Aus dem Institut für Tier- und Umwelthygiene – ITU
des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

und

Leibniz-Institut für Agrartechnik und Bioökonomie – ATB
der Fachabteilung Technikbewertung

Assessment of the influencing factors regarding loss of methane potential due to manure storage in dairy cow and fattening pig farms

Inaugural-Dissertation
zur Erlangung des Grades eines
Doktors der Naturwissenschaften
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vorgelegt von
Julio Elias Hilgert
Mechanical Engineer aus Peritiba, Brasilien

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"We can only see a short distance ahead, but we can see plenty there that need to be done."

(Alan Turing)

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List of abbreviations

AD – Anaerobic digestion

BMP – Biochemical methane potential

CH_4 – Methane

CO_2 – Carbon dioxide

DM – Dry matter

GHG – Greenhouse gas

Mtoe – Million tons of oil equivalent

NFC – Non-fiber carbohydrate

TAN – Total ammonia nitrogen

VFA – Volatile fatty acids

VS – Volatile solids

Chapter 1: Introduction

Methane (CH_4) emissions are a significant contributor to global warming due to their potency as a greenhouse gas (GHG), possessing 27 times the global warming potential of carbon dioxide (CO_2) over a 100-year timescale (IPCC, 2021). Manure management is a prominent source of these emissions in agricultural landscapes (UNEP - CCAC, 2021). Liquid manure, when stored, undergoes anaerobic digestion (AD), a biochemical process that not only leads to the natural degradation of organic matter but also results in the formation and emission of CH_4. In recent years, the emphasis on understanding and mitigating CH_4 emissions from manure storage has increased, given its implications for climate change and sustainable agriculture (EU, 2020; IPCC, 2019). Factors influencing the magnitude of these emissions are multifaceted, varying from storage conditions and manure chemical composition to microbial communities involved in the AD process.

Storage conditions, especially the temperature and the length of storage, play a critical role in CH_4 formation and emissions from manure. Manure is generally exposed to daily and seasonal fluctuations in environmental temperature during storage. The effects of these temperature shifts are profound due to the temperature-dependent behavior of the microbes and methanogenic communities that catalyze the AD process (Feng et al., 2018; Hafner & Mjöfors, 2023; IPCC, 2019). The length of manure storage profoundly influences CH_4 production and emissions, with the initial stages marked by rapid CH_4 formation due to the breakdown of readily degradable organic matter (Aguirre-Villegas & Larson, 2017; B. Amon et al., 2006; VanderZaag et al., 2018). As storage continues, the degradation process shifts towards more complex organic compounds, gradually decreasing CH_4 formation (Johannesson et al., 2017). Additionally, the chemical composition of manure significantly dictates the amount of CH_4 produced during storage. The volatile solids (VS) content, including carbohydrates, proteins, and fats, plays a crucial role (Triolo et al., 2011). A higher concentration of VS in manure has the potential to produce more CH_4 (Møller et al., 2004). Conversely, compounds like ammonia, a by-product of protein decomposition, can hinder the activity of methanogenic bacteria and thereby reduce CH_4 production (Yang et al., 2018).

The overarching aim of this study is to examine the impact of manure storage conditions and chemical composition on the loss of CH_4 potential due to CH_4 formation and emissions, specifically in dairy cows and fattening pig farms. Assessing the CH_4 potential loss can bring new insights into the degradability of the organic matter due to storage conditions and manure chemical composition and provide strategies to mitigate CH_4 emissions from manure management. The derived research questions that shaped this work are as follows:

Can the manure storage temperature indirectly influence CH_4 emissions by causing significant alterations in the chemical composition of the dairy cows and fattening pig liquid manure, especially concerning the accumulation of volatile fatty acids (VFA) during storage? (Publication A)

Considering the manure storage conditions, how do the biochemical methane potential (BMP) and the chemical characteristics of manure from various stages of the liquid manure storage change in commercial dairy cows and fattening pig farms? (Publication B)

Can mathematical models to predict manure BMP based on manure composition and storage conditions be developed? Can such models enhance our understanding of organic matter degradation and support the design of effective climate change mitigation measures? (Publication B)

Chapter 2: Literature

This introductory chapter provides a comprehensive overview of CH_4 formation and emission from manure management, beginning with a broad contextualization of global warming issues and international agreements before focusing on the specific area of manure management and potential mitigation strategies, notably biogas production (2.1). Then follows a general discussion of manure management practices, emphasizing the procedures related to liquid manure management (2.2). After, the biochemical mechanism of organic matter in the liquid manure management environment is explained with a particular focus on AD (2.3). Finally, it is examined how various factors, such as storage conditions and the manure chemical composition, influence the CH_4 formation and emission during manure storage (2.4).

2.1 - CH_4 formation and emission in manure management and the missed opportunities in biogas production

In 2015, the international community signed the Paris Agreement to limit global warming to below 2°C, preferably below 1.5°C. It sets out a framework for countries to reduce their greenhouse gas (GHG) emissions, adapt to the impacts of climate change, provide financial and technological support to developing countries, and establish and implement contributions to reach the target (UN, 2016). The Intergovernmental Panel on Climate Change supported this political engagement and defined a near-term strategy to avoid future increases in the average global temperature. Among other objectives, the plan emphasizes the need for a significant reduction of GHG, primarily CH_4 emissions. It also recommends accelerating the transition to clean and renewable energy sources and promoting changes in agriculture to enhance carbon sequestration and decrease emissions (IPCC, 2021).

There are several reasons why tackling CH_4 emissions has become a priority. Firstly, a significant rise in anthropogenic CH_4 concentration in the atmosphere has been observed, increasing from about 1,775 ppb in 2006 to 1,857 ppb in 2018, with pre-industrial levels estimated at 720 ppb in 1750 (Etheridge et al., 1998; Nisbet et al., 2019; Saunois et al., 2020). Recent increases in anthropogenic CH_4 concentration in the atmosphere have been attributed to biogenic sources (Schaefer et al., 2016).

Secondly, CH_4 has a relatively short half-life of approximately 8.6 years in the atmosphere, which means the impacts of reducing its emissions can be felt more rapidly than those of other GHGs (Muller & Muller, 2017). However, even after its removal from the atmosphere, CH_4's warming effect partially lingers because it may contribute to the build-up of other GHGs (Mar et al., 2022).

Lastly, the average global warming potential of non-fossil CH_4 is estimated to be 27.2 times greater than CO_2 over a 100-year time horizon and 80.8 times greater over a 20-year time horizon (IPCC, 2021). As a result, anthropogenic CH_4 emissions are projected to increase the planet's heat storage by 0.97 Wm^{-2}, while CO_2 emissions account for a 1.68 Wm^{-2} increase (Stocker, 2014). Overall anthropogenic CH_4 concentration in the atmosphere is believed to be responsible for a 0.5°C increase in average global temperature compared to measurements from 1850-1900 (IPCC, 2021).

Leveraged by these factors, initiatives, and political strategies to substantial and sustained reduce CH_4 emissions arise (Leonard, 2014); for instance, the European Union target to decrease anthropogenic CH_4 emissions by 50% from 2020 to 2050, potentially mitigating global temperature change by 0.18°C by 2050 (EU, 2020). Effectively executing the political strategies necessitates significant social and economic transformations, particularly in sectors that contribute most significantly to CH_4 emissions.

The economic sectors that significantly contribute as sources of anthropogenic CH_4 emissions are agriculture with 40% - mainly from livestock enteric fermentation and manure management (32%) - and fossil fuel with 35% of the emissions (UNEP-CCAC, 2021). Enteric fermentation and manure management increased their contributions from 102 $TgCH_4yr^{-1}$ in 2000–2006 to 115 $TgCH_4yr^{-1}$ in 2017 (Jackson et al., 2020). This information is consistent with the observed global meat production increased from 315 million tonnes in 2010 to 349 million tonnes in 2019, representing a 10.8% increase over the decade. For instance, pig meat production increased from 110.4 million tonnes in 2010 to 124.2 million tonnes in 2019, representing a 12.5% increase. In 2010 global milk production was approximately 688 million tonnes, and by 2019 it had increased to about 829 million tonnes, representing a 20.5% increase over the decade (FAO, 2021).

Consequently, particular focus has been given to tackling agricultural CH_4 emissions; for instance, Germany's climate action plan 2050 defined that the agricultural sector must reduce its GHG emissions by 31-34% of the 1990 levels by 2030 (BMU, 2016). While enteric fermentation accounts for most CH_4 emissions in livestock production, manure management presents feasible emission mitigation options on commercial farms, especially biogas production (Kupper et al., 2020; Mohankumar Sajeev et al., 2018).

Biogas is a mixture of gases produced by the AD or fermentation of organic matter that can be used as a renewable energy source. When combusted, it can generate heat and electricity and serve as a fuel for transportation and other industrial processes. Biogas composition depends on the substrate, but generally, it includes between 55% and 70% CH_4, from 30% to 45% CO_2, and trace amounts of other gases such as hydrogen sulfide (H_2S) (Deublein &

Steinhauser, 2008). It is called biomethane when purified to concentrations equal to or higher than 98% CH_4. The lower heating value (LHV) of CH_4 is 50 MJ/kg, which is released during combustion. Utilizing anaerobic digesters to process animal waste can generate biogas while simultaneously decreasing CH_4 emissions from the manure during storage and field use (Kebreab et al., 2006; Sommer et al., 2004).

The total world biogas installed capacity in 2021 was 21 GW, corresponding to about 1% of renewable energies installed capacity globally. However, it increased 64% since 2012 (IRENA, 2022). Animal manure is used as substrate in around one-third of all energy generated by biogas (IEA, 2020). The International Energy Agency (IEA) predicts that the total global biogas production will increase to 75 Mtoe by 2040, twice the production in 2018 (IEA, 2020). This dual capacity of animal manure in agriculture - both a GHG emitter and a potential renewable energy source - facilitates a synergistic relationship between emissions reduction and avoiding fossil fuel combustion for energy production.

2.2 – Manure management

Manure storage is needed in agriculture to create a cleaner and healthier environment for livestock and meet regulations regarding nitrogen losses (EEC, 1991). Systems for managing manure are engineered to contain and convey substantial quantities of biomass from animal barns to outdoor storage areas and fields, where it will be utilized as fertilizer (Malomo et al., 2018). In Germany, manure application to fields is subject to certain restrictions and guidelines to minimize environmental impact (BMELV, 2017). Farmers are generally prohibited from applying manure during winter when the risk of runoff and leaching is highest. Instead, manure is typically applied during the growing season, either as a pre-sowing application or as a top dressing.

Several manure management systems include solid-bedding, liquid-manure, and composting systems. Solid-bedding systems use straw or other materials to absorb and store manure. Liquid-manure systems involve collecting and storing liquid manure in tanks or lagoons. Composting systems collect and store manure in a contained area, allowing it to decompose over time (Andersen et al., 2015; Varma et al., 2021).

According to the German National Inventory, liquid manure (slurry) systems were used on 52.37% of dairy farms and 77.79% of pig farms in 2019 (German Environment Agency, 2021). Other estimations show that liquid manure management systems exist in 32% and 38% of Western Europe and North American dairy farms, respectively (Gerber et al., 2013; Petersen, 2018). Liquid manure management is also predominant in pig farms in these regions (Dennehy et al., 2017; IPCC, 2019). For both animal categories, liquid manure can be transported from the housing to the storage by pumping systems (Bernal et al., 2015; Dalby et al., 2021). While liquid systems yield higher CH_4 emissions than solid systems (IPCC, 2019), they simultaneously enhance biogas production. This enhancement is due to anaerobic processes that thrive in liquid systems characterized by low oxygen availability, promoting biogas generation (Achinas et al., 2020).

2.3 – Degradation of the organic matter in the liquid manure management environment

Manure primarily consists of animal feces and urine, but the composition can vary significantly due to differences in the proportion of water, feeding remains, bedding material, dust, and animal species Sommer et al., 2004). Different values of liquid manure dry matter (DM) content can be found in the literature; Weinfurtner (2011) analyzed several literature sources and verified that for dairy cattle manure, DM ranges from 2.0 to 12.0 % of fresh matter. These figures may depend on the season, feeding, and manure age. From this DM, approximately 75% is VS, and it also has a pH between 7 and 8 and a total nitrogen content of 1 to 4 g kg^{-1} (Kupper et al., 2020). The composition of pig liquid manure is slightly different, with DM in the range of 1.5 to 12% (Scheftelowitz & Thrän, 2016), but higher total ammonia nitrogen (TAN) content than cattle liquid manure, possibly due to the more significant presence of fibrous material in cattle liquid manure (Dalby et al., 2021; Petersen et al., 2016). The characteristics of manure vary globally, often due to other feeding and management practices, resulting in more diluted manures in Asia and highly concentrated ones in Dutch farms (Sommer et al., 2013).

When animal manure is stored in a liquid form, it undergoes various chemical processes, mainly AD, which produces CH_4 (Li et al., 2012). AD is a sequence of biochemical processes involving a variety of microorganisms that, in the absence of oxygen, degrades organic matter, leading to CH_4 and CO_2 production during manure storage (Amon et al., 2007; Baral et al., 2018). AD is the biochemical process used to produce biogas in biogas facilities; however, there are some similarities; this study focuses on the AD process that occurs naturally due to the degradation of organic matter during manure storage. AD comprises a process sequence, for instance, hydrolysis, acidogenic fermentation, acetogenesis, and methanogenesis. Figure 1 presents a scheme representing the main anaerobic degradation pathways in liquid manure storage.

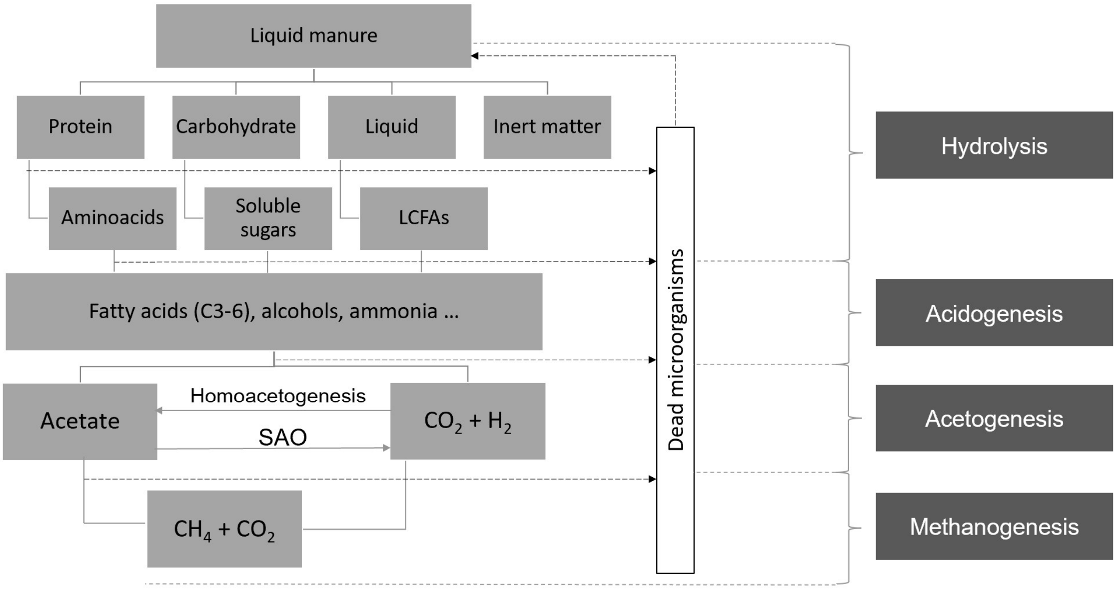

Figure 1 – The main degradation pathways in AD of liquid manure. Adapted from Dalby et al. (2021) and Wu et al. (2017).

During hydrolysis, large organic molecules such as proteins, carbohydrates, and lipids are broken down into simpler compounds, such as sugars, amino acids, and fatty acids, through the action of a diverse community of microorganisms that include bacteria, fungi, and protozoa (Harirchi et al., 2022; Prabhakaran et al., 2016). The microorganisms produce enzymes (amylases, proteases, and lipases) that, using water as the reactant, dissociate the large organic molecules in manure into simpler compounds, which other microorganisms can absorb for further digestion (Uddin & Wright, 2022).

The simpler compounds formed in hydrolysis then undergo further decomposition in the second stage, acidogenesis, where acid-forming bacteria convert them into organic acids (such as acetic acid, propionic acid, and butyric acid) alcohols, and CO_2 (Ai et al., 2018). The primary bacteria involved in acidogenesis are called acidogenic bacteria; they use various metabolic pathways to produce organic acids (Wainaina et al., 2019).

In the process of acetogenesis, specific bacteria use the organic acids generated in the preceding stage and transform them into acetic acid, hydrogen, and CO_2 through various biochemical pathways (Angelidaki et al., 2011; Müller et al., 2010). These pathways, including the Wood-Ljungdahl and acrylate, are complex processes involving multiple enzymatic reactions that convert compounds like CO_2 or pyruvate/lactate into acetic acid.

CH_4 production during methanogenesis occurs through complex biochemical reactions involving various enzymes and metabolic pathways. The methanogenesis process can generally be divided into two main routes: acetoclastic and hydrogenotrophic methanogenesis (Khanal, 2009). In acetoclastic methanogenesis, acetic acid is converted directly into CH_4 and

CO_2 by a group of methanogenic archaea known as acetoclastic methanogens. In hydrogenotrophic methanogenesis, hydrogen and CO_2 are converted into CH_4 and water by a different group of methanogenic archaea known as hydrogenotrophic methanogens.

Methanogens are more sensitive to environmental factors such as pH, temperature, and substrate availability than other microorganisms involved in the process, like hydrolytic and acidogenic bacteria (Dalby et al., 2021; Habtewold et al., 2018; Hou et al., 2017).

The AD is the process that leads to CH_4 production during manure storage, however during liquid manure storage, aerobic processes also occur, particularly at the surface where there is exposure to air; moreover, air can be present in bulk due to rain and mixing, pumping, emptying, and filling operations (Blanes-Vidal et al., 2012; Dalby et al., 2021). Nevertheless, it is considered that in the absence of storage, the GHG emissions associated with manure handling can be disregarded (Rotz, 2018).

The presence of air can include the activity of aerobic bacteria that break down organic matter through aerobic decomposition, which produces CO_2 and other by-products (Møller et al., 2004a; Sommer et al., 2000). During the initial storage stages, aerobic bacteria can break down organic compounds into simpler molecules, generating some heat. As oxygen is depleted, AD becomes dominant, which is a more efficient process for CH_4 formation and is the primary process during manure storage (Girard et al., 2013).

Considering the manure management phases before it reaches biogas facilities or in farms where manure is stored without such facilities, it is predominantly understood that methanogens in liquid manure originate from the gut of the animals (Dalby et al., 2021; Ozbayram, 2020; Söllinger et al., 2018). Nonetheless, due to diverse environmental influences, methanogen communities undergo constant adaptations leading to shifts in their abundance, activity, and taxonomy (Dalby et al., 2021; Habtewold et al., 2017; Ozbayram, 2020). Studies show that in relatively fresh liquid manure, methanogenic communities might not be prevalent while CO_2 production continues through fermentation, thus, resulting in lower CH_4/CO_2 ratios (Sommer et al., 2017). Furthermore, the microbial communities dominating aged manure differ from those in fresh manure (Barret et al., 2013; Dalby et al., 2021; Habtewold et al., 2018a).

Factors such as the availability of substrates, inhibitors, physical conditions, and the duration of storage significantly impact the prevailing microbial community in AD (Dalby et al., 2021). In farms without a biogas facility, the remaining liquid manure in storage after clearing can act as an inoculum, leading to a rapid rise in CH_4 production compared to scenarios without an inoculum (Sokolov et al., 2019). Conversely, in biogas facilities, the inoculum is either added or retained during the operation. The BMP test, a well-established laboratory method, can evaluate the potential for CH_4 and biogas production of animal manure during AD (VDI, 2016).

2.4 – Influencing factors on CH_4 formation and emissions during manure storage

The majority of the studies investigating the degradability of manure due to storage usually focus on the increase of CH_4 production during biogas operation, and less research has been done on the CH_4 formation and emission during storage (Batstone et al., 2002; Dalby et al., 2021). According to the Dalby et al. (2021) study, anaerobic conditions and degradable organic matter are the main factors contributing to CH_4 emissions from stored liquid animal manure. As liquid manure is stored for months, the combination of these factors results in the production of CH_4. Among the factors influencing the CH_4 formation during liquid manure storage, it is possible to mention the storage temperature, the changes in the chemical composition of manure, and the storage duration.

2.4.1 – Storage temperature

On farms, manure is typically stored in areas not sheltered from climatic elements, thus directly experiencing the impact of ambient temperature. The storage temperature can substantially influence the biochemical processes occurring during manure storage, including CH_4 formation and emission (IPCC, 2019). Consequently, understanding the effect of the storage temperature on the CH_4 emissions during manure storage provides crucial insights for more effective mitigation strategies.

Earlier research has established a connection between storage temperature and CH_4 emissions from stored manure. In a study by Sommer & Husted (1995), it was measured CH_4 emissions from dairy and pig manure storages for one year; they verified that the emission rates increased significantly with storage temperatures and that the presence of a natural crust reduced CH_4 emissions, but even the crust effect declined with the rise of temperature. The lab-scale studies made by Massé et al. (2003) and Massé et al. (2008) assessed the impact of slurry characteristics, temperature, and storage duration on CH_4 emissions from dairy cow and swine manure. These studies concluded that CH_4 emissions depend on the interaction between these factors and recommended short storage periods and the use of below-ground storage tanks to maintain the temperature lower during warmer seasons in Canadian farms.

More recently, a study by Feng et al. (2018) stored liquid dairy and pig manure for 52 days at temperatures from 15 to 30 °C before biogas production. They concluded that the CH_4 emissions during storage were substantially higher for slurries stored at 30 °C. In a study with cattle manure, Im et al. (2020) investigated the effects on CH_4 emissions and the subsequent biogas production potential according to the storage temperature (ranging between 15 to 35° C) for 80 days. Their study showed that CH_4 emissions were higher when the temperature reached 35° C, while emissions were almost halved at temperatures below 20 °C. Cárdenas

et al. (2021) also studied the CH_4 emissions from stored dairy slurry in different seasons. The sample stored during summer reached a cumulative emission of 0.148 $kgCH_4\ kg_{VS}^{-1}$, whereas the winter sample reached 0.0011 $kgCH_4\ kg_{VS}^{-1}$, showing that temperature and storage duration are essential, influential factors on CH_4 emissions from the slurry.
These studies have found that lower storage temperatures can lead to a significant reduction in emissions. However, there are still gaps regarding our understanding of emissions under psychrophilic conditions (between 5 °C and 25 °C), which are more common for manure storage Dalby et al. (2021). Moreover, prior studies provide limited insights into the interactions between fermentation products such as VFA and storage temperature (Feng et al., 2018; Im et al., 2020). While the significant impact of temperature on CH_4 formation and emission is widely recognized, a more thorough understanding of the specific effects of storage temperature and seasonal variations on manure storage is needed.

2.4.2 – Manure chemical composition

The chemical composition of manure significantly dictates the CH_4 production and formation during storage. Usually, scientific literature evaluates manure chemical composition in terms of DM, VS, pH, and TAN, sometimes is possible to find the VS components represented by VFAs, crude protein, crude fat, non-fiber carbohydrates (NFC), cellulose, hemicellulose, and lignin. Manure chemical composition varies over the storage time due to the biochemical process leading to organic matter degradation. The significative changes occur because of the AD process that naturally occurs during the storage process and the exposition to environmental conditions, for instance, precipitation, solar irradiation, and ambient temperature. The animal diet and digestion system are other factors that influence manure's chemical composition. Generally, CH_4 emissions are higher from pig manure than from cow manure (Feng et al., 2018).

As an example of how connected and essential the changes in the manure chemical composition for CH_4 emissions from manure storage, the study by Massé et al. (2008) stored two different dairy manure samples (A and B) for 370 days at 20 and 10 °C, initially, they verified that manure B had notably lower levels of VFAs and a higher pH compared to manure A. The biogas generated from manure B stored at both tested temperatures consisted of 53 and 70% CH_4, whereas manure A reached 10 and 58% CH_4. They concluded that this difference was due to Manure B already having an active microbial community capable of metabolizing organic matter and converting VFAs into CH_4. Besides, Manure A exhibited a higher DM concentration compared to Manure B, potentially compounds like ammonia or VFAs at levels that could suppress methanogenic activity, in line with the findings of their previous study Massé et al. (2003). A derivate study by Wood et al. (2012) also showed that CH_4 emissions adjusted by the VS decrease exponentially as DM content increase. On the other hand, Habtewold et al. (2017) verified that cumulative CH_4 emissions from dairy cow manure were reduced by ~70% as the DM content decreased from 9.5 to 0.3%. The seeming contradiction among these studies underscores the imperative for additional research to improve our comprehension of the role of chemical composition in CH_4 emissions during manure management.

In biogas production, specific chemical components, such as carbohydrates, protein, and fats, typically result in amplified biogas production due to these compounds' biodegradability during AD (Raposo et al., 2020). Nonetheless, alongside these benefits, some components can impede CH_4 formation. For example, protein decomposition generates ammonia which, when present in high concentrations, can hinder the activity of methanogenic bacteria (Yang et al., 2018). Regarding the fibrous content in manure, lignin is known to be non-degradable in anaerobic environments because the extracellular enzymes require oxygen to depolymerize (Khan & Ahring, 2019). This fact also influences the degradability of cellulose since hemicellulose and lignin protect cellulose from being degraded (Liao et al., 2005).

It is essential to consider the AD that occurs due to animal management practices and storage conditions, and understanding how these practices might alter the chemical composition of the manure and hence impact the CH_4 formation during storage is also vital (Johannesson et al., 2017). Besides, statistical models are available to predict manure's BMP (Triolo et al., 2011), but no study has been dedicated to verifying the BMP of stored manure samples.

2.4.3 – Storage period

The length of manure storage significantly influences CH_4 formation and emission (Johannesson et al., 2017; Sawamoto et al., 2016). Initial stages of storage are characterized by a higher rate of CH_4 formation, attributable to the quick breakdown of easily degradable organic matter such as sugars and amino acids (Aguirre-Villegas & Larson, 2017; B. Amon et al., 2006; VanderZaag et al., 2018). These elements represent the readily available fraction of the manure, and their degradation begins soon after storage. As the storage period extends, the focus of degradation shifts towards more complex organic compounds, such as fibers, fats, and proteins. The AD of these last compounds tends to occur slower, leading to a gradual decrease in CH_4 formation over time, affecting the subsequent CH_4 emissions (Johannesson et al., 2017).

Some studies have examined how storage length influences CH_4 production in manure storages. Gopalan et al. (2013) evaluated the BMP of beef feedlot manure as it aged on pads and in stockpiles from Canadian farms, examining the changes in degradable and non-degradable organic fractions. They discovered that BMP generally significantly decreased with aging for both manure types. In contrast, Johannesson et al. (2017) conducted a comprehensive study on the biogas properties of outdoor-stored dairy cattle manure under temperate climate conditions but did not evaluate the degradation process occurring between different stages of the manure management chain. However, they confirmed that storage significantly impacts solid concentrations and decomposition rates. Lastly, in a non-peer-reviewed article de Buisonjé & Verheijen (2014) assessed the differences in BMP and organic matter composition among pig slurries at different stages of the manure management chain. Their findings indicated a 74% decrease in CH_4 potential when comparing pig manure samples stored for 32 and 120 days.

Besides, some studies verified that the degradation of manure chemical composition over time is usually significantly connected with the storage temperature. In the Sawamoto et al. (2016) study, dairy cow manure from two farms was incubated at different temperatures (5, 15, 25, and 35 °C) and with varying dilution rates. They verified the CH_4 production during approximately 210 days concluding that after this period, the CH_4 production for manure stored at 5 °C was insignificant, whereas 25 and 35 °C were about 2 and 2.5 higher than at 15 °C, confirming that when exposed to higher temperatures, it produces CH_4 at higher rates, i.e., taking less time to reach CH_4 produced.

A significant knowledge gap lies in identifying how the duration of manure storage influences CH_4 formation in commercial farms since storages receive a continuous influx of recently excreted manure in the storage tanks until it is removed (Sawamoto et al., 2016). While it is recognized that manure's composition changes over time due to ongoing biological activity (Johannesson et al., 2017), the specifics of how these alterations influence the loss of BMP across varying storage periods remain unclear.

Chapter 3: Publications

3.1 Publication "A"

Methane Emissions from Livestock Slurry: Effects of Storage Temperature and Changes in Chemical Composition

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Methane Emissions from Livestock Slurry: Effects of Storage Temperature and Changes in Chemical Composition

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Abstract: Livestock production contributes to releasing methane into the atmosphere. Liquid manure management offers significant opportunities to reduce these emissions. A better understanding of the factors controlling methane emissions from manure is necessary to select effective mitigation strategies. Our study aimed to identify the influence of storage temperature and the associated change in chemical composition on methane emissions from dairy and fattening pig manure. Storage temperature affects microbial activity and induces changes in chemical composition that are key influences in methane emissions. Dairy and fattening pig manure samples were stored at five different temperatures (5–25 °C) for 90 days in a laboratory-scale experiment to measure the methane production. The chemical composition of the slurry samples was analyzed, and the biochemical methane potential (BMP) tests were performed before and after storage. For pig manure stored at 25 °C and 20 °C, methane emissions accounted for 69.3% and 50.3% of the BMP, respectively. Maximum methane emissions for dairy slurry were observed at 25 °C but remained at a low level. Analyses of the accumulation of volatile fatty acids (VFAs) during storage are presented in few studies, this work revealed a potential inhibition of methane production, where the accumulation of VFAs was most elevated in samples stored at 20 °C and 25 °C. This partly counteracted the increase in methane emissions expected from the higher temperatures. The degree of VFA and dissociated fatty acids accumulation in dairy cattle slurry should be assessed for more accurate estimations of methane emissions from slurry stores.

Keywords: GHG emissions; manure management; pig manure storage; dairy manure storage; biochemical methane potential

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1. Introduction

The Paris Agreement aims to limit global warming to well below 2 °C or preferably even to below 1.5 °C, but this goal cannot be achieved without economic and social transformation [1]. Triggered by this decision, nearly half of the European Union countries have prepared national climate laws to change their economic activities toward net-zero emissions [2]. Among the sources of greenhouse gas (GHG) emissions are agriculture and land use, and in 2019, these sources contributed to approximately 20% of these emissions (11 billion tons of CO_{2eq}) [3]. Livestock production systems account for 40% of these emissions, mainly from enteric fermentation and manure management. Methane (CH_4) accounts for 70% of GHG emissions from manure management [4]. Considering a time horizon of 100 years, non-fossil CH_4 has an average global warming potential (GWP) 27.2 times higher than CO_2; given a 20-year time horizon, the average GWP of non-fossil CH_4 is 80.8 [5]. Although CH_4 does not stay in the atmosphere as long as other GHGs, it still contributes substantially to the warming effect because it is continuously produced and emitted in great amounts. Consequently, the International Panel on Climate Change (IPCC) argues in their global warming scenarios that strong and sustained reductions in CH_4 emissions would limit the warming effect [5]. For the first time, the agricultural sector is mandatorily required to reduce its GHG emissions. In Germany, the Climate Action Plan 2050 states that the agricultural sector should reduce emissions by 31 to 34% of the 1990 levels by 2030 [6].

Manure management offers a range of technically feasible options for emission mitigation that can be implemented on commercial farms [7]. Manure consists mainly of a mixture of feces and urine, but its composition can vary widely due to different proportions of water, feeding leftovers, bedding material, and dust [8]. Animal diet and performance also have a crucial impact on manure composition and on the consequent emissions from manure management [9]. Manure management systems are different in the world regions but can be defined as a set of activities that include collecting, handling, storing, treating, and utilizing manure on-farm [10,11]. According to the latest estimations, liquid manure management systems are present in 32% and 38% of the dairy farms in Western Europe and North America, respectively [12,13]. Liquid manure management also dominates on pig farms in these regions [14,15]. For both animal categories, liquid manure has less than 15% dry matter (DM), allowing it to be transported by pumping systems [16]. However, liquid systems lead to an increase in methane emissions when compared with solid systems [15], because methane results from anaerobic processes, which are favored in liquid systems with low oxygen availability [17]. First, the organic matter is transformed into low-molecular-weight components such as volatile fatty acids (VFAs), which are further processed to produce methane and carbon dioxide. According to the review carried out by Kupper et al. [18], the average methane emissions were 1.21 $kg_{CH4}\ kg_{VS}^{-1}\ h^{-1}$ and 1.84 $kg_{CH4}\ kg_{VS}^{-1}\ h^{-1}$ for cattle and pig slurry stored in tanks, respectively (kg_{VS} stands for kilogram of volatile solids). Most of the literature presents environmental conditions, most notably the temperature, manure management on the farm, and chemical composition, as the main factors influencing methane emissions from liquid manure storage. Some studies verified the seasonal effect of temperature and showed that seasonal average temperatures above 15 °C lead to higher methane emissions [19–22]. There is a consensus that residual old manure left after the removal of slurry hosts adapted microorganisms that cause immediate production of methane when inoculating fresh manure [21,23,24]. The abundance of easily degradable carbon in fresh manure is considered to increase methane emissions from slurries [25–27].

The IPCC Guidelines for National Greenhouse Gas Inventories recommend methane conversion factors (MCFs) to estimate emissions from different manure management systems and climate conditions. MCF reflects how much of the theoretical methane production potential of the volatile solids content in a substrate (B_0) will be emitted. Experimentally, the B_0 values can be determined with the biochemical methane potential (BMP) test [28]. The animal category and diet influence B_0 values. For instance, cow manure tends to have a higher dry matter and fiber content than pig manure [18]. B_0 values for dairy cows are 240 $L_NCH_4\ (kg_{VS})^{-1}$ and for pigs 450 $L_NCH_4\ (kg_{VS})^{-1}$ [15], where normal liter L_N is a unit of mass for gases equal to the mass of 1 L at a pressure of 1 atmosphere and at a standard temperature of 0 °C. For dairy cow and fattening pig liquid systems, MCF ranges from 71 to 80% when the manure is stored under warm conditions (26 °C to 28 °C), much higher than the factors from 17% to 25% for storage at cool temperatures (10 °C to 14 °C) [15].

In the specific case of the calculations of the MCFs used for manure management methane emissions, the IPCC guidelines Tier 2 consider all countries from Western Europe sharing the same manure characteristics, e.g., B_0 and volatile solids (VS) for the animal categories, differentiating each country by the average storage temperature, manure management system and retention time. This methodology reports an uncertainty range of 20% for the emission factors [15], but ignoring local practices could lead to inaccurate decisions on mitigation strategies [29]. To improve the quality of the obtained data, countries are advised to develop and use a Tier 2 method with MCF, B_0, and VS values that reflect specific local conditions [15]. Many studies have suggested measures and methodologies to improve the accuracy of national inventories [21,25,30–34].

As found by Dalby et al., information is scarce about the effect of temperature on methanogens in manure stored under psychrophilic conditions (between 5 °C and 25 °C), although this is the most common storage temperature range [30]. In this direction, Im et al. investigated the temperature range from 15 to 35 °C for stored solid cattle manure for 80 days [35]. Their study showed that the highest CH_4 emissions occurred at a storage temperature of 35 °C, while emissions were almost halved at temperatures below 20 °C [35]. Feng et al. stored liquid dairy and pig manure for 52 days at temperatures from 15 to 30 °C before biogas production [36]. They concluded that the methane emissions during storage were substantially higher for slurries stored at 30 °C [36]. Additionally, Cardenas et al. studied the methane emissions from stored dairy slurry in different seasons [20]. The sample stored during summer reached a cumulative emission of 0.148 kg_{CH4} kg_{VS}^{-1}, whereas the winter sample reached 0.0011 kg_{CH4} kg_{VS}^{-1}, showing that temperature and storage duration are important influential factors on methane emissions from the slurry. These studies confirm that a more in-depth understanding of the influence of slurry storage temperature on the level of methane emissions is needed. It is necessary to assess the temperature influence on methane emissions from manure management that reflects the temperature storage range considering a country specific approach. In addition, other products that are formed during microbial degradation processes in the course of slurry storage can influence methane release, while formation of these products also depends on storage temperatures. Studies that take into account interactions between fermentation products such as volatile fatty acids and storage temperature are limited. Novelty of the present study lies in a detailed investigation of the effects of storage temperature on methane emissions accompanied by changes in chemical composition during storage of dairy cow and fattening pig manure, and subsequent effects on the biochemical methane potential.

In this work, it is evaluated if the storage temperature has a direct effect on the microbial activity leading to methane emissions and, in addition, whether it can have an indirect effect through relevant changes in the chemical composition, especially the accumulation of VFAs during storage of dairy and fattening pig liquid manure. Furthermore, results are expected to confirm the MCF values calculated from the IPCC methodology for different storage temperatures. Hence, the present study investigated the influence of storage temperatures between 5 and 25 °C on CH_4 emissions from liquid dairy manure and fattening pig manure to enhance the understanding of methane emissions during the slurry storage period.

2. Materials and Methods

To answer the research question, primary quantitative data for the cumulative methane yield from pig and cattle slurry samples were collected in an experimental approach, where slurry samples were incubated under 5 different controlled temperatures (5–25 °C) for 90 days. Then, an inoculum was added to the substrates to assess the residual BMP under anaerobic conditions at 37 °C.

2.1. Dairy Manure, Fattening Pig Manure

Dairy manure samples were collected at the Educational and Experimental Institution for Animal Breeding and Husbandry-LVAT, Groß Kreutz, Brandenburg, Germany. The barn is a free-stall dairy barn with dimensions of 36 m × 18 m, that keeps 51 Holstein Friesian cows. The floor of the barn is approximately 1/5 slatted floor and 4/5 solid floor. The lactating cows are typically fed a mixture of maize and grass silages, rye forage, alfalfa, straw, rapeseed cake, and soybean meal. The chemical composition of the feed is estimated as 13.0% crude protein, 20.8% crude fiber, 3.8% crude fat, and 5.9% crude ash, and the total energy content is 18.8 MJ/kgDM. A mechanical system of scrape alleys cleans the floor and moves manure to a pumping pit approximately once every hour. The sample collection was conducted on 28 September 2020. Using a shovel, a ten-liter sample of fresh manure was collected from 10 different points on the cow alley in a way that both urine and feces were collected.

Fattening pig manure samples were collected at the Educational and Experimental Institution for Animal Breeding and Husbandry, LVAT Ruhlsdorf, Brandenburg, Germany. The compartment of the barn where samples were taken presents conventional housing conditions (slatted floors) with dimensions of 15 m × 10 m, where 19 fattening pigs with an age of approximately 170 days were kept. Fattening pigs are typically fed a ration of rye, triticale, barley, soybean meal, rapeseed meal, peas, and sunflower meal. The chemical composition of the ration is 14% crude protein, 4.7% crude ash, 4% crude fiber, and 1.9% crude fat, and the total energy content is 12.8 MJ/kgDM, where DM stands for dry matter. The slatted floor drains manure to a preliminary storage area under the barn. Manure remains for approximately two weeks in the preliminary storage, after which the manure is directed to an outdoor storage area. Two-week-old manure samples were collected on 19 November 2020. The samples were taken from three points within the preliminary storage under the floor using a pump. Twenty liters of manure were collected.

Immediately after collection, the samples were stirred, and the temperature was measured. The samples were kept in cooling boxes and transported to the biogas laboratory at the Institute for Agricultural Engineering and Bioeconomy. In the laboratory, subsamples for the storage experiment were kept in insulated cooling boxes for approximately 12 h until the experiment was started. Other subsamples were stored frozen at −18 °C before chemical analyses were carried out.

2.2. Physical–Chemical Analysis

The temperature and electrical conductivity of manure samples were measured immediately after sampling on the farm with a thermometer (Hamster ET2, Elpro, Buchs, Switzerland) and a handheld pH meter (Multiline P3 pH/LH, WTW, Weilheim, Germany), respectively. The pH value was measured directly in the sample by immersing the electrode (Sen Tix 81, WTW, Weilheim, Germany) [37]. Fresh manure samples were stored at −18 °C and gently defrosted before the chemical analysis and the batch anaerobic digestion tests. The dry matter (DM) content was verified by drying, at 105 °C, until a constant weight was reached; subsequently, the ash content was determined by dry combustion at 550 °C in a muffle furnace (CWF 1100, Carbolite Gero GmbH & Co. KG, Neuhausen, BW, Germany) [38]. The contents of alcohols (C1 to C4) and volatile fatty acids (C2 to C6) were determined by cold-water extraction, followed by gas chromatography (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a PERMABOND FFAP capillary column (Machery-Nagel GmbH & Co. KG, Düren, Germany) and a flame ionization detector [38]. The sum of volatile acids is given as acetic acid equivalent (AAeq). In this work, a methodology described by Weissbach and Kuhla was used to correct DM values (DM_{co}) and VS values (VS_{co}) for losses of volatile compounds during oven drying considering the pH value and the content of volatile components [39].

The content of carbon, nitrogen, sulfur, and hydrogen was verified employing an elemental analyzer (Vario EL, Elementar Analysensysteme GmbH, Hanau, Germany) by using the principle of catalytic raw combustion under oxygen supply and higher temperatures [38]. The content of crude protein was determined by multiplying the elemental nitrogen detected by 6.25. The crude fat level was verified gravimetrically, following the Weibull–Stoldt method, after acidic hydrolysis using 3N hydrochloric acid and by extraction with petroleum ether, at 90 °C, for 1 h using the AnkomXT10-Extractor (Ankom Technology Corp., Macedon NY, USA). Analysis of acid detergent fiber (ADF) and neutral detergent fiber (NDF) were conducted following the methodology of Van Soest et al. (1991), and the Ankom 2000 fiber analyzer system with filter bag technology (Ankom Technology Corp., Macedon, NY, USA) was employed [40]. The content of acid detergent lignin (ADL) was measured gravimetrically after the addition of 72% sulfuric acid to the bag from ADF analysis for 3 h, drying the sample, and incinerating the sample in a muffle furnace, at 600 °C, for 2.5 h [41,42].

Total ammoniacal nitrogen (TAN) was converted to ammonia by magnesium oxide and, using steam distillation (Vapodest 20 Gerhardt, Apeldoorn, The Netherlands), transferred to a distillation receiver containing boric acid [38]. The chemical oxygen demand (COD) analyses were carried out following standard methods [37].

2.3. Experimental Procedures

Figure 1 shows a scheme with the sequence of the experiments and analyses executed during this study.

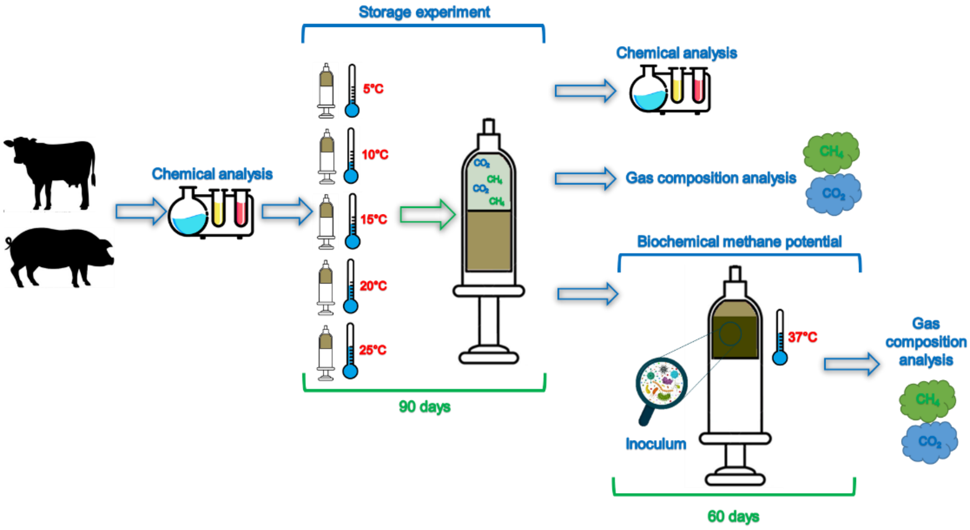

Figure 1. Scheme of the experimental design used in this study.

2.3.1. Storage Experiments

The storage experiments were conducted using freshly sampled manure. The cow manure experiments started one day after collection, and pig manure storage started on the same day. The experiments were set up under anaerobic conditions according to the methodology of [43]. Approximately 60 g of the manure samples was placed in a 100 mL glass syringe. After weighing, the syringes were closed with the piston, and the inside air was withdrawn until the solid substrate reached the outlet, ensuring anaerobic conditions. Between the plunger and the syringe, silicone paste ensured a gas-tight seal. The samples were placed in incubators where constant temperatures were maintained for 90 days. The temperatures chosen to conduct the tests were 5 °C, 10 °C, 15 °C, 20 °C, and 25 °C. These temperatures were chosen to cover the most common range for outdoor storage in temperate climates [21]. For each type of manure, storage at different temperatures was conducted at the same time, but in different incubators; 3 replicate determinations were performed for each temperature. During the incubation, the gas volume was determined by measuring the displacement of the plunger with a ruler in millimeters at least 5 times per week. The volumes of gas production obtained during the experiments were converted to standard temperature and pressure conditions (dry gas, 0 °C, 1013 hPa) and divided by the mass of volatile solids of the substrate. The composition of the produced gas was measured with a gas analyzer system with CH_4 (Advanced Gasmitter, Sensors Europe GmbH, Erkrath, Germany) and CO_2 (MonoGas Analyzer, Pronova Analysentechnik GmbH & Co. KG, Berlin, Germany) infrared sensors. During the experiment, gas analysis was performed whenever the substrate produced approximately 30 mL of gas, less often for the samples kept at 5 °C (once in the whole period, for dairy and pigs) and more often for the samples kept at 25 °C (nine times for dairy and seven times for pig).

Gas composition and volume were measured for 90 days. After the experiment, each sample was divided into two subsamples, one subsample directed to chemical analyses to verify composition changes after storage and the other subsample used to assess the residual BMP. Agitation was performed during the volume and gas composition measurements. Methane production was expressed in terms of L_NCH_4 per kg of VSco ($L_Nkg^{-1}{}_{VSCO}$).

Comparison with IPCC Methodology Tier 2

The experimentally obtained values for MCF during the storage of manure at different temperatures were compared with the MCF values suggested by the IPCC guidelines. The comparison was not possible for storage at 5 °C, since the guidelines are not designed for that temperature. The experimentally determined MCF values were from liquid dairy and fattening pig manure at the defined storage temperature. The MCF values obtained from the IPCC guidelines were those representing of Western Europe, which were converted to ($L_Nkg^{-1}{}_{VSC}$) [15].

2.3.2. Biochemical Methane Potential Tests

The BMP test is a technique used to assess the methane production potential and the biodegradability of biomass. The BMP test was performed according to the standard procedure [28]. The inoculum with active methanogenic microorganisms was a mixture of digestate from laboratory batch experiments and two large-scale agricultural biogas plants that were operated with livestock manure, energy crops, and crop residues as feedstock under mesophilic temperature conditions. This slurry was sieved with a standard sieve (mesh size 3 mm) to avoid large particles and then stored in a tank, at 37 °C, and stirred once a week. Inoculum was used to evaluate the biochemical methane potential of cow manure (DM 5.71%FM, VS 64.98%DM) and pig manure (DM 3.94%FM, VS 65.75%DM) after the storage experiment.

The syringes were filled with 30 g of inoculum and a quantity of substrate that kept the ratio of volatile solids between inoculum and substrate between 2 and 3. As in the storage experiment, in the BMP test, the displacement of the piston was recorded. The manure of each replicate of the storage experiment was analyzed separately for its methane production potential. In addition, 3 replicates with inoculum only were incubated as blank samples, and 3 replicates with cellulose as substrate were tested to verify the activity of the inoculum. The gas composition was measured periodically, approximately twice a week in the first 14 days and once per week thereafter. The batch tests were completed when the daily rate of biogas during three consecutive days was <0.5% of the total biogas produced up to that time [28]; for the tests conducted, 40 to 60 days were required depending on the sample. The volume of the biogas produced in each sample was corrected for the gas volume produced by the inoculum. Agitation was applied during the volume and gas composition measurements.

2.4. Data Analysis

2.4.1. Statistical Analysis

The significance of differences between the temperature of storage and the dependent variables (methane emissions from manure stored and chemical composition, i.e., DM, VS, pH, alcohol content and VFA content) were verified by Welch's analysis of variance (ANOVA). Additionally, Welch's ANOVA was applied to verify the effects of the storage temperature on the kinetic parameters of the equations. When significant effects were evident, the Games–Howell post hoc test, using the 0.05 p-level, was applied for multiple comparisons of means. The statistical analysis was performed using the software R [44], and the package stats version 4.0.2 was used for the kinetics analysis [44].

2.4.2. Kinetics Analysis

Kinetics analysis can reveal how fast the degradability of slurry occurs and whether the methanogenic community is well adapted to the environment. For the storage experiment, a logistic expression (Equation (1)) was used to regress the experimental methane production against time [45,46]. This expression estimates the half-life of the methane emissions, which means the time at which half of the potential methane is emitted. The curve obtained is symmetrical around the inflection point.

$$y(t) = \frac{y_m}{1 + exp[-R_m \cdot (t - t_{50})]} \tag{1}$$

where $y(t)$ is the cumulative specific methane yield at time t ($\mathrm{L_N CH_4 kg^{-1}{}_{VS}}$), y_m is the maximum specific methane yield at theoretically infinite digestion time ($\mathrm{L_N CH_4 kg^{-1}{}_{VS}}$), R_m is the maximum specific methane production rate ($\mathrm{L_N CH_4 kg^{-1}{}_{VS} day^{-1}}$), t is the time (days) and t_{50} is the half-life (days).

For the BMP experiments, the kinetics analysis was performed using a first-order differential Equation (2) and a modified Gompertz Equation (3). The first-order differential equation is used to model the degradability of substrates because it allows the estimation of the substrate degradation constant (*k*).

$$y(t) = y_m \cdot \left(1 - e^{(-k_1 t)}\right) \tag{2}$$

where $y(t)$ is the cumulative specific methane yield at time t ($\mathrm{L_N CH_4 kg^{-1}{}_{VS}}$), y_m is the maximum specific methane yield at theoretically infinite digestion time ($\mathrm{L_N CH_4 kg^{-1}{}_{VS}}$), t is the time (days) and k is the first-order constant ($\mathrm{day^{-1}}$).

The modified Gompertz equation allows us to estimate the lag phase time λ and the maximum specific methane production rate R_m [46]. The curve obtained has a fixed inflection point and is asymmetrical around the inflection point [47–49]. The negative lag times estimated from this equation were assumed to be 0 [50].

$$y(t) = y_m \cdot exp\left\{-exp\left[\frac{R_m \cdot e}{y_m} \cdot (\lambda - t) + 1\right]\right\} \tag{3}$$

where $y(t)$ is the cumulative specific methane yield at time ($\mathrm{L_N CH_4 kg^{-1}{}_{VS}}$), y_m is the maximum specific methane yield at theoretically infinite digestion time ($\mathrm{L_N CH_4 kg^{-1}{}_{VS}}$), R_m is the maximum specific methane production rate ($\mathrm{L_N CH_4 kg^{-1}{}_{VS} day^{-1}}$), λ is the lag phase, and t is the time (days).

3. Results and Discussion

3.1. Manure Characteristics

The physical and chemical characteristics of dairy and pig manure are presented in Table 1. The chemical composition of the samples was in the range typically reported in the literature [18]. The temperature in loco reflected the environmental conditions during sampling and the housing and manure management system of the farms. The dry matter values of dairy and fattening pig manure are comparable to the values shown in literature

[20,51]. The low dry matter content in pig manure was likely caused by the use of cleaning and drinking water in the animal houses and by the sample being taken from an intermediate storage, whereas the cow manure was taken from the barn floor [52]. Methane production occurs in a pH range from 6.5 to 8.5, with an optimum between 7.0 and 8.0 [53]. The pH value of the dairy manure samples was connected with slightly elevated concentrations of organic acids, but it is still in the range reported in other studies [21,32]. The pH of the pig manure samples were as well in line with values from the literature [31,54]. The most prominent fatty acid in both manures was acetic acid. Based on VS, the VFA content constituted 5% and 53% of the volatile solids in cow manure and pig manure, respectively. The pH value and the concentration of VFAs interact and may result in an ''inhibited steady state'' in well-buffered systems, where methane formation occurs stably but with a low methane yield [55]. According to Drosg et al., if the VFA concentration is above 4.0 g/L in mesophilic anaerobic digestion plants, this VFA concentration is typically regarded as an indicator of process imbalance, and therefore, inhibition of methane production occurs [56].

Table 1. Physicochemical composition of the manure samples collected from the dairy cow and fattening pig barns.

Animal Category	Dairy Cow Manure	Fattening Pig Manure
Temperature in loco (°C)	16.0	18.9
EC (mS/cm)	9.88	24.1
DM (in %FM)	11.74	1.68
VS (in %DM)	86.07	58.13
VS (in %FM)	10.10	0.98
pH	6.61	7.79
TAN (in mg/kgFM)	866.9	2578.1
Methanol (in g/kg)	0.06	0.00
Ethanol (in g/kg)	0.09	0.00
Acetic acid (in g/kg)	4.06	2.4
Propionic acid (in g/kg)	0.89	0.34
i-Butyric acid (in g/kg)	0.05	0.04
Butyric acid (in g/kg)	0.32	0.00
i-Valeric acid (in g/kg)	0.05	0.06
Valeric acid (in g/kg)	0.06	0.00
Sum of VFA as acetic acid (in g/kg)	5.10	2.74
COD in mg/kgFM	111,729.2	8400.8
Crude fat (in %DM)	1.52	1.34
NDF (in %DM)	54.29	3.21
ADF (in %DM)	32.81	1.40
ADL (in %DM)	9.71	0.64
N (in %DM)	2.46	2.68
C (in %DM)	44.2	30.54
S (in %DM)	0.24	1.35
H (in %DM)	3.87	2.56
Crude protein (in %DM)	15.38	16.75

The content of ashes in dairy manure is comparable with many other individual studies [57]; for pig manure, the content of ashes is in accordance with Kupper et al., 52.6 %DM for manure stored in a lagoon [18]. The content of TAN and crude protein are similar to values for dairy manure and pig slurry stored in tanks [18]. The values for crude fat and fibers are reported in a few individual studies and cannot be compared.

3.2. Methane Emissions during Storage

3.2.1. Dairy Manure

The cumulative methane emissions from cow manure stored under different temperature conditions are presented in Figure 2. The average coefficient of variation was 10.6%. The average methane concentration in biogas from dairy manure was 15.3%. This low CH_4/CO_2 ratio is supported by Sommer et al., who affirm that fresh slurry does not have an active methanogenic community still, and then mostly CO_2 is produced [58]. The maximum methane release occurred during storage, at 20 and 25 °C, reaching 4.90 $L_N CH_4$ $(kg_{VSCO})^{-1}$, the minimum of 0.23 $L_N CH_4$ $(kg_{VSCO})^{-1}$ was determined for storage, at 5 °C. In comparison, the highest cumulative methane emissions found in these experiments corresponded to only approximately 2% of the biochemical methane potential of lactating cow manure reported in other studies [35,59]. BMP measurements were conducted to measure the maximum methane production from these samples.

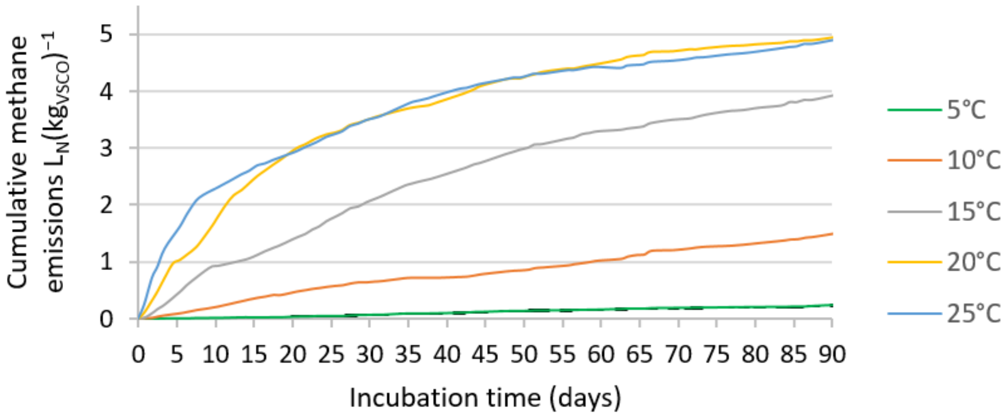

Figure 2. Cumulative methane emissions during the storage of dairy manure at different temperatures.

Table 2 shows the chemical composition of the samples after 90 days of storage. The results revealed an increase in the concentration of VFA in the samples and a decrease in pH when compared with the initial values; these trends were enhanced at higher storage temperatures. The concentration of VFAs was well above the inhibition levels for methanogens [56]. This storage effect was also observed in previous research. Massé et al. evaluated the methane emissions from 100 kg dairy manure stored in storage pilots for one year, the initial pH of the sample was 6.41 [32]. The dry matter content of samples A and B was 10.4% and 7.1%, respectively, and the storage temperatures were 10 °C and 20 °C. After 90 days, only sample B had significant methane emissions; during this period, the VFA concentration increased for sample A and decreased for sample B. They concluded that because sample A was more concentrated than sample B, it may have components such as VFAs in concentrations that could inhibit methanogenic activity. In other publication Massé et al. stored dairy manure, collected under the slatted floor, in a tank of 232 L capacity, with low (4.2%FM) and high (9.2%FM) total solids (TS) content for 272 days, at 10 °C and 15 °C [60]. They observed that dilution and higher temperature contributed to higher methane content in the gas from the low TS sample (approximately 70%) than from the high TS (approximately 25%). These studies confirm that the low methane emissions observed may be related with dry matter content around $10\%_{FM}$ and the concentration of VFAs that inhibit methane emissions.

Table 2. Chemical composition of dairy and fattening pig manure samples stored at temperatures of 5, 10, 15, 20, and 25 °C. g$_{AAeq}$ stands for grams of acetic acid equivalent. The significance differences according to the Games–Howell test are reported through the indices "^{a}", "^{b}", "^{c}", "^{d}", "^{e}", "^{ab}", "^{abc}", "^{bc}".

Dairy Manure					
Storage temperature (°C)	5	10	15	20	25
DM (in %$_{FM}$)	12.04 ± 0.37	12.44 ± 0.36	11.74 ± 0.20	11.34 ± 0.22	11.46 ± 0.37
VS (in %$_{DM}$)	85.44 ± 0.26	86.10 ± 0.62	84.98 ± 0.07	84.58 ± 0.56	84.72 ± 0.89
VS (in %$_{FM}$)	10.29 ± 0.35	10.71 ± 0.39	9.98 ± 0.16	9.59 ± 0.21	9.71 ± 0.39
pH-average	6.52 ± 0.16 ^{a}	6.26 ± 0.08 ^{ab}	5.97 ± 0.08 ^{bc}	5.90 ± 0.11 ^{bc}	5.65 ± 0.20 ^{c}
Alcohols (in g/kg)	0.24 ± 0.06	0	0	0	0
Acetic acid (in g/kg)	5.95 ± 0.66 ^{b}	7.95 ± 0.20 ^{ab}	8.63 ± 1.05 ^{ab}	9.81 ± 1.38 ^{ab}	10.31 ± 1.57 ^{a}
Propionic acid (in g/kg)	2.31 ± 0.06 ^{b}	2.46 ± 0.12 ^{b}	2.78 ± 0.44 ^{ab}	3.07 ± 0.34 ^{ab}	3.28 ± 0.43 ^{a}
Butyric acid (in g/kg)	1.93 ± 0.12	2.59 ± 0.30	3.07 ± 0.62	2.43 ± 0.31	2.98 ± 0.72
Valeric acid (in g/kg)	0.20 ± 0.03 ^{c}	0.46 ± 0.18 ^{bc}	0.62 ± 0.27 ^{abc}	0.85 ± 0.06 ^{ab}	1.01 ± 0.22 ^{a}
VFA-Sum as acetic acid (in g$_{AAeq}$/kg)	9.27 ± 0.58 ^{c}	12.13 ± 0.62 ^{b}	13.65 ± 2.15 ^{abc}	14.76 ± 1.87 ^{abc}	16.23 ± 2.45 ^{a}
Fattening Pig Manure					
Storage temperature (°C)	5	10	15	20	25
DM (in %$_{FM}$)	1.50 ± 0.01 ^{b}	1.48 ± 0 ^{b}	1.49 ± 0.09 ^{abc}	1.24 ± 0.01 ^{c}	1.11 ± 0.03 ^{d}
VS (in %$_{DM}$)	52.81 ± 0.29 ^{b}	52.66 ± 0.10 ^{b}	51,67 ± 1.40 ^{ab}	42.70 ± 0.92 ^{c}	35.32 ± 1.01 ^{d}
VS (in %$_{FM}$)	0.79 ± 0.01 ^{b}	0.78 ± 0 ^{b}	0.77 ± 0.07 ^{abc}	0.53 ± 0.02 ^{c}	0.39 ± 0.02 ^{d}
pH-average	7.69 ± 0.05 ^{c}	7.84 ± 0.10 ^{bc}	7.84 ± 0.08 ^{bc}	8.15 ± 0.03 ^{b}	8.29 ± 0.04 ^{a}
Acetic acid (in g/kg)	3.04 ± 0.03 ^{c}	3.40 ± 0.05 ^{b}	3.65 ± 0.02 ^{a}	1.5 ± 0.05 ^{d}	0.33 ± 0 ^{e}
Propionic acid (in g/kg)	0.47 ± 0	0.48 ± 0	0.34 ± 0	0.03 ± 0	0.0
Butyric acid (in g/kg)	0.07 ± 0	0.05 ± 0	0.03 ± 0	0.0	0.0
Valeric acid (in g/kg)	0.11 ± 0	0.09 ± 0	0.06 ± 0	0.0	0.0
VFA-Sum as acetic acid (in g$_{AAeq}$/kg)	3.53 ± 0.02 ^{b}	3.87 ± 0.06 ^{a}	3.98 ± 0.02 ^{a}	1.53 ± 0.05 ^{c}	0.33 ± 0 ^{d}

Another explanation for the observed low methane emissions is presented by Zhang et al., who showed that in a mesophilic mixed culture, the inhibition of hydrogenotrophic methanogens is caused by the concentration of free acetic, propionic and butyric acids [61,62]. They tested the specific methanogenic activity against pH, acid concentration and the concentration of free acids and concluded that the free acids are the key factor in inhibiting methanogenesis. The results obtained by this study showed that the concentration of free acetic acids does not surpass the thresholds for total inhibition mentioned by Zhang et al., but partial inhibition is not eliminated [61,62]. Further studies are needed to verify to what extent the storage temperature and the cumulative concentration of the different free acids could potentialize the inhibitory effect on methanogenic activity.

Another possibility for the low methane emissions is that fresh dairy manure does not present an adapted inoculum community, and that the lag phase for the development of these microorganisms may take longer than the 90 days. A study presented by Sommer et al. showed that fresh cattle slurry incubated at 20 °C with adapted inoculum took more than 100 days of lag phase before starting to emit significantly [24]. Additionally, a recent study from Lendormi et al. regarding acclimation of microbial community to psychrophilic anaerobic digestion showed that among five swine manure samples, the most efficient took 2 months of storage to acclimate [63]. The methanogens present in fresh dairy manure in our study, from rumen, may have not adapted to the conditions of the environment, and the low methane emissions were verified. Future studies could verify which is the main cause of the low methane emissions observed.

Statistical analysis of the chemical composition of the fresh and stored samples revealed no statistically relevant changes in DM$_{FM}$ (F = 4.37, $p = 0.07$), VS$_{FM}$ (F = 4.31, $p = 0.07$), or VS$_{DM}$ (F = 3.41, $p = 0.12$). The analysis of pH (F = 14.88, $p < 0.05$) and VFAs (F = 44.82, $p <$

0.05) revealed that with higher storage temperatures, there was a trend to decrease pH and to increase VFA concentration. The combined effect of high temperatures and dry matter content during manure storage was also verified by El-Mashad et al., who tested the production of VFAs during a one-month storage of dairy manure with 2%, 4%, and 9% total solids concentrations at 15 °C, 25 °C, and 35 °C [64]. The samples with higher DM concentrations produced more VFAs (gCOD/L) and less biogas (mL/g_{VS}). El-Mashad et al. also verified that temperature had a positive effect on methanogenic activity, especially for samples with lower DM content [64]. The effect of temperature on the VFA concentration during manure storage may be further studied to understand the mechanisms related with the inhibition of methane emissions and the adaptability of the microorganisms to degrade manure.

Table 3 presents the kinetics analysis of the cumulative methane emissions during the storage of dairy manure. A significant effect of the temperature of storage on the methane yields was found (F = 160.84, $p < 0.001$). Below 15 °C, the storage temperature significantly reduced the methane emissions for dairy manure (0.210 ± 0.009 $L_N\ kg_{VS}^{-1}$ at 5 °C and 1.552 ± 0.238 $L_N kg_{VS}^{-1}$ at 10 °C), while there was almost no difference in methane emissions from manure stored at 20 and 25 °C. There were significant effects of the storage temperature on the maximum cumulative methane production (F = 225.74, $p < 0.001$), the maximum specific methane production rate (F = 69.364, $p < 0.001$), and the half-life (F = 108.02, $p < 0.001$). The rate of methane production showed a tendency to be higher at 20 and 25 °C for dairy manure. The half-life decreased with the increase in the storage temperature for cow manure, showing that lower storage temperatures, in addition to allowing fewer methane emissions, occur at a slower pace.

Table 3. Logistic curve coefficients for the cumulative methane production from dairy and fattening pig manures during 90 days of storage. The significance differences according to the Games–Howell test are reported through the indices “^{a}”, “^{b}”, “^{c}”, “^{d}”, “^{e}”, “^{ab}”, “^{abc}”.

Storage Temperature (°C)	Maximum Cumulative Production ($L_N CH_4\ kg^{-1}_{VS}$)	Maximum Specific Production Rate ($L_N CH_4\ kg^{-1}_{VS}\ d^{-1}$)	Half-Life (d)
Dairy Manure			
5	0.210 ± 0.009 ^{c}	0.071 ± 0.004 ^{b}	43.85 ± 4.29 ^{ab}
10	1.552 ± 0.238 ^{b}	0.048 ± 0.001 ^{c}	42.35 ± 1.98 ^{a}
15	3.741 ± 0.305 ^{a}	0.071 ± 0.002 ^{ab}	28.47 ± 1.46 ^{b}
20	4.620 ± 0.562 ^{a}	0.096 ± 0.006 ^{a}	16.65 ± 0.78 ^{c}
25	4.273 ± 0.270 ^{a}	0.088 ± 0.001 ^{abc}	14.64 ± 2.55 ^{c}
Fattening Pig Manure			
15	36.145 ± 4.926 ^{b}	0.044 ± 0.004 ^{b}	61.2 ± 4.2 ^{b}
20	196.530 ± 21.734 ^{a}	0.044 ± 0.003 ^{b}	79.6 ± 5.2 ^{a}
25	175.933 ± 15.088 ^{a}	0.072 ± 0.002 ^{a}	47.2 ± 1.4 ^{c}

A comparison between the methane emissions of the dairy manure samples stored for 90 days and the MCF obtained from the IPCC (2019) reveals that the incubated manure samples produced lower emissions than IPCC estimates for commercial farms. According to the IPCC methodology, dairy manure stored at 25 °C, 20 °C, 15 °C, and 10 °C should result in methane emissions 36.5, 21.8, 18.0 and 28.3 times higher than those observed at the respective temperatures. The reason for the low methane emissions may be the partial inhibition of methanogenesis observed during the storage experiment. Enteric methane is produced mainly by hydrogenotrophic methanogens that may not be able to survive in the colder and harsher environment of the manure, and instead, the growth of other methanogens adapted to this environment could be needed which were not present in the fresh excreta collected for the storage experiment [65,66].

3.2.2. Fattening Pig Manure

Figure 3 presents the cumulative methane emissions for fattening pig manure stored at different temperatures. The average overall coefficient of variation was 12.2%. The average methane share in biogas for pig manure was 74.9%. The highest average methane yield was 166.19 L_NCH_4 $(kg_{VSCO})^{-1}$, observed at 25 °C, and the lowest was 1.28 L_NCH_4 $(kg_{VSCO})^{-1}$ when manure was kept at 5 °C. Different from the methane yields verified in cow manure storage, the emissions from pig manure responded more strongly to the higher temperatures. The higher methane production is justified by the chemical composition, as pig manure typically has more easily degradable material per content of dry matter than cow manure [67]. Another reason for this difference is the higher content of ammonia in pig manure. Ammonia could have avoided the drop in pH, maintaining the optimum pH for methanogens [68]. Additionally, previous studies identified lignin as a chemical component that reduces methane yields [69,70]. Lignin is not degradable compared with other organic compounds present in manure, thus decreasing methane production and controlling VS degradation during the anaerobic digestion process [70].

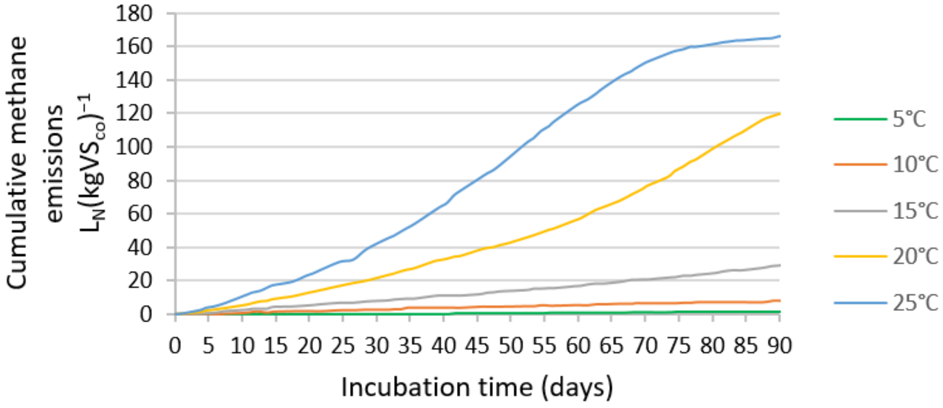

Figure 3. Cumulative methane emissions during the storage of fattening pig manure at different temperatures.

Table 2 shows the chemical composition of the pig manure samples after a 90-day storage period. The statistical analysis showed that the content of DMFM (F = 271.59, $p < 0.05$), VSFM (F = 271.23, $p < 0.05$) and VSDM (F = 205.67, $p < 0.05$) decreased with warmer storage conditions. The analysis of the pH (F = 56.30, $p < 0.05$) and VFAs (F = 19153, $p < 0.05$) revealed that higher storage temperatures tended to increase the pH and decrease the VFA concentration, i.e., opposite to what was observed during the storage of dairy manure.

Differently than observed for cow manure, the low solids concentration in pig manure resulted in comparatively low VFA concentrations in g/kg of pig manure, although the VFA share of the VS in pig manure was very high. As a consequence, the inhibition levels of VFAs and free fatty acids were not clearly exceeded in pig manure. Thus, more methane was released during storage, especially at higher temperatures, which in turn decreased the VFA concentration. As observed by Popovic and Jensen, the total VFA concentration in pig slurry decreased significantly during storage, at 5 and 25 °C, with the most rapid losses at 25 °C, because of the conversion of VFAs to methane [71].

Table 3 shows the kinetic analysis of the results obtained for the cumulative methane emissions for fattening pig manure stored for 90 days at 15, 20, or 25 °C. Statistical analysis showed that there were significant differences between the factors (F = 410.34, $p < 0.001$). The highest values for maximum methane production (F = 149.13, $p < 0.001$) were reached at temperatures of 20 °C and 25 °C, where cumulative methane production was 195.15

and 175.93 L_NCH_4 $(kg_{VSCO})^{-1}$, respectively. The highest value was observed for 20 °C, probably due to a limitation in the model that could not catch the stabilization of the curve. The rate of methane production (F = 131.33, $p < 0.001$) showed the highest value at 25 °C. The half-life showed a significant difference (F = 52.60, $p < 0.01$), with the lowest value for the sample stored at 25 °C. The modeled methane emissions for pig manure stored at 5 °C and 10 °C are not shown because of the poor fitting of the curve caused by very low gas production. Overall, outcomes were in line with other studies that recommend frequent removal of slurry from indoor storage to colder outdoor storage as a possible methane emissions mitigation strategy [29,32].

The use of the logistic function to model methane emissions during storage is justified by the flexibility of this curve to the different profiles of methane emissions under different storage temperatures. However, further studies are needed to develop a model that can describe methane emissions during storage at different temperatures. The graphical representations of the models and the experimental data for the storage experiments can be found in the Supplementary Information.

For fattening pig manure, the observed experimental MCF values were close to the estimates of the IPCC methodology. The experimental results at 25 °C, 20 °C and 15 °C were, respectively, 1.66, 0.96 and 3.36 times the MCF values of the IPCC methodology for these temperatures. Here, the results may also support the necessity of country-specific MCFs, and as expressed by Sommer et al., the models should consider the different temperatures inside animal houses and outdoor storage [8].

3.3. Methane Yield during the Biochemical Methane Potential Test

3.3.1. Dairy Manure

The BMP results after storage are presented in Figure 4. The average overall coefficient of variation was 6.3%. The average methane share in biogas for dairy cows was 59.7%. Table 4 shows the kinetics analysis for the BMP experiment with the residues from the dairy manure storage experiment as substrate. There were significant differences between the storage temperatures (F = 15.865, $p < 0.01$). The first-order decay ranged from 0.04 to 0.10 d^{-1} (F = 88.366, $p < 0.001$), indicating that there was a slow degradation compared with values obtained for different silage crops [47].

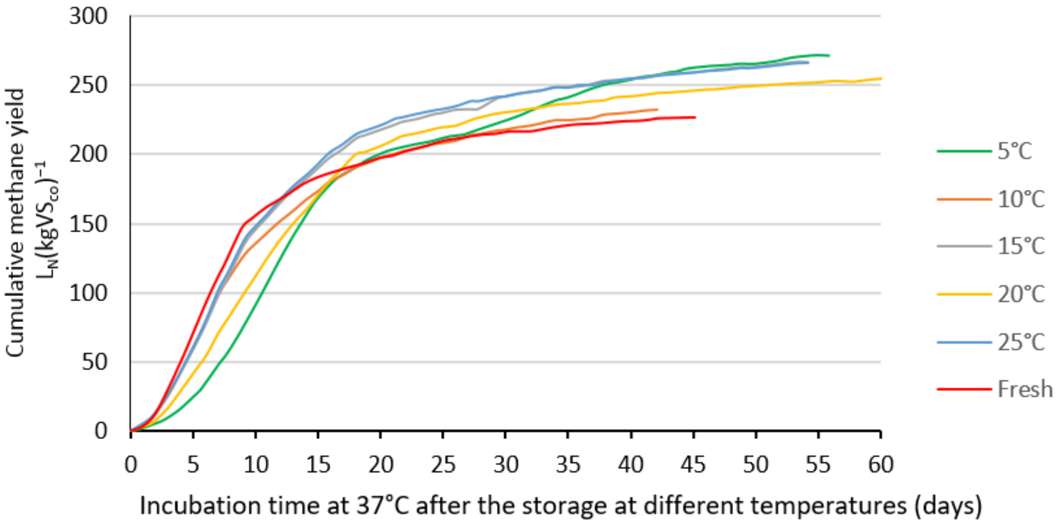

Figure 4. Cumulative methane yield during the BMP test of the residues of the dairy manure stored at different temperatures.

Table 4. Methane production characteristics of the BMP tests using dairy and fattening pig manures stored for 90 days at different temperatures as substrate. The significance levels of the Games–Howell test results are reported through "^{a}", "^{b}", "^{c}", "^{d}", "^{e}", "^{ab}", "^{abc}". The goodness of fit of the fitted equations is reported in the Supplementary Material.

	First-Order Differential Equation	**Modified Gompertz Equation**		
Temperature Storage (°C)	**First-Order Decay (d^{-1})**	**Maximum Specific Methane Yield (L_N kg$^{-1}_{VS}$)**	**Maximum Specific Methane Production Rate (L_N kg$^{-1}_{VS}$ d^{-1})**	**Lag Phase (d)**
Dairy Manure				
Fresh	0.10 ± 0.001 ^{a}	216.97 ± 22.096 ^{ab}	17.26 ± 1.222	0.952 ± 0.174 ^{b}
5	0.04 ± 0.006 ^{c}	259.92 ± 35.572 ^{ab}	12.98 ± 1.757	2.969 ± 0.459 ^{a}
10	0.07 ± 0.001 ^{b}	223.00 ± 4.964 ^{a}	14.35 ± 0.355	0.841 ± 0.055 ^{ab}
15	0.07 ± 0.002 ^{b}	253.51 ± 6.665 ^{b}	14.93 ± 0.013	0.887 ± 0.107 ^{b}
20	0.06 ± 0.016 ^{abc}	241.58 ± 8.920 ^{ab}	15.33 ± 1.014	2.783 ± 2.908 ^{ab}
25	0.08 ± 0.002 ^{b}	253.44 ± 2.504 ^{b}	15.60 ± 0.287	0.991 ± 0.324 ^{b}
Fattening Pig Manure				
Fresh	0.020 ± 0.002 ^{c}	238.76 ± 8.88 ^{a}	13.42 ± 0.34 ^{b}	7.85 ± 0.27 ^{a}
5	0.088 ± 0.009 ^{ab}	261.72 ± 22.96 ^{a}	20.91 ± 3.05 ^{abc}	1.84 ± 0.07 ^{c}
10	0.096 ± 0.004 ^{a}	272.57 ± 23.12 ^{a}	22.63 ± 0.65 ^{a}	1.54 ± 0.16 ^{c}
15	0.098 ± 0.004 ^{a}	233.02 ± 13.80 ^{a}	23.49 ± 1.25 ^{a}	2.36 ± 0.11 ^{b}
20	0.076 ± 0.002 ^{b}	149.13 ± 9.03 ^{b}	6.91 ± 0.35 ^{c}	-
25	0.026 ± 0.006 ^{c}	90.29 ± 19.99 ^{b}	2.70 ± 0.43 ^{d}	-

Using the modified Gompertz equation, the maximum specific methane yield (F = 13.196, $p < 0.01$) was observed from the manure previously stored at 5 °C and the minimum from the fresh sample. The maximum specific methane production rate (F = 5.0444, $p = 0.06$) presented a maximum value of 17.26 L_N kg$^{-1}_{VS}$ **d^{-1}** for the fresh sample and a minimum of 12.92 L_N kg$^{-1}_{VS}$ **d^{-1}** for the sample stored at 5 °C, which is in line with the results obtained for the first-order decay, although these differences only approached statistical significance. The lag phase (F = 8.652, $p = 0.02$) was maximum for the sample stored at 5 °C (2.969 d) and minimum for the sample stored at 10 °C (0.841 d).

3.3.2. Fattening Pig Manure

Figure 5 shows the cumulative methane yields of the stored pig manure measured during the BMP tests. The average overall coefficient of variation was 9.3%. The average methane share in biogas from fattening pig manure was 75.0%. Table 4 shows the kinetics analysis for the BMP experiment with the residues from the fattening pig manure storage experiment as substrate. There were significant differences between the methane yields for different storage temperatures (F = 44.628, $p < 0.001$). The group that included fresh manure and temperatures in the range 5–15 °C had similar results that were different from the yields for storage at 20 °C and 25 °C. Storage of pig manure at temperatures above 15 °C, corresponding to storage in the barn for a longer period, led to higher methane emissions than storage at lower temperatures. For storage at 25 and 20 °C, the emissions during storage represented 69.6 and 50.3% of the potential emissions, respectively.

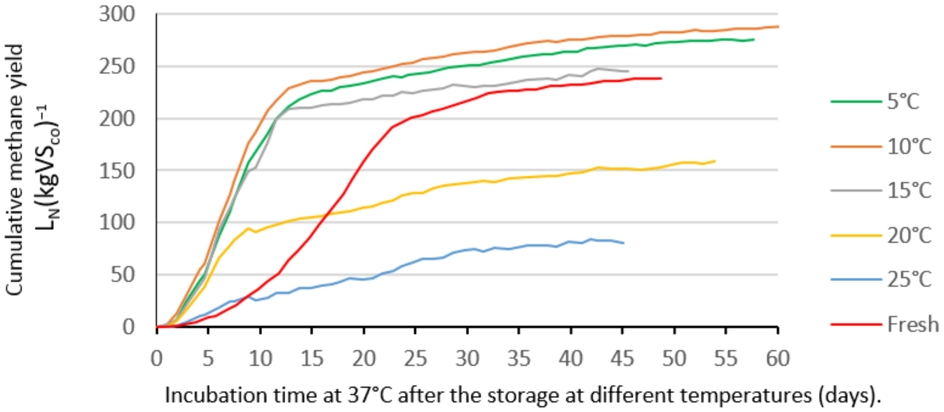

Figure 5. Cumulative methane yield during the BMP test of the residues of the fattening pig manure stored at different temperatures.

The variation in the first-order decay (F = 327.44, $p < 0.001$) was from 0.02 d^{-1} for the fresh sample to 0.098 d^{-1} for the sample stored at 15 °C. In comparison with dairy manure, these results showed the important influence of prior storage temperature on BMP results. One reason for the higher decay constant at 15 °C could be the highest concentration of VFAs for this variant. VFAs are usually easily degradable and quickly converted to methane if they do not reach inhibitory concentrations. In particular, acetic acid is a direct precursor for methane formation.

The maximum specific methane yield of the stored pig manure was significantly influenced by the storage temperature (F = 41.822, $p < 0.001$). No statistically significant differences between fresh manure and pig manure stored at 5, 10, and 15 °C were found, but significant differences between these and the samples stored at 20 and 25 °C were observed, confirming that considerable organic matter degradation takes place during storage at temperatures of 20 and 25 °C, as also seen by Sommer et al. [72]. These results are in line with the results published by Feng et al., where pig manure was stored for 52 days at 15 °C, 20 °C, 25 °C, and 30 °C prior to biogas production [36]. They reported that for storage at 25 °C and 30 °C, the losses of CH_4 reached 4.7% and 46% of the B_0 value, respectively. As an implication, manure stored at temperatures of approximately 25 °C for longer periods may emit more CH_4 during storage than during subsequent digestion in biogas facilities. These results confirm the negative environmental impact of manure storage and show that biogas production would be a good option to reduce this impact [73].

The maximum specific methane production rate was influenced by the storage temperature (F = 390.51, $p < 0.001$), and the fresh manure and the manure stored at 5 °C, 10 °C and 15 °C presented higher rates, indicating that easily degradable compounds were not lost during storage at lower temperatures, and these compounds contributed to the methane production potential during the BMP test. The lag phase (F = 229.73, $p < 0.001$) showed that the fresh sample took more days to start the methane emissions than the samples stored for 90 days, which appears to be evidence that the methane production potential developed during storage. Samples stored at 20 °C and 25 °C showed a rapid onset of methane formation, so a lag phase was not detectable [50].

Overall, these results show that dairy and pig manure have significant methane potential emission and that there is a necessity of bringing more sustainable practices to the livestock production in order to reduce the environmental impact.

4. Conclusions

In this study, experiments were performed to assess methane emissions during 90 days of storage of dairy and fattening pig manure under temperatures from 5 °C to 25 °C.

After this period, the residual methane potential was verified by BMP tests, at 37 °C. During the storage of dairy manure, methane emissions were low, presumably due to inhibition of methanogenic activity through the accumulation of VFAs or the necessity of adapted methanogens that are not present in the very fresh manure. The concentration of VFAs were progressive higher according to the increase in storage temperature. The total methane emissions during storage at 25 °C accounted for only 2% of the maximum methane production potential. The dry matter content associated with the decomposition of organic matter and the accumulation of VFAs may have led to a pH decrease and inhibition of methanogenic activity, resulting in low methane emissions. Further studies could reveal if and under which conditions the accumulation of VFAs also occurs on commercial farms.

During the storage of fattening pig slurry at 20 °C and 25 °C, methane emissions accounted for 50.3% and 69.6% of the maximum methane potential, respectively. The experiments showed that slurry storage under warm conditions must be avoided. Some practices could be used to mitigate methane emissions, for instance, transportation of slurry from the barn to the outside storage, promoting storage during the cold seasons, when field application is not possible. In addition, biogas production is an important option to mitigate methane emissions from manure during subsequent storage.

By comparing the experimental data with the MCF values suggested by the IPCC guidelines [15], it was possible to identify differences mainly regarding dairy manure methane emissions. Although it is acknowledged that the likely inhibition of methane emissions in the dairy manure samples may not occur on commercial farms if fresh manure is mixed with older manure with adapted methanogens, it may be important to consider the different storage temperatures during the different stages of the manure management chain for both animal categories.

Further studies need to confirm that similar results can be applied to the manure management chain of commercial farms, with methods that could verify methane emission rates in loco and the relationships with management. They may improve methane emissions estimations by providing MCF values that reflect regional conditions, such as the manure storage temperature profile, chemical composition, and storage period. Better estimations of methane emissions in national emission inventories could improve the opportunities to make targeted choices on mitigation strategies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su14169934/s1.

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Supplementary information

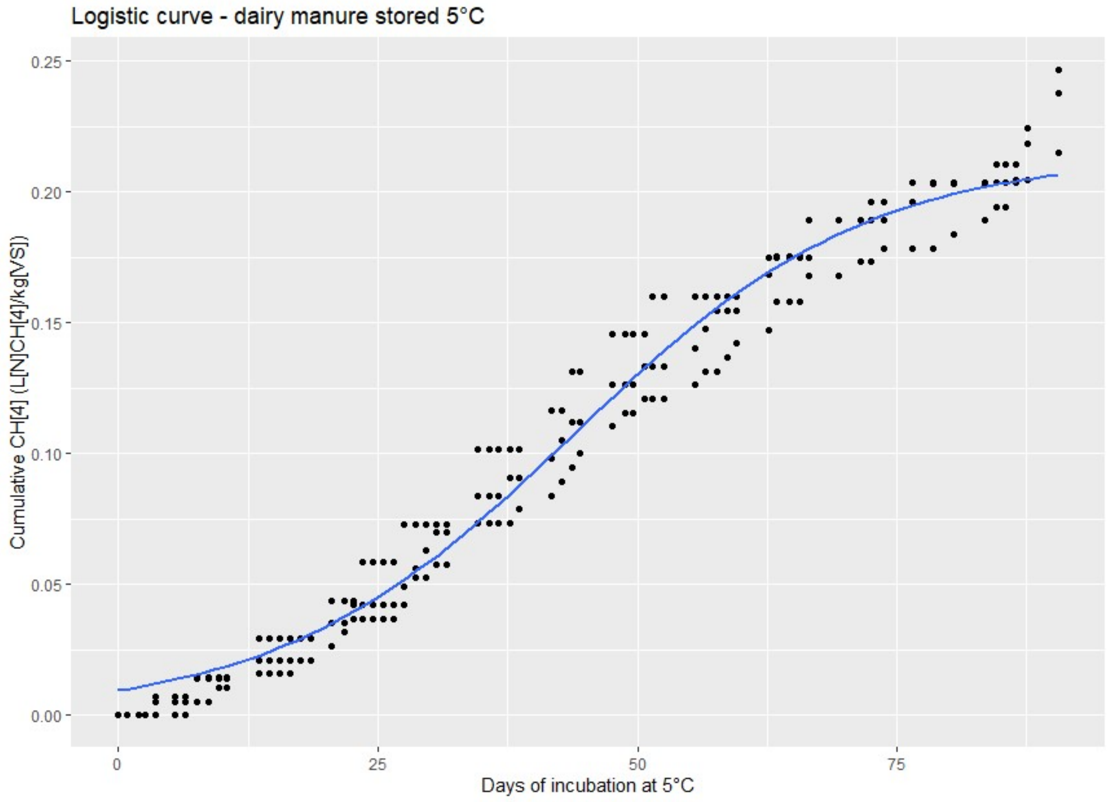
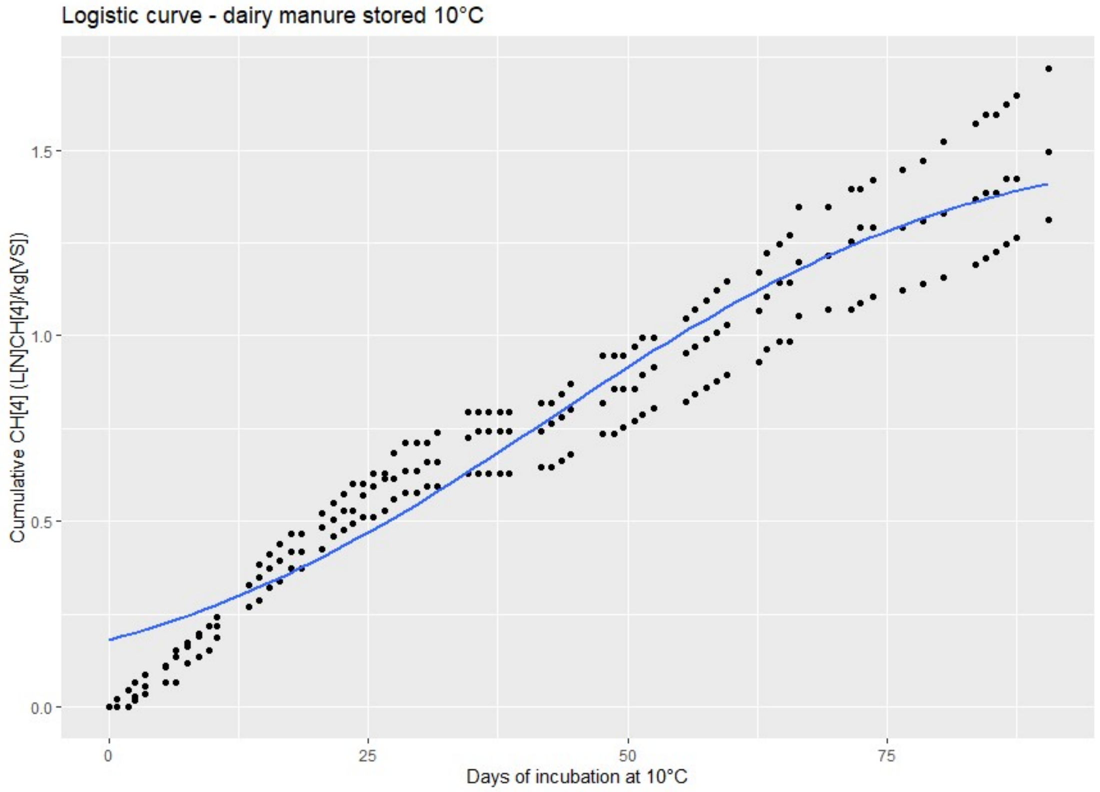


Fig S.1 – Graph showing the measured (black dot) cumulative methane emissions for dairy cow manure stored at 5°C for 90 days and the modelled logistic curve (blue line).



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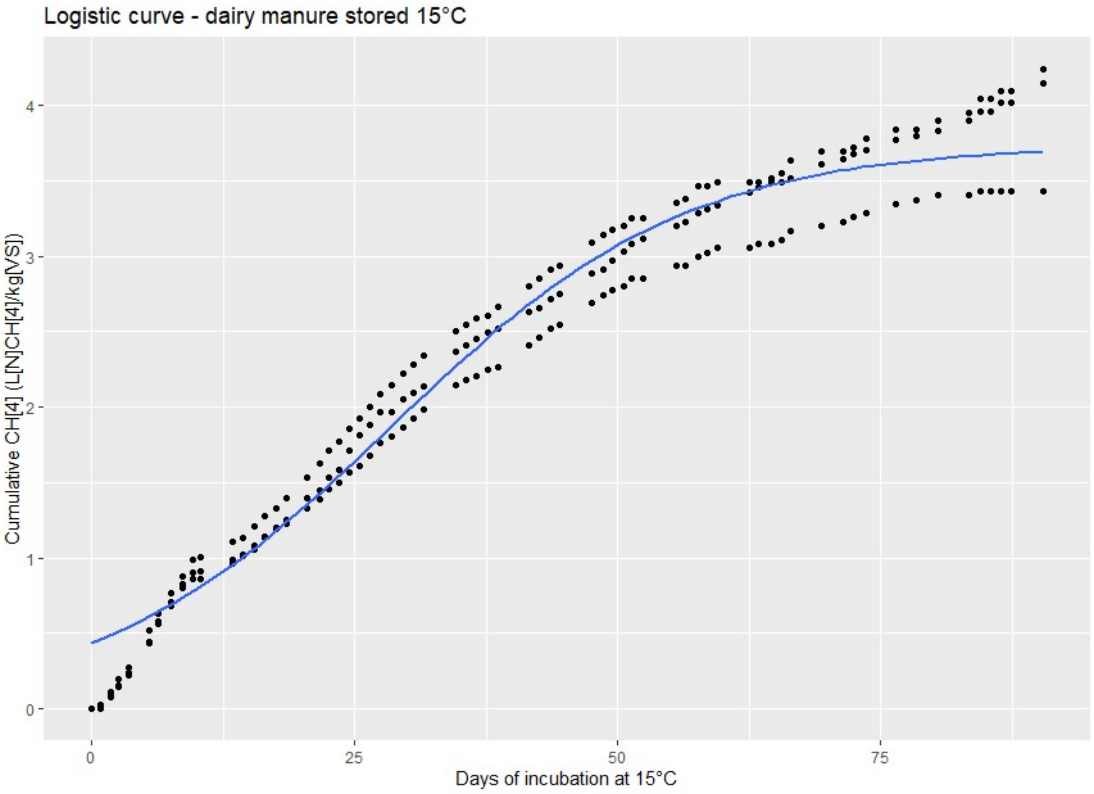

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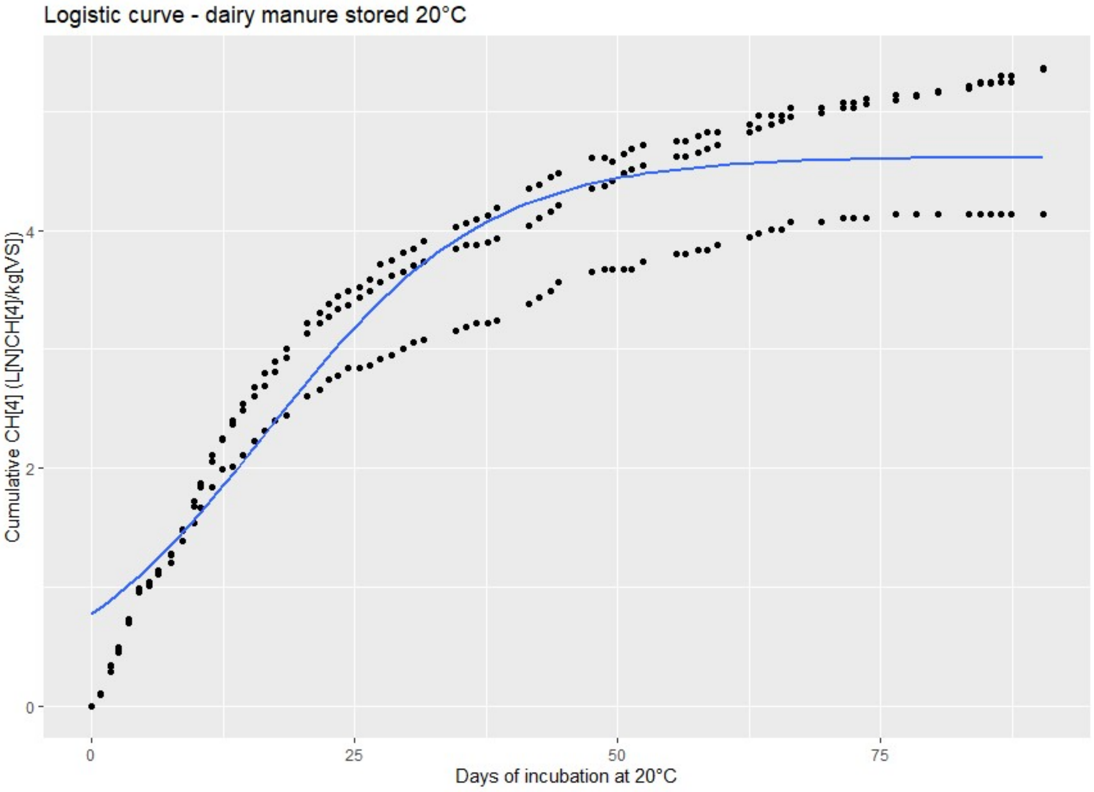

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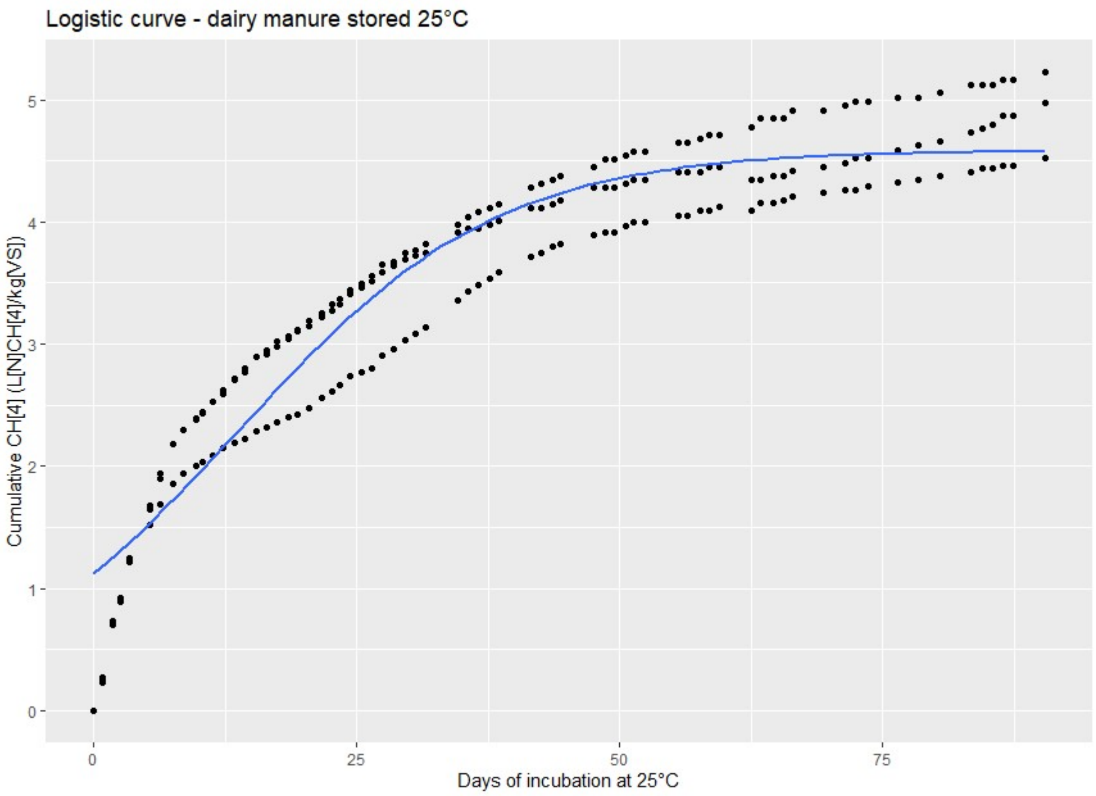


Fig S.5 – Graph showing the measured (black dot) cumulative methane emissions for dairy cow manure stored at 25°C for 90 days and the modelled logistic curve (blue line).

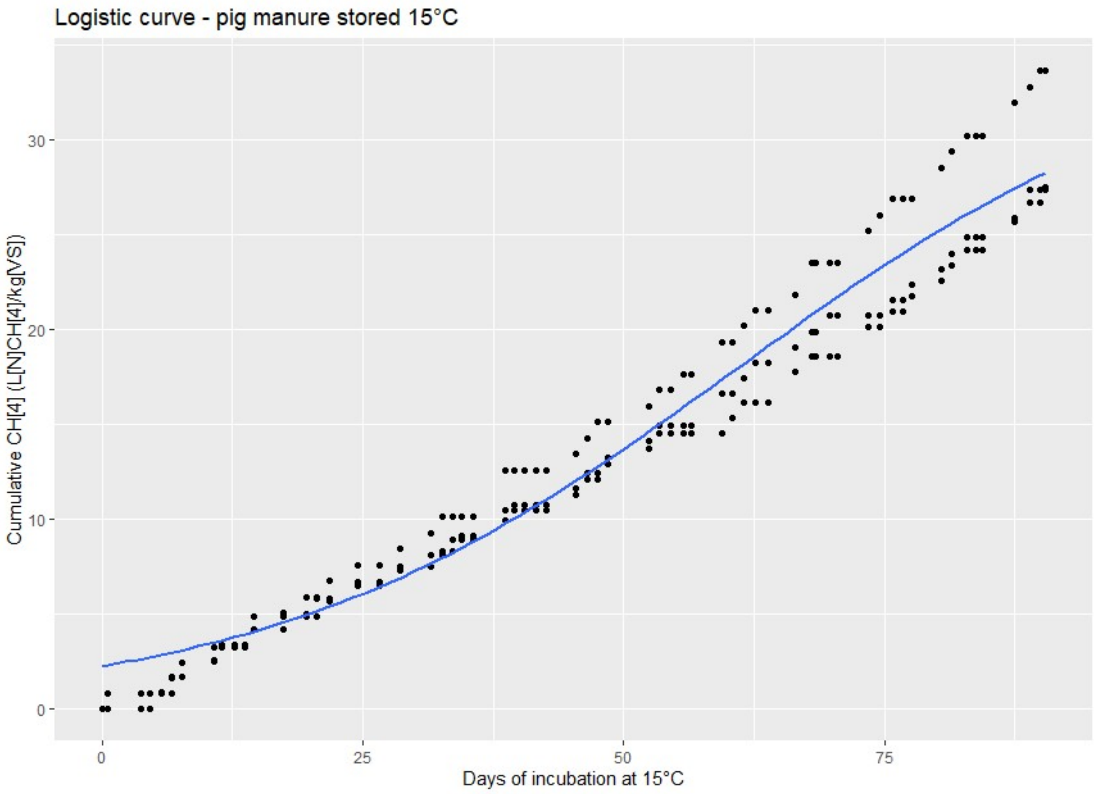
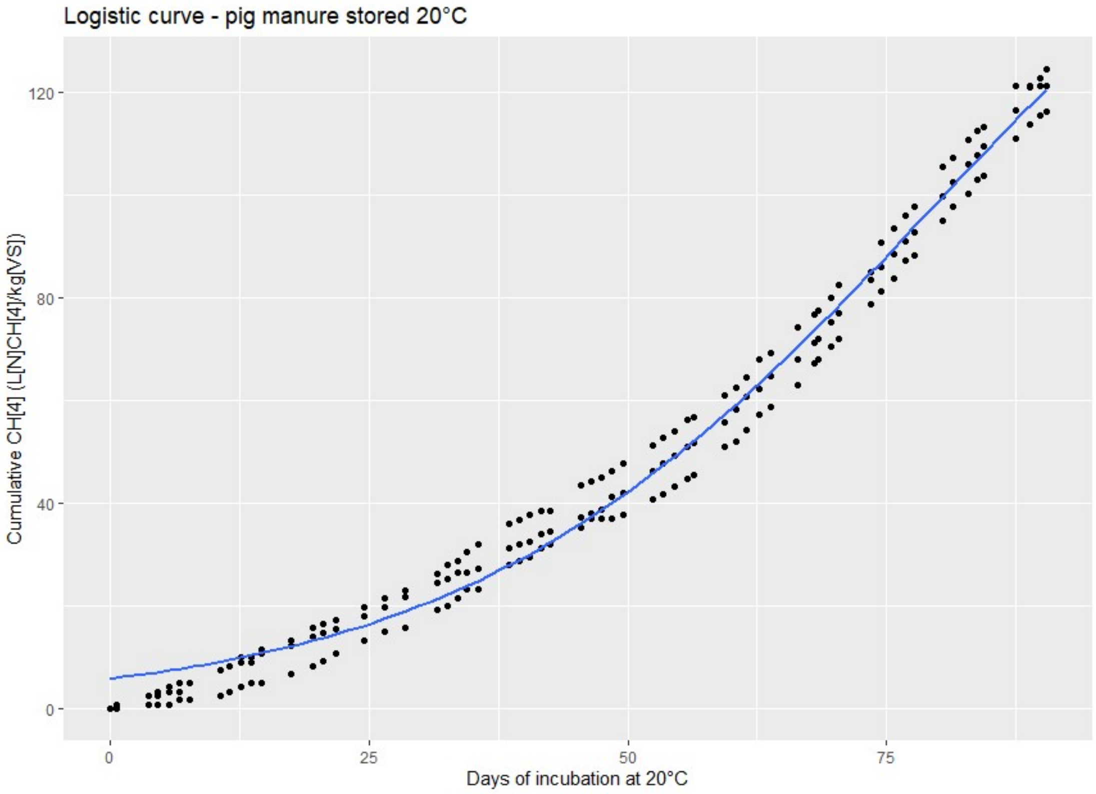


Fig S.6 – Graph showing the measured (black dot) cumulative methane emissions for fattening pig manure stored at 15°C for 90 days and the modelled logistic curve (blue line).



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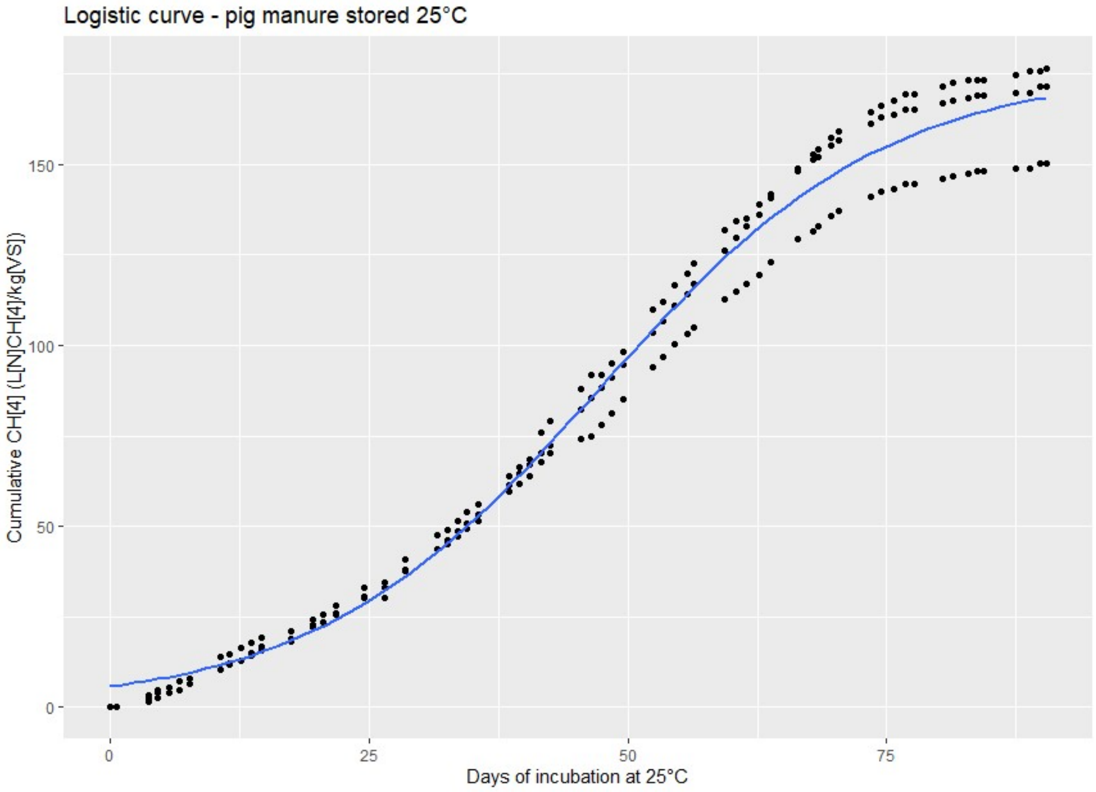

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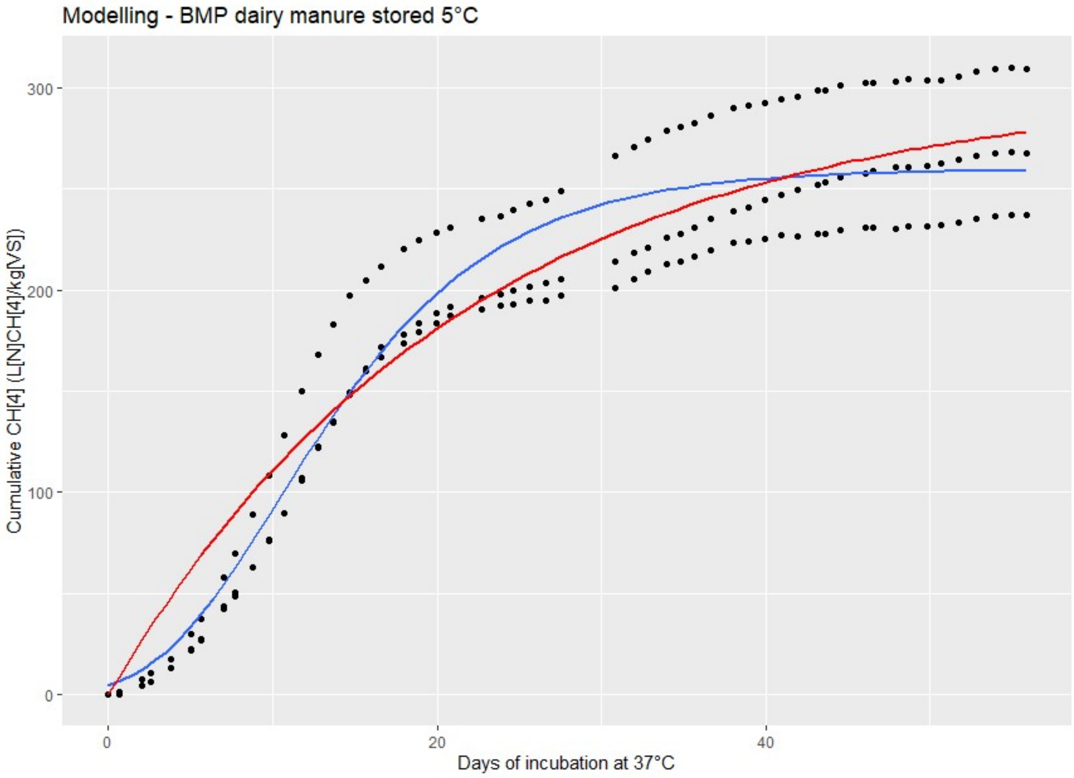
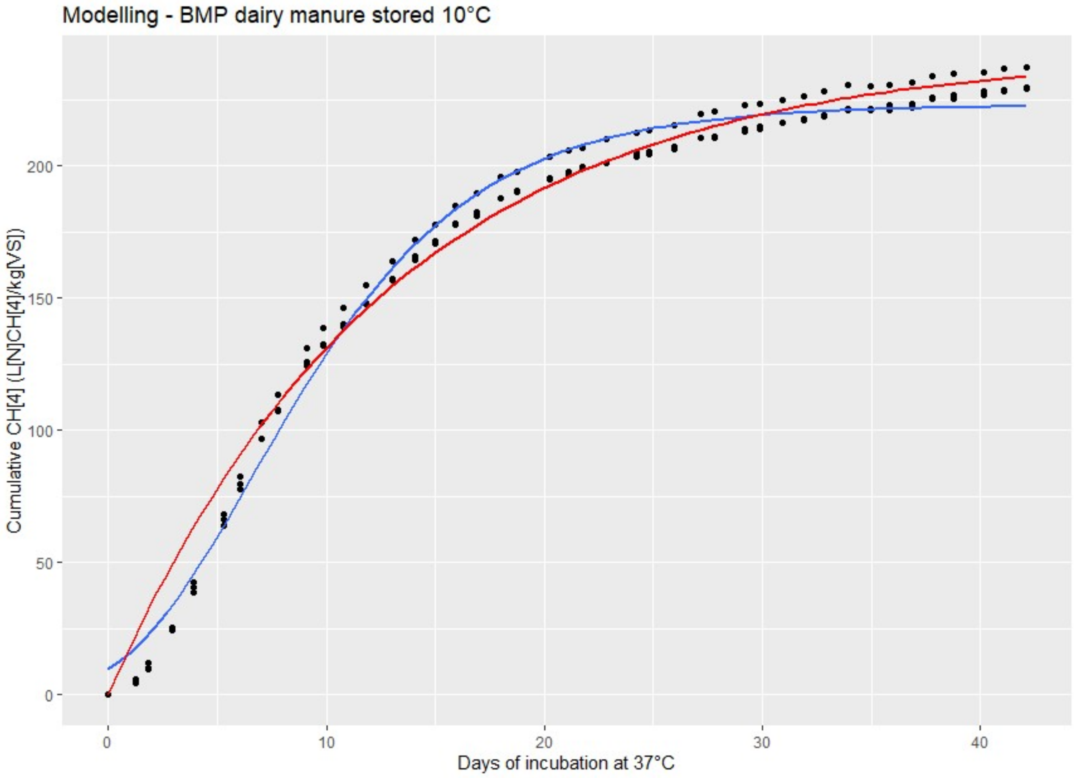


Fig S.9 – Graph showing the measured (black dot) cumulative methane emissions for the BMP test with the residues of stored cow manure at 5°C for 90 days, the modelled modified Gompertz equation (blue line, AIC: 1515.8) and, the modelled first order differential equation (red line, AIC: 1545.9).



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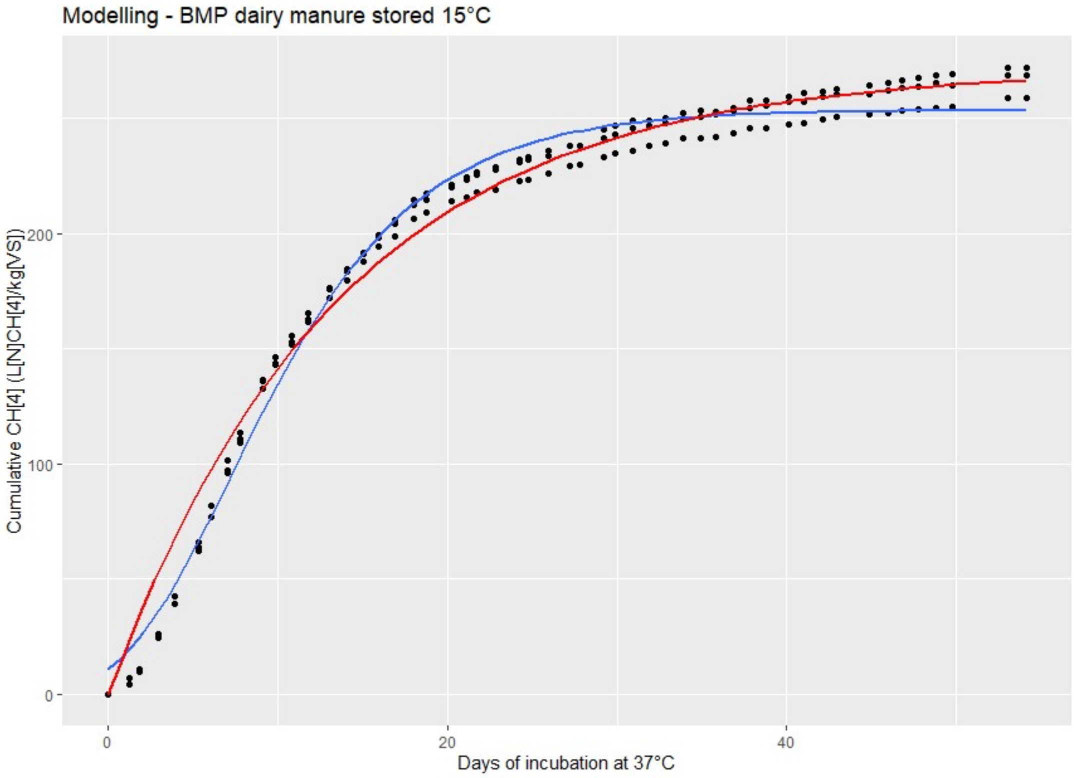

Fig S.11 – Graph showing the measured (black dot) cumulative methane emissions for the BMP test with the residues of stored cow manure at 15°C for 90 days, the modelled modified Gompertz equation (blue line, AIC: 1116.7) and, the modelled first order differential equation (red line, AIC: 1169.0).

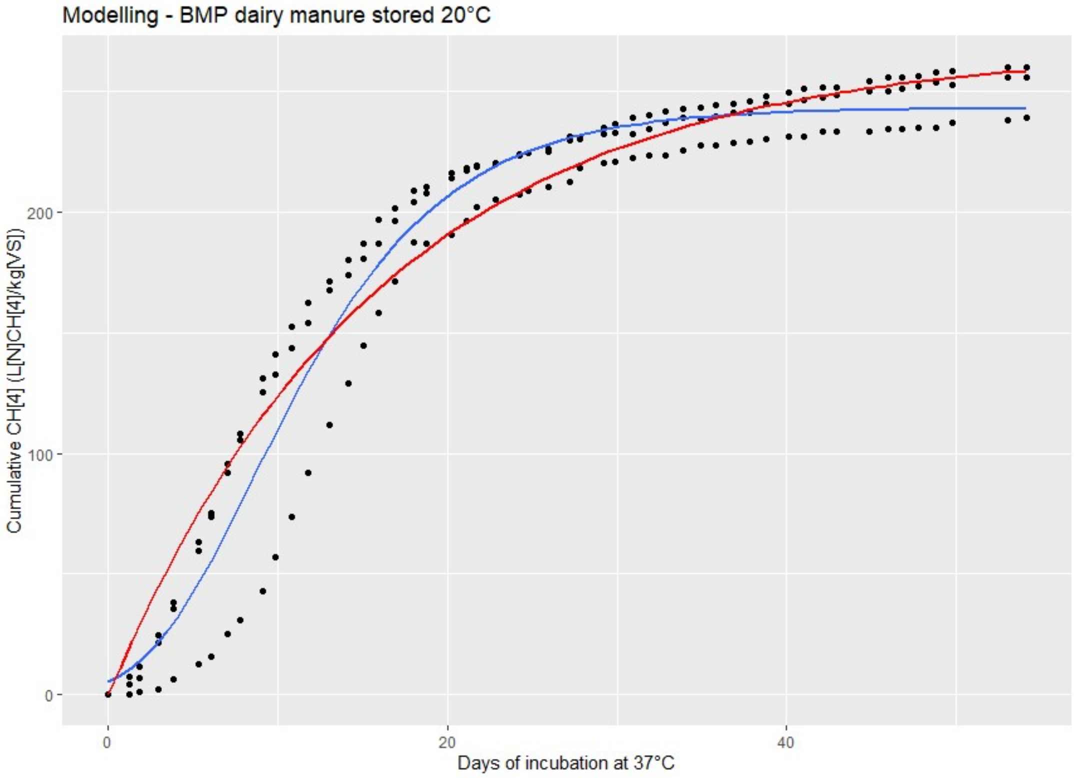

Fig S.12 – Graph showing the measured (black dot) cumulative methane emissions for the BMP test with the residues of stored cow manure at 20°C for 90 days, the modelled modified Gompertz equation (blue line, AIC: 1329.1) and, the modelled first order differential equation (red line, AIC:1392.1).

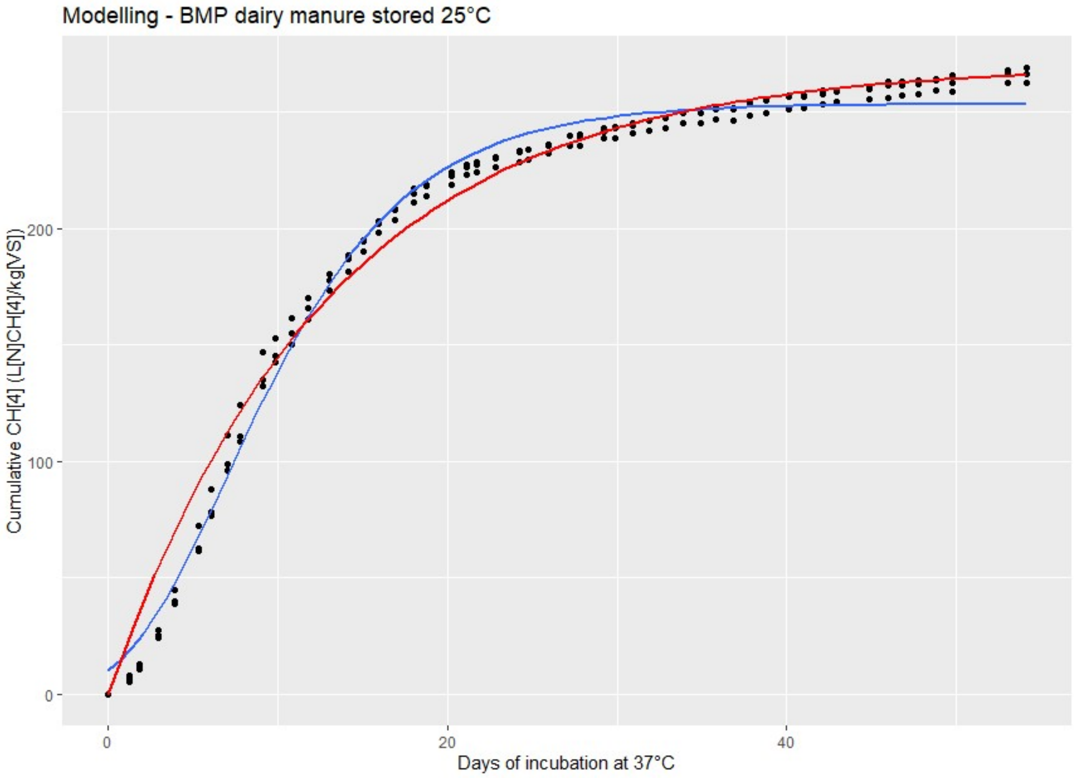

Fig S.13 – Graph showing the measured (black dot) cumulative methane emissions for the BMP test with the residues of stored cow manure at 25°C for 90 days, the modelled modified Gompertz equation (blue line, AIC: 1073.4) and, the modelled first order differential equation (red line, AIC: 1165.5).

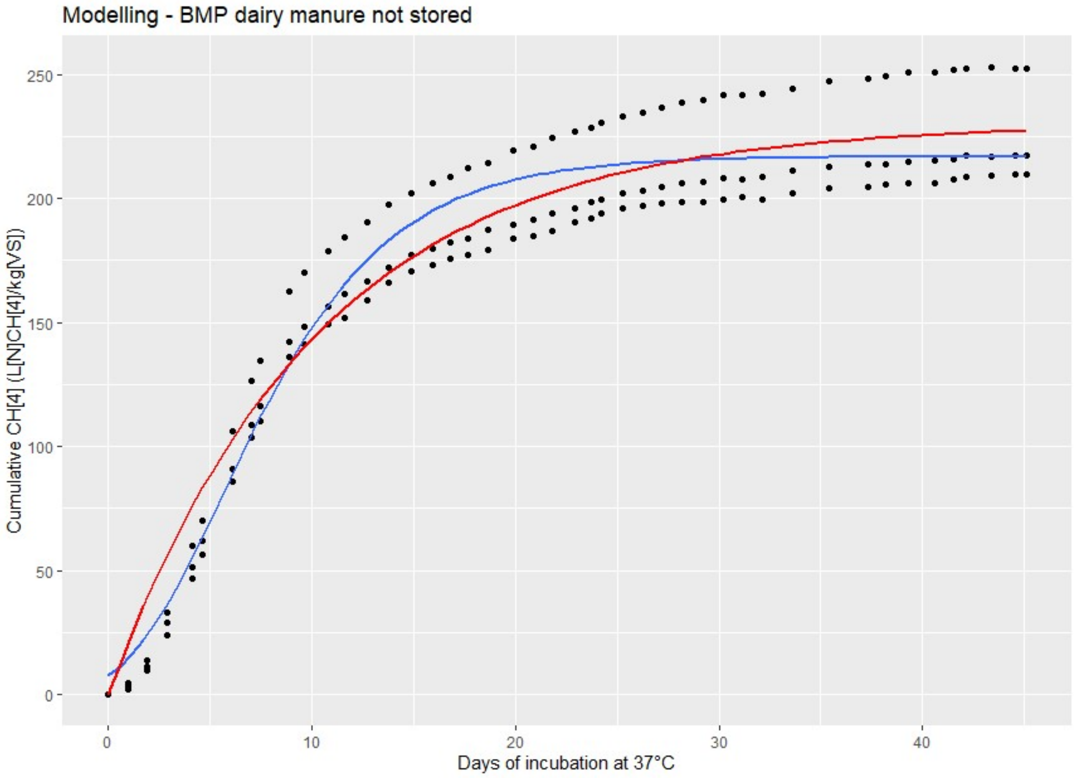

Fig S.14 – Graph showing the measured (black dot) cumulative methane emissions for the BMP test with cow manure not previously stored, the modelled modified Gompertz equation (blue line, AIC: 1152.4) and, the modelled first order differential equation (red line, AIC: 1163.7).

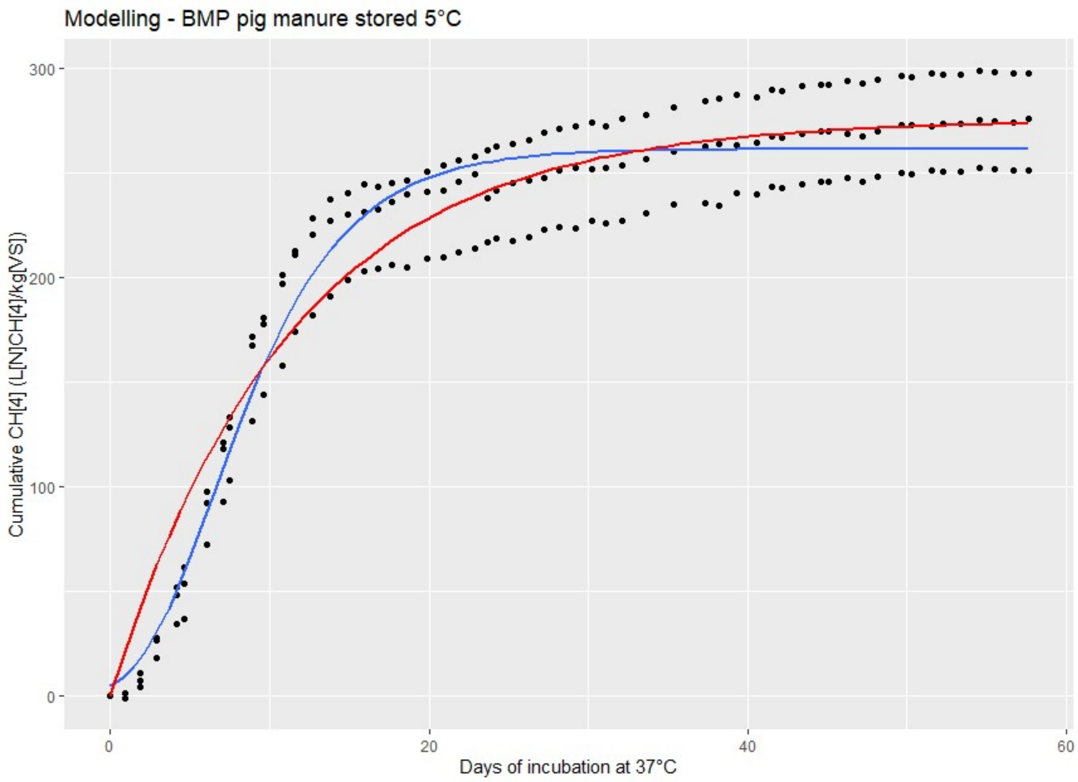

Fig S.15 – Graph showing the measured (black dot) cumulative methane emissions for the BMP test with the residues of stored pig manure at 5°C for 90 days, the modelled modified Gompertz equation (blue line, AIC: 1521.0) and, the modelled first order differential equation (red line, AIC: 1557.1).

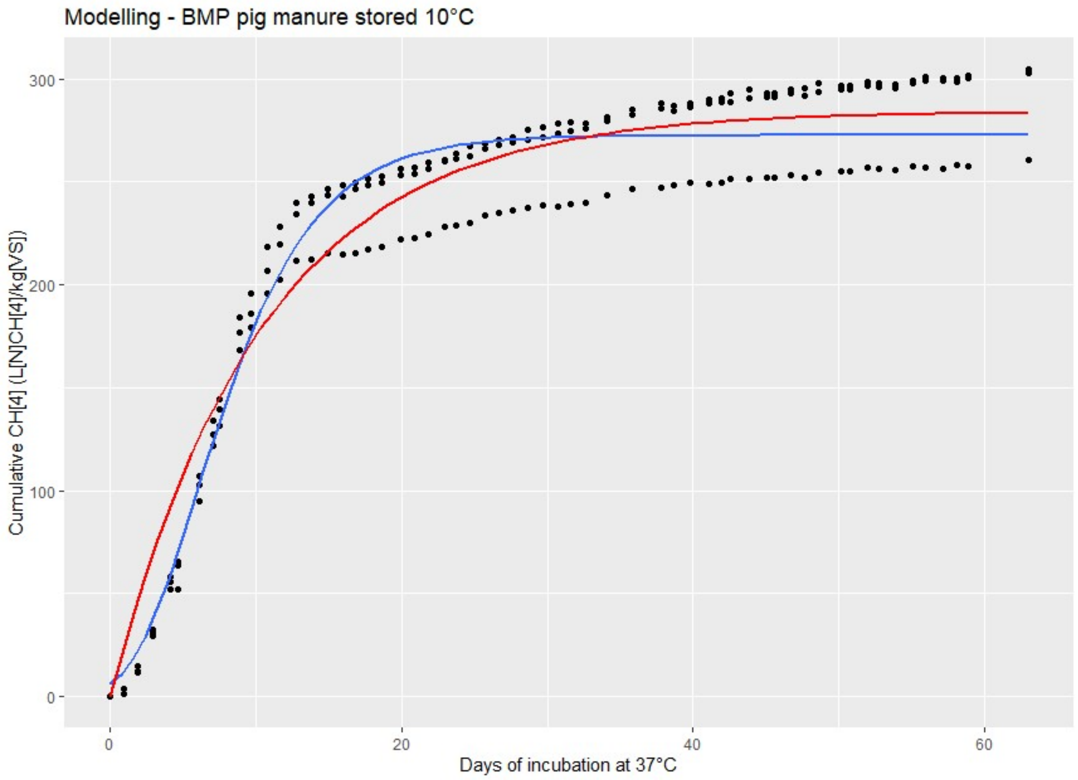

Fig S.16 – Graph showing the measured (black dot) cumulative methane emissions for the BMP test with the residues of stored pig manure at 10°C for 90 days, the modelled modified Gompertz equation (blue line, AIC: 1553.0) and, the modelled first order differential equation (red line, AIC: 1585.3).

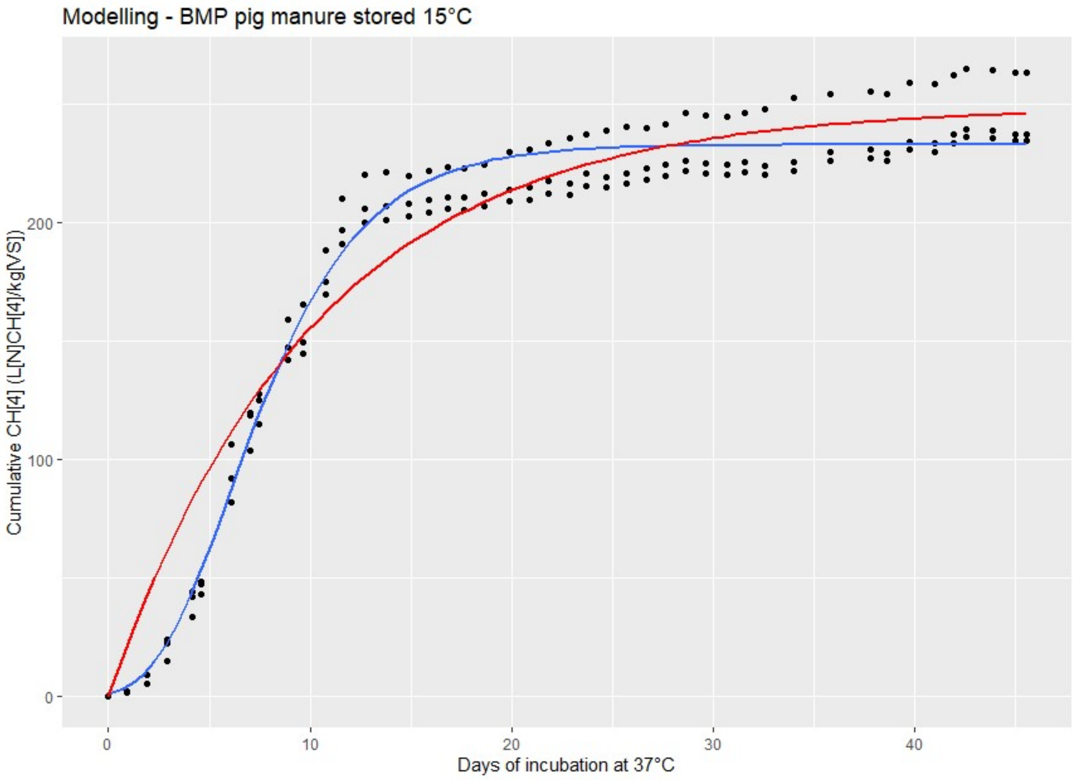

Fig S.17 – Graph showing the measured (black dot) cumulative methane emissions for the BMP test with the residues of stored pig manure at 15°C for 90 days, the modelled modified Gompertz equation (blue line, AIC: 1062.5) and, the modelled first order differential equation (red line, AIC: 1180.1).

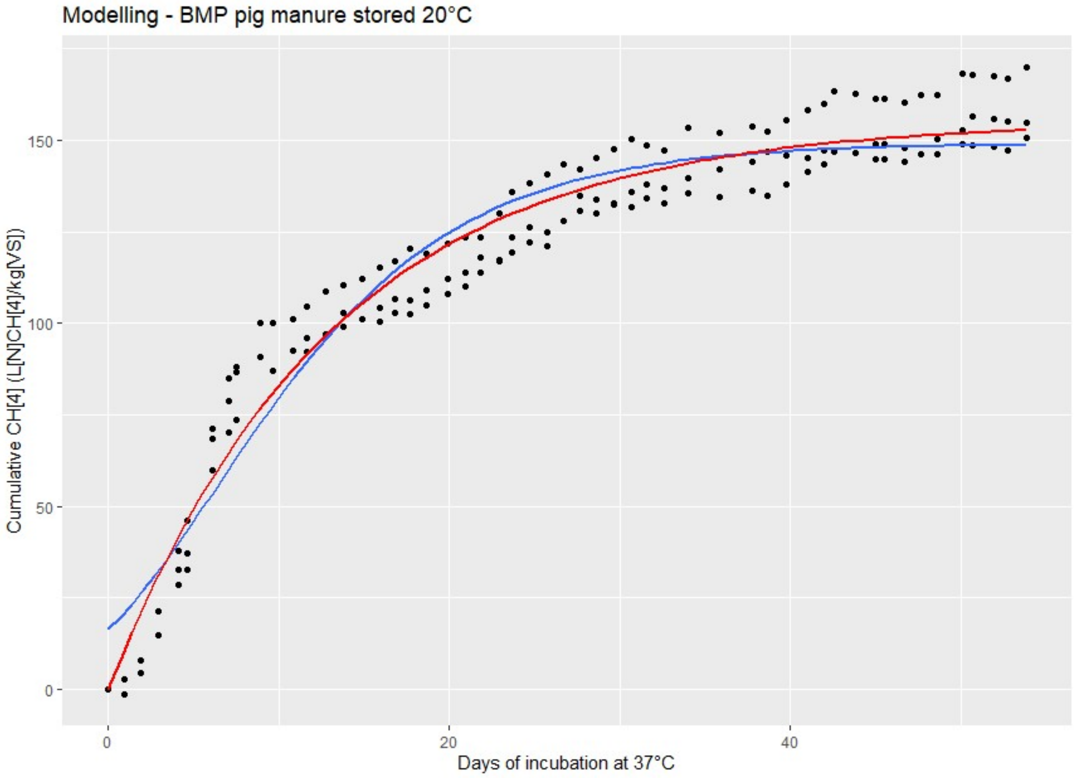

Fig S.18 – Graph showing the measured (black dot) cumulative methane emissions for the BMP test with the residues of stored pig manure at 20°C for 90 days, the modelled modified Gompertz equation (blue line, AIC: 1231.2) and, the modelled first order differential equation (red line, AIC: 1160.7).

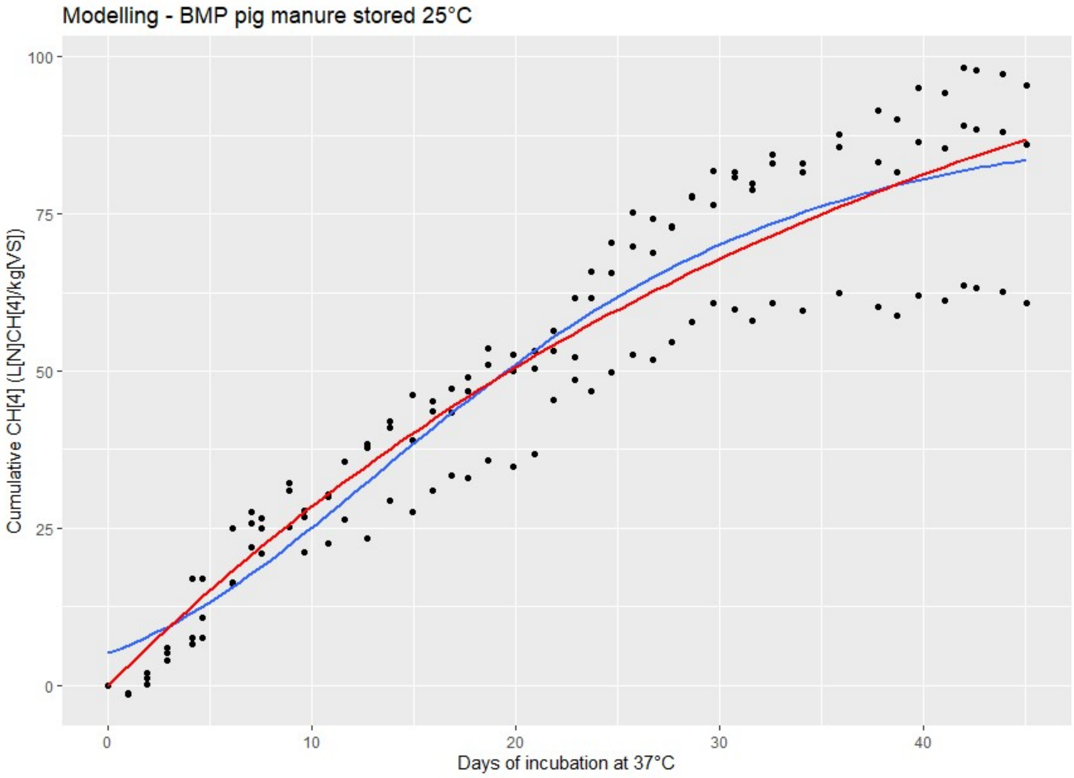

Fig S.19 – Graph showing the measured (black dot) cumulative methane emissions for the BMP test with the residues of stored pig manure at 25°C for 90 days, the modelled modified Gompertz equation (blue line, AIC: 972.3) and, the modelled first order differential equation (red line, AIC: 968.6).

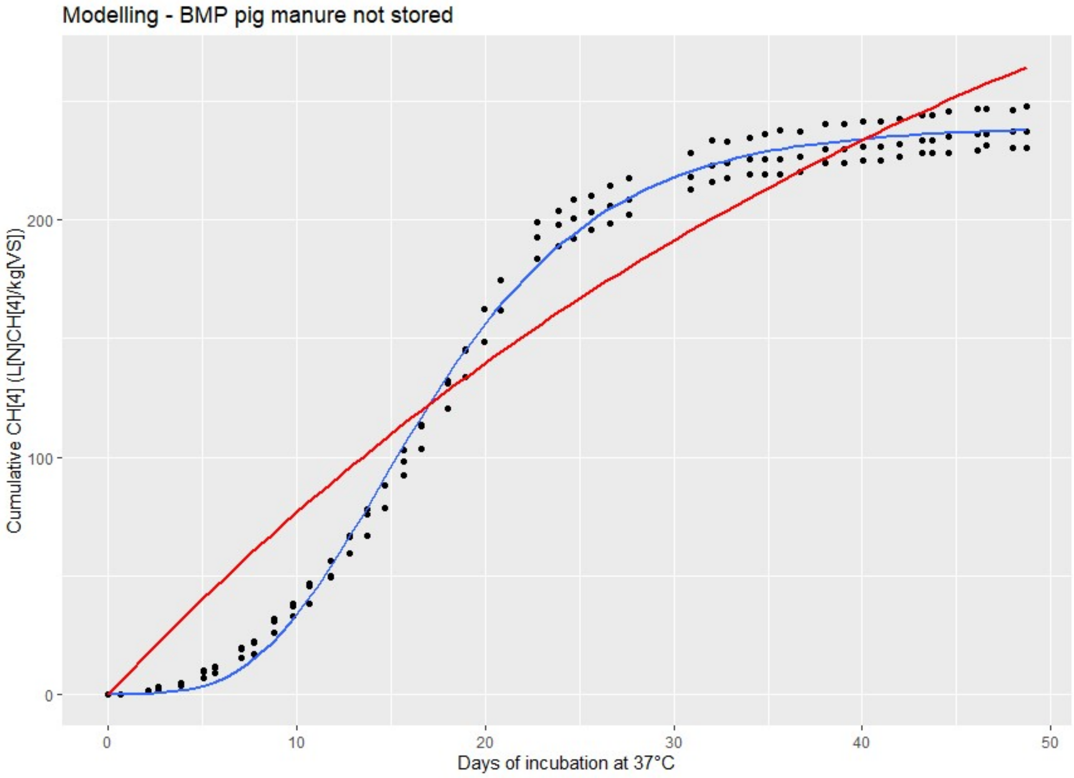

Fig S.20 – Graph showing the measured (black dot) cumulative methane emissions for the BMP test with pig manure not previously stored, the modelled modified Gompertz equation (blue line, AIC: 950.3) and, the modelled first order differential equation (red line, AIC: 1312.5).

3.2 Publication "B"

Assessment of the biochemical methane potential of in-house and outdoor stored pig and dairy cow manure by evaluating chemical composition and storage conditions

Julio E. Hilgert, Christiane Herrmann, Søren O. Petersen, Federico Dragoni, Thomas Amon, Vitaly Belik, Christian Ammon, Barbara Amon

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Research Paper

Assessment of the biochemical methane potential of in-house and outdoor stored pig and dairy cow manure by evaluating chemical composition and storage conditions

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ABSTRACT

Biogas production is a suitable option for producing energy from dairy and pig manure types. During manure storage, organic matter degradation results in methane emissions decreasing the potential biogas yield. The present research advances the understanding of the biochemical methane potential (BMP) and the chemical characteristics of manure collected year-round from sequential stages of the liquid manure management chain of commercial dairy cow and pig farms. To this end, manure samples from six livestock farms in Germany were analyzed. The results showed that changes in chemical composition during storage led to a 20.5% decrease in the BMP of dairy manure from the barn to outdoor storage. For fattening pig manure samples, there was a 39.5% decrease in the BMP from intermediate to outdoor storage. An analysis of BMP according to manure age showed that pig manure degrades faster than dairy manure; the importance of promptly feeding manure to the biogas plant in order to avoid significant CH_4 emission losses and reduction in energy producing capacity was highlighted. The best BMP predictors for dairy manure were the contents of dry matter, volatile solids and lignin, whereas best BMP predictors for pig manure were dry matter and volatile fatty acid (VFA) content. Prediction models performed well for samples from outdoor storages; refinements for predicting BMP of less aged samples presenting lower chemical variability would be necessary.

1. Introduction

In 2018, methane in the atmosphere reached levels 2.6 times higher than the preindustrial level (Saunois et al., 2020). The Intergovernmental Panel on Climate Change (IPCC)'s sixth assessment report states that atmospheric CH_4 caused a 0.5 °C global temperature increase until 2019 compared to 1850–1900 levels (IPCC, 2021). Anthropogenic CH_4 contributes 0.97 Wm^{-2} to planetary heat storage, while CO_2 contributes 1.68 Wm^{-2} (Stocker, 2014). CH_4, with a nine-year atmospheric lifetime (Prather et al., 2012), has a GWP 79.7 times higher than CO_2 over 20 years time scale and 27 times higher over 100 years (IPCC, 2021). In addition, CH_4 emissions correspond to only 3.9% w/w of the yearly CO_2 emissions (Saunois et al., 2020). Reducing anthropogenic CH_4 emissions can lower their concentrations and global warming impact, which meets IPCC near-term strategy urging significant reductions by 2030–2040. Leveraging this strategy, the European Union (EU) aims to cut CH_4 emissions by 50% from 2020 to 2050, reducing global temperature change by 0.18 °C until 2050 (EU, 2020).

Primary anthropogenic CH_4 sources include agriculture (40%; 32% from manure and enteric fermentation, 8% from rice cultivation), fossil fuels (35%), and waste (20%) (UNEP-CCAC, 2021). A recent assessment has shown that emissions from enteric fermentation and manure management increased their contributions from 102 $TgCH_4yr^{-1}$ in 2000–2006 to 115 $TgCH_4yr^{-1}$ in 2017[1] (Jackson et al., 2020). Meat production increased 46% from 236.4 million tons in 2000 to 346.1 million tons in 2018, with pig meat at 35% (Ritchie & Roser, 2017); 690 million tons of dairy milk (81% of global production) were made in 2019 (OECD et al., 2020). Therefore, changes in the livestock sector are needed to achieve emission reductions consistent with temperature increases below the 2 °C targets proposed by the Paris Agreement.

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[1] Tg means teragrams a unit of mass equivalent to one trillion grams.

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A range of emission mitigation technologies is available for manure management systems that may, additionally, provide extra revenue streams for farmers, such as their applications as fertilizer and for biogas generation. The biogas resulting from manure is a renewable energy source; the digestate that remains after anaerobic digestion is usable as a valuable fertilizer. According to the EU's long-term decarbonization strategy, the annual production of biogas should increase four or five times by 2050 relative to the 2020 levels. Cooperation among farmers and communities will be essential for maximizing the potential of biogas production from diffuse sources in agriculture and for reaching the proposed target (EU, 2020).

Manure management systems (MMSs) are designed for storing and transporting large volumes of biomass types from animal barns to outdoor storages, and from there to the fields. Manure storage is needed in order to apply manure during vegetation to create a cleaner and healthier environment for livestock and to meet regulations regarding nitrogen losses in agriculture (EEC, 1991). According to the German National Inventory, liquid manure (slurry) systems were used on 52.37% of dairy farms and 77.79% of pig farms in 2019 (German Environment Agency, 2021). Anaerobic degradation is a sequence of biochemical processes that lead to CH_4 production during manure storage (Amon et al., 2007). The sequence is composed of hydrolysis, acidogenic fermentation, hydrogen-producing acetogenesis, and methanogenesis. The strictly anaerobic process leads to the degradation of organic matter and the production of mainly CH_4 and CO_2. These processes are accelerated in anaerobic digestion (AD), where conditions for anaerobic degradation are optimised.

Chemical composition of in organic matter plays vital roles in potential CH_4 production, and the contents of the different components may be used that predict the potential of feedstock for biogas production. Previous studies have highlighted the importance of the chemical composition of manure to biogas production. Amon et al. (2007) verified that significant protein content in dairy manure is associated with higher CH_4 yields during anaerobic digestion than those of samples with less protein. In comparison, lignin content tends to reduce the specific CH_4 yield. However, predictions of CH_4 production potential of manure depends on the anaerobic degradation occurring before treatment, as determined by animal and manure management practices (Triolo et al., 2011), as well as storage conditions (Clemens et al., 2006; Wood et al., 2012).

Dairy manure has a lower content of biodegradable carbon than pig manure, reducing the biochemical methane potential (BMP). In a study by Triolo et al. (2011), the differences between pig and dairy cow manure were evident due to the higher content of organic nitrogen in dairy manure than in pig manure. In contrast, in pig manure, most nitrogen is in the form of total ammonia nitrogen (TAN). The lignocellulose contents were higher in cow manure than in pig manure, mainly due to the differences in their diets. The studies by Triolo et al. (2011) and Amon et al. (2007) presented mathematical models aimed at predicting the BMP from the manure chemical composition.

In a recent review, Raposo et al. (2020) presented different mathematical models to predict BMPs from different feedstock (energy crops, food and crop wastes, municipal solid waste, and manure). The researchers verified that proteins and lipids positively affect the CH_4 potential, whereas nonstructural carbohydrates present different impacts on BMP, depending on the feedstock. Regarding fibers, anaerobic digestion can degrade a limited fraction of lignin. Usually, hemicellulose positively impacts BMP, differing from cellulose, which commonly harms CH_4 potential due to the effect of crystallinity and the combined effect with lignin.

To our knowledge, this study is the first to assess the BMP and the chemical characteristics of manure collected year-round from sequential stages of the liquid manure management chain of commercial dairy cow and fattening pig farms, and to model the BMP according to the manure management stages and the chemical composition. Accurately estimating the CH_4 potential from animal manure is critical for designing successful climate change mitigation measures. Although many feedstocks have been studied regarding the BMP, there is a need for documenting the effect of manure storage conditions on CH_4 potentials and chemical composition. In addition, understanding the progressive degradation of organic matter along the manure management chain is relevant for the potential mitigation of the environmental impacts of livestock systems. The aims of this work are (1) to characterize the chemical compositions and BMPs of liquid dairy cow and fattening pig manure samples from different stages on the manure management chain (MMC) and (2) to model BMP according to the manure composition along the MMC of cattle and pig slurry systems.

2. Materials and methods

2.1. Dairy cow manure and fattening pig manure

Dairy cow manure samples were collected form four farms, and fattening pig manure samples were collected from two farms. All farms offered the possibility to collect manure samples from the barn and from outdoor facilities. Table 1 shows the main characteristics of the dairy and pig farms that contributed to this study, including information about the herd, feeding, housing and storage, and manure management are available. The manure management systems of the farms exhibit typical traits for Germany, as liquid manure systems are predominant on dairy cattle and pig farms: manure is typically stored inside the barn and moved to an outside store at regular intervals (German Environment Agency, 2021). Three dairy farms are located in Brandenburg (DE01, DE02 and DE04), while one is in Lower Saxony (DE03). They all have Friesland-Holstein cows stabled in freestall barns. This breed and this housing system are common in Germany (59% and 81% of the dairy farms, respectively) (Tergast et al., 2022). Regarding the fattening pig farms (DE05 and DE06) they are all located in Brandenburg and they have full or partial slatted floors as the vast majority of German pig farms, which is consistent with slurry management (Rohlmann C, 2022).

For both animal categories the sample collection was performed in different positions along the MMC. For dairy farms, barn samples were collected from the walking alley while operating scrapers, which allowed the collection of feces and urine from the walking alley. Samples from the pumping pit in the dairy farms were collected using a 1-m long sampling device (Stainless Steel Zone Sampler, Hartwig Instruments BV, Rotterdam, The Netherlands). Samples were collected from different depths in the pit and mixed before packing. The same instrument was used for collection of samples from outdoor storage. Since the outdoor storage tanks were high and wide, samples from the top or bottom layers were collected depending on the level of slurry in the tank at the visiting time.

Samples collected in pig farms varied according to the design of the manure management system. On farm DE05, the manure was stored under a barn in pits with a pull-plug system that were emptied approximately every three months; samples were collected using a bucket positioned in the outlet stream every minute during discharge to outdoor storage. On farm DE06, the samples were collected under the slatted floor using a pump (Unistartk/K 2001-B, ZUWA-Zumpe GmbH, Laufen, Germany). Pig manure samples were collected from outdoor storage by following the same procedure as that for cow manure. Samples collected under the slatted floor of pig farms are referred to as barn samples, and samples collected from the pumping pit in pig farms are referred to as intermediate-storage samples.

At every spot, 5-liter samples were collected. Immediately after collection, the samples were stirred, placed in cooling boxes and transported to the biogas laboratory at the Leibniz Institute for Agricultural Engineering and Bioeconomy in Potsdam, Germany. In the laboratory, 1-L subsamples were stored frozen at −18 °C for physical–chemical analyses and BMP tests.

Table 1
Characteristics of the dairy cow and fattening pig farms where manure samples were collected.

Dairy cow farms						Fattening pig farms			
	Units						Units		
Farm ID	[-]	DE01	DE02	DE03	DE04	Farm ID	[-]	DE05	DE06
Herd Info									
Animal breed	[-]	Friesland–Holstein	Friesland–Holstein	Friesland–Holstein	Friesland–Holstein	Annual production	pigs/ year	500	1000
Number of milking cows in barn	head	205	380	112	350	Average weight at delivery	kg	120	130
Feeding									
Grass silage	%FM	4	29.3	37	50	Dry or wet feeding	[-]	Dry	Dry
Maize products	%FM	61	36.1	41	27	Feed ration	[-]	Rye, triticale, barley, soybean meal, rapeseed meal, peas, and sunflower meal	Barley/husked (35%), rye (20.6%), wheat (31%), soybean meal (9.3%), and soybean hulls (4.1%)
Roughage and byproducts	%FM	25	9.1	4	10				
Concentrates and minerals	%FM	0	8.3	17	6				
Other	%FM	10	17.2	–	7				
Housing characteristics									
Barn dimensions (length × width)	m	38.8 × 17.65, 35 × 71.5	19 × 75, 19 × 33, 25 × 60	42 × 53	23 × 75, 43 × 75	Barn dimensions (length × width)	M	17 × 64 + 17 × 64	76 × 16
Bedding type in the lying boxes	[-]	Straw and lime	Barley straw	no	Straw (barley/rye) and lime (2%)	Bedding type in the lying boxes	%	50	100
Manure collection system	[-]	Pumping pit and scrapers	Pumping pit, ring channels and scrapers	Pumping pit, ring channels and scrapers	Pumping pit and scrapers	Manure collection system	[-]	Pit	Pit
Manure management	[-]	Stored before field application	Stored before field application	Stored before field application	Stored before field application	Manure management	[-]	Stored and field applied	Stored and field applied
Outdoor Storage									
Manure storage conditions	[-]	Liquid with crust	Liquid with tent and solid crust	Liquid with crust	Liquid with crust	Manure storage conditions	[-]	Liquid with crust (clay additive)	Liquid with crust
Storage type	[-]	Glassed steel	Glassed steel	Concrete	Metal and concrete	Storage type	[-]	Metal	Concrete
Storage period	[-]	November to April	November to April	November to April	November to May	Storage period	[-]	November to April	November to April
Average diameter	m	24	25.7	33	25.1 and 23.5	Average diameter	m	14.6	15
Height	m	7	4.2	6	5,	Height	m	6	5

2.2. Physical–chemical analysis

Electrical conductivity, pH, and sample temperature were measured immediately after collection with an electrical conductivity meter, pH meter (Multiline P3 pH/LH, WTW, Weilheim, Germany) and thermometer (Hamster ET2, Elpro, Buchs, Switzerland), respectively. The pH value was obtained by immersing the electrode (Sen Tix 81, WTW, Weilheim, Germany) (DIN38404). The frozen samples were gently defrosted before the chemical analysis and batch anaerobic digestion tests occurred. To determine the dry matter (DM) content, samples were dried at 105 °C to a constant weight, followed by dry combustion at 550 °C to determine the ash and volatile solids (VS) content in a muffle furnace (CWF 1100, Carbolite Gero GmbH & Co. KG, Neuhausen, BW, Germany) (VDLUFA, 2006). The contents of volatile fatty acids (VFAs) (C_2, C_3, C_4, C_5, and C_6) and alcohols (C_1, C_2, C_3, and C_4) were verified by cold-water extraction and gas chromatography (Agilent Technologies Inc., Santa Clara, CA, USA), including a PERMABOND FFAP capillary column (Machery-Nagel GmbH & Co. KG, Düren, Germany) and a flame ionization detector (VDLUFA, 2006).

An elemental analyzer was used to assess the contents of hydrogen, nitrogen, sulfur and carbon (Vario EL, Elementar Analysensysteme GmbH, Hanau, Germany); this determination adopted the principle of raw catalytic combustion under high temperatures and oxygen supply (VDLUFA, 2006). Crude protein was determined by multiplying elemental N by 6.25. Analyses of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were conducted according to Van Soest et al. (1991) using the Ankom 2000 fiber analyzer system with filter bag technology (Ankom Technology Corp., Macedon, NY, USA) (VDLUFA, 2012a). The content of acid detergent lignin (ADL) was measured gravimetrically after adding 72% sulfuric acid to the bag from ADF analysis for 3 h, drying the sample, and incinerating the sample in a muffle furnace at 600 °C for 2.5 h (VDLUFA, 2012b, 2012c). The amounts of the different fibrous fractions were assessed as follows: cellulose was determined by calculating the difference between ADF and ADL, and hemicellulose was determined as the difference between ADF and NDF. Lignin was defined as ADL by assuming that the fraction of lignin-bound nitrogen was insignificant. The crude fat level was verified according to the Weibull–Stoldt method using the AnkomXT10-Extractor (Ankom Technology Corp., Macedon, NY, USA).

Total ammonia nitrogen (TAN) was converted to ammonia by magnesium oxide, collected by steam distillation (Vapodest 20 Gerhardt, Apeldoorn, The Netherlands), and then transferred to a distillation receiver containing boric acid (VDLUFA, 2006). Chemical oxygen demand (COD) analyses were conducted by following standard methods (DIN38409-2, 1987). More details about the physical and chemical parameters are found in (Hilgert et al., 2022).

2.3. Biochemical methane potential tests

BMP tests were performed to evaluate the CH_4 production potential of the collected manure samples according to the standard procedure VDI (2016). The inoculum was a mixture of digestate from laboratory anaerobic digestion batch experiments and digestate from two commercial biogas plants running with crop residues, animal manure, and energy crops as feedstock. The inoculum had methanogenic microorganisms adapted to mesophilic temperature conditions. The digestate was sieved (mesh size 3 mm) to avoid large particles, stored in a tank at 37 °C and stirred once a week. The inoculum was not standardized over several batches; therefore, a possible effect of the inoculum on CH_4 production could not be fully ruled out. Hence, blank samples were included to evaluate this influence, as well samples with cellulose tested the activity levels of the microorganisms.

The experimental setup used 100-mL glass syringes filled with inoculum and substrate in quantities that guaranteed volatile solids ratios of approximately 2; the ratio was controlled by weighing (Precision balance EG4200-2NM, Kern & Sohn GmbH, Balingen, Germany). The glass syringes were closed; so that the piston movement displaced the air inside, ensuring anaerobic conditions. Silicone paste (Silicon paste medium viscous, Carl Roth GmbH + Co. KG, Karlsruhe, Germany) was applied between the piston and the syringe to avoid leakages during handling and measurement of gas production. Five replicates were set up for each manure sample.

The samples were incubated at 37 °C. To evaluate the volume of gas produced during incubation, the piston displacement was recorded at least 4 times per week. The biogas volume produced by the blank samples was subtracted from the measured gas production of each sample. Biogas production was expressed in norm liters per kg of volatile solids ($L_N\ kg_{VS}^{-1}$); that is, the volume of biogas production was based on normal conditions, which included a dry gas temperature of 273 K and pressure of 1013 mbar (VDI 4630., 2016). The gas composition (CH_4 and CO_2) was measured twice per week in the first 14 days and once per week in the following days (for CH_4: Advanced Gasmitter, Sensors Europe GmbH, Erkrath, Germany; for CO_2: MonoGas Analyzer, Pronova Analysentechnik GmbH & Co. KG, Berlin, Germany). Standard gas (60% CH_4 and 40% CO_2, Air Liquide Deutschland GmbH, Düsseldorf, Germany) was used to verify the calibration of the equipment every time the gas composition was measured. The agitation of the batches occurred during volume and gas composition measurements. The completion of the BMP tests respected the stopping criteria of the VDI procedure (i.e., daily biogas production during three consecutive days was lower than 0.5% of the total biogas produced until that time) (VDI 4630., 2016). Depending on the sample, the stopping criteria were reached after 45 and 70 days.

2.4. Data analysis

2.4.1. Dry matter correction

The VFA content was expressed as acetic acid equivalents following division by factors based on molarity. To account for the losses of volatile components during sample processing, a methodology from Weissbach and Kuhla (1995) was used to include the VFA content in the calculation of the DM.

In order to account for the dry matter loss from dairy cow manure during storage, the dry matter content of outdoor storage samples was corrected based on the ash content of manure samples collected in the barn. The assumption considered was that the ash content during storage is constant (Larney et al., 2005).

2.4.2. Kinetics analysis

Kinetic analyses were performed to extract the degradation parameters of the samples using a first-order differential Eq. (1) and a modified Gompertz equation Eq. (2). The first-order differential equation was used to model the degradability of substrates which allowed the estimation of the substrate degradation constant k.

$$y(t) = y_m \bullet \left(1 - e^{(-k_1 t)}\right) \tag{1}$$

where $y(t)$ is the cumulative specific CH_4 yield at time t $\left(L_N CH_4 kg_{VS}^{-1}\right)$, y_m is the maximum specific CH_4 yield at a theoretically infinite digestion time $\left(L_N CH_4 kg_{VS}^{-1}\right)$, t is the time $(days)$ and k is the first-order decay constant (day^{-1}).

The modified Gompertz equation allowed the estimation of the lag phase time (λ) and the maximum specific CH_4 production rate (R_m) (Zwietering et al., 1990). The curve obtained had a fixed inflection point and was asymmetric around the inflection point (Herrmann et al., 2016; Lo et al., 2010; Morais et al., 2021). The negative lag times estimated from this equation were assumed to be 0 (Dalgaard & Koutsoumanis, 2001).

$$y(t) = y_m \bullet exp\left\{ - exp\left[\frac{R_m \bullet e}{y_m} \bullet (\lambda - t) + 1\right]\right\} \tag{2}$$

where $y(t)$ is the cumulative specific CH_4 yield at time t, $\left(L_N CH_4 kg_{VS}^{-1}\right)$, y_m is the maximum specific CH_4 yield at a theoretically infinite digestion time $\left(L_N CH_4 kg_{VS}^{-1}\right)$, R_m is the maximum specific CH_4 production rate $\left(L_N CH_4 kg_{VS}^{-1} day^{-1}\right)$, λ is the lag phase (*days*), and t is the time (*days*).

To evaluate the effect of storage time on the BMPs of the manure samples, the average storage time was estimated based on the last time the storage was emptied before the day of sample collection. The period in days was divided by two to estimate the average storage time. Eq. (1) and Eq. (2) were adapted to more accurately represent the obtained information, in Eq. (1) essentially turning the subtraction of the terms inside the brackets into an addition, and in Eq. (2) by removing the negative sign of the double-exponential term. By comparing the Akaike Criteria for these curves, the best fit was determined. Furthermore, the parameters derived from these equations were assessed to confirm if they retained the same significance as those used in BMP curves.

2.4.3. *Statistical analysis*

Statistical data analysis was performed using R software (R Core Team, 2022). The significance levels of differences in the physicochemical compositions and the kinetic parameters from the manure samples at different positions on the manure management chain were verified by Welch's analysis of variance (ANOVA). When significant effects were identified, the Games–Howell post hoc test, using a 0.05 p-level, was applied for multiple mean comparisons. A principal component analysis (PCA) was carried out with the y_m and the chemical components as input to explore possible positive and negative relationships.

Descriptive statistics using multilevel multiple linear regression tests were performed to develop mathematical models for predicting the potential y_m of livestock manure samples from the storage systems. The chemical component contents of the samples and the stage of storage where the samples were collected, were used as predictors. Statistical techniques to reduce the number of predictors were used. Graphical representations of these analyses are given in the Supplementary Material. To verify which manure chemical components could explain most of the sample variability, PCA and correlation coefficients were calculated for barn as well as intermediate and outdoor storage systems to reduce the collinearity of the variables. If two predictors were strongly correlated (correlation higher than 0.7), the presence of both in a model could decrease the model significance, and one of them was chosen based on higher correlation with the principal components and potential to simplify the model. After predictor selection, a stepwise regression using Akaike information criterion was performed to select the model that showed the best fitting with the obtained BMPs. To identify and exclude outliers, Cook's distance was used to estimate the influence of a data point when performing a least-squares regression analysis. Interactions between lignin and other chemical components were tested to verify whether the interaction had some influence on the BMP. The obtained models were validated by plotting the results against the obtained kinetic parameters.

3. Results and discussion

3.1. *Manure chemical characteristics*

Table 2 shows the samples' physicochemical parameter means and the significant differences when present for pig and cow manure specimens in different stages on the MMC. For dairy manure, the average dry matter contents were higher for barn storage samples than for outdoor storage samples (F = 9.67 and p = 0.004), which could be explained by the dilution and degradation of organic matter during storage. For pig manure, dry matter was lower for barn samples relative to outdoor storage (F = 9.13 and p = 0.012), which may have been caused by the

Table 2

Kinetic parameters for CH_4 production and chemical characteristics of dairy cow manure and fattening pig manure samples. y_m is the maximum specific CH_4 yield at a theoretically infinite digestion time from the Gompertz equation $\left(L_N CH_4 kg_{VS}^{-1}\right)$, R_m is the maximum specific CH_4 production rate from the Gompertz equation $\left(L_N CH_4 kg_{VS}^{-1} day^{-1}\right)$, λ is the lag phase from the Gompertz equation (*days*) *and k is the first-order decay constant from the first-order differential equation* (day^{-1}). *The significance levels of the Games–Howell test results are reported with the letters a, b and c.*

	DM (%)	VS (%$_{DM}$)	pH	EC (µS/cm)	VFAs (%$_{DM}$)	TAN (mg/kg$_{FM}$)	XP (%$_{DM}$)	XF (%$_{DM}$)	NFC (%$_{DM}$)	Lig (%$_{DM}$)	Cel (%$_{DM}$)	Hem (%$_{DM}$)	C/N	CH_4 content (%)	y$_m$ – Gompertz	R$_m$ - Gompertz	λ - Gompertz	K - EDO
Dairy cow manure																		
Kinetics parameters for CH_4 production and chemical characteristics of dairy cow manure collected from barn storage systems																		
Mean	9.59^{a}	81.08^{a}	6.87	12.04^{b}	6.59^{a}	1306.4	16.67^{a}	1.91	14.77	8.06	19.79^{a}	19.87^{a}	16.79	61.69	277.52^{a}	21.56^{a}	0.46	0.13
SE	0.37	1.04	0.08	0.55	0.38	82.26	0.4	0.13	0.69	0.24	0.34	0.45	0.32	0.33	4.87	0.53	0.09	0.03
Kinetic parameters for CH_4 production and chemical characteristics of dairy cow manure collected from outdoor storage systems																		
Mean	8.16^{b}	61.78^{b}	6.89	14.22^{a}	4.09^{b}	1310.25	12.45^{b}	1.79	13.15	6.88	13.66^{b}	13.83^{b}	17.29	63.13	223.69^{b}	13.89^{b}	n.a.	0.09
SE	0.3	3.88	0.1	0.69	0.96	55.77	0.69	0.12	0.93	0.49	1.11	1.02	0.69	0.61	7.7	1.45	n.a.	0.01
Fattening pig manure																		
Kinetics parameters for CH_4 production and chemical characteristics of fattening pig manure collected from barn storage systems																		
Mean	1.12^{a}	58.72	7.27	16.43	2.83	1546.5^{a}	19.42	2.73^{b}	22.61^{a}	2.56^{b}	3.89	7.53^{b}	11.87	70.57	312.66^{a}	22.76^{a}	0.36	0.09^{a}
SE	0.12	2.26	0.05	1.28	0.77	134.89	0.76	0.48	1.18	0.4	0.64	1.38	0.58	1.11	20.66	2.47	0.41	0.01
Kinetics parameters for CH_4 production and chemical characteristics of fattening pig manure collected from intermediate storage systems																		
Mean	4.00^{ab}	70.79	7.07	17.71	7.13	2573.8^{ab}	18.58	5.56^{a}	14.07^{b}	5.85^{a}	9.29	17.44^{a}	13.65	71.25	230.26^{b}	15.42^{ab}	0.86	0.08^{ab}
SE	1.02	3.4	0.15	1.31	3.05	528.51	1.09	0.43	0.46	0.73	1.61	2.09	1.69	1.55	10.88	2.77	0.39	0.01
Kinetics parameters for CH_4 production and chemical characteristics of fattening pig manure collected from outdoor storage systems																		
Mean	2.68^{b}	60.14	7.4	15.37	1.82	2238.7^{b}	18.81	3.64^{b}	14.30^{b}	5.85^{a}	6.29	11.26^{ab}	11.97	69.5	165.73^{c}	9.20^{b}	n.a.	0.06^{b}
SE	0.42	2.65	0.05	2.26	0.39	106.29	0.79	0.22	1.27	0.95	1.16	1.6	1.6	1.07	16.98	1.51	n.a.	0.01

problem of obtaining representative samples, especially for pig manure, where the nonhomogeneous nature has a tendency to cause natural stratification during storage (Ndegwa & Zhu, 2003). VS concentrations of cow manure for barns and outdoor storage were significantly different ($F = 22.125$ and $p < 0.001$), consistent with organic matter degradation during storage. This difference was verified in the study of Browne et al. (2015), where dairy slurry specimens were stored at 9 °C and 20 °C for 26 weeks. The VS concentrations based on fresh matter decreased by 5% and 17% for the specimens stored at 9 °C and 20 °C, respectively. The differences in VS among the sampling positions were not significantly different for pig manure.

Although the average pH values of cow and pig manure samples did not present significant differences among the storage systems, there was a pH increase with increasing storage time. This result was in agreement with the studies performed by Sommer & Husted (1995) and Teixeira Franco et al. (2018), where the manure pH value tended to increase with a decrease in VFA concentration explained by conversion into CH_4 and the release of CO_2. For cow manure, the VFAs seemed to degrade according to the progress in the MMC ($F = 5.24$ and $p = 0.032$). The abovementioned study by Browne et al. (2015) showed that the VFA concentration in 20 °C stored dairy cow slurry markedly decreased from the 17th week; the VFA concentration of manure stored for 26 weeks at 9 °C remained relatively constant. The VFAs in pig manure did not present significant differences among the storage systems ($F = 1.864$ and $p = 0.233$); however, the concentrations tended to decrease from intermediate storage to outdoor storage. These results confirmed the hypothesis of organic matter degradation because acetogens degraded VFAs to acetate, CO_2, and hydrogen, which were further converted by methanogens to CH_4 and CO_2 (Gerardi, 2003). This finding was in line with the results of Teixeira Franco et al. (2018), in which stored cow manure with low total solids (TS) content and relatively high pH levels leads proliferates methanogenic consumption and, consequently, VFA consumption.

The TAN content for dairy manure was similar among the storage systems ($F = 0.001$ and $p = 0.973$). For pig manure, the TAN content from the barn sample was lower than that of the samples from the other storage systems ($F = 7.912$ and $p = 0.0169$), while the protein content showed similar values for all the storage systems ($F = 0.231$ and $p = 0.798$). In contrast, the protein contents of dairy manure samples presented significant decreases ($F = 27.64$ and $p < 0.001$) from barn storage to outdoor storage, suggesting at some degradation during storage.

The content of crude fat (X_F) was not significantly different between the cow manure storage systems ($F = 0.566$ and $p = 0.458$). For pig manure, the samples from the intermediate storage had higher contents of X_F than the contents from the other storage systems ($F = 10.191$ and $p = 0.007$).

The content of nonfiber carbohydrate (NFC) was low in dairy manure samples from both stages ($F = 1.851$ and $p = 0.184$), showing that the digestive process of the cattle removed most of the easily digestible carbon. For pig manure, the barn samples had higher contents of NFC than the outdoor and intermediate storage samples ($F = 21.842$ and $p < 0.001$). Regarding the fiber content, in cow manure, the contents of hemicellulose ($F = 27.38$ and $p < 0.001$) and cellulose ($F = 27.66$ and $p < 0.001$) were lower in the outdoor samples than in the barn samples. The lignin contents in cow manure samples was not significantly different ($F = 4.053$ and $p = 0.055$) between the sampling storage systems, agreeing with studies by Susmel & Stefanon (1993), Gerardi (2003) and Muhammad Nasir & Mohd Ghazi (2015), who showed that lignin was a challenging component to degrade by anaerobic microbial communities. For pig manure, samples from the barn presented lower values for lignin ($F = 10.181$ and $p = 0.005$) and hemicellulose ($F = 7.310$ and $p = 0.013$) than those from the other sampling positions. This fact agrees with the results from other cow manure studies and could show the difficulties of degrading lignin by microorganisms. The cellulose contents in pig manure samples exhibited significant differences among the storage systems ($F = 5.249$ and $p = 0.035$); however, the post hoc analysis revealed no significant differences. The C/N ratio did not present significant differences between the storage systems for cow manure ($F = 1.37$ and $p = 0.2469$) and pig manure ($F = 0.458$ and $p = 0.650$).

3.2. Biochemical methane potentials

The comparisons of the maximum y_m showed that barn samples have a higher BMP than aged samples, and this was true for cow manure ($F = 36.38$ and $p < 0.001$) as well as pig manure ($F = 14.342$ and $p < 0.001$). Degradation of organic matter by anaerobic microorganisms during storage possibly decreased the CH_4 production potential through emissions to the atmosphere. There was a 20.4% decrease in the y_m for dairy cow manure from the samples collected in the barn storage system relative to those collected in the outdoor storage system. The study by Browne et al. (2015) observed a similar decrease in the specific CH_4 yield of stored dairy cow manure at 20 °C after 18 weeks of storage. For fattening pig manure, there were 47.1% decreases in the y_m values, showing a faster degradation of organic matter in pig manure than in cow manure. This sharp decrease in the CH_4 potential for pig manure during storage was in agreement with the study by de Buisonjé & Verheijen (2014) who stored pig manure for different periods and verified a 74% decrease in the CH_4 potential when comparing pig manure samples stored for 32 and 120 days.

The cow manure samples from barns presented higher maximum production rates (R_m) values than the outdoor samples ($F = 24.64$ and $p < 0.001$). A similar behavior was seen for pig manure, i.e., with samples from the barn storage system presenting higher rates than those from the outdoor storage system ($F = 10.412$ and $p = 0.006$). The difference was probably due to samples from the outdoor storage system being more degraded than the barn samples. Additionally, the trend for the decay constant, showed a decreasing tendency according to the position on the manure management chain for both animal manure types. However, no significant differences appeared in the cow manure samples ($F = 2.39$ and $p = 0.141$), whereas for pig manure the samples from the barn had a higher decay constant than those from outdoor storage ($F = 4.269$ and $p = 0.046$). The lag-phase evaluation suggested a shorter lag-phase periods for older samples, but there were no significant differences with both animal categories (cow $F = 2.11$ and $p = 0.163$) (pig: $F = 0.605$ and $p = 0.560$). The analyses of the differences between different seasons and farms were not significant for either of the animal manure types.

3.3. Effect of storage time (or aging) on BMP

Fig. 1 and Fig. 2 show BMPs from dairy cow and fattening pig manure samples, respectively, plotted against the average storage time. For both manure types there is evidence for a fast decrease in the BMP at the beginning of the storage and declining rates with increasing storage time, agreeing with the rates presented in Table 2, and with the observations of de Buisonjé & Verheijen, (2014). Also, Gopalan et al. (2013) conducted an analysis of the average BMPs of beef feedlot manure samples. The researchers found that fresh samples (<8 h old) had BMPs of 350 mL CH_4 g_{VS}^{-1}, pad samples (aged between 3 and 8 weeks) had BMPs of 270 mL CH_4 g_{VS}^{-1}, and stockpile samples (aged between 8 and 12 months) had BMPs of 140 mL CH_4 g_{VS}^{-1}. Similarly, Hashimoto et al. (1981) demonstrated a decrease in the BMPs of beef cattle manure samples with increasing storage time. Specifically, the BMP for fresh manure was 260 mL CH4 g_{VS}^{-1}, and the BMP for manure aged between 6 and 8 months was 210 mL CH_4 g_{VS}^{-1}.

The faster decrease in pig manure could be related to the higher content of NFC compared to cow manure; since in cattle this component is already digested in the rumen and gut (Amon et al., 2007). The study of Feng et al. (2018), in which the CH_4 emissions from cattle and pig manure specimens were measured in storages prior to digestion, showed that the degradation was more intense for pig manure samples. Another reason can be related to the controlled environmental temperature in the

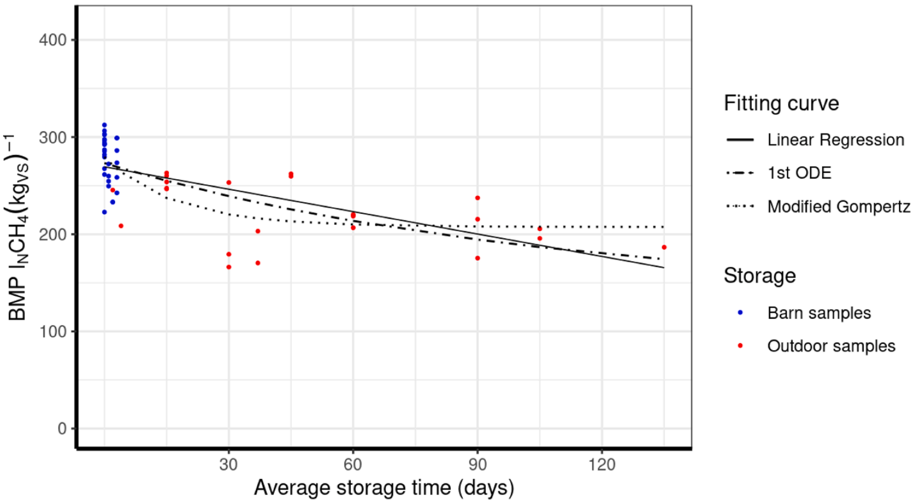

Fig. 1. BMPs of dairy cow manure samples collected from barn and outdoor storage systems by considering the storage time. Outliers were excluded based on Cock's distance.

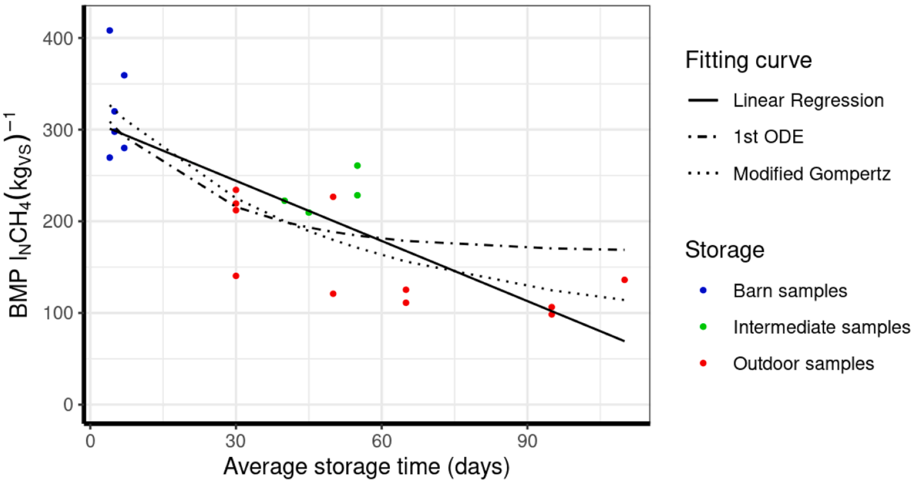

Fig. 2. BMPs of fattening pig manure samples from barn, intermediate and outdoor storage systems by considering the storage time. Outliers were excluded based on Cock's distance.

pig barns of approximately 20 °C, which will increase in the activity of microorganism involved in the degradation of organic matter (Hilgert et al., 2022).

Manure samples from different stages in the manure management process could undergo changes in chemical composition and CH_4 potential in two phases. The initial phase was marked by a high TS content that caused the bacteria to hydrolyze fibers to produce VFAs, producing mainly acetic acid; this phenomenon decreased the pH values to levels that could inhibit the methanogens. The second phase occurred when manure was stored in a more liquid-like setting, and it was characterized by an increase in methanogen growth and VFA consumption, breaking down VFAs into CH_4 and CO_2. The literature confirmed this process for both pig and cow manure types (Conn et al., 2007; Teixeira Franco et al., 2018).

The adapted Modified Gompertz Equation presented the best fitting criterial for both animal categories when compared with the adapted first-order equation and linear regression. More details on the goodness of fit of the non-linear and linear analysis are presented in the Supplementary Material. Despite the good fitting, it is evident that the curve fitting is limited due to variations in samples and conditions from different barns, such as feeding, housing, manure storage, and management systems. Additionally, uncertainties regarding precise storage times and factors influencing storage (e.g., temperature) contribute to these limitations.

Furthermore, non-linear models are generally effective in representing biological degradability of organic matter (Herrmann et al., 2016). Concerning the rate parameter (R_m cow: 4.1, R_m pig: 0.25) of the adapted Modified Gompertz Equation they are lower in comparison to those in BMP tests (Table 2), since storage takes place at lower temperatures and without the addition of inoculum. Compared with the results by Hilgert et al. (2022), where fresh manure samples were stored at different temperatures for 90 days, the rates of methane production during storage are higher than those obtained by the fitting curves. Future studies with broader sample size and further investigations regarding residual BMP may confirm the significance of other parameters.

3.4. *Principal component analysis (PCA): Relationships between y_m and chemical components*

A separate PCA was performed for each animal category, and samples from different positions in the manure management chain were included. The results of these analyses are presented in Fig. 3 and Fig. 4. The position of the vector for each individual component describes its

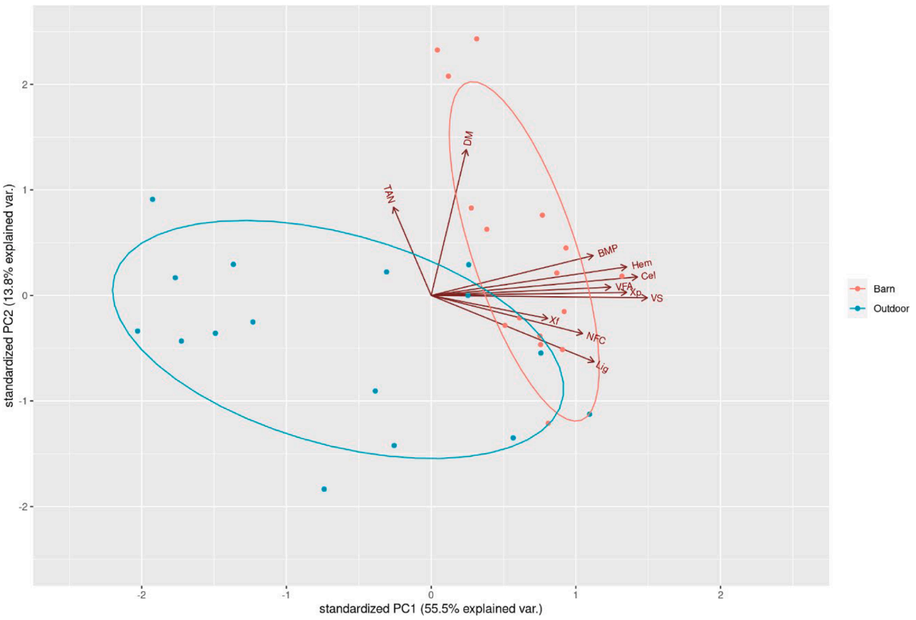

Fig. 3. PCA between BMP and the chemical components for dairy cow manure samples collected in the barn and outdoor storage systems.

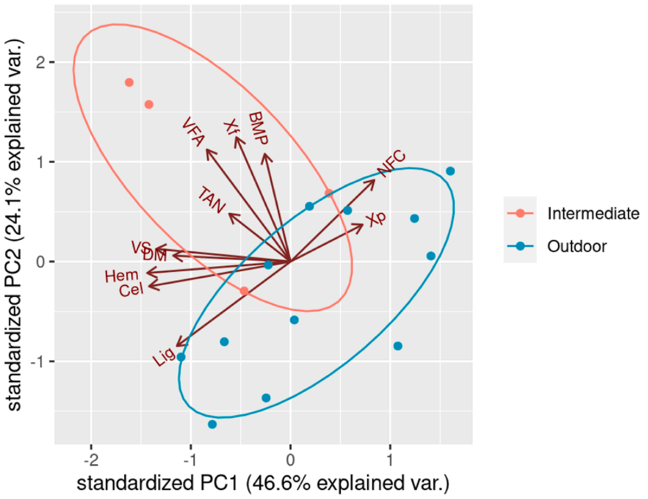

Fig. 4. PCA between BMP and the chemical components for fattening pig manure samples collected in the intermediate and outdoor storages.

association with the other variables. Components with correlation indices exceeding 0.7 with the PCs were highly correlated, and components with correlations below −0.7 were strongly negatively correlated. As shown in Fig. 3 and Fig. 4, each dot represented a sample, and ellipses delimit samples from the different positions in the MMC. For this analysis, fresh and pumping pit dairy cow samples were grouped as barn storage samples. For pig manure, in Fig. 4, only samples from intermediate and outdoor storage systems were considered due to the aforementioned difficulties of sampling in barns.

In Fig. 3, two principal components of dairy cow manure explained 69.3% of the total variation (55.5% and 13.8%, respectively). The components that were highly correlated with PC1 were volatile solids (0.99), hemicellulose (0.89), VFAs (0.82), cellulose (0.94), crude protein (X_p) (0.89) and lignin (0.74). BMP also presented a high correlation with PC1 (0.74); the analysis showed that samples with highly correlated with PC1 tended to have higher BMPs, besides dry matter was strongly correlated with PC2 (0.92). As in the previous sections, this analysis indicated that barn storage samples tended to have higher CH_4 potentials than outdoor storage samples, as indicated by the ellipses in the graphs.

This analysis was partially in agreement with a review performed by Raposo et al. (2020), stating that many models predicting CH_4 potentials from the initial chemical composition of different feedstocks (for instance, energy crops and animal manure) revealed that the contents of hemicellulose and X_p were positively related to BMP. The content of volatile solids was a good indicator of organic matter but not clearly positively related to BMP. The contents of lignin and cellulose were reported to be negatively related to the BMP. The studies by Amon et al. (2007) and Triolo et al. (2011), in which models predicted methane potentials, showed lignin was negatively correlated with BMP. Cellulose content was negatively correlated with methane potential for animal manure according to a study by Triolo et al. (2011).

Fig. 4 shows the principal components to explain the variances in the fattening pig manure samples. PC1 explains 46.6% of the variance, and PC2 explains 24.1% of the variance. The correlation analysis with PC1 revealed strong correlations with the contents of hemicellulose (-0.97), cellulose (-0.96), volatile solids (-0.91) and dry matter (-0.79). Regarding PC2, the correlated components were (X_f) (0.84) and VFAs (0.76). When identifying the samples from each stage of storage, samples from the intermediate storage tended to have a tendency for higher BMP and organic component levels, such as X_f and VFAs; samples from the outdoor storage tended to have higher contents of lignin, X_p and NFC. The content of crude fat was often related to high BMPs in substrates relative to protein or carbohydrate contents (Raposo et al., 2020).

3.5. *Predicting y_m from stored dairy cow manure*

Details about the PCA and the correlation matrix for selecting the chemical components as predictors for y_m for dairy cow manure are shown in the Supplementary Material. For barn samples, the main principal components explained 54.2% of the variability; the contents of volatile solids, X_p, lignin, and hemicellulose were the chemical components most correlated with the main principal components. The correlation matrix for the barn samples showed that all components were strongly correlated with y_m, which could have occurred because of the low variability levels between the samples; many of them were collected under similar conditions. A strong negative correlation was found between the dry matter and lignin content (-0.73), indicating that samples with lower dry matter levels had higher lignin contents. A positive correlation was seen between the volatile solids contents and the NFC, indicating that for these samples, NFC were important components of the volatile solids contents.

For the outdoor storage samples of dairy cow manure, the main principal components presented 74.6% of the sample variability. The highest correlations occurred between PC1 and volatile solids (-0.99), VFA (-0.88), X_p (-0.90), NFC (-0.81), lignin (-0.84), cellulose (-0.95) and hemicellulose (-0.91). For PC2, the dry matter content presented a strong correlation (0.88). By analyzing the correlation matrix, it was possible to verify that the content of volatile solids was strongly correlated with many of the organic components; X_f was an exception, possibly because the content of fat in dairy manure was low.

According to the results from the PCA, the components initially selected to be part of the model were volatile solids, X_p, lignin, hemicellulose, and dry matter. After the stepwise analysis, the obtained models are presented in Eqs. (3) and (4).

$$y_{m(dairybarn)} = 347.2 + 2.96DM - 1.2VS + 0.22Lig \quad (3)$$

$$y_{m(dairyoutdoor)} = 275.8 - 11.71DM + 3.2VS - 21.43Lig \quad (4)$$

The model predicting y_m for dairy cow barn samples had a significant intercept ($p = 0.01$), which could be explained by the low variability levels of the barn samples, whereas the predictors have low significance ($p > 0.05$). However, the predictors for y_m from the outdoor dairy manure samples were statistically significant, and the dry matter content negatively influenced the BMP ($p < 0.05$). At an advanced degradation state, dry matter was mainly composed of lignin and ash, both being nondegradable components, because a high dry matter content was related to a low BMP. Lignin negatively affected the CH_4 potential of the sample ($p < 0.001$), as in the models presented in the studies by Amon et al. (2007) and Triolo et al. (2011). The content of volatile solids positively impacted the CH_4 potentials of the outdoor samples ($p < 0.001$), likely because more organic material was available for degradation by anaerobic digestion.

Fig. 5 shows a graph where the maximum BMP values obtained from the kinetic model are plotted against the models presented in Eq. (3) and Eq. (4). The multiple R-squared value of the model is 0.829, and the adjusted R-squared value is 0.775, indicating that the multiple regression equations could reasonably explain the variation in the CH_4 yields from the manure samples. Interactions between lignin and other chemical components were analyzed to verify whether the presence of lignin could prevent the degradation of other organic components, for instance, cellulose ($p = 0.980$), hemicellulose ($p = 0.732$), VFAs ($p = 0.075$), X_f ($p = 0.246$), X_p ($p = 0.253$), and NFC ($p = 0.843$). The results of these analyses were not significant for the model presented in Eq. (3) and Eq. (4).

3.6. Predicting y_m from stored fattening pig manure

A statistical analysis of the chemical components as predictors for y_m for fattening pig manure is shown in the Supplementary Material. For the intermediate storage samples, the main principal components explained 91.5% of the variability, and all the chemicals were strongly related to the principal components. Due to the small sample size, many chemical components established strong correlations in the correlation matrix. The final selection determined the contents of dry matter, VFAs, hemicellulose and lignin as the main predictors for the BMPs of the intermediate storage samples.

For the fattening pig manure outdoor storage samples, the main principal components presented 74.4% of the sample variability. The highest correlations were found between PC1 and dry matter (0.73), volatile solids (-0.82), NFC (-0.79), lignin (0.99), cellulose (0.95) and hemicellulose (0.97); for PC2, VFA (0.77) and X_f (0.94) presented strong correlations. After analyzing the correlation matrix for outdoor storage pig manure samples, the predictors selected were the contents of lignin and X_f.

Following this selection, a stepwise regression resulted in the position and the content of dry matter and VFA being the variables that best predicted y_m for fattening pig manure. The model equations are shown in Eq. (5) and Eq. (6) for intermediate and outdoor samples, respectively.

$$y_{m(pigintermediate)} = 222.02 - 6.76DM + 4.94VFA \quad (5)$$

$$y_{m(pigoutdoor)} = 186.78 - 30.9DM + 30.21VFA \quad (6)$$

The model predicting y_m for fattening pig manure from the intermediate storage samples had a significant intercept ($p < 0.01$) that was explained by the low variability of the intermediate storage system. Other predictors for samples from the intermediate storage systems were not significant ($p > 0.05$). By evaluating the model for the outdoor storage system samples, the contents of dry matter and VFAs were significant ($p < 0.05$). This result could be the effect of the larger sample size and the higher variability levels of the samples because they came from two different farms.

Fig. 6 shows a comparison between the BMP values obtained from the kinetic model plotted against the data obtained from the models presented in Eq. (5) and Eq. (6). The multiple R-squared value of the model is 0.696, and the adjusted R-squared value is 0.527, indicating that the multiple regression equations poorly explained the variations in the CH_4 yields from the pig manure samples. Additionally, for the obtained model, interactions between lignin and other chemical components were analyzed; however, these analyses revealed no significant

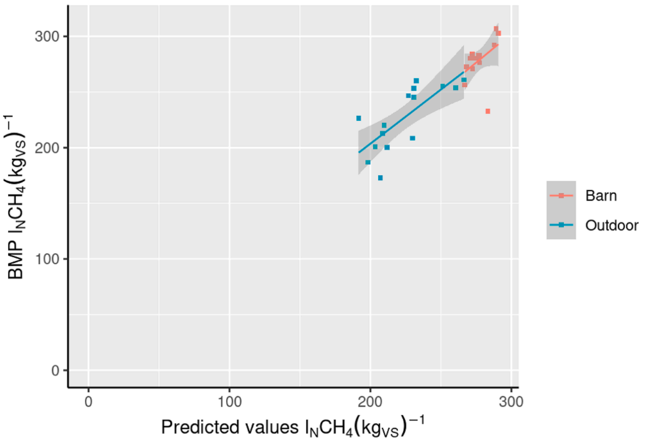

Fig. 5. Validation of the model to predict CH_4 production potential from manure management from dairy farms (Multiple R^2: 0.829, adjusted R^2: 0.775, $p < 0.001$).

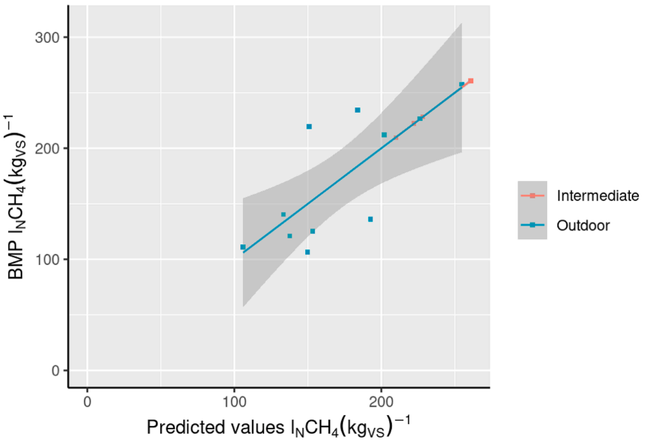

Fig. 6. Validation of the model to predict CH_4 production potential from manure management from fattening pig farms (Multiple R^2: 0.696, adjusted R^2: 0.527, $p = 0.032$).

results for the model presented in Eq. (5) and Eq. (6), for instance, with cellulose (p = 0.334), hemicellulose (p = 0.199), VFAs (p = 0.434), X_f (p = 0.087), X_p (p = 0.105), and NFC (p = 0.410).

The analysis of the models for the different animal manure types showed different intercepts for the samples from the less aged manure relative to the older samples, suggesting that important degradation of the organic matter occurred during storage. This phenomenon was in accordance with the strategy of exporting manure as soon as possible to biogas facilities to avoid losses in potential CH_4 sources to the atmosphere (de Buisonjé & Verheijen, 2014; Browne et al., 2015; Møller et al., 2022).

4. Conclusions

This study investigates the BMP of dairy cow and fattening pig manure samples from sequential stages of the liquid MMS of commercial farms, examining changes in the chemical compositions of these samples to evaluate organic matter degradation during storage. An empirical model is proposed to predict stored animal manure BMP. Results show significant organic matter degradation during storage, led to CH_4 emissions, with a up to 20.5% decrease in BMP for dairy manure and 39.5% decrease for fattening pig manure. The loss of degradable organic matter was twice as high in pig manure compared to dairy manure. The study suggests rapidly transferring manure to biogas facilities to maximize energy yield and minimize CH_4 emissions. The model highlights the importance of lignin for dairy manure and VFAs for pig manure in predicting BMP. Further refinement of the model is needed for better short-term stored manure BMP prediction.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.wasman.2023.05.031.

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Supplementary information

Table 1 - Parameters of the equations used to fit the data, Akaike criteria, R^2 of the linear regression for the data regarding BMP of dairy cow manure storage (p values included).

Adapted Gompertz Equation	**Adapted 1st order differential Equation**	**Linear regression**
y_m=207.5 ($p<0.001$)	y_m=136.6 ($p<0.001$)	Intercept=269.4 ($p<0.001$)
R_m=4.1 (p=0.070)	k=0.00954 ($p<0.001$)	time=-0.769 ($p<0.001$)
λ=-41.5 (p=0.038)		
		R^2=0.433
AIC=495.7	AIC=500.4	AIC=504.8

Table 2 - Parameters of the equations used to fit the data, Akaike criteria, R^2 of the linear regression for the data regarding BMP of pig manure storage (p values included).

Adapted Gompertz Equation	**1st order differential Equation**	**Linear regression**
y_m=67.36 (p=0.5)	y_m=167.06 ($p<0.001$)	Intercept=309.74 ($p<0.001$)
R_m=0.25 (p= 0.71)	k=0.041 (p=0.13)	time=-2.1 ($p<0.001$)
λ=-48.53 (p= 0.07)		
		R^2=0.65
AIC=227.99	AIC=230.76	AIC=230.06

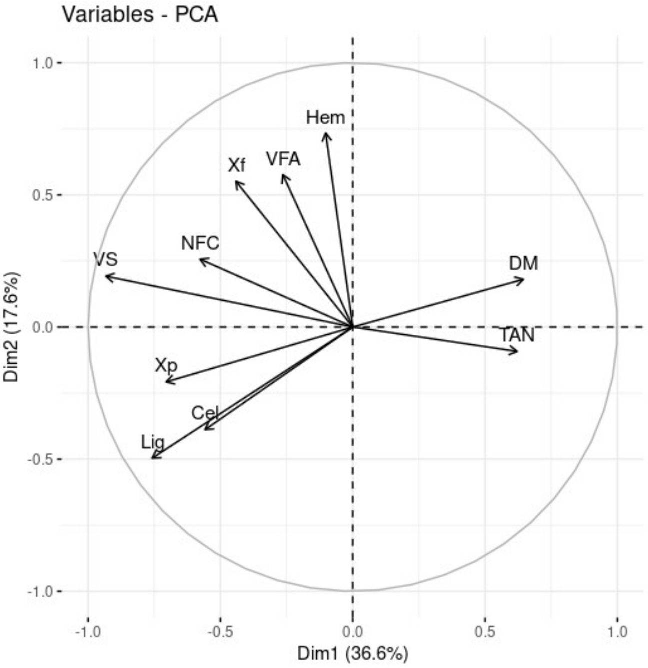

Fig. 1 - Principal component analysis with the chemical components of the barn samples.

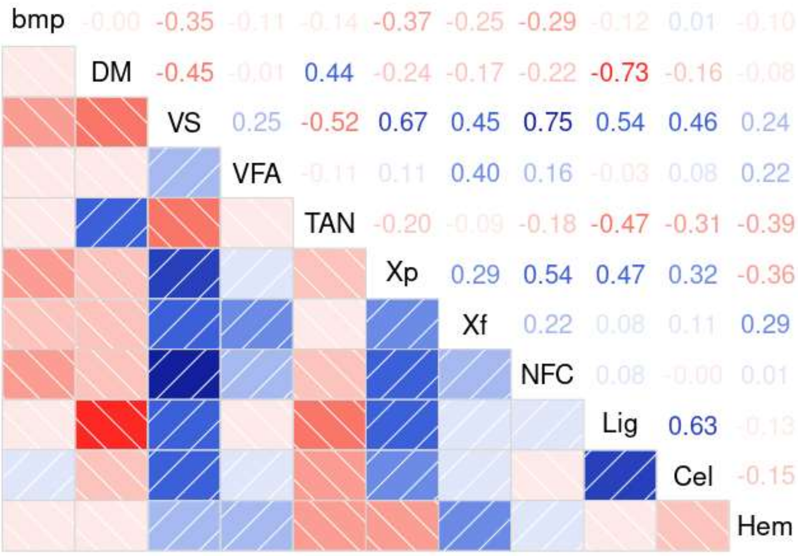

Fig. 2 - Correlation matrix between chemical composition parameters of dairy cow manure from the barn and biochemical CH_4 potential test.

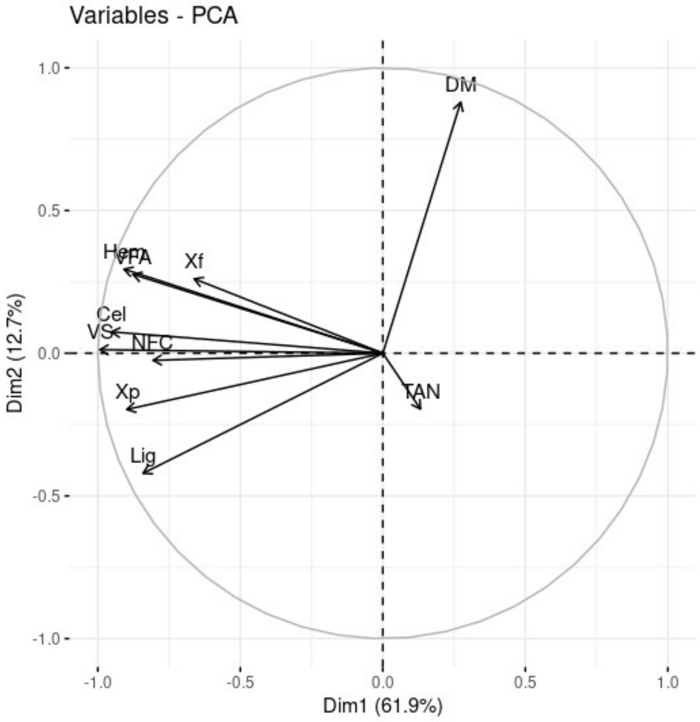

Fig. 3 - Principal component analysis with the chemical components of the barn samples.

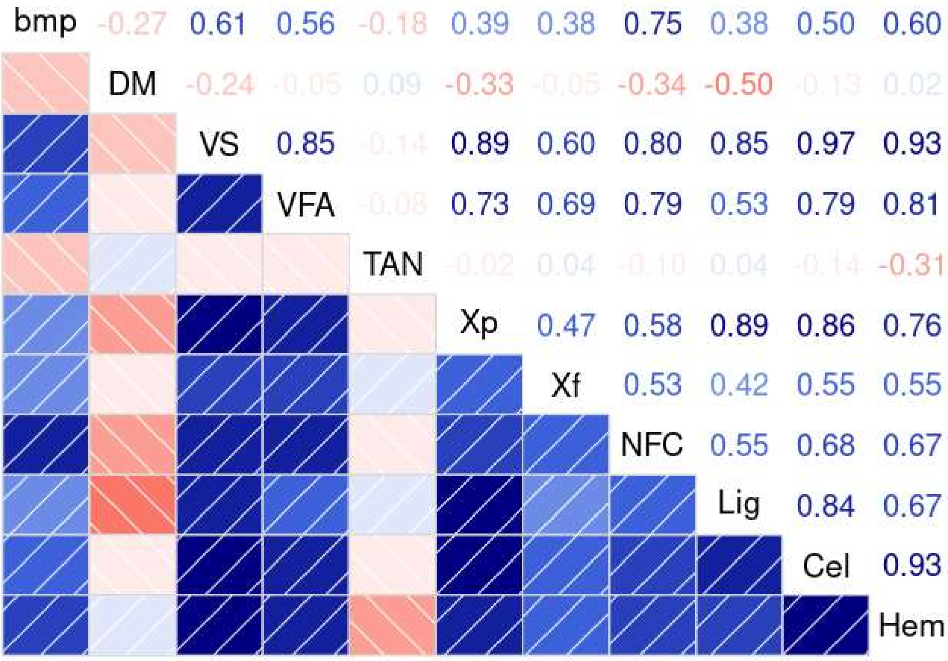

Fig. 4 - Correlation matrix between chemical composition parameters of dairy cow manure from the outdoor storage and biochemical CH_4 potential test.

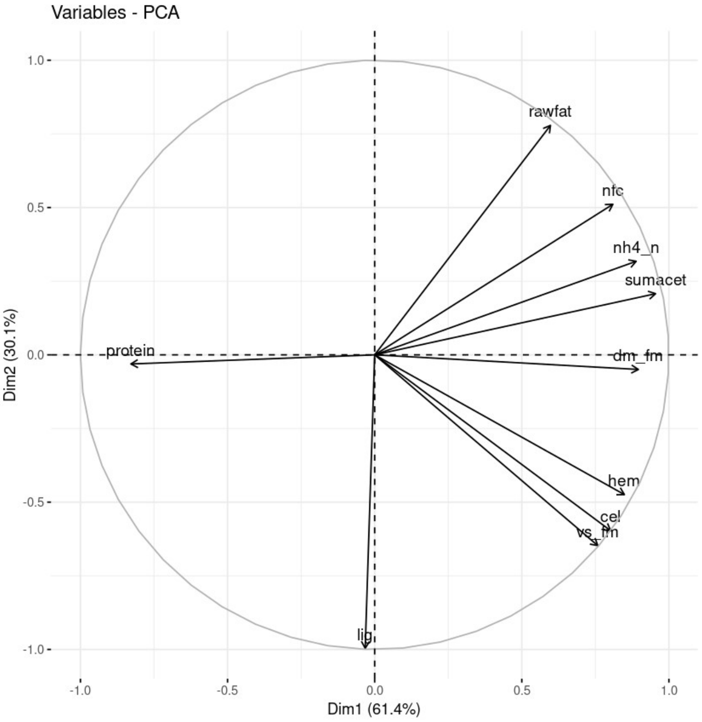

Fig. 5 - Principal component analysis with the chemical components of the fattening pig intermediate storage manure samples.

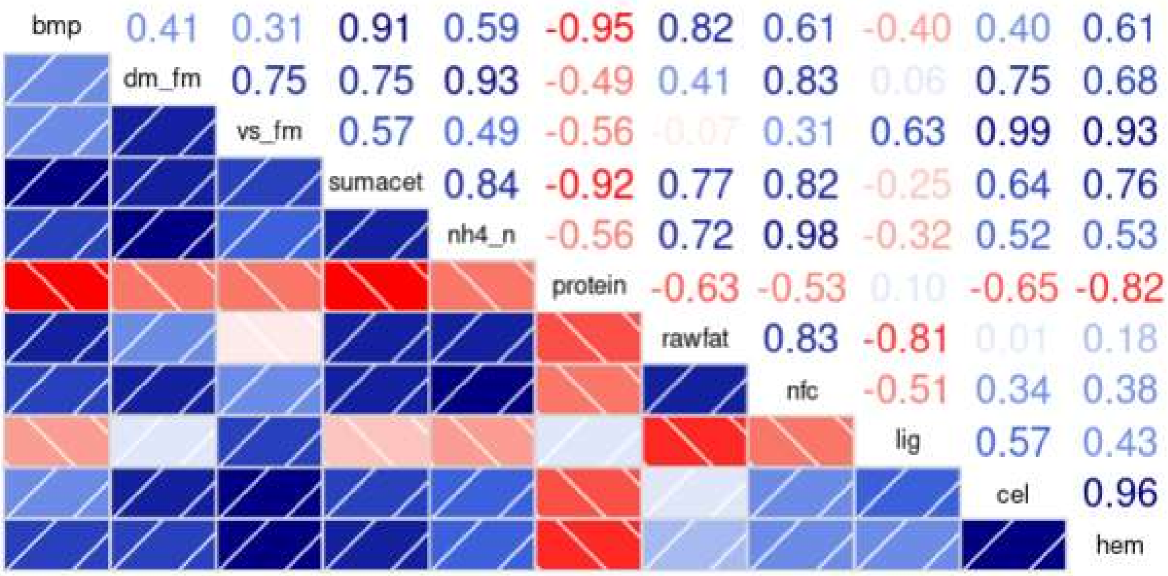

Fig. 6 - Correlation matrix between chemical composition parameters of fattening pig manure from the intermediate storage and biochemical CH4 potential test.

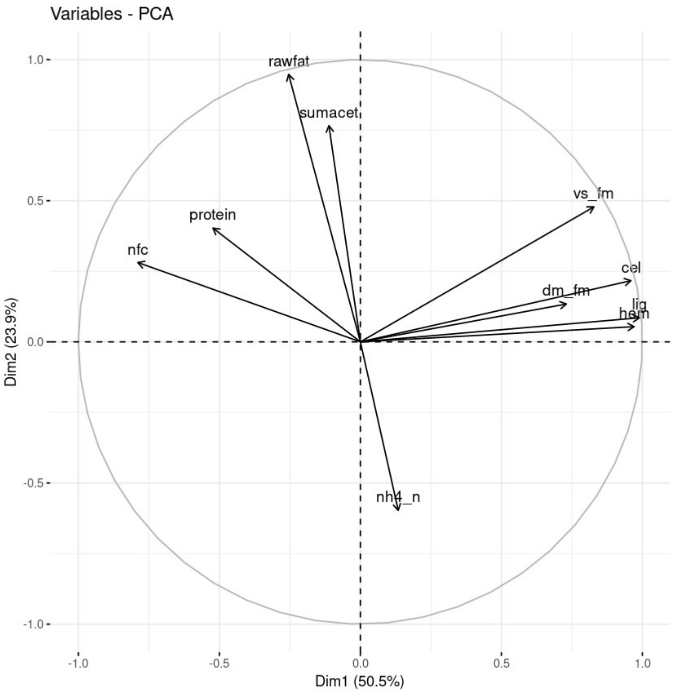

Fig. 7 - Principal component analysis with the chemical components of the fattening pig outdoor storage manure samples.

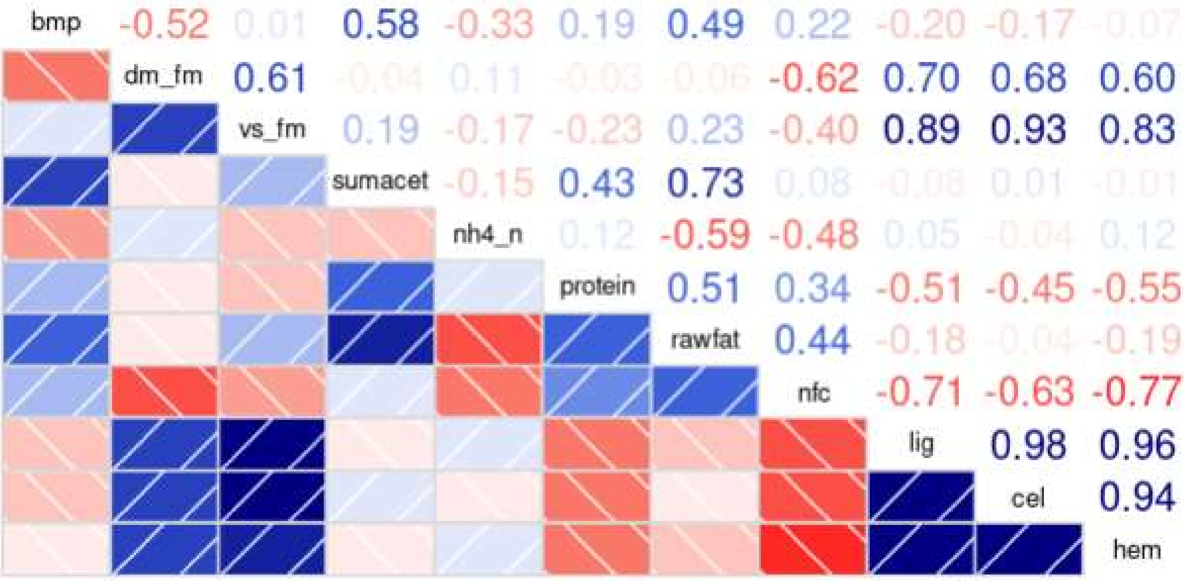

Fig. 8 - Correlation matrix between chemical composition parameters of fattening pig manure from the outdoor storage and biochemical CH_4 potential test.

Chapter 4: Discussion

This segment of the thesis discusses the obtained results in the context of the proposed research questions and the hypotheses posed in the first chapter. It also includes a critical reflection and classification of the results in the literature. The research questions that led this thesis were (1) can the manure storage temperature indirectly influence CH_4 emissions by causing significant alterations in the chemical composition of the dairy cows and fattening pig liquid manure, especially concerning the accumulation of VFA during storage? (2) Considering the manure storage conditions, how do the BMP and the chemical characteristics of manure from various stages of the liquid manure storage change in commercial dairy cows and fattening pig farms? (3) Can mathematical models to predict manure BMP based on manure composition and storage conditions be developed? Can such models enhance our understanding of organic matter degradation and support the design of effective climate change mitigation measures?

The first study (Publication A) results indicated that pig manure stored at 25 °C and 20 °C produced more CH_4 during the incubation than the samples stored at lower temperatures. Their CH_4 cumulative emission reached 69.3% and 50.3% of the BMP values for the fresh sample, respectively. While maximum cumulative CH_4 production for dairy slurry remained low during the observed period, the cumulative CH_4 emission for the samples stored at 25 °C reached approximately 2% of the BMP values for the fresh samples. The chemical analysis of the dairy cow manure after incubation revealed the accumulation of VFAs during storage. The highest accumulation of VFAs was observed in samples stored at 20 °C and 25 °C, which potentially inhibited CH_4 production and somewhat offset the anticipated increase in CH_4 production due to the elevated temperatures.

Some possible reasons for the inhibition of CH_4 production during manure storage were found in the literature. Firstly, according to a study by Zhang et al. (2018), the inhibition of methanogens, specifically hydrogenotrophic ones, can be caused by the concentration of free acetic, propionic, and butyric acids. Despite, in the presented first study, the concentration of free acetic acids did not surpass total inhibition thresholds, partial inhibition could not be excluded. On the other hand, a recent study by Sun et al. (2023), using samples from a municipal wastewater treatment plant, revealed that the decay of methanogens was due more to low pH levels (below 6.5) than the high concentrations of VFA compounds observed during the acidic failure of an AD.

Secondly, most methanogens found in liquid manure have their origins in the digestive system of the animal, and the microbial community in fresh dairy manure might not be well-adapted to the storage conditions (Dalby et al., 2021; Habtewold et al., 2017, 2018; Ozbayram, 2020). Studies by Lendormi et al. (2022) and Sommer et al. (2004) suggested that manure from different animals takes varying times to acclimate, the methanogens present in fresh dairy manure in this study may not have enough time to adapt to the storage conditions, leading to an extended lag phase for the development of methanogens that were more than 90 days, resulting in low CH_4 production and emission.

Thirdly, the accumulation of VFA could result from a relatively high DM content in the samples. A study by El-Mashad et al. (2011) showed that manure with higher DM content produced more VFAs and less biogas, particularly at higher temperatures, confirming the results achieved by Massé et al. (2008) and Massé et al. (2003), where also samples with higher DM contents produced less CH_4 than the more diluted samples. A recent study by Teixeira Franco et al. (2018) stored different cow manure samples for 120 days at 25 °C with increasing DM levels and verified that more concentrated samples led to lower losses of the original CH_4 potential. As the DM content presented by the manure samples in the first study was 11.74 %, the influence of the high DM cannot be ruled out. Despite offering three plausible explanations, the obtained results did not conclusively identify the primary cause of CH_4 production inhibition in the dairy cow manure samples. Therefore, further research possibly evaluating different levels of VFA, DM, and analysis of the microbial activity in dairy manure may determine the underlying mechanism inhibiting CH_4 production.

In the second study (Publication B), aspects regarding the BMP and the compositional changes of dairy and pig manure from different stages of the liquid manure storage throughout the year were investigated. The findings revealed that during manure storage, the decomposition of organic matter culminates in CH_4 emissions, thereby reducing the overall potential yield of CH_4. Specifically, the BMP of dairy manure fell by 20.5% from the barn to outdoor storage, while fattening pig manure decreased by 39.5% from intermediate to outdoor storage. The study underscores the faster degradation of pig manure compared to dairy manure, emphasizing the need to rapidly export manure into biogas plants to prevent substantial CH_4 emissions and diminished energy production capabilities or to outdoor storage, where the temperatures are generally lower.

The analysis of the dairy cow and fattening pig manure samples from different stages in the manure management chain showed changes in the chemical composition. For dairy manure, there was a decrease in the average DM and VS contents; their concentrations were higher for samples stored in barns compared to outdoor storage, likely due to the dilution and degradation of organic matter during storage (Tavares et al., 2014; Triolo et al., 2011). However, for pig manure, the DM was lower in barn samples compared to outdoor storage, possibly due to challenges in obtaining samples and natural stratification during storage (Boltianskyi et al., 2016; Ndegwa & Zhu, 2003).

The pH values of both cow and pig manure samples did not significantly differ among storage systems, but there was an increase in pH with longer storage time, possibly due to the decrease in VFAs concentration and conversion into CH_4 and CO_2, consistent with organic matter degradation due to the AD (Gerardi, 2003; Møller et al., 2004; Paul & Beauchamp, 1989; Teixeira Franco et al., 2018). As for fiber content in cow manure, both hemicellulose and cellulose were found in lower quantities in outdoor samples than in barn samples. There were no significant differences in lignin content in cow manure between the different storage systems, which is consistent with previous research suggesting that lignin is difficult for anaerobic microbial communities to degrade (Gerardi, 2003; Nasir & Ghazi, 2015; Susmel & Stefanon, 1993).

The maximum BMP was found to be higher in fresh barn samples as compared to aged samples for both cow and pig manure. Specifically, a 20.4% decrease in BMP was observed for dairy cow manure from barn storage system samples compared to outdoor storage system samples. Likewise, pig manure showed a sharper reduction in BMP of 39.5% during storage; this decrease could be attributed to its higher NFC content than cow manure. This is because these carbohydrates are already digested in the rumen and gut in cattle. Feng et al. (2018) and Sommer et al. (2007) observed more intense degradation in pig manure samples. This could be due to controlled environmental temperatures in pig barns, which foster increased microbial activity that accelerates organic matter degradation.

Although in this study, it was not possible to verify significant differences in the BMPs due to the seasons of storage, the storage temperatures profiles are intrinsically connected with the air temperature, and this needs to be counted as an essential factor influencing the microorganisms' activity, it may be possible that in tropical conditions the degradation could be more intense and at a higher rate (Cárdenas et al., 2021; Hafner & Mjöfors, 2023). The constant influx of fresh manure in the storage tanks possibly influenced the difficulty of verifying significant differences among the seasons on the remaining BMP.

The BMP from dairy cow and pig manure samples plotted against average storage time indicated a rapid initial decrease in BMP followed by a slower decline with increasing storage time. This trend agrees with earlier studies by de Buisonjé & Verheijen (2014), Gopalan et al. (2013), and Hashimoto et al. (1981), where BMPs of cattle and pig manure decreased with increasing storage time.

In the second study (Publication B), statistical models aimed at forecasting the BMP of dairy cows and fattening pig manure at various stages in the manure storage were introduced, utilizing manure chemical composition data. Based on my research, this is the first model of its kind; a previous one was proposed by Triolo et al., 2011, focusing on biogas production where data from fresh manure samples were used.

The most reliable BMP predictors for dairy manure were the contents of DM, VS, and lignin. The CH_4 potential of the sample was negatively impacted by lignin, as evidenced in the research conducted by Amon et al. (2007) and Triolo et al. (2011), possibly due to the non-degradability of lignin in anaerobic conditions and that it prevents degradation of components for instance cellulose and hemicellulose. Conversely, the CH_4 potentials were positively influenced by the presence of VS, probably due to the availability of a more significant amount of organic material for the AD process. In contrast, the most effective predictors for pig manure were the DM and VFA content. The prediction models demonstrated good accuracy for samples derived from outdoor storage; however, further model refinement would be necessary to predict the BMP of less mature samples that exhibit lower chemical variability.

The assessment of the CH_4 potential loss during manure storage indicated a considerable degradation of organic matter during storage, which led to CH_4 emissions. Notably, pig manure experienced a more significant loss of degradable organic matter due to CH_4 formation and emission than dairy cow manure. For manure samples stored at different temperatures, the research noted a lesser loss in the CH_4 potential of dairy manure, potentially due to the inhibitory effect of accumulated VFAs or lack of adapted methanogens in fresh manure during storage. Future studies may elucidate why CH_4 formation was inhibited during storage and whether strategies can be designed using this inhibition.

This study suggests that quickly transporting manure to biogas facilities or outdoor storage could help maximize energy yield and reduce CH_4 emissions. The study also underscored the potential predictive role of lignin in dairy manure and VFAs in pig manure for BMP. Although an empirical model was developed, it needs further refinement for improved prediction of BMP in short-term stored manure. If validated on commercial farms under various regional conditions, such as storage temperature profile, chemical composition, and storage duration, these findings could help enhance national emission inventories and formulate targeted mitigation strategies.

Chapter 5: Zusammenfassung

Bewertung von Einflussfaktoren auf den Verlust des Methanpotenzials bei der Lagerung von Flüssigmist in Milchvieh- und Mastschweinebetrieben

In verschiedenen nationalen und internationalen Verordnungen und Übereinkommen wird die Notwendigkeit, Treibhausgasemissionen (THG), insbesondere Methanemissionen (CH_4) zu reduzieren, unterstrichen. Im Sektor Landwirtschaft stellen vor allem die enterische Fermentation von Nutztieren und das Güllemanagement bedeutende Quellen dieser Emissionen dar. In der Schweine- und Milchviehhaltung sind Flüssigmist-Systeme am weitesten verbreitet und für einen Anstieg der CH_4-Emissionen verantwortlich. Die vorliegende Studie untersucht weiterhin das Potenzial der CH_4-Produktion aus Flüssigmist als erneuerbare Energiequelle und als effektive Strategie zur Reduzierung von THG-Emissionen, und verdeutlicht die Bedeutung eines besseren Verständnisses der Faktoren, die den möglichen CH_4-Verlust aus Güllelagern regulieren. Im Rahmen der vorliegenden Studie wurden die Auswirkungen von Lagerbedingungen und Veränderungen in der chemischen Zusammensetzung auf die CH_4-Bildung und -Emissionen aus Milchvieh- und Mastschweinegülle analysiert.

Um den Einfluss der Lagertemperatur auf die CH_4-Produktion und die Effekte der Güllelagerung auf das biochemische Methanpotential (BMP) zu überprüfen, wurden zwei verschiedene experimentelle Methoden angewandt. In der ersten Untersuchung wurden Proben ohne Inokulum bei verschiedenen Temperaturen gelagert und die CH_4-Freisetzung gemessen. Bei der Lagerung von Schweinegülle bei 25°C und 20°C betrug die CH_4-Freisetzung 69,3% bzw. 50,3% des BMP. Die höchste CH_4-Freisetzung für Milchvieh-Gülle wurde bei 25°C beobachtet, blieb jedoch insgesamt auf einem niedrigen Niveau. Während der Lagerung von Milchviehgülle wurde eine Hemmung der CH_4-Produktion festgestellt. Im Rahmen der Studie werden mehrere mögliche Gründe für diese Hemmung angeführt, weiterführende Untersuchungen zur Bestätigung dieser Hypothesen sind jedoch zu empfehlen.

In einer zweiten experimentellen Studie wurden die BMP von Flüssigmistproben, die im Verlauf eines Jahres entlang der Managementkette des Flüssigmists auf Milchvieh- und Mastschweinebetrieben genommen wurden, bewertet. Die Ergebnisse zeigten eine Reduzierung des BMP von Milchviehgülle um 20,5% aufgrund von Veränderungen in der chemischen Zusammensetzung im Verlauf des Transfers vom Stall zum Außenlager. Ebenso ergab sich ein Rückgang des BMP um 39,5% für Proben von Mastschweinegülle im Vergleich der Zwischenlagerung im Stall und des Außenlagers. Bei der Untersuchung des BMP in Bezug auf das Alter der Gülle wurde festgestellt, dass Schweinegülle bei der Lagerung schneller abgebaut wird als Milchviehgülle.Um die BMP aus der chemischen Zusammensetzung vorherzusagen, wurden mathematische Modelle entwickelt und die effektivsten Vorhersageparameter für jede Nutztierkategorie identifiziert. Für weniger gealterte Proben mit geringerer Variabilität ihrer chemischen Zusammensetzung sind weiterhin Verbesserungen zur Vorhersage der BMP erforderlich. Insgesamt verdeutlicht die vorliegende Studie die Notwendigkeit, Flüssigmist schnell zu einer Biogasanlage oder zu Außenlagern zu transportieren. Solche Maßnahmen können dazu beitragen, signifikante CH_4-Emissionen zu verhindern und einen Rückgang der Energieproduktionskapazität des Flüssigmistes zu vermeiden.

Chapter 6: Summary

Assessment of the influencing factors regarding loss of methane potential due to manure storage in dairy cow and fattening pig farms

The necessity to decrease greenhouse gas (GHG) emissions, mainly methane (CH_4), is underscored in diverse national and international regulations and conventions. Agriculture, particularly livestock enteric fermentation and manure management, is a significant source of these emissions. In pig and dairy cattle farming, liquid-manure systems are the most prevalent and responsible for an upsurge in CH_4 emissions. This study further investigates the potential of CH_4 production from manure as a renewable energy source and an effective strategy to decrease GHG emissions, emphasizing the importance of better understanding the factors that regulate CH_4 potential loss from manure storage. This study scrutinized the effect of storage conditions and changes in chemical composition on CH_4 formation and emissions from dairy and fattening pig manure.

Two different experimental techniques were designed to verify the influence of storage temperature on CH_4 production and the effects of manure storage on biochemical methane production (BMP). In the first method, samples were stored without inoculum at varying temperatures, and CH_4 production was measured. In pig manure stored at 25°C and 20°C, CH_4 production amounted to 69.3% and 50.3% of the BMP, respectively. The highest CH_4 production for dairy slurry were observed at 25°C but remained low. The production of CH_4 in dairy manure was found to be inhibited during storage. The study suggested several potential reasons for this inhibition, though further research is recommended to confirm these hypotheses.

The second set of experiments, evaluate the BMP of manure samples collected year-round from different stages on the manure storage in dairy cow and fattening pig farms. The findings indicated a 20.5% reduction in the BMP of dairy manure due to alterations in chemical composition during the transfer from the barn to outdoor storage. Similarly, there was a 39.5% drop in the BMP for samples of fattening pig manure in the transition from intermediate to outdoor storage. When examining BMP relative to the age of the manure, it was observed that pig manure decomposes quicker than dairy manure. Mathematical models were developed to predict BMP from the chemical composition, and the most effective predictors for each livestock category were identified. Improvements to predict BMP for less aged samples with reduced variability in their chemical compositions would be necessary. Overall, this study highlights the necessity of promptly transporting manure to either a biogas plant or outdoor storage facilities. Such measures can help prevent significant CH_4 emissions and avoid a decrease in energy production capacity.

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Chapter 8: List of publications and oral presentations

8.1 Research articles

Hilgert, J.E.; Amon, B.; Amon, T.; Belik, V.; Dragoni, F.; Ammon, C.; Cárdenas, A.; Petersen, S.O.; Herrmann, C. (2022). Methane Emissions from Livestock Slurry: Effects of Storage Temperature and Changes in Chemical Composition. Sustainability 2022, 14, 9934. https://doi.org/10.3390/su14169934

Hilgert, J. E., Herrmann, C., Petersen, S. O., Dragoni, F., Amon, T., Belik, V., Ammon, C.; Amon, B. (2023). Assessment of the biochemical methane potential of in-house and outdoor stored pig and dairy cow manure by evaluating chemical composition and storage conditions. Waste Management, 168, 14-24. https://doi.org/10.1016/j.wasman.2023.05.031

Petersen, S. O., Ma, C., **Hilgert, J. E.**, Mjöfors, K., Sefeedpari, P., Amon, B., André Aarnink, Balázs Francó, Federico Dragoni, Karin Groenestein, Steen Gyldenkærne, Christiane Herrmann, Nicholas J. Hutchings, Ib S. Kristensen, Jing Liu, Jørgen E. Olesen, Rodhe, L. (2024). In-vitro method and model to estimate methane emissions from liquid manure management on pig and dairy farms in four countries. Journal of Environmental Management, 353, 120233. https://doi.org/10.1016/j.jenvman.2024.120233

8.2 Oral and poster presentations at conferences

Hilgert, J. E. Assessment of the influencing factors of methane emissions from liquid livestock manure management systems combining experiments and modelling. Colloquium at PhD day in ATB, Potsdam, Germany, 11.11.2020

Hilgert, J. E., Amon, B., Herrmann, C. (2021). Einfluss der Lagertemperatur auf das Biogasbildungspotenzial von Milchvieh- und Mastschweinegulle – Oral presentation - Biogas in der Landwirtschaft – FNR. Online. 29-30 September 2021.

B. Amon, C. Herrmann, F. Dragoni, A. Cardenas, T. Amon, C. Ammon, **J. Hilgert**, S. Petersen. (2022). M4Models: influencing factors of methane emissions from dairy cow and fattening pig manure management systems – Poster presentation – International Conference in Manure Management and Valorization – manuREsource 2022. s' Hertogenbosch, The Netherlands. 11-12 May 2022.

Hilgert, J. E., Amon, B., Dragoni, F., Petersen S. O., Herrmann, C. Assessing chemical composition and methane production characteristics of livestock manure during a year –

Poster presentation - International Conference on Zero Greenhouse Emission in High Productive Agriculture – ZEA. Copenhagen – Denmark. 03-05 May 2022.

Hilgert, J. E., Herrmann, C., Petersen, S. O., Dragoni, F., Amon, T., Belik, V., Ammon, C.; Amon, B. (2022). Influence of the temperature of storage on biogas production from dairy cows and fattening pigs' liquid manure – Oral presentation – AgEng LandTechnik. Berlin, Germany. 22-23 November 2022.

Hilgert, J. E. Assessment of the influencing factors on methane emissions during storage of dairy cow and fattening pig liquid manure. Colloquium at PhD day in ATB, Potsdam, Germany, 06.12.2022.

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Chapter 11: Conflict of Interest

In the context of this work, there are no conflicts of interest due to contributions from third parties.

Chapter 12: Declaration of Independence

I hereby certify that I have prepared this thesis independently. I certify that I have used only the sources and aids indicated.

Berlin, den 03.05.2024 Julio Elias Hilgert

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