these results show that these microorganisms were sufficiently active to permit spent cow bedding to be digested without a specific addition of solid inoculum. The slightly faster start-up was not found significant enough to justify the use of solid inoculum when using spent cow bedding, a substrate which, in any case, needs a long time to be degraded.
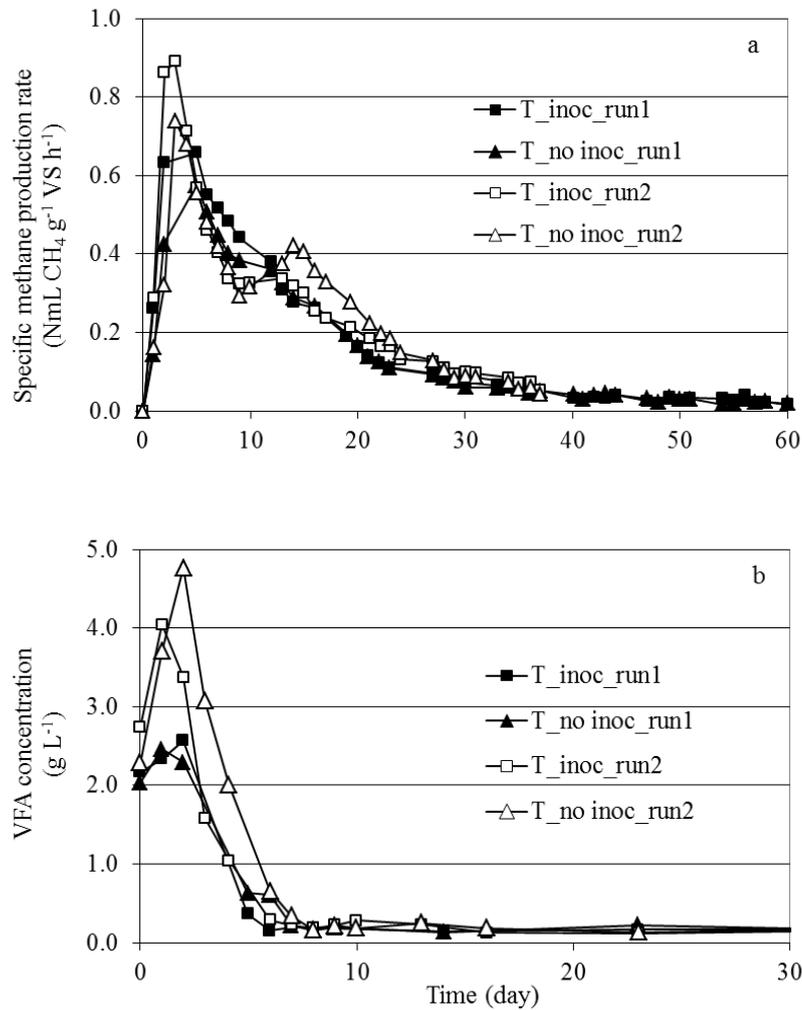
Figure 25 : Comparison of thermophilic conditions with (T_inoc) and without (T_no inoc) solid digestate, in run 1 and run 2: (a) specific methane production rate; (b) VFA concentration

4.1.3.2 *Comparison of thermophilic vs mesophilic conditions*

Reactor performances

Figure 26a compares the SMP rates in LBRs inoculated with solid digestate and run under thermophilic (T_inoc) or mesophilic (M_inoc) temperatures. Operating under thermophilic temperature increased the initial degradation kinetics as suggested by the higher SMP in thermophilic conditions during the first 7-8 days. The difference in the peaks reached was related to
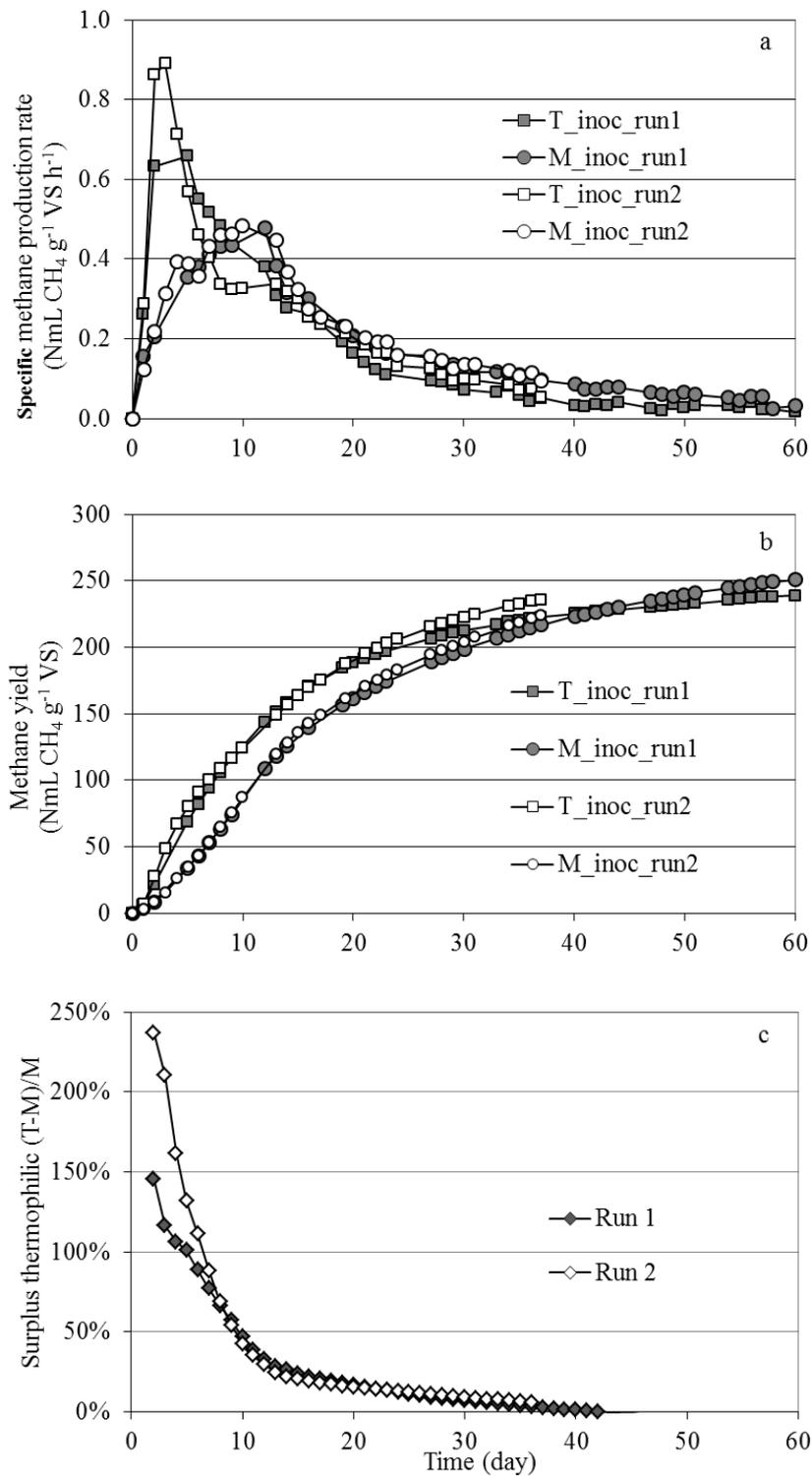
Figure 26 : Comparison of mesophilic (M_inoc) and thermophilic (T_inoc) conditions, in run 1 and run 2: (a) specific methane production rate; (b) methane yield; (c) additional methane yield in thermophilic conditions

the use of two different spent bedding samples between runs. After 15 days, the SMP rates for both runs and conditions were similar.

Figure 26b presents the methane yield (MY) for both temperature conditions and runs. Run 2 was carried out to confirm SMP rates and MYs, but a technical problem required the interruption of the experiment after 37 days. However, MYs measured in runs 1 and 2 were very similar, thus indicating that the inoculum had already adapted in run 1 and that the results were repeatable. More precisely, after 37 days the errors on the MYs between the two runs (calculated as |x1 - x2| / M(x1,x2) with x1 and x2 the measures and M the mean value of the measures) were 3.5% an 2.1% for thermophilic and mesophilic reactors, respectively. Because of the higher initial SMP

rates, the MY in thermophilic conditions were higher and only after about 42 days of operation the same MY was reached in thermophilic and mesophilic reactors. The additional amount of methane produced in thermophilic, in comparison to mesophilic conditions, is depicted in Figure 26c. Initially, the surplus was significant in both runs but it rapidly decreased to reach an average value of 27% at 13 days and 0% at 42 days. Methane yield at 42 days corresponded to 80% of the BMP (run 1). This means that if 80% of the potential energy were recovered, then thermophilic temperatures would not offer any advantage over mesophilic temperatures. However, for lower BMP value, the interest in using thermophilic conditions by reducing batch duration should be assessed economically.

In batch digesters fed with corn stover and operated at 20% TS, Shi et al. (2013) also reported similar MY in thermophilic and mesophilic conditions after 38-45 days of operation. However, when treating easily-degradable substrates such as vegetable waste, Hegde and Pullammanappallil (2007) reported better performance under thermophilic conditions with a significant reduction of the time (10 days) to reach the 95% of the BMP. The particular behaviour observed when treating spent cow bedding suggests that a thermophilic temperature had an impact mainly on its easily-degradable fraction (higher methane production rates over the first 7-8 days) and not on the slowly-degradable one (similar SMP rates after 12 days). Since the easily-degradable compounds represent only a small fraction of spent cow bedding which is known to be rich in lignocellulosic material (Riggio et al., 2017a), the advantage of operating under thermophilic conditions was thus extremely

limited. It is interesting to note that the degradation kinetics recorded were not influenced by nitrogen inhibition (N-NH$_4^+$ kept below 0.9 g L^{-1}) and only the effect of temperature on kinetics was observed. Nitrogen inhibition should be considered as a further problem requiring a solution in thermophilic conditions.

Higher VFA concentrations were observed in thermophilic condition as a consequence of a faster hydrolysis (Figure 27a). However, after 5 days very low concentrations were measured in thermophilic condition while 12 days were needed to degrade completely the accumulated VFAs in mesophilic condition. The delay observed was due to an accumulation of propionic acid in mesophilic condition (Figure 27b). In fact, thermophilic temperature favours the consumption of propionic acid because it lowers the Gibbs free energy of the reaction (Amani et al., 2010). The high alkalinity in the system (higher than 5 g CaCO$_3$ L^{-1} at start-up), hampered a too important drop of the pH which remained between 7.3 and 8.2 considering both runs and temperature conditions.
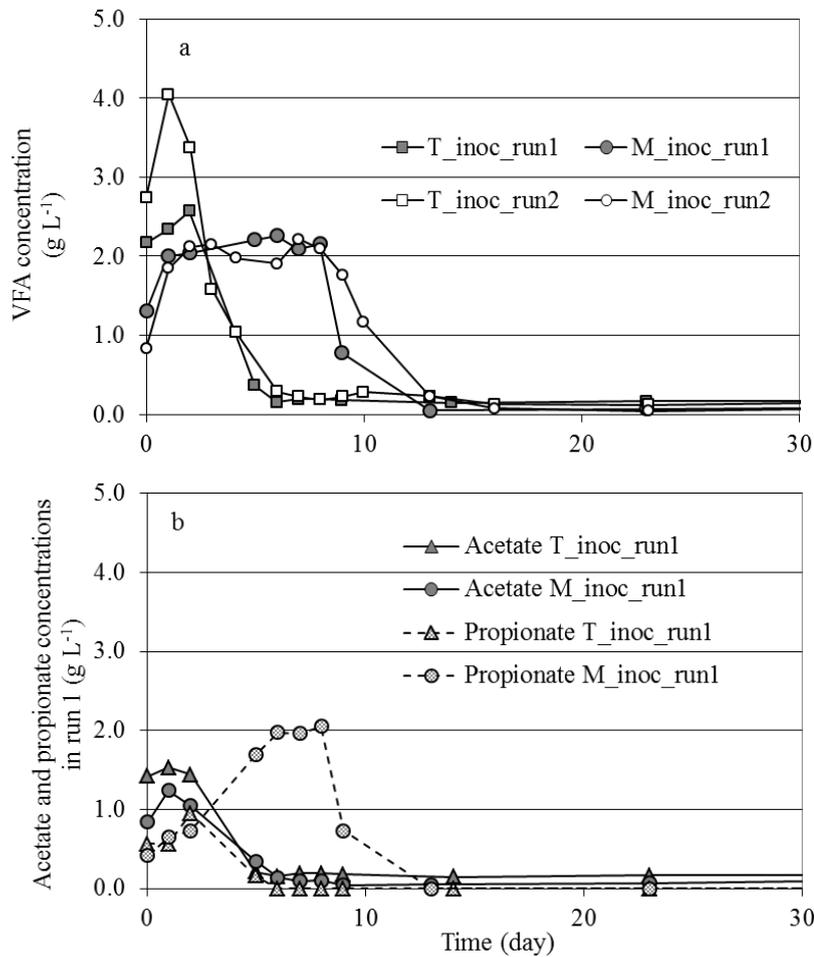
Figure 27 : Comparison of mesophilic (M_inoc) and thermophilic (T_inoc) conditions: (a) VFA concentration in run 1 and run 2; (b) acetate and propionate concentrations in run 1

Electricity production

Methane production rates have a significant impact on methane conversion into electricity through CHP units, mainly on account of their technical constraints (i.e. minimum methane content and maximum combustion power). Figure 28a depicts the combustion power (P_{biogas}) associated to the cumulated biogas of the four digesters at the inlet of the CHP. First, to prevent dropping below the minimum methane content (i.e. 45%), the use of biogas produced from a new batch can start only when the methane content is high enough and, hence, a part of the initial biogas produced is not exploited.
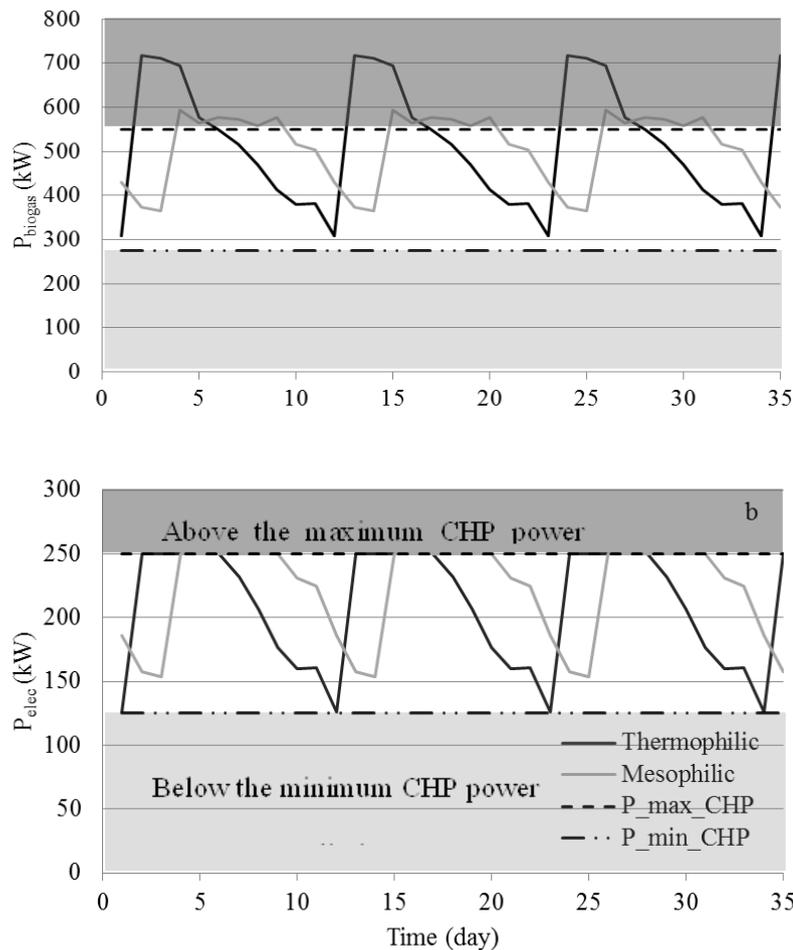
Figure 28 : Comparison of a simulated industrial plant operated in mesophilic and thermophilic conditions. The simulation, based on the data from run1, consider the treatment of 9,400 tons/year of spent cow bedding, the installation of a combined heat and power (CHP) unit of 250 kW$_{el}$ and the use of 4 digesters staggered in time with a batch duration of 44 days. (a) heat power contained in the cumulated biogas at the inlet of the CHP unit; (b) electrical power produced considering the CHP power working range: the maximum (P_max_CHP) and the minimum (P_min_CHP)

For both temperatures, the amount of methane lost is quite similar: the difference is a mere 1.4%. In addition, in thermophilic conditions, high SMP rates during the first days of each batch cause important fluctuations of the total methane flow at the entrance of the CHP unit (Figure 28a). When P$_{biogas}$ overtakes the maximum power accepted by the CHP (i.e. 550 kW$_{comb}$ or 250 kW$_{el}$), the surplus biogas is burned in a torch and then lost if no storage is provided. As a consequence, about 7.9% of the methane is not converted into electrical power in thermophilic conditions compared to the 2.3% in a mesophilic environment. Another interesting aspect is showed by Figure 28b which

illustrates the electrical power (P_{el}) produced by the CHP from biogas collected in mesophilic and thermophilic conditions. In mesophilic conditions, the CHP is operated at its P_{nom} for a longer than in thermophilic conditions (i.e. 54% and 36% of the time, respectively). As a consequence, a better exploitation of the biogas energy can be achieved in mesophilic conditions, since η_{el} is the highest at P_{nom} whereas it decreases for lower powers.

To sum up, higher methane production rates in thermophilic conditions during the first days of the batch process are proved to induce, on one hand, the loss of a part of the biogas produced because the CHP power limits are overtaken, and, on the other hand, a poorer exploitation of the biogas's combustion power due to variable electrical efficiency. Based on this simulation, 5.9% less electrical energy can be produced for an annual period in thermophilic conditions (1.84×10^6 kW·h$_{el}$) as opposed to mesophilic conditions (1.95×10^6 kW·h$_{el}$). This result represents a further drawback when using thermophilic temperatures in a discontinuous process, in addition to the higher energy consumption, higher investment cost because of the use of thermo-resistant materials, as well as further biological issues as nitrogen inhibition.

4.1.4   Conclusions

The digestion of spent cow bedding, a slowly-degradable substrate, was compared at mesophilic and thermophilic conditions. Thermophilic temperature increased methane production but this effect was restricted to the start-up period (degradation of the easily-degradable fraction), with a reduced advantage for the cumulated methane recovered over the long term. Furthermore, higher kinetics during the first days caused higher fluctuation of the methane flow at the inlet of a CHP unit, with consequent lower electrical energy production. These issues should be considered when assessing advisability of implementing thermophilic conditions for the digestion of spent cow bedding in LBRs.

CHAPTER 5

Study of the co-digestion of spent cow bedding with an easily-degradable substrate and of the VFAs management during acidification

In the previous chapter the treatment of spent animal bedding as a sole substrate was studied and some optimization strategies were established. In this chapter, the co-digestion of spent animal bedding with an easily-degradable substrate is discussed since such configuration can occur seasonally at industrial scale. The system setting used in this section was modified and a new design, simulating the presence of other rectors as in a sequential system at industrial scale, was considered. In this chapter, the influence of the addition of different fractions of an easily-degradable substrate in such a system is tested and compared (section 5.1). Afterwards the effect of the reactor flushing with different amount of leachate is studied and a strategy to manage VFAs in sequential process proposed (section 5.2).
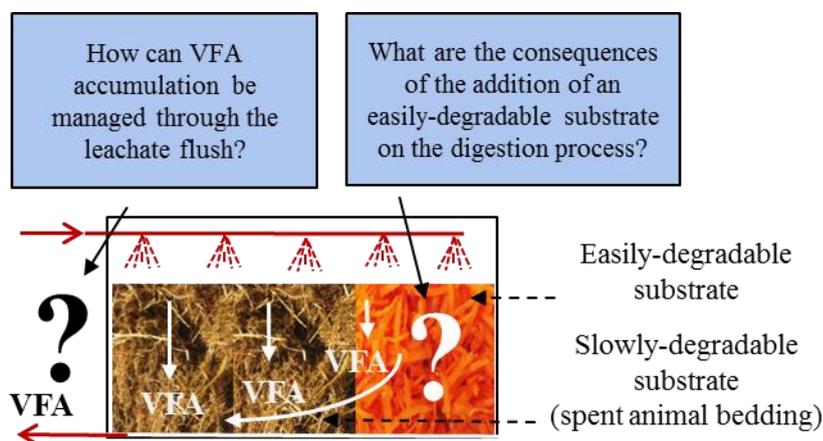
Figure 29 : Graphical abstract of the fourth issue faced in this PhD thesis: the extent of inhibition when adding a significant fraction of an easily-degradable substrate and the use of the leachate flush to manage volatile fatty acid accumulation

5.1 Co-digestion of spent cow bedding with an easily-degradable substrate

5.1.1 <u>Introduction</u>

Spent animal bedding was shown to be easily digested in a LBR (see chapter 2). However, at industrial scale, spent animal bedding is often mixed with other substrates. The substrate composition is often seasonal and easily-degradable waste or acidic waste such as ensiled maize, generating a high production of VFAs at the beginning of the batch operation, can be added. It is then important to manage properly this acidification phase and assess properly the amount of easily-degradable substrate that can be mixed without affecting too much the reactor performance.

Single-stage systems, where leachate is recirculated within the same reactor, were shown to suffer of longer methanogenesis phase leading even to complete failure if the initial inoculation is not adapted to the substrate. Massive inoculations are then made to treat easily-degradable substrate. For example, Kusch et al. (2012) digested maize silage at a S/I of 0.5 compared to spent horse bedding easily digested at a S/I of 10.

In addition, Dearman et al. (2006) showed that single-stage system suffered of VFAs accumulation and lower methane yield when compared to sequential system exchanging leachate and loaded with the same substrate mixture: 65% food waste, 25% digested biosolids and 10% seeds on a weight basis. Sequential systems are then more adapted to treat easily-degradable waste avoiding massive inoculation. In addition, this configuration represents the one often used on Naskeo sites.

The objective of this test was then to compare the influence of the addition of an increasing amount of easily-degradable substrate on the digestion process of a LBR operated as in a sequential system.

5.1.2 Materials and Methods

Three different co-digestion mixtures were tested. In each reactor 360 g of dry matter was loaded and the mixture fractions were calculated on a TS basis. In addition to a fixed amount of cereal residues (15% TS), different proportions of rapidly and slowly-degradable substrates (carrots and spent cow bedding, respectively) were added in the following proportions on a TS basis: 10% - 75% in condition L, 25% - 60% in condition M and 40% - 45% in condition H (Table 11).

Table 11: Composition of the co-digestion mixture used in each condition. For each substrate used, the amount of fresh matter added and the share that it represents in term of TS are provided

		Low (L)	Medium (M)	High (H)
Spent cow bedding	g	1011	808	607
	% (TS)	75%	60%	45%
Carrots	g	244	611	976
	% (TS)	10%	25%	40%
Cereal	g	244	244	245
	% (TS)	15%	15%	15%

These mixtures were inoculated with solid digestate using an inoculation ratio of 13% TS (digestate TS / total TS) as reported for previous experiments. Reactors were loaded and bulk phase brought close to water saturation through continuous recirculation of leachate for about 10-15 minutes. This latter was then extracted, the reactors were closed and the experiment started. The liquid phase used for extraction was the mixture of a leachate and water buffered with $NaHCO_3$ in order to keep total alkalinity at 11.4 g $CaCO_3$ L^{-1} (partial alkalinity at 9.4 g $CaCO_3$ L^{-1} and initial pH of 8.6). It was kept at room temperature and the reactors were flushed every day (excepted during the week-end) with 360 mL of the liquid phase: the leachate flush ratio was of 1 L $kg^{-1}TS$ d^{-1}. The leachate was sprinkled on the bulk, let percolating and then collected after three hours for analysis.

The substrate and leachate properties are reported in Table 12. Further details about the system used, its set-up, the analysis carried out and the protocol used are provided in section 5.2.

Table 12 : Characterization of the substrates loaded: total solids (TS), volatile solids (VS) of spent cow bedding, cereal residues, carrots and digestate

	Spent cow bedding	Carrots	Cereal residues	Digestate
TS	24.3% +/- 0.6%	12.4% +/- 0.1%	89.6% +/- 0.4%	15.9% +/- 0.5%
VS	20.6% +/- 0.8%	11.6% +/- 0.1%	71.9% +/- 0.5%	10.4% +/- 0.3%
VS/TS	84.7% +/- 3.8%	93.8% +/- 1.0%	80.2% +/- 0.7%	65.1% +/- 2.7%

5.1.3 Results and discussion

Figure 30 a-b shows the methane production rate and the methane content in the biogas. Methane production rate is modified by the increasing addition of easily-degradable fraction: the higher this fraction the longer the lag phase and the lower the methane peak reached.
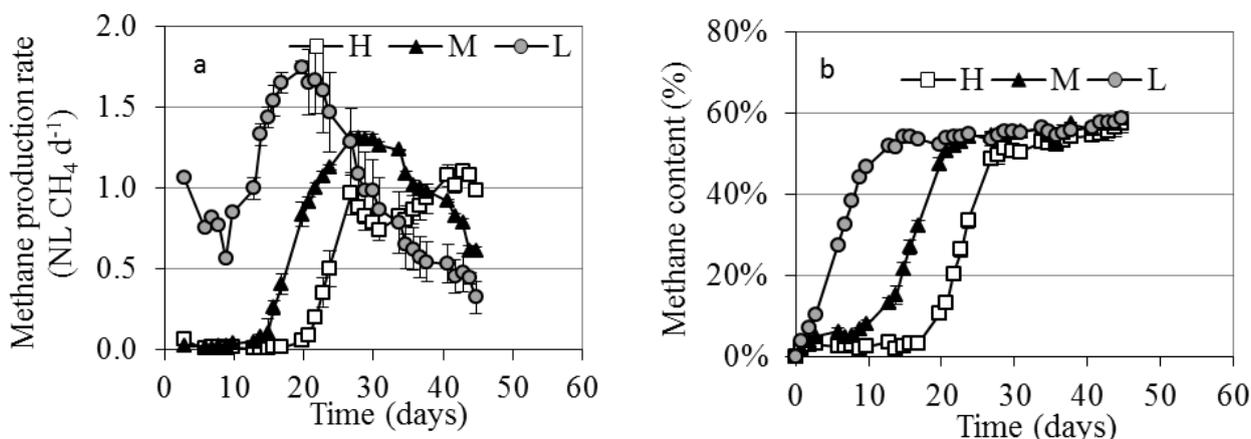
Figure 30 : Biogas parameters monitored during the digestion process considering three different fractions of easily-degradable substrate loaded in the LBRs: (H), medium (M) and low (L). (a) Methane production rates and (b) methane content in the biogas.

The methane content clearly shows a methanogenesis inhibition: no inhibition is observed with 10%TS of carrots, the lag phase lasts 9 days with 25%TS of carrots and 18 days with 40%TS of carrots. The methane content stabilized between 55% and 60% after 10 days from the end of the lag phase in all the conditions.

Figure 31 shows respectively the VFA concentrations and the pH in the leachate after percolation through the bulk and extraction from the reactors. The VFA peak were higher when a

bigger amount of easily-degradable fraction was loaded in the reactor: 14 g COD L^{-1} in condition L, 20 g COD L^{-1} in condition M and 24 g COD L^{-1} in condition H. In addition, significant VFA concentrations (> 1g COD L^{-1}) were extracted for a longer time when adding easily-degradable substrate: 21 days, 34 days and 44 days respectively. Since the same amount of leachate was used to flush the reactors all along the process, higher VFA concentrations mean that the total amount of VFAs extracted were higher when increasing the amount of carrots added: 60.9 ± 1.1 g COD in condition H, 41.8 ± 2.1 g COD in condition M and 19.7 ± 1.8 g COD in condition L.
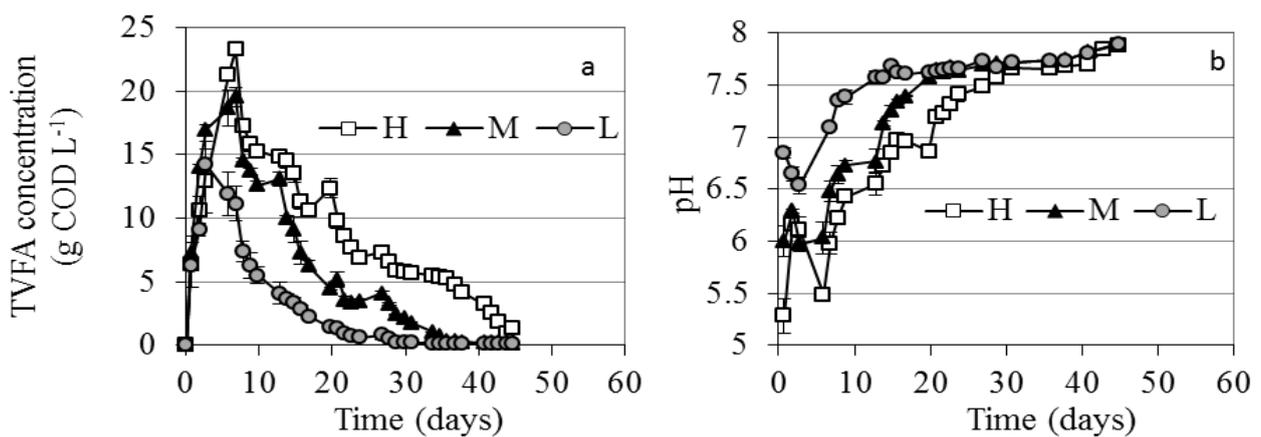
Figure 31 : Parameters monitored in the leachate during the digestion process considering three different fractions of easily-degradable substrate loaded in the LBRs: high (H), medium (M) and low (L). (a) Volatile fatty acids concentration and (b) pH of the leachate in LBRs with different fractions of easily-degradable substrate

The high concentrations in VFAs were reflected also by the pH level which reached very low values especially in conditions M and H, respectively 5.9 and 5.3, while the minimum was 6.5 in condition L. Low pH values were related to the low methane content and to the duration of the methanogenesis lag-phase.

5.1.4 Conclusions

The increase of the fraction of easily-degradable substrate in total mixture leads to increasing inhibition due to the accumulation of VFAs and low pH. 10% TS of easily-degradable fraction did

not cause any particular inhibition and methane was rapidly produced. From 25%TS of easily-degradable matter, the inhibition was significant and the time to recover longer, meaning that the addition of such an amount should be done with caution at industrial-scale. However, independently of the percentage added, the system recovered its methanogenic capacity thanks to the flushing. Flushing is then important in order to overcome reactor acidification.

5.2 Leachate flush strategies to manage volatile fatty acids accumulation in leach-bed reactors

5.2.1 Introduction

Anaerobic digestion (AD) is a very efficient biological process facilitating the treatment of organic solid waste and the production of biogas which can be used for energy purposes. In relation to total solids (TS), the reactor operating modes are divided in three main groups: wet (TS < 15%), semi-dry (10% < TS < 20%) and dry (TS > 20%) (Karthikeyan and Visvanathan, 2012a). Dry AD processes are the best-suited to an agricultural context where solid waste such as cereal residues and spent animal bedding have high TS content and fairly low biodegradability. Among dry processes, leach-bed reactors (LBRs) present economic and technological advantages thanks to their simple design and easy operation. A LBR usually consists of a batch digester in which the solid substrate is loaded with a wheel loader, and a liquid tank storing a leachate which is discontinuously sprinkled over the substrate top during the whole process. Therefore, leachate plays important roles by helping increasing the moisture content, improving mass transfer and diluting inhibitory compounds (Degueurce et al., 2016d). In comparison to other technologies, LBRs are recognized for their many advantages: high loadings of solid waste, reduced water consumption, unnecessary digestate post-treatment, reduced investment costs and greater biological stability compared to classic wet processes (Karthikeyan and Visvanathan, 2012a). However, this batch system entails certain disadvantages: i) discontinuous biogas production caused by frequent loading and emptying of the digester ; ii) incomplete degradation of the substrate, mainly due to its heterogeneity, leachate channeling and progressive bulk compaction (André et al., 2015); iii) accumulation of intermediate compounds formed during the first days of a batch operation due to the initial high loading of fresh substrate. In this sense, volatile fatty acids (VFAs), one of the main intermediates of the anaerobic process, represent a major problem for the proper management of LBRs. In fact, they may be produced in quite high concentrations which can cause inhibition and decrease methane production. In LBRs treating agricultural waste, VFA accumulation can represent an important problem,

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especially if easily-degradable substrates (e.g. fruits or vegetable waste), produced seasonally in large quantities, are mixed to slowly-degradable substrates such as spent animal bedding. The management of VFAs will be different in relation to the process configuration adopted. In a LBR with internal recirculation of the leachate, the VFAs accumulated must be consumed in the same reactor and, often, considerable amounts of digestate are used to increase the reactor inoculation with methanogens in order to prevent acidification (Kusch et al., 2012). VFAs produced in a LBR can also be extracted and eliminated separately by coupling the LBR to an external AD reactor wherein VFAs are consumed (Viétez and Ghosh, 1999), making this configuration particularly advantageous. In this last case, leachate management plays a very important role because it can enhance the transport and degradation of VFAs.

In the literature, several coupling strategies have been tested. The use of an external liquid AD reactor such as a UASB (up-flow anaerobic sludge-blanket) reactor (LBR-UASB coupling) has been proposed for the treatment of easily-degradable compounds (i.e. food waste, municipal solid waste and grass silage) (Browne et al., 2013; Han and Shin, 2004; Nizami et al., 2010; Shin and Han, 2000; Xu et al., 2011). In such a configuration, one or more LBRs were operated as acidogenic reactors with small batch durations, while the UASB was used as a methanogenic reactor, as in a two-stage process. However, the use of a specific methanogenic reactor is not the only option for managing VFAs when treating easily-degradable substrates. The coupling of two LBRs (LBR-LBR coupling) was proposed in a sequential process where the VFAs produced in a freshly-loaded LBR (new) were consumed in a more mature LBR (old) at the end of the digestion. First used in its simplest configuration by Hall and Hawkes (1985) to treat manure, a more complex configuration known as SEBAC (sequential batch anaerobic composting) was then developed in the USA by Chugh et al. (1999) and Chynoweth et al. (1991) to treat municipal solid waste (MSW). They suggested to use three LBRs, the new and the old one coupled until stabilization of the new reactor, and a third one already stabilized (with an incubation time between new and old) with internal leachate recirculation. With no particular modification in the coupling strategy (new-old) yet paying

more attention to the coupling period, further work was carried out in Australia (Dearman and Bentham, 2007; Nopharatana et al., 1998) and in Thailand (Tubtong et al., 2004) in the treatment of MSW, food waste, the organic fraction of MSW (OFMSW) and market waste.

Although the LBR-LBR coupling was proven to be less efficient than the LBR-UASB coupling in terms of VFA conversion, the use of a continuous external methanogenic reactor increases costs, demands more accurate process control and results in a more complex design (Poggi-Varaldo, 2005). The latter constitutes a real drawback for LBR systems whose simplicity is one of their main advantages, in particular in a farm-scale context. Moreover, the co-digestion of rapidly- and slowly-degradable substrates, which can occur seasonally, requires a long digestion time to recover the energy potential of the slowly-degradable organic compounds. This makes the LBR-LBR coupling a very beneficial option for treating this kind of mixture.

Very few data are available in the literature to help understanding and optimizing leachate and VFA management in a LBR-LBR system. Only Dearman and Bentham (2007) and Tubtong et al. (2004) analysed in details the effect of leachate recirculation rate in a LBR-LBR system. They both pointed out the importance of increasing the volume of the leachate exchanged between a new and a mature reactor, observing an increase in the methane production rate.

In the literature, the extraction and the consumption of VFAs (from and in LBRs respectively) have always been studied simultaneously as a consequence of the chosen experimental design which consists in the direct coupling of two (or more) LBRs. Additionally, this configuration does not permit the clear assessment of the impact of VFA accumulation on the process performance. However, since extraction and consumption of VFAs are crucial aspects for understanding and managing the leachate flush in a LBR, a novel approach is proposed in the present study by always injecting a new leachate with known properties (i.e. with or without VFAs). This strategy permitted to provide separate data on both VFA extraction and consumption in LBRs.

Consequently, the objectives of this paper are the following: to test the influence of the leachate flushing rate on the VFA extraction and the overall substrate degradation performance in a

new reactor, and to study the consumption of VFAs in mature reactors. Based on the above, the final goal is to propose practical guidelines on the leachate flush strategy in order to improve the VFA management for the treatment of a mixture of rapidly- and slowly-degradable substrates in a LBR-LBR system.

5.2.2   Materials and Methods

Experimental apparatus

Six LBRs were operated in parallel. Each reactor consisted in a 7 L jacketed glass cylindrical reactor of 14.5 cm diameter and 43 cm internal height. The LBRs were kept in mesophilic condition (37°C) by water recirculation from a thermo-regulated bath (Figure 32). A mesh with 1 mm holes was placed at 10 cm from the bottom of the reactors to hold the solid substrate. This allowed creating a 1.7 L volume at the bottom of the reactors for temporary leachate storage. Solid substrate occupied 3 L and the head space was of 2.3 L. A tube connecting the headspace and the volume under the mesh was added in order to facilitate percolation by equalizing the pressures in case of compaction of the bulk solid. A peristaltic pump was used for injection of fresh leachate on the top of the bulk and a sprinkler was added for a homogeneous distribution on the entire surface. The same pump was used to extract the leachate which accumulating at the bottom of the reactor. A Tedlar Biogas bag and a valve were added to the biogas circuit in order to regulate the pressure during the injection and the extraction process. A port in the biogas circuit allowed the collection of gas samples for analysis.
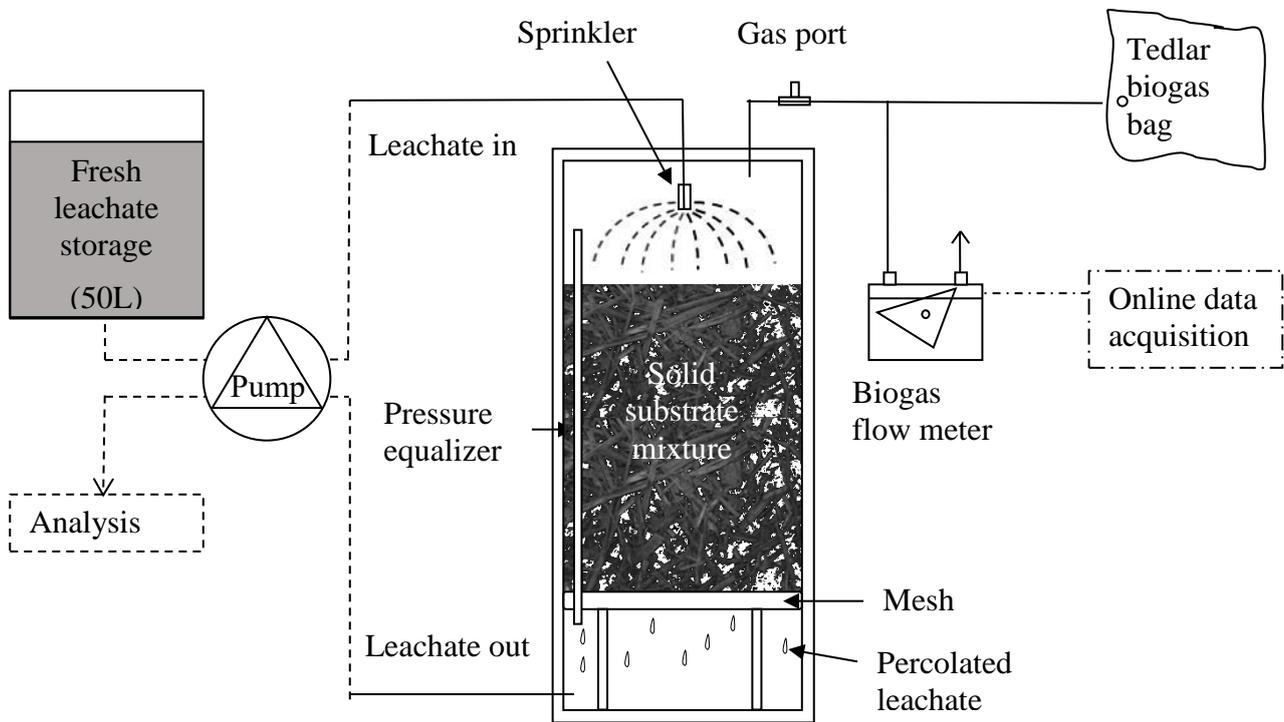
Figure 32 : Experimental design. Fresh leachate without volatile fatty acids (VFAs) is stored in a 50 L volume. A fixed amount of leachate is pumped into the leach-bed reactor (LBR) through a peristaltic pump. After percolation through the bed, the leachate enriched in VFAs is pumped out and analysed.

The substrate loaded in the reactors was a mixture of spent cow bedding and cereal waste (collected from a dairy farm in the South of France and kept at -20°C before use), both of which are commonly available in a rural context and made-up the slowly-degradable substrates. Additionally, carrots from a supermarket were added as a model for an easily-degradable co-substrate. The mixture contained, on a TS basis, 45% of raw spent cow bedding, 15% of raw cereal residues and 40% of carrots. These latter were freshly grated to roughly 3-cm long bits with a Moulinex ME415 vegetable mincer before being mixed in with the rest. A total mass of 360 g TS or 2,100 g RM (raw matter) was loaded into each reactor and compacted. The bulk density was about 630 kg m^{-3}. The spent cow bedding, thanks to its porous structure, permitted good leachate percolation (Demirer and Chen, 2008; Myint and Nirmalakhandan, 2009; Riggio et al., 2017a; Xu et al., 2011). In order to ensure digester inoculation, solid digestate from a previous batch was mixed to the solid bulk before loading and represented 13% of the mixture (digestate TS/total TS), as previously done by Riggio et

al., (2016). The leachate used for flushing the solid bulk was collected at an industrial agricultural site using sequential LBRs to treat mainly spent cow bedding and green waste. This was diluted 1:1.7 (vol/vol) and stored at 37°C throughout the whole experiment in order to maintain microorganism activity as high as in a real system. The properties of the substrates and leachate before loading are summarized in Table 13.

Table 13 : Characterization of the substrates loaded: (a) total solids (TS), volatile solids (VS), and BioMethane potential (BMP) of spent cow bedding, cereal residues, carrots and digestate; (b) total and soluble chemical oxygen demand (CODt and CODs), volatile fatty acid (VFA), total and partial alkalinity and pH of the leachate

a		Spent cow bedding	Cereal residues	Carrots	Digestate	Leachate
TS/RM	%	22.6 ± 0.6	89.8 ± 0.2	10.7 ± 0.3	19.2 ± 0.4	-
VS/RM	%	19.3 ± 0.3	70.9 ± 0.4	9.9 ± 0.2	10.3 ± 0.5	-
VS/TS	%	85.5	78.9	93.0	53.6	-
BMP	NmL CH$_4$ g^{-1} VS	184 ± 5	228 ± 16	385 ± 7	24 ± 12.3	-
b						
DCOt	g COD L^{-1}	-	-	-	-	15.8
DCOs	g COD L^{-1}	-	-	-	-	13.3
VFA (acet. acid)	g L^{-1}	-	-	-	-	0.2
Total Alkalinity	g CaCO$_3$ L^{-1}	-	-	-	-	15
Partial Alkalinity	g CaCO$_3$ L^{-1}	-	-	-	-	7.3
pH	-	-	-	-	-	8.0

Experimental procedure

Every day except for the week-ends the reactors were flushed once: a given volume of fresh leachate was injected into the reactor and drawn off 3 hours later (i.e. after percolation) from the bottom of the reactor in order to be analysed. The injection of three different volumes of fresh leachate was tested in duplicate (Figure 33): high (H1-H2) with 720 mL flush^{-1}, medium (M1-M2)

with 360 mL flush[-1] and low (L1-L2) with 180 mL flush[-1]. Only one flush per day was effectuated in each reactor. These volumes corresponded to a volume of leachate injected per day and per amount of total solids loaded of, respectively, 2.0, 1.0 and 0.5 L kg^{-1} TS d[-1], for one flush per day (or, to facilitate comparison with the literature: 0.34, 0.17 and 0.09 L kg^{-1} RM d[-1] or 0.22, 0.11 and 0.05 L L^{-1}$_{subst}$ d[-1]). These leachate flush-rates were chosen to be representative of those applicable at industrial scale.
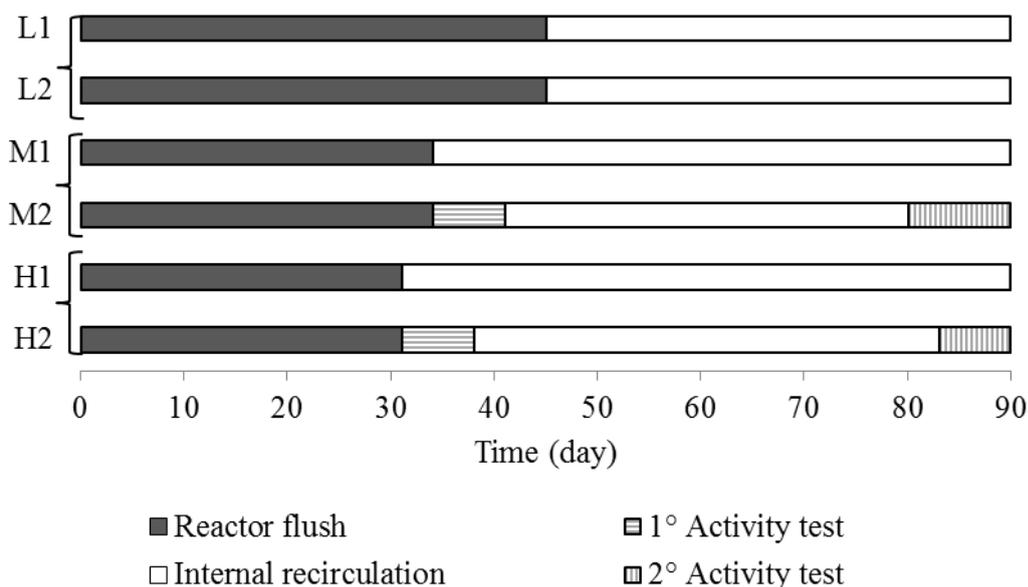
Figure 33 : Experimental planning and configuration. Three conditions are tested in duplicate: in L1–L2 a low leachate flush rate is applied (0.5 L kg^{-1} TS d[-1]), in M1–M2 a medium leachate flush rate is applied (1.0 L kg^{-1} TS d[-1]) and in H1– H2 a high leachate flush rate is applied (2.0 L kg^{-1} TS d[-1]). During the "reactor flush" period, a fresh leachate is pumped in and out every day while, during the "internal recirculation" period, the same leachate is recirculated through the bed. Additionally, M2 and H2 are used to carry out an activity test.

The reactors were flushed until no more VFAs were observed in the extracted leachates: 31 days in condition H, 34 days in condition M and 45 days in condition L. Starting from this moment and until day 90, an internal recirculation mode was adopted: the leachate was recycled within the same reactor and never renewed. Throughout the internal recirculation mode, the amount of leachate recirculated was the same as during the reactor flush.

Given the good repeatability of the pairs H and M, one of each pair (H2 and M2) was used to carry out two activity tests. These tests were done by adding acetic acid to the fresh leachate and then flushing the reactor with 360 mL of the prepared solution (as in condition M). Two different acetic acid concentrations were tested: 5 g L^{-1} in H2 and 10 g L^{-1} in M2. The first test was done at 31 days and 34 days for H2 and M2, respectively, whereas the second test at the end of the digestion process for both conditions.

Sampling and analysis

Characterization of the initial substrates was carried out in triplicate and the average values with standard deviations were reported. The TS content was measured after drying at 105 °C (378 K) for 24 h, followed by 3 h of calcination at 550 °C (823 K) for determination of VS. BioMethane Potential (BMP) tests were carried out on the raw samples in batches of 500 mL inoculated with anaerobic sludge from a sugar industry anaerobic treatment plant of, in accordance with the procedure described by Riggio et al. (2017a).

The industrial leachate used for flushing was characterized in terms of partial and total alkalinity, VFA concentration, pH, and total and soluble chemical oxygen demand (CODt and CODs, respectively) (Table 14).During the digestion process, after every reactor flush, pH, VFAs and CODs were measured. Specifically, pH, alkalinity and CODt were measured in the raw leachate, while VFAs and CODs were measured in a leachate previously filtered at 0.45 μm. The pH was measured with Mettler Toledo InPro 4260i probes connected to a Mettler Toledo pH M300 operational manual transmitter. Partial alkalinity was measured at a pH of 5.75 and total alkalinity at a pH of 4.30, as advised by Hill and Jenkins (1989). VFAs were analysed in a VARIAN I-MET-0084 gas chromatograph with helium as the gas vector. COD was measured using 0-1.500 mg L^{-1} Aqualytic tube tests after 2 h of oxidation at 160 °C in a HACH COD reactor with readings via Aqualytic Multi Direct spectrometer. During digestion, biogas volume was recorded manually once a day using the Ritter flow meter Milligascounter MGC-1 V3. Biogas flow was recorded every 5

min only during the activity tests, with the help of a home-made software connected to the Ritter flow meter. Biogas composition was analysed with a micro-GCPRO CP-4900 gas chromatograph using helium as the gas vector. Finally, biogas volume was expressed in standard conditions: 273.15 K and 101,325 Pa.

Table 14 : Characterization of the substrates loaded: total solids (TS), volatile solids (VS), and BioMethane potential (BMP) of spent cow bedding, cereal residues, carrots and digestate; total and soluble chemical oxygen demand (CODt and CODs), volatile fatty acid (VFA), total and partial alkalinity and pH of the leachate

		Spent cow bedding	Cereal residues	Carrots	Digestate	Leachate
TS	$\%_{RM}$	22.6 ± 0.6	89.8 ± 0.2	10.7 ± 0.3	19.2 ± 0.4	-
VS	$\%_{RM}$	19.3 ± 0.3	70.9 ± 0.4	9.9 ± 0.2	10.3 ± 0.5	-
VS/TS	%	85.5	78.9	93.0	53.6	-
BMP	NmL CH_4 g^{-1} VS	184 ± 5	228 ± 16	385 ± 7	24 ± 12.3	-
CODt	g COD L^{-1}	-	-	-	-	15.8
CODs	g COD L^{-1}	-	-	-	-	13.3
VFA (acetic acid)	g L^{-1}	-	-	-	-	0.1
Total Alkalinity	g $CaCO_3$ L^{-1}	-	-	-	-	15.0
Partial Alkalinity	g $CaCO_3$ L^{-1}	-	-	-	-	7.3
pH		-	-	-	-	

Data processing

Conditions H and M represent the average values of the pairs (H1-H2 and M1-M2, respectively) with their variation range (maximum and minimum values). Since H2 and M2 were used to carry out activity tests (on days 31 and 34, respectively), H and M only correspond to H1 and M1 after these days (without error bars). On the other hand, since L1 and L2 showed significant differences, both reactors are described for the whole operation.

During activity tests, the biogas associated with the degradation of acetic acid was determined by subtracting the volume of biogas produced by the reactor receiving acetic acid from the blank reactor run without it (i.e. H2 minus H1 and M2 minus M1). Three successive injections of acetic acid were made to evaluate the methanogenic activity. The average values of the three tests are presented with their standard deviation.

5.2.3   Results and discussion

5.2.3.1   Reactor performances

Operating periods

The following parameters were monitored along timeline: pH (Figure 34a), methane production rate (Figure 34b) and total VFA (TVFA) concentration in the extracted leachate (Figure 34c). As a representative example, the TVFA composition of condition H is also reported (Figure 34d). As an aid, it should be noted that the amount of VFAs extracted through a flush represents only a fraction of the total VFAs present in the bulk phase. As a consequence of that, TVFA concentration and pH in the solution are only indicative of the actual amounts in the bulk. Overall, the experiment can be divided into three main periods, which can be identified by analysing the biogas directly produced by the solid phase along with the characteristics of the leachate after percolation through the bulk.

Period I (0-15 days): Acidification

Period I was characterized by a strong acidification, with pH dropping below 6 (Figure 34a), an absence of methane production indicating a complete methanogenesis inhibition due to low pH (Figure 34b), and high TVFA concentrations in the leachate (Figure 34c). During the first days of the process, the CODs of the extracted leachate was slightly higher than the COD of VFAs, meaning that other metabolites (e.g. alcohol or more complex acids) were also produced in acidic

conditions, as suggested also numerous authors (Cadavid-Rodriguez and Horan, 2014; Cysneiros et al., 2012; Dogan et al., 2008; Selvam et al., 2010). These metabolites represented a maximum (condition H) of 4% of the total COD degraded. CODs was exclusively composed by VFAs after the first 3 days. As a consequence, for a clearer presentation of the results, only VFAs will be considered in this work. During the first 3 days, a significant production of hydrogen (average of all reactors: 27.6 ± 1.1 NmL H_2 g^{-1} VS of fresh matter), was measured. This is typical during an acidification phase as reported also by Chugh et al. (1999) and Han and Shin (2004) during the initial days of digestion in LBR treating unsorted municipal solid waste and food waste, respectively.

The highest TVFA concentration (24 g COD L^{-1}) was reached with the lowest leachate flush-rate applied (digesters L1 and L2) and corresponded to roughly twice the concentration observed for the highest leachate flush-rate (digester H). This indicates that the dilution factor played an important role in TVFA concentrations in the extracted leachate and, as a consequence, on its pH. Condition H is used as an example to illustrate the composition of the extracted VFAs (Figure 34d) during this period, since their relative composition was similar for the three conditions. The main VFAs produced were butyric, acetic and, to a lesser extent, propionic acids in period I, while all the other VFAs (iso-butyric, valeric and iso-valeric) were recorded at very low concentrations. Bearing in mind that no VFA conversion into methane took place, Figure 34d shows that butyric acid was produced in great amount only during the earliest days; acetic acid in less quantity yet for a longer time (as suggested by a less sharp peak); whereas propionic acid was produced almost constantly throughout. The predominance of butyric and acetic acids in an acidification phase has already been reported in acidogenic LBRs (Cadavid-Rodriguez and Horan, 2014; Cysneiros et al., 2012). The high percentage of butyric acid extracted and the production of H_2 in the first days are a clear indication of a dark fermentation pathway, which was then interrupted because of an increase in the pH due to the daily flush. At the end of period I, the pH was higher than 6.8 for all the conditions,

TVFA concentrations dropped significantly to values lower than 12 g COD L^{-1} and methane production was lower than 0.2 NL CH$_4$ d^{-1}.

Period II (15-35 days): Methanogenesis establishment

This period was characterized by the establishment of the methanogenic activity until the maximum methane production rates were reached (Figure 34b). In the first days of this period, pH in the leachate switched towards values higher than 7 and proved that methanogenesis inhibition in phase I was effectively linked to a low pH. In fact, pH higher than 7 are known to be favourable for anaerobic digestion (Jha et al., 2011). However, it is reminded that the pH measured in the extracted leachate only partially represents the effective pH in the reactor bulk, which might be little lower inside the bulk. In period II, TVFA concentrations were lower than in period I and decreased all along. TVFA decrease was related not only to their extraction but also to their conversion into methane (Figure 34b). A slightly higher concentration of propionic acid was observed in this period compared to period I. In fact, propionic acid is known to be more difficult to degrade than the other VFAs and its complete degradation occurred only after depletion of both butyric and acetic acid (Öztürk, 1991).

Figure 34b shows that the methane production kinetics were similar for all the conditions. Similarly, the methanogenesis lag-phase were also comparable across all the conditions but L1 which means that leachate flush rate had a very little influence on this parameter: the methanogenesis lag-phases were about 15 days on average with only a difference of 2 to 3 days between conditions H and L2 (L1 will be discussed later). Literature reports a stronger influence of the leachate flush rate on the methanogenesis lag-phase: Tubtong and Towprayoon (2010) observed a difference of 10 days between two LBRs treating market waste and flushed once every two days with 0.8 and 2.3 L kg^{-1} TS flush^{-1} (15 and 25 days respectively), while Dearman and Bentham (2007) observed a difference of 20 days between two LBRs treating organic fraction of municipal solid waste and flushed once per week with 0.8 and 1.6 L kg^{-1} TS flush^{-1} (30 and 50 days

respectively). Although the real reasons of these differences are difficult to define as many factors could play a role, the minor flush frequency (0.5 flush day^{-1} or 1 flush week^{-1} compared to 1 flush day^{-1} in this study) causing a delayed mobilization of the VFAs and accumulation in the bulk phase could be the cause.

Even if high leachate flush rates showed little effect on methanogenesis lag-phase some differences were observed when decreasing it to value close 0.5 L kg^{-1} TS d^{-1}, as for reactors L1 and L2. In fact, a reduced volume of leachate injected could induce differences in the VFA extraction capacity because of the heterogeneity of the matrix and the channeling. Altogether, this suggests that reducing too much leachate flush rate degrades the extraction capacity and leads to variable results.
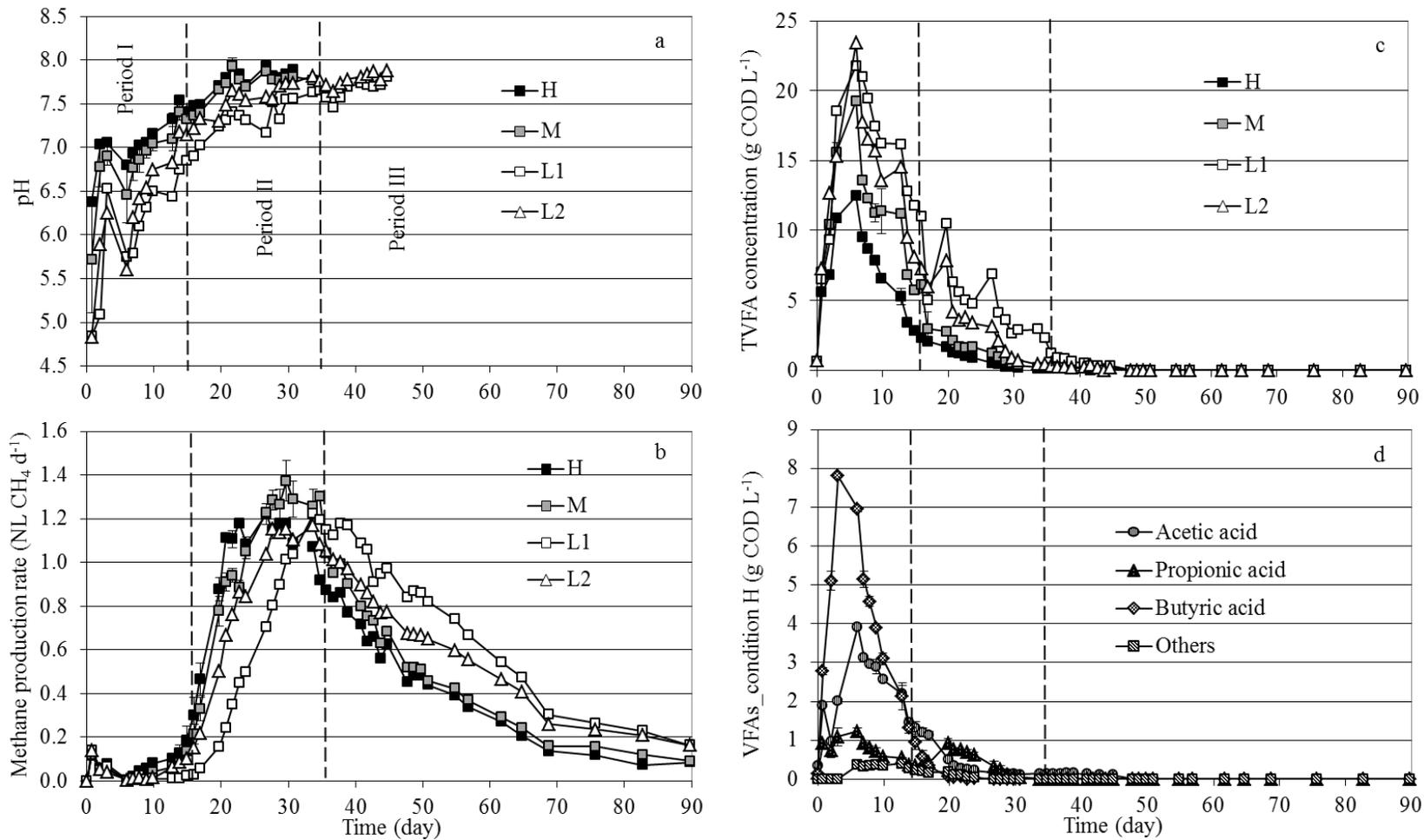
Figure 34 : Parameters measured during the digestion process: (a) pH; (b) methane production rate; (c) total volatile fatty acids (TVFA) concentration; (d) volatile fatty acids (VFAs) concentration (condition H). H and M refers to the mean value of H1- H2 and M1 - M2, respectively, during period I and period II while, they correspond to only H1 and M1, respectively, during period II

Period III (15-35 days): Degradation completion

This period is characterized by a constant decrease in the methane flow rate until the batches were stopped. No more VFAs were extracted from the bulk through leachate recirculation and the pH stabilized at about 7.7. Higher methane production rates were observed during this period when decreasing the leachate flush rates. During the last 20 days, the methane flow rates were low indicating the end of the degradation. The experiment was then stopped at day 90.

Activity tests were performed on H2 and M2 by injecting acetic acid (through the addition of fresh leachate) at two periods, after the peak of the methane production rate (34 and 31 days for M2 and H2, respectively) and right before stopping the batch (90 days) (Figure 34). Three injections were realized in each condition. A mass balance between the VFAs injected with the fresh leachate and the VFAs extracted allowed determining that 36% ± 3% of VFAs were hold in the bulk after each injection (Table 15).

Table 15 : Activity test. Reactor H2 corresponds to a duplicate operated with a high flush-rate and M2 to a duplicate operated at a medium flush-rate.

	Reactor used	Acetic acid concentration g L^{-1}	Acetic acid mass hold % (w/w)	Peak duration hour
1° test	H2	5	37% ± 1%	12
	M2	10	39% ± 1%	22
2° test	H2	5	35% ± 0%	22
	M2	10	32% ± 1%	35-50
Total			36% ± 3%	

Figure 35 presents the biogas flow rate due to acetic acid consumption (average of the 3 injections). The results show that the biogas production rate was about four-fold higher in the first period than at the end of the batch treatment, indicating that methanogenic activity was higher when the injections were carried out right after the biogas peak rather than at the end of a batch. Indeed,

by sprinkling a leachate with an acetic acid concentration of 10 g L^{-1}, the amount of acetic acid held in the bulk was consumed faster, namely one day for the 1st injection and two days for the 2nd injection. The reason of a reduced methanogenic activity over time is linked to the reduction of available substrates for methanogens during the digestion process.
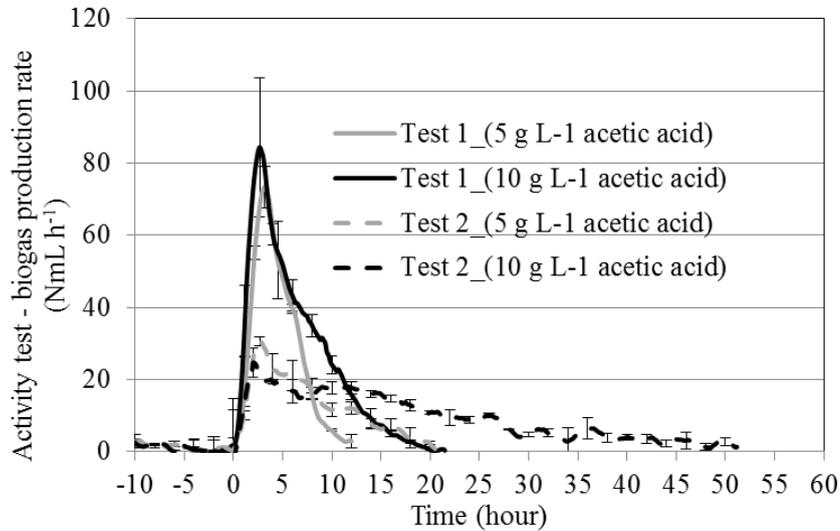
Figure 35 : Activity test: biogas production rate after injection of leachate containing acetic acid. 1st injection is effectuated after the methane peak (after about 30 days) and 2nd injection at the end of digestion (after about 80 days). Two acetic acid concentrations in the leachate were tested: 5 g L^{-1} and 10 g L^{-1}.

Subtrate degradation

Figure 36a presents the daily production of H$_2$ and CH$_4$ collected in the biogas and VFAs collected in the extracted leachate. Reactors L1 and L2 have been used as representative examples and H$_2$, CH$_4$ and VFAs converted into COD for clearer comparison. During period I, the most abundant metabolites collected were VFAs (along with H$_2$ only in the earliest days); during period II, CH$_4$ collection corresponded to a decreasing VFAs extraction; whereas during period III, only CH$_4$ was collected. During periods I and II, the extracted VFAs represent only part of the total VFAs produced through acidification of the substrate, since it is not possible to assess the real quantity of VFAs accumulated within the solid bulk phase. However, since no VFAs were extracted, there were no VFAs retained in the bulk during period III, and the accumulated amounts

of H_2, VFAs and CH_4 presented in Figure 36b corresponded to the quantity of substrate degraded (while, before this period, they corresponded only to the COD accessed and available to the operator, i.e. inferior to the real substrate degradation).
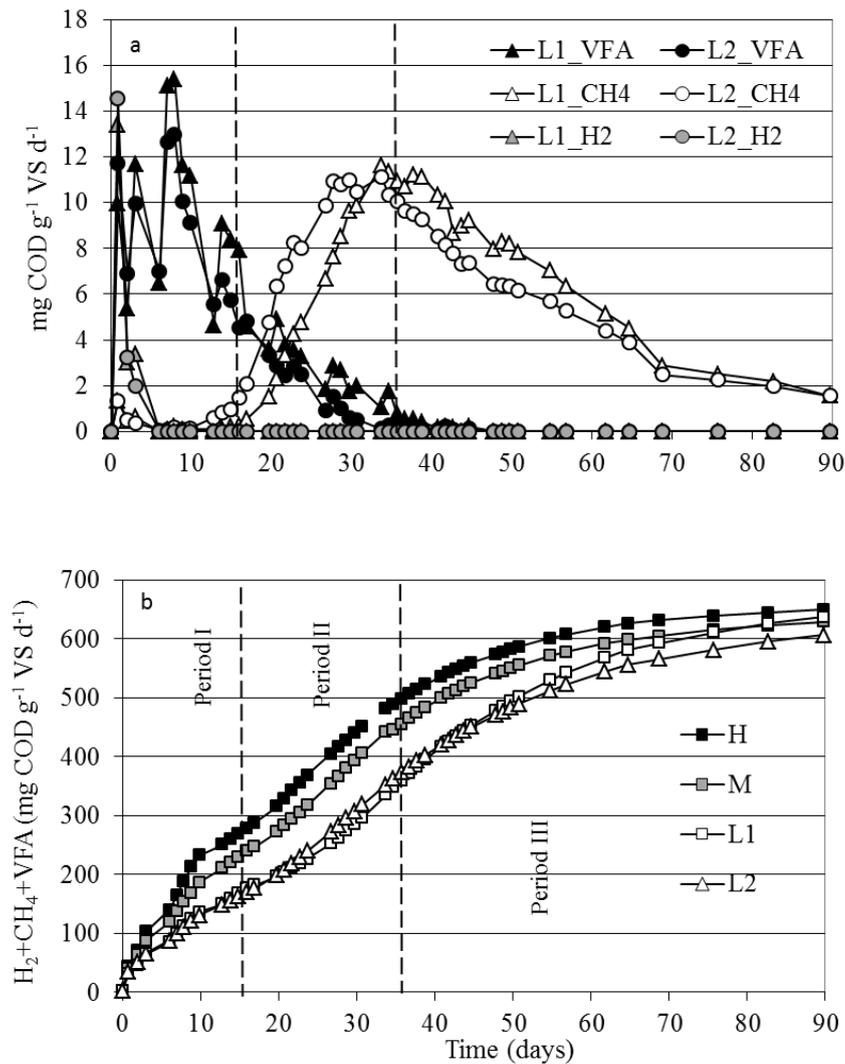
Figure 36 : Dynamic presentation of all the metabolites extracted or produced during the digestion process: (a) extraction rate of volatile fatty acids (VFAs), and production rate of methane (CH_4) and hydrogen (H_2) in conditions L1 and L2; (b) accumulated amounts of VFAs, CH_4 and H_2 over time. H and M refers to the mean value of H1- H2 and M1 - M2, respectively, during period I and period II while, they correspond to only H1 and M1, respectively, during period III

The comparison of the collected COD in Figure 36b and the BMP corresponding to the substrate mixture calculated based on the BMPs of the single substrate and their proportion in the mixture (255 NmL CH_4 g^{-1} VS, i.e. 728 mg COD g^{-1} VS, given a conversion factor of 350 NmL

CH$_4$ g^{-1} COD) permitted the determination of the percentage of the BMP reached along with time. At 35 days, 34%, 29%, 21% and 20% of the BMP were reached in conditions H, M, L1 and L2, respectively (Table 16). A significant difference was still measurable at 60 days: 81%, 77%, 72% and 69% in conditions H, M, L1 and L2, respectively. Since mesophilic processes with slowly-degradable compounds are usually not operated for more than 60 days at industrial scale, condition H proves to be a better condition to apply in order to increase substrate degradation within this limited time frame. In a coupled system (LBR-LBR coupling), the increase of the leachate flush-rate would mean a better overall methane production rate, as effectively observed by Tubtong et al. (2004) and Dearman and Bentham (2007). After 90 days of operation, the degradation curves converged towards similar values due to the similar loading, thus further confirming the accuracy of the measurements. By this time, between 81% and 87% of the BMP was reached.

despite the differences observed in the methane produced and the VFAs extracted (Figure 36a). It means that the VFA extraction and the delayed methane production were equivalent (in COD terms) and that VFA extraction was essential to make the COD entrapped in the bulk available during period II.

Table 16 : COD$_{H2+CH4+VFA}$ -to-BMP ratio: evolution over time of the ratio of the metabolites collected as hydrogen (H$_2$), methane (CH$_4$) and volatile fatty acids (VFAs) to the BioMethane Potential (BMP) of the loaded substrates (expressed in COD).

COD$_{H2+CH4+VFA}$ -to-BMP ratio				
Day	H	M	L1	L2
15	34%	29%	21%	20%
35	65%	59%	45%	48%
45	74%	70%	60%	60%
60	81%	77%	72%	69%
90	87%	84%	85%	81%

It is worth noticing that the overall degradation kinetics in L1 and L2 were very similar

Extraction of VFAs from LBRs

Table 17 reports the amount of total COD extracted as VFAs, CH_4 and H_2 during the 90 days of the experiments. Hydrogen production constituted only 3% of the overall COD, a small fraction of total substrate degradation. CH_4 represented the highest fraction of the total COD, with values ranging between 56% and 70%. High amount of VFAs (up to 41% in condition H) were extracted: the higher the leachate flush-rate, the higher the amount of VFAs extracted and the lower the amount of CH_4 produced in the digester.

However, the percentage of VFAs extracted and the leachate flush-rate were not directly proportional (e.g. by doubling the amount of leachate injected, the mass of VFAs extracted increased by only 23% when passing from condition M to H). This suggests that an optimum should be found between the amount of leachate used to flush, the amount of VFAs extracted and the VFA concentration in the extracted leachate. These parameters should then be assessed when conceiving a process using LBRs, but they will not be considered in this context.

Table 17 : Overall amount of volatile fatty acids (VFAs), methane (CH_4) and hydrogen (H_2) collected after 90 days, expressed in gram of chemical oxygen demand (COD) per gram of volatile solids (VS) initially loaded in the reactor. The percentage values are calculated based on the final amount of COD collected in each reactor

	H1[1]		M1[1]		L1		L2	
	g COD g^{-1} VS	%	g COD g^{-1} VS	%	g COD g^{-1} VS	%	g COD g^{-1} VS	%
H_2	18.0	3%	18.0	3%	17.3	3%	17.1	3%
VFA	258.2	41%	209.7	34%	191.8	31%	160.7	27%
CH_4	355.4	56%	383.6	63%	410.6	66%	411.6	70%
Total	631.7	100%	611.3	100%	619.7	100%	589.4	100%

[1] because of the activity test, only the values of H1 and M1 are provided

Figure 37 a-b show the daily and the accumulated mass of VFAs that were extracted from the bulk thanks to the flush. These figures show that most of the VFAs were extracted during period I, only a small fraction in period II, while almost no VFAs were extracted in period III. VFAs are known to inhibit hydrolysis at very high concentrations, while their extraction permits to reduce it (Cadavid-Rodriguez and Horan, 2014). Such reduced VFA inhibition in the first stage of the process is therefore essential to decrease the overall batch duration and achieve higher performance. Hence, a high leachate flush-rate is important during period I.
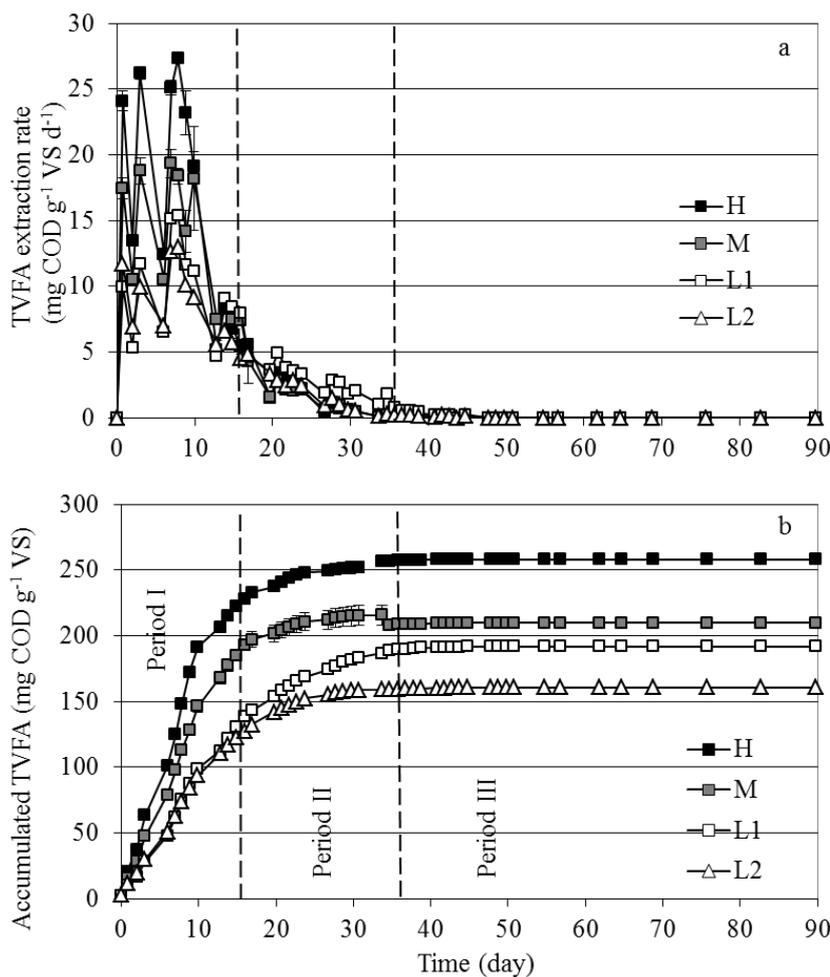
Figure 37 : Dynamic presentation of total volatile fatty acids (TVFA) extracted: (a) extraction rate of TVFA; (b) accumulated TVFA extracted over time. H and M refers to the mean value of H1- H2 and M1- M2, respectively, during period I and period II while, they correspond to only H1 and M1, respectively, during period III

Furthermore, a high leachate flush-rate in period I will reduce the methanogenesis lag-phase problem observed in L1 and L2. Considering that the problems of homogenous percolation and channeling are bigger at industrial scale than at lab scale, the reduction of the leachate volume injected is not recommended during this phase.

The very small amount of VFAs extracted in period II and the very similar methane production kinetics obtained (Figure 34b) in all three conditions indicate that the leachate flush-rate does not have a great impact in this period and that, consequently, the lowest and least expensive leachate flush-rate could be chosen. It would also be of interest to lower the amount of VFAs extracted as well as the problem connected to their consumption. To this end, several authors have even advised to switch to an internal recirculation mode (zero extraction) in this period, once specific conditions confirming a stabilized methanogenesis have been reached, such as: a pH above 6.5 (Chugh et al., 1999), a methane production rate higher than 0.5 L $CH_4 kg^{-1}$ VS d^{-1} (Dearman and Bentham, 2007) and a methane content in biogas exceeding 30% (Tubtong and Towprayoon, 2010).

Consumption of external VFAs in LBRs

After extraction, VFAs have to be converted into methane and the treatment of the large amounts of VFAs extracted (e.g. 41% of the degraded COD in condition H) has to be properly managed. Nonetheless, the availability of readily-degradable substrates like VFAs can be considered an advantage, since their conversion and subsequent methane production can be easily controlled. This would permit a smoothing-out of the methane flow-rate sent to the cogeneration engine (an important issue at plant scale (Degueurce et al., 2016d)) but also, in a more global context, adapting the biogas production to a demand-driven biogas supply (Hahn et al., 2014).

Figure 37a permits estimating the maximum quantity of COD to be sent to a methanogenic reactor (if no storage volumes are considered) for conversion into methane. The maximum values are reached during period I when the most VFAs were extracted: 27 mg COD g^{-1}VS d^{-1} in condition H, 19 mg COD g^{-1}VS d^{-1} in condition M and 15 mg COD g^{-1}VS d^{-1} in condition L. These organic

compounds could be easily treated via a very common process, such as an UASB (Browne et al., 2013; Han and Shin, 2004; Nizami et al., 2010; Shin and Han, 2000; Xu et al., 2011). However, a LBR-LBR coupling (VFAs are consumed in a LBR) will be considered since this would allow to keep the process simple, a real advantage for a process intended for rural context. In this case study, lower TVFA concentrations than those found in period I were chosen for activity tests (Figure 35 and Table 15) as if leachate storage tanks had been added to the system, thus leading to a dilution of the organic load sent to the LBR. Indeed, as shown in Figure 35, the methanogenic activity of H2 and M2 was almost two-fold higher right after the biogas peak as compared to the value reached at the end of digestion. It is interesting to note that during injection 1 the maximum biogas production rate doubled, rising from 80 NmL h^{-1} to 160 NmL h^{-1} (the small volume produced in comparison to the head space did not reveal any difference in the methane content), whereas during injection 2 the maximum biogas production rate increased from 10 NmL h^{-1} to 35 NmL h^{-1}, much lower than the previous rate (160 NmL h^{-1}). Therefore, for more efficient VFA degradation in LBRs, VFAs should be injected a few days after the biogas production peak, when no more VFAs are extracted and when hydrolysis becomes the limiting step in the LBR (beginning of period III). Clear details about the methanogenic activity of the mature LBR used in coupled systems are not given in the literature since the attention was mainly focused on the stabilization of the new reactor (Chynoweth et al., 1991; Dearman and Bentham, 2007; Tubtong and Towprayoon, 2010). However, reactor maturity and methanogenic activity must surely have impacted their results via the VFA consumption rate.

Figure 35 also shows that by injecting a leachate with acetic acid at a concentration of 5 g L^{-1}, the maximum biogas rate was already reached. In fact, by using a leachate with an acetic acid concentration of 10 g L^{-1}, similar biogas rate were obtained up to 6-7 h after injection and only the total time to consume the entire amount of acetic acid varied between the two conditions (5 and 10 g L^{-1}) (Table 15). Although injecting a leachate with a higher VFA concentration clearly increased the amount of VFAs held in the bulk phase, a concentration of 5 g L^{-1} was found to be enough to maintain a high biogas production rate and not to drop below the half the height of the biogas peaks

(at 6-7 hours). Thus, an injection of a leachate with an acetic acid concentration varying between 5 and 10 g L^{-1} at a frequency of every 6-7 hours seems to be the optimal strategy to allow the maximum consumption of VFAs and maintain a high biogas production rate. Finally, along with this frequency, small volumes per injection seem reasonable. In this study, sprinkling 360 mL of leachate over the bulk substrate (18.5 cm high and 14.5 cm in diameter) resulted in a retention of only 36% ± 3% of the mass of the acetic acid injected into the reactor (Table 15). Even if this percentage was dependent on the geometry of the reactor, meaning that this result cannot be generalized, higher volumes of leachate would probably have increased only the channeling with no significant impact on the mass retained in the bulk. This view is further corroborated by the increasing compaction of the bulk over time and the consequent reduction of percolation efficiency (André et al., 2015).

Overall strategy for VFA management in LBRs

A LBR treating a mixture of easily and slowly-degradable substrates passes through three periods. Table 18 summarizes the recommendations to properly manage VFAs based on the results obtained in each of them. During initial acidification (period I), a large amount of VFAs are produced which must be extracted rapidly. Hence, a high leachate flush-rate, ≥ 2 L kg^{-1} TS d^{-1}, has to be applied in order to reduce the risk of inhibiting hydrolysis while increasing the overall substrate degradation rate. During methanogenesis establishment (period II), less amounts of VFAs are present in the substrate bulk and their extraction can be reduced (≤ 1 L kg^{-1} TS d^{-1}) or even stopped (internal recirculation), making sure to avoid negative impact on the methanogens growth activity. Once the biogas peak is reached, a reactor could be left to complete its degradation. As such, the leachate flush would no longer play any role in VFA transfer and could be stopped. However, a LBR could also be used to consume external VFAs (coming from other reactors). In this case, leachate injection should be regulated in order to keep a high biogas production rate in order to convert the majority of external VFAs. To exploit the highest methanogenic activity,

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external VFAs should be injected right after the methane peak, when a clear lack of easily-degradable substrate is observed. Moreover, by using a leachate containing only acetic acid, an injection every 6-7 hours with a concentration between 5 and 10 g L^{-1} was found to be the best strategy. Small volumes are also advised (here 360 mL flush^{-1} was tested).However, since different combinations of volume per flush and frequency can give similar results (Degueurce et al., 2016d), the most suitable combination must be found in relation to the technical possibilities linked to the digester and substrate properties (e.g. digester geometry and percolation capacity). Similarly, more complex mixtures of organic acids than straightforward acetic acid should be tested.

Table 18 : Recommendations about volatile fatty acids (VFAs) management in a sequential leach-bed reactor (LBR) process

	Period I	Period II	Period III
Description	Acidification	Methanogenesis establishment	Degradation completion
Days *	0-15	15-35	>35
VFA	Extraction	Extraction / Internal consumption	Possible addition (from other LBRs)
Aim	Reduce the hydrolysis inhibition and the methanogenesis lag phase	Find a balance between extraction and internal consumption	Maintain a high methane production rate
Issue	Extract the maximum of VFAs / be able to consuming them all (in other reactors)	Extract the minimum of VFA (let them be consumed in the reactor) /do not delay establishment of methanogenic population	Maximize the VFA concentration in the bulk (max of VFA sent / min VFA out the bulk) / avoid VFA inhibition
Leachate management advise	High rate : ≥ 2 L kg^{-1} TS d^{-1}	Medium rate: ≤ 1 L kg^{-1} TS d^{-1} Attention to the reduced homogeneity in percolation [2] (Switching to a self-recirculation mode when methanogenesis is established)	Acetic acid concentrations between 5 and 10 g L^{-1}, injection every 6-7 h and small volumes per recirculation[3] (Last days stop leachate addition to complete VFA consumption in the bulk)

*these ranges are average for the three conditions tested

[2] Tested by other authors

[3] Volume conditions were not tested. However, they seem the best solutions

Effect of frequency

In the previous experiment the frequency of flushing was kept constant (i.e. once per day). However, its influence could play an important role and its impact on the VFA extraction capacity was studied more in detail in a separate context.

1,200 g of spent cow bedding (28.1% TS and 22.9% VS) and 400 g ensiled maize (12.3% TS and 11.7% VS) were loaded in two different reactors. 1500 mL of water were added in each reactor and digestion started as in a single-stage process (see section 2.1.2 for system design). After 5 days, a high concentration of VFAs was measured in the leachate, the reactors were stopped and initial liquid extracted. Afterward, the reactors were flushed following different strategies: the first was flushed 12 times by injecting 200 mL (corresponding to 0.5 L kg^{-1} TS flush^{-1}) and the second 10 times with 800 mL (corresponding to 2 L kg^{-1} TS flush^{-1}). After that phase, 1 L of water was injected in each reactor and let recirculate for over 1 hour continuously in order to homogenise the remaining VFAs and to identify its amount. The cumulated amounts of VFAs extracted, respectively in the reactor flushed with 200 ml and in the one flushed with 800 mL, were 11.1 g and 12.8 g. A difference of only 13% allowed us to compare the two extraction strategies.

Figure 38 shows the cumulated mass of VFAs extracted over the cumulated amount of water used for extraction. The results highlight that using a bigger volume of water for extraction allowed extracting more VFAs. However, for a given amount of water used, bigger amount of VFAs were extracted flushing with a smaller volume. This means that an optimum should be found between VFAs extracted and leachate pumped (in an industrial system), and that the frequency is a very important parameter affecting the extraction efficiency (since a same amount of VFAs can be extracted using a smaller amount of water but split in several flushes). This test allowed also to quantify the amount of VFAs extracted compared to the

total amount of VFAs accumulated: by using a leachate flush rate of 0.5 L kg^{-1} TS flush^{-1} about 9% of the total VFAs in the bulk were extracted with one flush while 14% were extracted with 2.0 L kg^{-1} TS flush^{-1}. That means that, after a flush, most of the VFAs are still in the bulk and only a very small fraction is washed out.
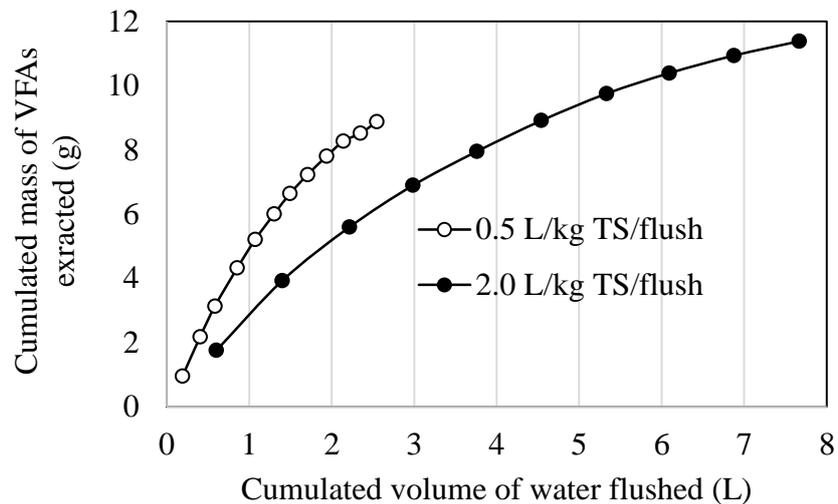
Figure 38 : Cumulated mass of VFAs extracted from a LBR after several flushes

5.2.4 Conclusion

A strategy of leachate management is proposed to optimize substrate degradation and methane production when co-digesting a mixture containing a high fraction of rapidly-degradable substrates in LBRs. In freshly-loaded reactors, leachate injection should be regulated to wash out most of the VFAs produced in order to reduce inhibition of the hydrolysis and digestion time. In mature reactors, leachate injection should focus on keeping a high methane production rate. Frequent injections straight after the methane production peak are advised to better consume external VFAs. Further studies should focus on a better understanding of, and improvements to, VFA consumption in mature LBRs.

CHAPTER 6

Discussion and perspectives

In this last chapter the major findings of the previous chapters are reported and discussed. This will allow having an overall view of the entire study, to draw the main conclusions and to open new perspectives.

6.1 Context

Anaerobic digestion is known worldwide to be an efficient bioprocess allowing the simultaneous stabilization of organic waste and energy recovery. Because of its advantages, AD is a no way around biotechnology and its role in the future energetic mix is undiscussed. As many other European countries, France is investing on its development to reach the objective fixed by the "Loi sur la transition énergétique" asking for 32% of the total energy production coming from renewable sources by 2030. Decentralized energy production is a very important development axis because it allows the spreading of energy sources on all the territory, which will directly reduce the loss connected to the energy transport and favour the local conversion of organic resources. The development of farm-scale anaerobic digestion plants is a very promising sector in France whose 90% of the available bioresources are estimated to originate from agricultural sector by 2030. 41% of those are animal dejections and 75% are estimated being under a dry state. Spent animal bedding, a mixture of animal faeces, urines and bedding material (most of the case straw in France) is then a very interesting waste to be considered for France decentralized energy production network. Moreover, the uncontrolled management of this waste can lead to environmental issues like soil and water pollution as well as odour nuisances and uncontrolled emission of methane.

Anaerobic digestion is able to efficiently solve these issues and leach-bed reactors, a dry discontinuous AD process, is adapted to this context and kind of organic substrates. Several small French companies recently decided to invest on its development to treat organic farm waste. However, the treatment of spent animal bedding in LBRs is not well known since this

technology was initially designed for the treatment of easily-degradable waste. As a consequence, many problems connected to the use of LBRs for the treatment of slowly-degradable waste have not been faced, and solved, yet. In addition, even if some industrial plants treating spent animal bedding exist, a lot of operational parameters should be still assessed to optimize this process and make it more profitable and competitive with respect to other technologies. In this context, research is required to answer industrial questions and help the implementation of farm-scale AD plants based on LBRs.

6.2   Digestion of spent livestock bedding in a single-stage LBR

In chapters 2, 3 and 4, the digestion of spent animal bedding in single-stage LBR was studied and some optimization levers were proposed to improve the methane production. These objectives were reached through: (i) the characterization of spent animal bedding and the identification of possible variability of its properties; (ii) the study of the LBRs advantages and limits as a process, as well as in relation to spent animal bedding digestion; (iii) the study of specific strategies to optimize methane production.

6.2.1   The substrate properties

The characterization of six different types of spent animal bedding (one from sheep, one from goat, one from horse and three from cows) allowed to sketch common properties of this kind of waste (Figure 39). Two very poorly studied types of spent animal bedding and two more common ones were chosen: the ones from sheep and goat belonged to the first group, and the ones form horse and in particular from cows to the second group. An overall analysis show that spent animal bedding has a high total solids content which enables them to be easy to handle, a VS/TS ratio close to 85% on average, which indicates a high organic matter content, and a balanced C/N ratio ranging between 20-28 (except for the spent horse bedding

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which displays a C/N ratio of 42), which means that spent bedding is a substrate whose digestion does not lack of nitrogen. Fibre analysis reveals that spent animal bedding is a lignocellulosic material, with presence of significant amount of cellulose, hemicellulose and lignin, but also a significant fraction of soluble compounds (between 20% and 37%). The co-existence of an easily- degradable fraction and a slowly-degradable one has a great impact on the dynamic of spent bedding degradation: a long digestion time and an initial high methane peak (further discussed later). Finally, the biomethane potential (BMP) measured for the substrates studied are very close, in a range between 192 and 239 NmL CH_4 g^{-1} VS, which indicates that their biodegradability is quite low compared to other substrates. However, expressed on a raw matter basis, spent animal bedding shows values ranging between 40 - 71 NmL CH_4 g^{-1} RM, which suggests that these substrates are interesting for energy recovery from an industrial point of view.

In spite of the common properties shared by different kinds of spent bedding, mulching, or the amount of straw used as bedding in relation to the number of animals (related to the husbandry practices), is found to be a parameter potentially affecting significantly the properties of spent animal bedding

In spite of the common properties shared by different kinds of spent animal bedding, some differences can appear within this waste category. A very important one is the mulching or the amount of straw used as bedding in relation to the number of animals, which a parameter strictly related to the husbandry practices. A low amount of straw can decrease the TS of the substrate, modify its physical structure, and then reduce the percolation efficiency (and degradation) in LBRs, while a high straw content can significantly increase the C/N which could lead to a non-optimal substrate for AD. Among the tested substrates spent horse bedding is found to be the one with the highest amount of straw. Even if its digestion did not

seem to be affected by the consequent high C/N ratio, mulching is a parameter that should be kept under control since it indirectly defines the faeces-to-bedding ratio.

6.2.2 Digestion of spent animal bedding in LBRs

The advantages and limits of the LBRs were studied together with the digestion of spent animal bedding and its degradation performance (Figure 39).

Digestion of spent animal bedding in LBRs is found to proceed efficiently without inhibition: the pH never drops below 6.5 and the methane content in biogas reaches values ranging between 55% - 60%. The initial accumulation of VFAs in the system, which characterizes a sensitive phase in the LBR process, is rapidly replaced by methane production whose peak appears straight after VFA consumption. Initial high biogas production (as well as the acids accumulation) is related mainly to the degradation of the easily-degradable fraction contained in the spent animal bedding while the significantly lower biogas production after 30 days is connected mainly to the degradation of its slowly-degradable fractions. Among all the types of spent animal bedding studied only spent goat bedding is characterized by a particularly high VFA accumulation (peak of ~11 g L^{-1}) and a significant lag phase while all the other types have a much more similar degradation process with low VFA peak of (~5 g L^{-1}) and almost no lag-phase. A reduced amount of active methanogens naturally present in the substrate could be the cause of this different behaviour. In this sense, a higher inoculation could be necessary when treating spent goat bedding in order to increase its methanogenesis kinetics and reduce its lag-phase.

Therefore, spent animal bedding of different types have been found to behave similarly when treated in LBRs which make it possible to consider them as similar substrates. However, despite of this generalization few exceptions as the one of spent goat bedding can significantly affect the process and should be taken into consideration
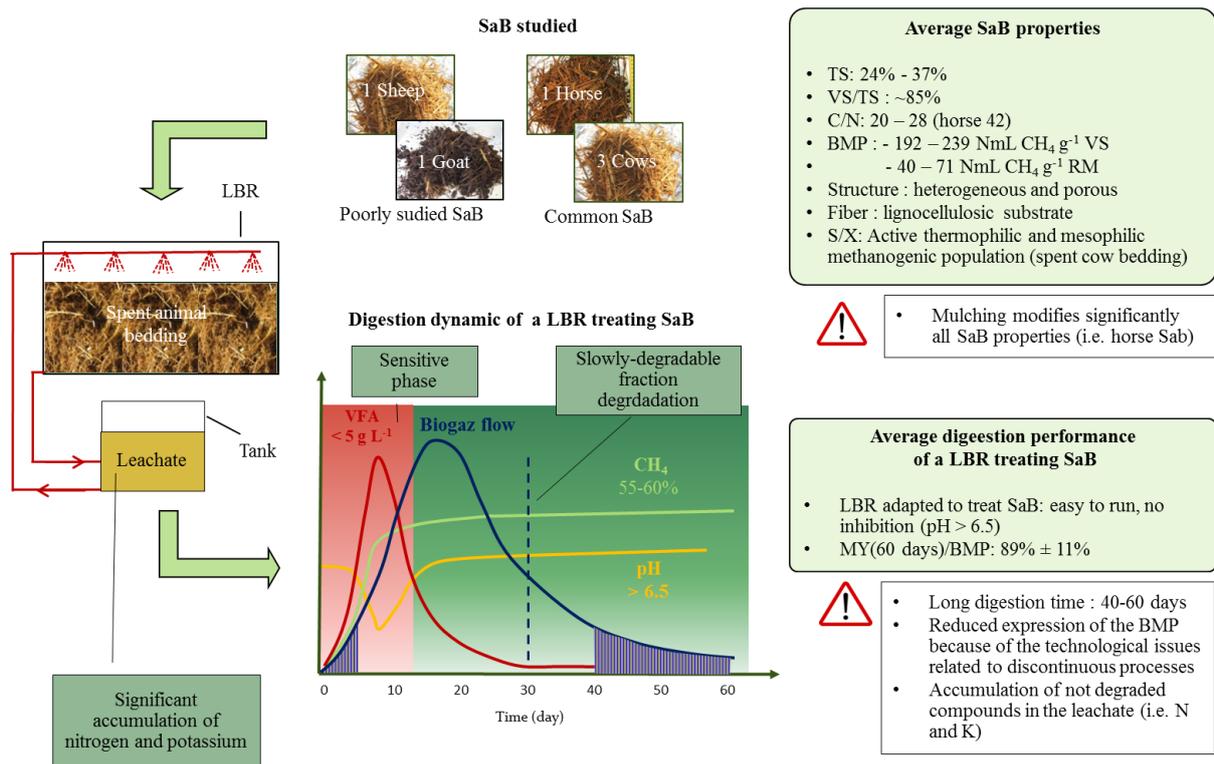
Figure 39 : Spent animal bedding (SaB) average properties and digestion performance in a leach-bed reactor (LBR)

Concerning the inoculation, it is also proved that spent cow bedding, the most common type of spent animal bedding, easily starts AD both in mesophilic and thermophilic temperature conditions without solid inoculum addition. This is a very interesting result since this proves that S/X of this substrate (considering it as a mixture of straw and faeces) is already sufficient and no further addition of methanogenic microorganism's inoculum is necessary. That can be of significant help when starting a new process when solid digestate is not available or, more generally, to reduce costs of a plant site.

Despite the different methane kinetics observed, the final degradation efficiencies of all the spent animal bedding tested are similar. The methane yield (MY)-to-BMP ratio is on average 89% ± 11% after 60 days of operation in LBRs. This proves that LBR is a suitable process to efficiently treat spent animal bedding.

Some drawbacks and optimization levers have been also identified in the digestion of spent animal bedding in a LBR. The long digestion time for these slowly-degradable substrates is recognized as the main weakness of this substrate when treated through AD. Shortening the digestion time could be an interesting option since, during the last 20 days, the methane production is really low and represents only 9% to 15% of the total methane production when a digestion time of 60 days is considered.

Further optimizations concern the start-up strategy. Indeed, this is a crucial phase during which methanogens grow in the digester and methane starts to be produced. However, the low initial methane content in the biogas during the start-up phase can hamper its combustion in a CHP unit. As an example, up to 8.1% of the total methane produced can be lost if a threshold of 40% of methane content in the digester head-space is considered as prerequisite prior injection in the CHP unit. Increasing methanogenesis rate can represent an interesting option since it allows a rapid increase of methane concentration in the biogas, but this can also induce a high methane production peak that could not be converted by the CHP because of the sudden flux of fuel to deal with. An optimum between these two parameters should then be found.

Another important aspect about LBRs is the water management and the reuse of the liquid fraction after an AD cycle. It has been highlighted that in LBRs a significant amount of water is needed and the required quantity is directly proportional to the initial substrate TS. As an example, considering an initial TS between 24% and 37% from 0.3 to 0.7 kg of water per kg of fresh matter is needed to run the process. This factor should not be underestimated at industrial scale, even if the advantage of this dry system remains relevant compared to a wet process.

Moreover, the reuse of the liquid fraction for the next AD cycle can cause accumulations of non-degraded compounds, and some of them can reach inhibitory

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concentration levels. With spent animal bedding the main concern are nitrogen and potassium accumulation. A final concentration in the liquid phase ranging from 1.7 to 4.0 g L^{-1} for K^+ and from 0.6 to 2.7 g L^{-1} for TKN with respect to the substrate treated was recorded at lab-scale (up to 5 g L^{-1} of $N-NH_4$ and 9 g L^{-1} of K^+ are measured in industrial leachate after several cycles). Their possible inhibitory effect should be better assessed and leachate dilution after several AD cycles should be considered as a possible option. However, the choice of a more appropriate system TS (i.e. the ratio of the volume of the tank containing the leachate and the total volume of the solid digesters) could delay the occurrence of inhibitory concentrations and the need of frequently diluting the leachate to bring these concentrations back to acceptable levels.

6.2.3 The optimization strategies

Once set the basic knowledge on the LBR process, the substrate properties and its digestion, the next step dealt with the optimization strategies. The main objective of those was to increase methane yield and methane rate: i.e. produce the maximum amount of methane in the shortest operation time and working volume. Two main operating parameters were selected, the inoculation (discussed in chapter 3) and the temperature (discussed in chapter 4); the first acting through an increase of the number of adapted microorganism able to degrade the substrate and the second by enhancing the activity of the already present micro-organisms. The optimization of the process certainly needed to deal with some technical and economic aspects related to LBR technical features. Those were then taken into account in order to propose the best option to be implemented at industrial-scale (Figure 40).
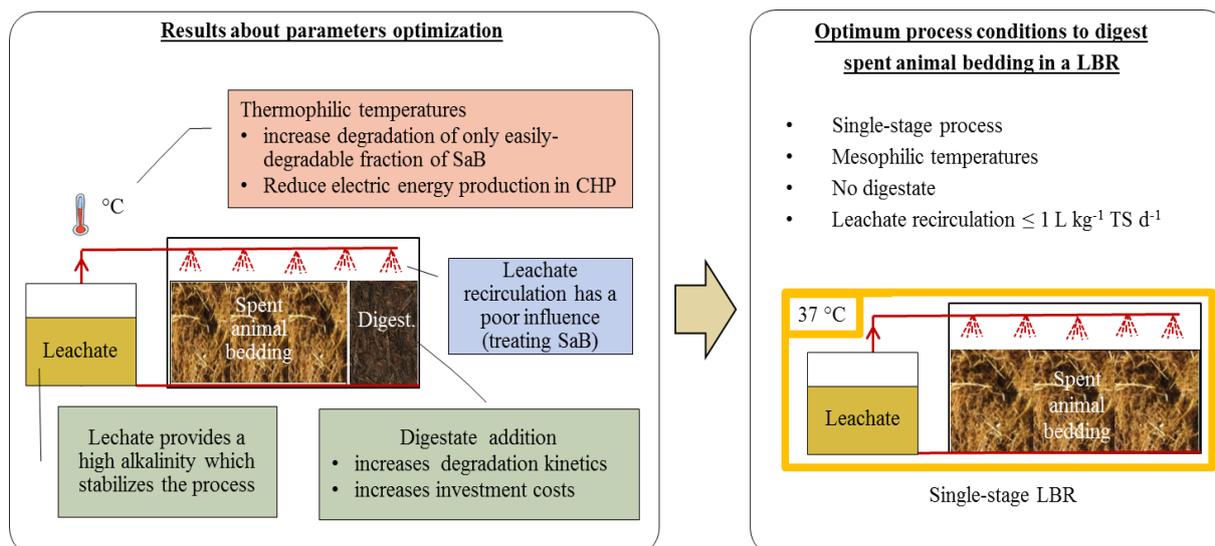
Figure 40 : Results of the parameters optimization and optimum process conditions to digest spent animal bedding in a LBR

Inoculation

Inoculation in LBRs is particularly important since it has to be repeated at each start-up phase, that is to say several times per year and per reactor at industrial scale. Its assessment is then critical. Inoculation can be done through the liquid leachate or the solid digestate. Leachate is shown to play mainly a role of process stability inside a reactor by providing the system with the needed alkalinity whereas digestate is found to act mainly on the biogas production. It is shown that 15.8% higher methane yield in 60 days could be reached by adding digestate (digestate TS/ total TS = 13%) compared to a simple leachate addition. This better depletion of the organic matter is an interesting result for a slowly-degradable substrate such spent cow bedding. However, the addition of digestate has to deal with the digester size since its addition leads to an increase of the digester working volume (if the same amount of fresh organic matter is treated). In chapter 3, it was shown that from an economical point of view, the use of the digestate or of the leachate gave the same economic results for two economical parameters: the internal rate of return and the payback period. This means that the economic impact due to the digester volume increase could be counterbalanced by the higher

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methane production. However, the investment cost significantly changed and for the addition of digestate (30% of the volume considering the same density for spent animal bedding and digestate), the initial investment was found 18% higher. These results suggest that the use of leachate is a better economic strategy than the addition of digestate since a higher investment could represent a real barrier to the feasibility of an industrial project. It is important to stress that such a strategy is possible because spent cow bedding already contains a significant amount of active methanogens. The addition of solid inoculum is then not necessary as it could be for other type of substrates (e.g. straw or maize silage). This is a rather great advantage connected to the use of spent animal bedding.

Thermophilic temperature

The temperature increase from mesophilic to thermophilic conditions was also evaluated on the digestion of spent cow bedding. The use of thermophilic conditions instead of mesophilic ones was listed as a possible method to increase methane production rate and reduce batch duration. The results showed that temperature effectively enhanced the methane production rate but this effect was mainly important on the easily-degradable fraction of the substrate and then on the first 10 – 15 days (only 14% higher methane yield in thermophilic condition after 15 days). Since spent animal bedding is mainly a lignocellulosic substrate (i.e. slowly-degradable substrate), the effect of temperature on the overall digestion was poor. Indeed, one of the main problems while treating spent animal bedding is to recover most of its potential energy because of its relatively low methane potential (measured through the BMP). Nonetheless, thermophilic and mesophilic temperature are showed equivalent in the attempt to reach the 80% of the BMP which is reached after 42 days of operation in both conditions.

In other words, the advantages of thermophilic over mesophilic temperatures concerning the methane yield are negligible after 42 days of digestion.

Furthermore, the use of thermophilic temperature and a consequent increase in the biogas production rate during the first days of digestion displays a significant impact on the biogas conversion in CHP unit. Because of the power operating range (i.e. between maximum and minimum powers) of the cogeneration engine, a too high fluctuation of the biogas production causes the loss of a part of the biogas, which is then sent to a torch. In the end, in thermophilic conditions, the loss of electric energy is estimated up to 5.9% (compared to the one produced in mesophilic condition). Furthermore, additional energy costs for maintaining a higher temperature in the digesters as well as the use of more expensive material for the construction of the structure in the case of thermophilic conditions would decrease even more its economic attractiveness. For this reason, thermophilic operating temperature to treat spent animal bedding is shown to provide no real advantage if spent animal bedding is considered as a resource. The search of an economic optimum between operation time and methane yield is then considered beyond the scope of the PhD thesis and not studied.

Leachate recirculation

Previous results (section 2.1.3.2) showed that the accumulation of VFAs is not particularly significant when digesting spent animal bedding as a sole substrate (maximum VFA concentrations generally lower than 5 g L^{-1} except for spent goat bedding which reached 11 g L^{-1}). Therefore, leachate recirculation does not influence significantly the digestion process and the modification of the leachate recirculation from 10 L kg^{-1} TS d^{-1} to 1 L kg^{-1} TS d^{-1} gave the same results in term of methane yield and methane production rate if the bulk water saturation is reached at the beginning. As a consequence, in order to reduce costs

related to pump use and maintenance the lowest leachate recirculation rate should be chosen and a value of 1 L kg^{-1} TS d^{-1} is considered an upper limit for this parameter.

6.3   The co-digestion of spent livestock bedding with an easily-degradable substrate in a sequential LBR system

Spent bedding is the substrate which is mainly found in countryside and for this reason the most used in farm-scale AD process. Usually, spent livestock bedding is showed to be easily digested in LBRs and does not involve acidification problems because of its high alkalinity. Co-digesting spent bedding with other more easily-degradable substrates seems then a possible and effective option to increase the methane production of the site and, as a consequence, its economic feasibility. Moreover, the addition of a second substrate is a common practice. Most of the time, this latter is an easily-degradable substrate that is added seasonally and sometimes can lead to acidification problems that should be further well controlled.

In chapter 5 the co-digestion of spent bedding with an easily-degradable substrate was studied. The system used was no more a single-stage digester but one simulating a sequential system as on a real site (the leachate was never recirculated within the same reactor and an external leachate always used). It is observed that methanogenesis inhibition is proportional to the fraction of easily-degradable substrate added (Figure 41): 10% (on a TS basis) of easily-degradable can be added to spent animal bedding without any particular risk of acidification, with 25% of TS, a 10 days lag-phase is observed while with 40% of easily-degradable the lag-phase reached 20 days. An increased accumulation of VFAs and the consequent decrease of the pH in the bulk in relation to the fraction of easily-degradable substrate added were found the main cause of inhibition. However, in any case the leachate flush allowed the system to recover and establish proper methanogenic conditions.
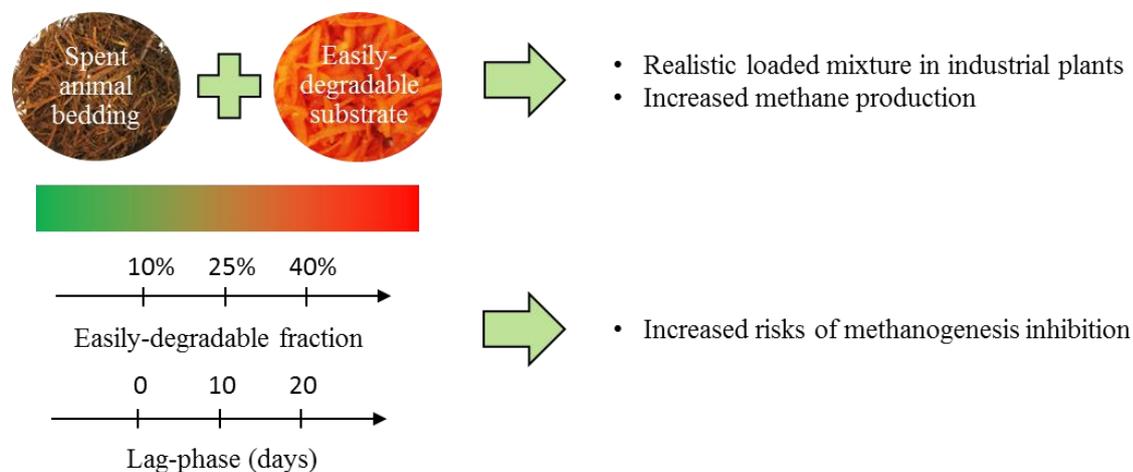
Figure 41 : Effect of the addition of a fraction of easily-degradable substrate to spent animal bedding

Coupling two reactors and flushing VFAs out of the inhibited reactor in order to consume them in a methanogenic one is shown in literature to be an effective strategy to treat

easily-degradable mixture and/or recover inhibited reactors. In chapter 5, the influence of the leachate flush rate on the recovery of a reactor with high risk of acidification (40% TS of easily-degradable substrate) was investigated (Figure 42). Three leachate flush rate conditions were tested as representative of the ones applicable at industrial scale: 2, 1 and 0.5 L kg^{-1} TS d^{-1}. This allowed to highlight that during the acidification phase, when no methane is produced, the leachate flush rate should be kept at its highest level ($\geq$ 2 L kg^{-1} TS d^{-1}) since the objective is to remove the VFAs produced in the bulk. This is shown to have a significant impact on the degradation rate of the substrate which at 35 days presented a degradation of 65% (COD$_{H2+CH4+VFA}$ -to-BMP) when flushing with 2 L kg^{-1} TS d^{-1} and 45%-48% when flushing with 0.5 L kg^{-1} TS d^{-1}. During the establishment of the methanogenesis, when methane production starts, the amount of VFAs extracted decreases considerably. For this reason a lower leachate flush rate is advised in this phase: $\leq$ 1 L kg^{-1} TS d^{-1}.

It should also be noted that at the end of this step, a large amount of VFAs is extracted from the bulk and that this could represent up to 41% of the total degraded COD. Good management of this great amount of VFAs is then particularly important in real systems since they could accumulate in the leachate storage and cause inhibition of the process. For this purpose, VFA consumption is at least as much important than VFA extraction. In order to better understand the consumption of external VFAs in LBRs, a leachate rich in acetic acid was used. The results showed that for an optimal acetic acid consumption in LBRs, this should be sent when the methanogenic activity in the batch is still high, i.e. right after the methane peak is reached. In this condition, their consumption time was reduced by half compared to an addition at the end of a batch. Furthermore, in order to keep a high methane production rate in methanogenic stable reactors, a leachate rich in easily-degradable compounds (like acetic acid) should be injected frequently (every 6-7 h in our conditions). In

addition, because of the bulk compaction and the problem of channelling, small volumes per flush are advised.

In the above mentioned tests, the frequency of recirculation was not considered and only the total volume recirculated was considered (frequency fixed at once per day). However, this parameter is very important and its role during extraction was highlighted in another test. Results in section 5.2.3.2 show that a big volume per flush could be replaced by smaller volumes recirculated more frequently. As an example, a frequency of 2 times per day with a leachate flush ratio of 0.5 L kg^{-1} TS $flush^{-1}$ (i.e. 1 L kg^{-1} TS d^{-1}) are proved to extract the same amount of VFAs than one flush of 2 L kg^{-1} TS $flush^{-1}$. The extraction efficiency should then be considered when fixing the recirculation strategies in the site and smaller volume sent more frequently could be preferred. However, a uniform percolation should be also ensured, otherwise the extraction efficiency could rapidly decrease as proved by the variability of the results obtained with a low leachate flushing rate 0.5 L kg^{-1} TS d^{-1}. Frequency and volume are then operating parameters that should be fixed together and an optimum looked in relation to the physical properties of the solid matrix and digester geometry.
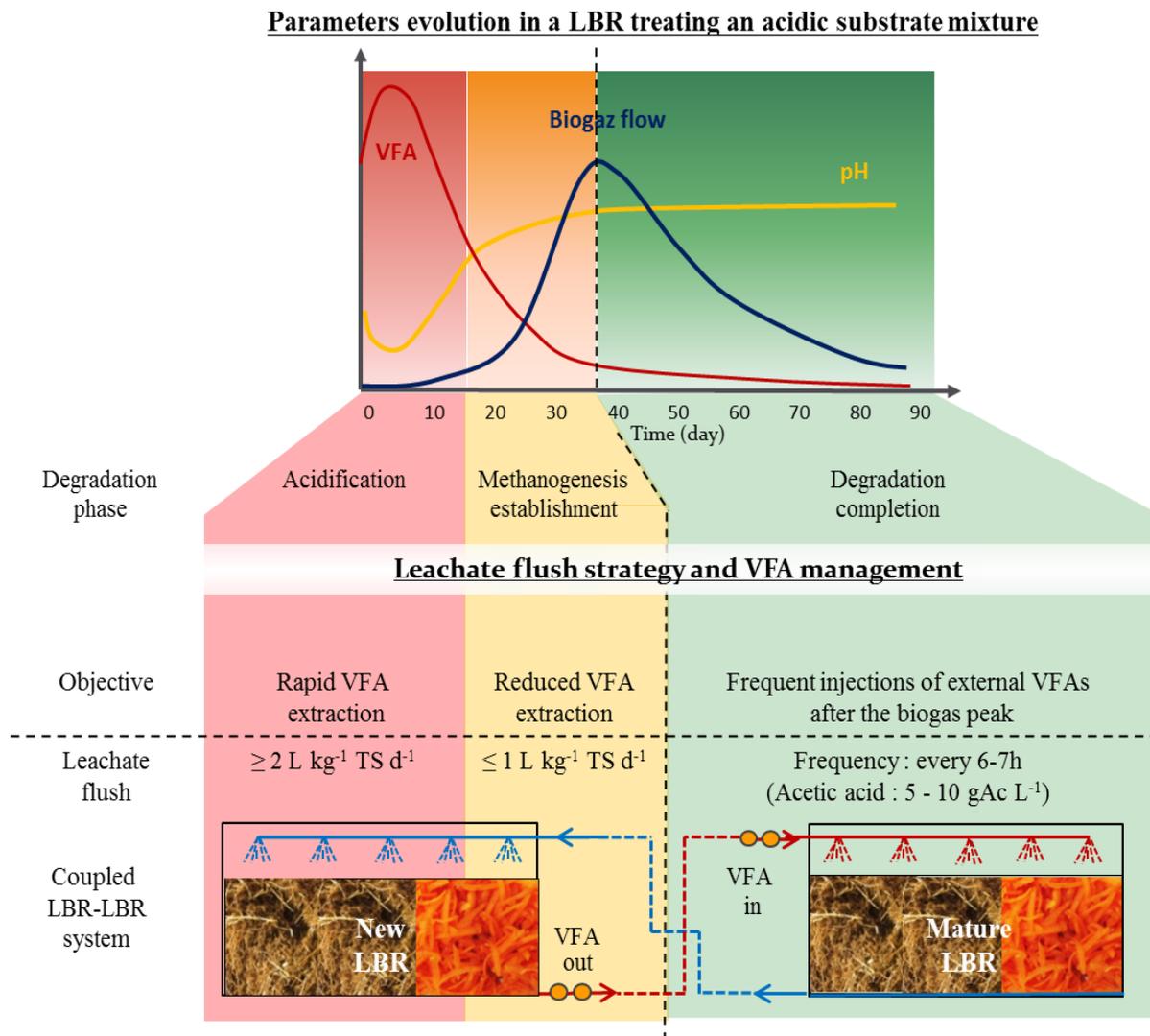
Figure 42 : Parameters evolution in a LBR treating an acidic mixture of spent cow bedding and an easily-degradable substrate; leachate flush and VFA management in relation to the phase of degradation; schematic functioning of a coupled LBR-LBR system: the new reactor from where VFAs are extracted and the mature reactor where VFAs are consumed

6.4 Perspectives

One of the major concerns in the LBR process optimization is the scale-up. Since LBR constitutes a dry and unmixed process, parameters linked to the physical properties of the bulk (e.g. particle size, medium porosity, etc.) highly impact the real performance at industrial site, much more than at lab scale. The difficulty in directly applying to the industrial scale the

results obtained at lab one is the major reason of delay in the development of dry AD processes in spite of the undiscussed simplicity of operation on field.

Phenomena related to the modification of the physical substrate properties during digestion, like compaction of the bulk, intimately affect the biological activity and the percolation in LBRs. As an example, leachate flush strategy developed for lab-scale process could not be applied directly at industrial scale and would probably need further adjustment making the search for the optimum leachate management a complicated quest at industrial-scale. Related to that, the treatment of easily-degradable compounds, whose performance has been shown to strictly depend on flush management can be difficult to optimize at this scale and empirical rules are often used.

The first action to undertake should be to consider physical substrate properties during experiments. Few authors already studied these aspects while working on spent cow bedding digestion in LBRs: (André et al., 2015) determined the relation between percolation and compaction all along the digestion and (Shewani et al., 2015) worked on the static and dynamic absorption of water in a LBR. However, a lot of questions still need to be answered: what is the relation between degradation and compaction? What is the optimum substrate column height in a LBR? What is the minimal amount of bulking agent required to ensure percolation even at later stage? All these are very important parameters to couple to biological tests like the ones carried out in this thesis.

The second important aspect is modelling. Modelling of AD in LBRs is considered one of the most complex models to develop in this field (Batstone et al., 2015) because of the higher number of parameters to consider compared to classic wet digestion, already efficiently described by ADM1 (Batstone et al., 2002). Beside the high number of parameters already considered in the ADM1, some others should be considered: physical substrate properties and

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their modification with TS content and digestion time, and data about water flow properties inside the bulk. Even if such a complex model was not yet developed, a first attempt to model the water flow through a bed composed of spent cow bedding and a coupling with a simplified AD biological model was tested by Shewani (2012). This work focused on the transfer of soluble compounds between solid and liquid phase during percolation. This interesting study, if well calibrated, could allow to finally answering problematic dealing with the optimum leachate management in percolation reactor and easily-degradable substrate digestion. However, calibration is a difficult step because of the need of including parameters coming from a full scale process.

Another important aspect of percolation systems such as LBRs is the role of immersion. Few authors already worked on that aspect (see paragraph 1.3.2) but a lot of questions remain unanswered. Immersion could be a very useful mean to solve problems connected to reach optimal initial bulk TS, but also to solve problems related to compaction and bad percolation which hamper degradation of the substrate. Also, the increase of the consumption of external VFAs, which is nowadays a very limiting aspect in sequential LBR process treating very easily-degradable mixture, could be improved by applying this strategy. Immersion is thought to be a very interesting approach that should be further investigated and considered seriously as a solution to all percolation problems observed and be an active part of a control strategy in LBR systems.

Finally, three PhD thesis (André, 2016; Degueurce, 2016; Shewani, 2016) recently completed in addition to this one were carried out simultaneously in France between 2013 and 2016 in order to solve the drawbacks connected to the implementation of LBR process and adaptation to spent animal bedding. Researches focused most of the time on different aspects of the process: Shewani (2016) on modelling water flows and mass transfer in LBRs,

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Degueurce (2016) mainly on the role of leachate properties and recirculation in single-stage system, André (2016) mainly on microbial community development and interaction in solid and liquid phase. Some research teams also focused on the use of the electrical tomography techniques to study the water flow inside real reactors and find correlation with substrate TS and degradation (André et al., 2016a; Degueurce et al., 2016b). Complementary results, most of them confirming each other, were produced in these past years and LBR technology surely made a big step towards a better understanding and practical implementation. The next step to validate this process, not only at lab scale but especially at industrial scale, should focus on the collection of all the results established in these last years and the design of new tests considering all the LBR aspects studied in these PhD works.

SUPPLEMENTARY MATERIAL

7.1 Statistical and modelling data on spent animal bedding characterization and digestion in LBR

Table 19 : Results of the ANOVA comparing the results of characterization and methane production of the substrates studied in leach-bed reactor

Parameters	Results	
Characterization		
TS	$[F(5,12) = 154, p=1.88e-10)]$	$p<0.05$
VS	$[F(5,12) = 157.5, p=1.65e-10)]$	$p<0.05$
Soluble	$[F(5,10) = 27.99, p=1.42e-05)]$	$p<0.05$
Cellulose	$[F(5,10) = 32.79, p=6.84e-06)]$	$p<0.05$
Hemicellulose	$[F(5,10) = 45.24, p=1.51e-06)]$	$p<0.05$
Lignin	$[F(5,10) = 9.95, p=0.00123)]$	$p<0.05$
C	$[F(5,12) = 5.191, p=0.00913)]$	$p<0.05$
N	$[F(5,12) = 85.12, p=2.05e-08)]$	$p<0.05$
P	$[F(5,12) = 41.89, p=3.46e-07)]$	$p<0.05$
BMP	$[F(5,12) = 25.25, p=5.58e-06)]$	$p<0.05$
In LBR		
Methane yield (volatile solids basis)	$[F(5,5) = 0.424, p=0.816)]$	$p<1$
Methane yield (raw matter basis)	$[F(5,5) = 4.407, p=0.0647)]$	$p<0.1$

Table 20 : Results of the modelling of the methane production in LBRs using the Gompertz equation

	Estimated Gompertz parameters			Confidence interval (CI)					
	μ_{max}	λ	A	μ_m CI95_low	μ_m CI95_up	λ CI95_low	λ CI95_up	A CI95_low	A CI95_up
	NmL CH$_4$ g^{-1} VS d^{-1}	days	NmL CH$_4$ g^{-1} VS	NmL CH$_4$ g^{-1} VS d^{-1}	NmL CH$_4$ g^{-1} VS d^{-1}	days	days	NmL CH$_4$ g^{-1} VS	NmL CH$_4$ g^{-1} VS
SB_sheep 1	7.40	1.38	174.7	6.93	7.87	0.62	2.14	171.7	177.7
SB_sheep 2	7.45	1.11	215.1	6.88	8.03	0.03	2.20	209.4	220.8
SB_goat 1	8.22	6.17	210.6	7.72	8.72	5.41	6.93	206.2	215.0
SB_goat 2	6.59	6.89	168.9	6.28	6.90	6.30	7.48	166.1	171.6
SB_horse 1	6.14	1.24	179.7	5.67	6.62	0.14	2.34	174.8	184.6
SB_horse 2	7.17	1.04	224.2	6.62	7.73	-0.12	2.21	217.5	230.9
SB_cow_h 1	6.56	3.53	179.0	6.01	7.10	2.51	4.55	172.6	185.4
SB_cow_h 2	6.48	4.13	175.7	6.04	6.91	3.32	4.94	170.7	180.8
SB_cow_g 1	-	-	-	-	-	-	-	-	-
SB_cow_g 2	5.82	5.03	162.5	5.37	6.27	4.08	5.98	156.8	168.1
SB_cow_m 1	7.58	4.01	208.7	7.05	8.10	3.16	4.86	202.4	215.1
SB_cow_m 2	6.71	4.12	181.6	6.22	7.20	3.23	5.01	175.9	187.3

μ_m : maximal methane production rate; λ: lag phase time; A: methane yield

Table 21 : Pearson correlation coefficients found among parameters from characterization, methane production in LBR and modelling

	A_{max}	BMP	C	cellulose	hemicellulose	lignin	MY (RM)
A_{max}	100.0%						
BMP	33.2%	100.0%					
C	-8.6%	-17.9%	100.0%				
cellulose	42.4%	87.5%	-4.2%	100.0%			
hemicellulose	8.9%	25.0%	21.2%	26.9%	100.0%		
lignin	-2.8%	-62.9%	47.5%	-47.5%	9.2%	100.0%	
MY (RM)	79.5%	22.7%	-51.2%	36.3%	5.6%	-15.9%	100.0%
MY (VS)	99.7%	31.2%	-5.8%	40.1%	6.5%	1.7%	76.3%
N	-35.2%	-70.6%	4.1%	-81.2%	-74.7%	23.0%	-38.4%
P	24.0%	27.8%	-72.4%	0.3%	-47.0%	-58.8%	41.6%
soluble	-36.8%	-59.7%	-23.6%	-75.8%	-76.6%	-2.0%	-26.1%
TS	38.4%	9.7%	-73.4%	20.3%	-5.0%	-25.5%	86.1%
VS	40.0%	11.0%	-72.4%	22.1%	0.3%	-24.7%	87.0%
λ	-34.1%	-4.2%	17.7%	-27.8%	-64.1%	-20.2%	-62.9%
μ_{max}	74.8%	18.8%	-35.0%	8.4%	-29.7%	0.2%	62.2%

	MY (VS)	N	P	soluble	TS	VS	λ	μ_{max}
A_{max}								
BMP								
C								
cellulose								
hemicellulose								
lignin								
MY (RM)								
MY (VS)	100.0%							
N	-32.0%	100.0%						
P	22.0%	21.2%	100.0%					
soluble	-35.3%	94.8%	44.4%	100.0%				
TS	34.0%	-25.7%	48.3%	-4.9%	100.0%			
VS	35.4%	-29.9%	45.5%	-9.3%	99.8%	100.0%		
λ	-31.1%	66.5%	25.7%	62.5%	-62.0%	-65.6%	100.0%	
μ_{max}	76.5%	5.8%	53.6%	9.0%	38.5%	37.7%	-3.8%	100.0%

μ_{m}: maximum methane production; λ: lag phase; A_{max}: maximum cumulative methane yield

7.2 Hypothesis for the economic simulation

- The average daily experimental values of methane yield presented in Figure 20b (conditions L and D) were used

- The biogas produced was considered exploitable when the concentration in methane reached 30 %.

- A coefficient of -7.5 % was applied to the experimental values of methane yield in order to simulate a worse degradation at farm-scale. On average, 86 % of the BMP is reached at lab-scale with spent bedding after 60 days of digestion while, at farm-scale, installations are close to 80 %, on average, (ADEME, 2014).

- The spent bedding and the digestate were considered to have a volumetric mass of 600 kg m^{-3}, as observed on site.

- The minimum number of digesters was set at four in order to ensure a constant biogas production over time. No maximum number was fixed.

- The only adjustable dimension in the digester volume was the length, with a maximum of 30 m. The working volume was fixed at 64 % of total volume.

- The total volume distribution was based on always preferring the minimum number of reactors. The addition of one more reactor took place when the maximum length was reached for all the reactors.

- Fixed electrical powers (for the Combined Heat and Power, CHP), ranging from 100 kW$_{el}$ to 300 kW$_{el}$ with 25 kW$_{el}$ steps, were used. Real CHP characteristics were reported for levels of (100, 150, 200, 250, and 300) kW$_{el}$ in accordance to manufacturer's technical specifications. The other intermediate power values used were simulated by limiting the nominal power of the next higher CHP unit. To simulate such cases, the efficiency of the CHP unit directly below the case studied and the cost of the directly above were used (e.g. for 125 kW$_{el}$, the efficiency of a 100-kW$_{el}$ CHP unit and the cost of a 150-kW$_{el}$ CHP unit were used).

- Electrical CHP power efficiency was considered to range between 39 % and 41 % in relation to the CHP size. The heat CHP power efficiency was fixed at 45 %.

- The number of hours operating at full-load was set at 8,000 hours per years as done by Caputo et al. (2005) and Lantz (2012).

- The feed-in tariffs used are those described by French legislation in government orders (Decree of 19 May, 2011) and (Decree of 30 July, 2013). To a base tariff set in relation to the CHP electrical power installed, two bonuses are added: one for energy efficiency and the other for the amount of animal manure used. The base tariff (for the electrical power considered in this work) was set between $0.1337 \, € \, kWh^{-1}_{el}$ for power $\leq 150 \, kW_{el}$ and $0.1267 \, € \, kWh^{-1}_{el}$ for power between $150 \, kW_{el}$ and $300 \, kW_{el}$; the energy efficiency bonus, independent of the electrical power, was set between 0 to $0.04 \, € \, kW^{-1}_{el}$ for energy efficiency values comprised between 35 % and 70 %; for electrical power $\leq 300 \, kW_{el}$; the animal manure bonus was set between 0 and $0.026 \, € \, kW^{-1}_{el}$ for 20 % and 60 % of animal manure used, respectively. Linear interpolation was applied for intermediate values. It is important to note that the animal manure bonus was always at its maximum value (since 100 % animal manure).

- The selling and purchase of spent bedding and digestate was not taken into account since in small agricultural projects the digestate produced is returned back to the farmers who brought the spent bedding initially. More details about the hypotheses made are reported in the supplementary material.

- The total time (N) used in Eq. 2 is 15 years, which corresponds to the number of years the project can benefit from advantageous electricity prices fixed by French legislation.

- The external process parameters chosen were selected for analysis since: the fraction of heat sold is the second source of revenue in co-generation AD plants (if the digestate is not sold, as in this case) and the preparation time for emptying and loading substrate from the digester is the most time-consuming activity in an LBR process (ADEME, 2010); the haulage distance has been described as a very important parameter in a lot of economic analysis and grants are always

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identified as the most important factor influencing a project's viability (ADEME, 2010; Massaro et al., 2015; Pantaleo et al., 2013; Walla and Schneeberger, 2008).

7.3   Sensitivity analysis

A sensitivity analysis was performed in order to identify which external process parameters (i.e. investment grants, fraction of heat sold, preparation time for loading and emptying the digesters and haulage distance) can affect the economic evaluation and whether differences exist in this respect between conditions D and L. The bar values presented in Figure 43 correspond to a variation of the IRR, or PP, in respect to the nominal value (e.g. (IRR-IRR$_{nom}$)/ IRR$_{nom}$). As an example, the impact of the variations in the investment grants on the IRR for 11.5 kt y^{-1} in condition L was studied (second bar from left side in Figure 43c). At its nominal value of 30 % (Table 22), the impact was zero since it corresponds to the reference case (IRR = IRR$_{11.5\,kt\,-\,L}$). By increasing its value to 50 % (> nominal), the upper range value (Table 22), the IRR increased by 71 % compared to the IRR$_{11.5\,kt\,-\,L}$. Similarly, a value of -43 % was found for the lower range limit (< nominal).

The sensitivity analysis showed that all four external process parameters selected had a great impact on economic performance, particularly on the IRR. Their variations in respect to the nominal values were often found symmetrical for nearly all capacities, which means that the evolutions of IRR and PP in relation to the external process parameters are quite linear in the ranges studied, without any optimum to be found. The heat sold and the substrate preparation had a minor impact overall whereas the haulage distance and the investment grants had a considerable effect. In particular, as also highlighted by Walla and Schneeberger (2008) and Massaro et al. (2015), the investment grants were found to be by far the most influential parameter since they directly affect the investment and, consequently, the PP .
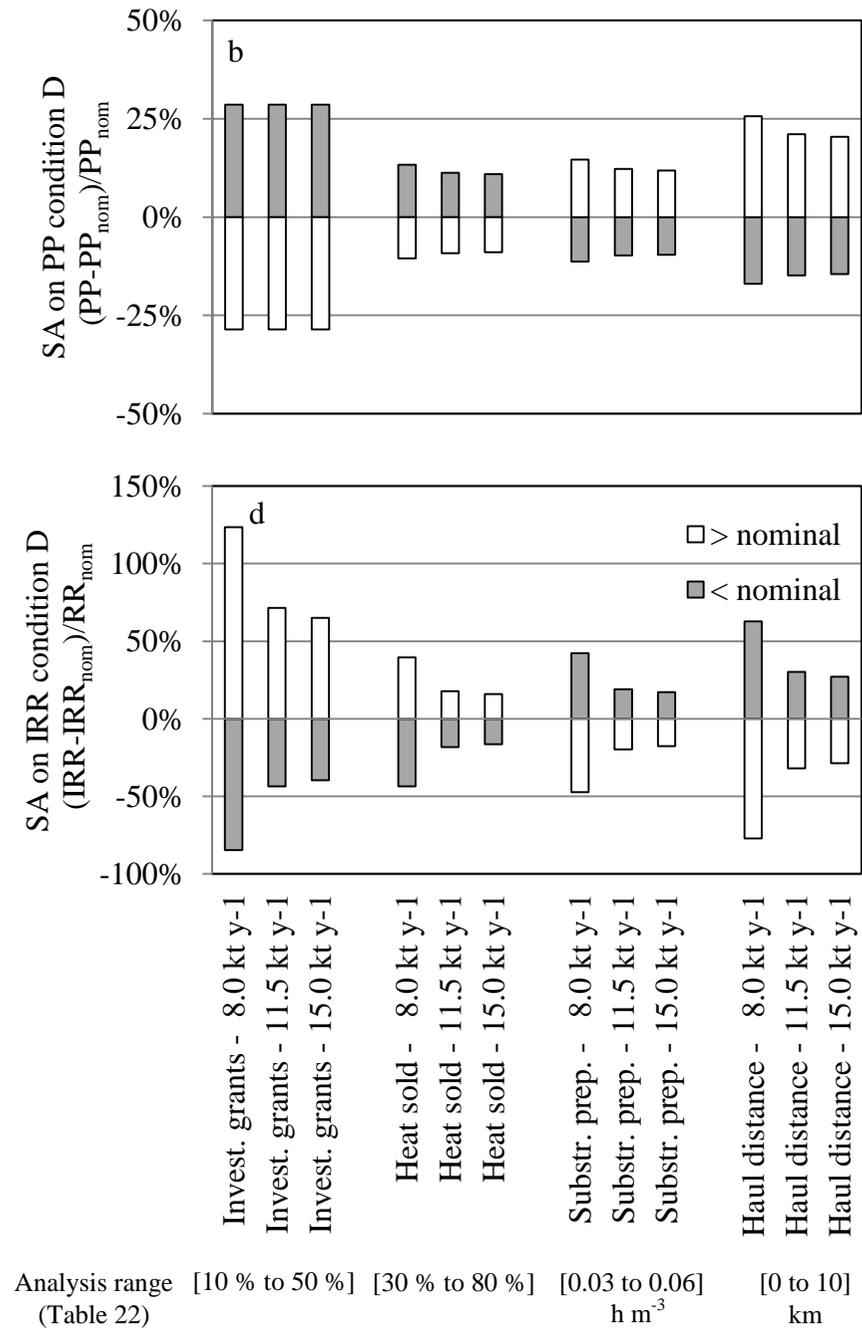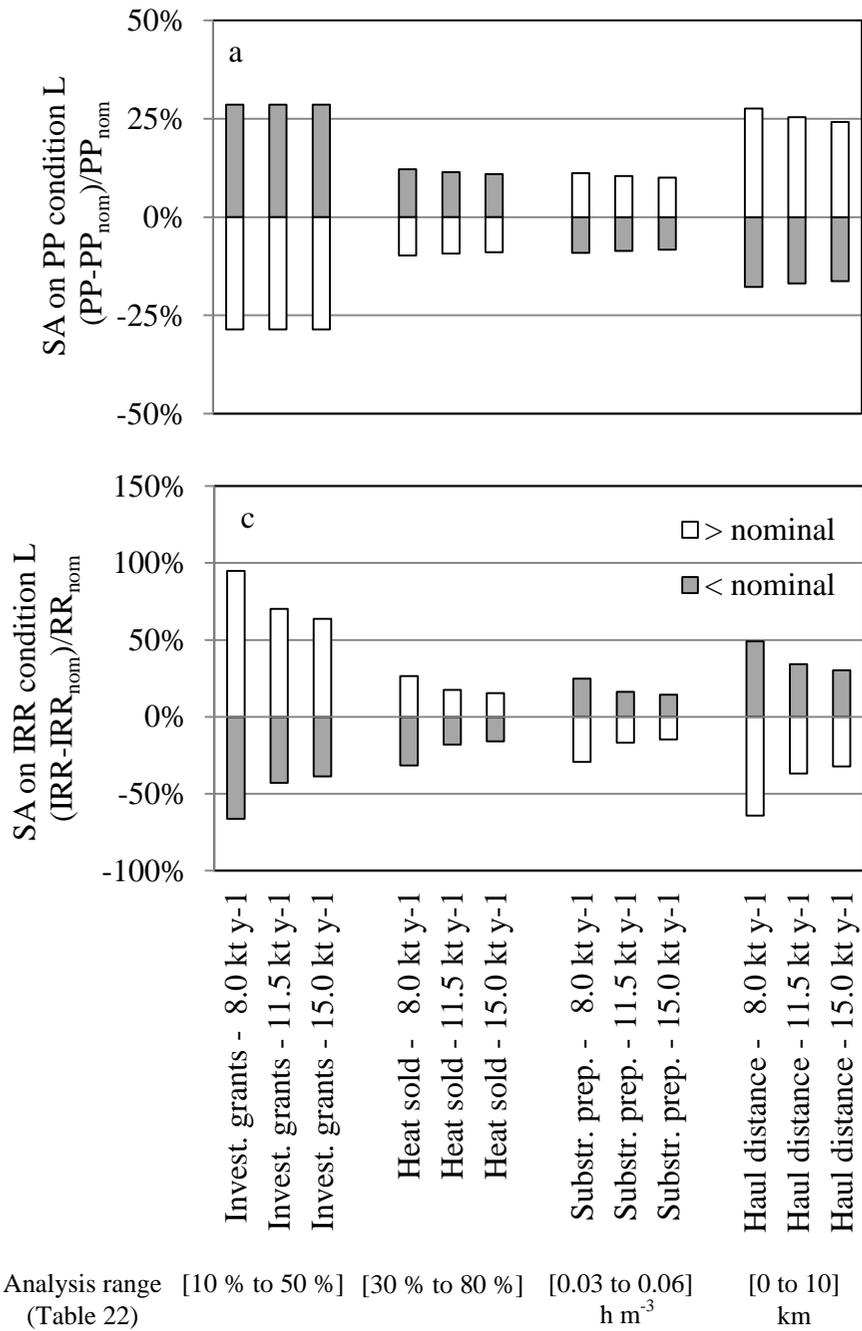
Figure 43 : Sensitivity analysis results: variability of the IRR and PP for three capacities: (8.0, 11.5, 15.0) kt y^{-1}. (a-b) Payback Period for condition D and L; (c-d) Internal Rate of Return for conditions D and L

and the IRR. This result highlights the importance that the subsidies still have on the development of this technology and, more generally, of the AD of solid waste. Economies of scale were found to affect the IRR (Figure 43 c-d) while more stable evolution characterized PP (Figure 43 a-b). The higher variations and its sensitivity to the capacity, make the IRR a crucial parameter.

In conditions D and L, while the relative difference in sensitivity to each parameter was similar, the parameters that most distinguished the two conditions were found to be substrate preparation and haulage distance. In fact, by adding digestate, the total substrate volume to be loaded and then emptied is higher; on the other hand, by using a leachate, less energy, is recovered from the same amount of matter transported.

Table 22 : Selected ranges for sensitivity analysis

			Nominal value	Range
Process parameter	Capacity	kt y^{-1}	11.5*	8.0 to 15.0
	Batch duration	day	50	40 to 60
External process parameter	Investment grants		30 %	10 % to 50 %
	Heat sold		55 %	30 % to 80 %
	Substrate preparation	h m^{-3}	0.03	0 to 0.06
	Haulage distance	km	5	0 to 10

*The sensitivity analysis of the other parameters was carried out also for 8.0 kt y^{-1} and 15.0 kt y^{-1}

Within the chosen ranges, these differences were not enough to justify choosing one start-up strategy over another. However, these differences could increase significantly for values out of the range limits. For example, for haulage distances above 10 km (up to 70 km

which is still an environmentally interesting solution (Massaro et al., 2015), the digestate option could turn out to be a better strategy than using a leachate. However, attention should be paid to the overall performance which will inevitably decline.

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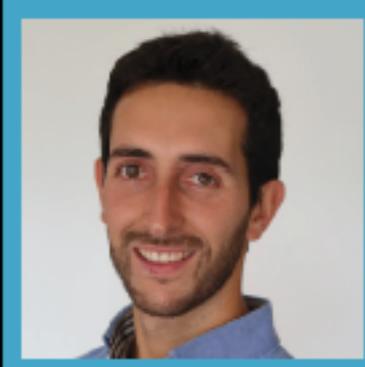
OPTIMIZATION OF THE PROCESS PARAMETERS CONTROLLING DRY ANAEROBIC DIGESTION OF SPENT ANIMAL BEDDING IN LEACH-BED REACTORS

Anaerobic Digestion (AD) is a process which allows the treatment of organic waste and the production of renewable energy. Specifically, dry AD allows the treatment of solid organic substrates offering several possibilities for the enhancement of agricultural waste such as spent animal bedding: a mixture of straw, feces and urine. Among the available biotechnologies in AD, a leach-bed reactor (LBR) is promising but poorly known process both at a scientific and industrial level. This PhD thesis aims to investigate and optimize the AD of spent animal bedding in LBRs.

Several issues have been studied: (i) the bio-physico-chemical characterization of spent animal bedding and its digestion potential in LBRs; (ii) the optimization of the start-up and the operating temperature of the digesters; (iii) the co-digestion of spent animal bedding with an easily-degradable substrate and the issues connected to the management of the volatile fatty acids (VFAs) produced.

The results showed that spent animal bedding is a valuable organic resource for AD in the agricultural context because of its properties: porosity, active methanogenic population, methane production, etc.. LBR proved to be efficient in the treatment of this organic matter, both as a sole substrate or in co-digestion with an easily-degradable one. Experimental results coupled with technical-economic simulations showed that the best operating conditions to digest spent animal bedding as a sole substrate are without digestate addition and at mesophilic temperature, while no particular benefit is found with leachate recirculation. However, the addition of an easily-degradable fraction to the spent animal bedding makes the leachate recirculation a critical parameter in order to manage the digestion process. This latter should then be adapted in relation to the digestion period in order to maximize the degradation efficiency.

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