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– Pflanzenernährung –

Reduction of Gaseous Emissions from Slurry by the Application of Alkaline Additives

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Abstract

The agricultural sector is undergoing a profound transformation process to meet international and national regulations and laws. These are being enacted by policymakers to limit the sector's tremendous gaseous emissions in an effort to reduce anthropogenic global warming and limit the effects of climate change. In this process, the improvement of slurry management strategies, i.e. the storage and application of slurry, is of particular importance, as this sector is one of the main sources of greenhouse gas emissions such as methane (CH_4) and nitrous oxide (N_2O) and of the environmentally harmful gas ammonia (NH_3). Therefore, this dissertation investigates and evaluates two different slurry treatment strategies based on the application of alkaline additives with the aim to reduce these emissions.

In the **first study**, a new mathematical tool is presented that allows the visualization of the main buffer systems in slurry. It facilitates the characterization of the buffer system and reveals their underlying dynamics. The knowledge gained contributes to the optimization of mitigation strategies relying on pH adjustment by the application of alkaline additives. This tool was successfully verified by more than 300 titrations of slurry stored at different temperatures and for different periods. The collected data revealed that buffer systems are subject to significant fluctuations during storage, which are greatly influenced by temperature. These fluctuations are caused by dynamic formation and degradation processes that are interrelated and thus form a complex system.

The **second study** of this dissertation implements and assesses the first slurry treatment strategy, which is based on the application of calcium hydroxide ($Ca(OH)_2$) as an alkaline additive to increase the pH value of the slurry. The study results demonstrated that during a storage period of 8 weeks and a pH value of 10, the emissions of CH_4 and CO_2 were reduced by 99% and these of N_2O by 60%, while a further increment did not cause a greater reduction. In addition, the framework conditions that must be adhered to for this strategy to be implemented successfully are outlined.

The **third study** presents the second slurry treatment strategy in which calcium cyanamide ($CaCN_2$) is utilized as a slurry additive to reduce gaseous emission during a long-term storage period of 26 weeks. It was demonstrated that $CaCN_2$ suppressed CH_4 emissions rapidly and long-lasting, resulting in a reduction potential of total greenhouse gas emissions by up to 99% over the entire storage period. Furthermore, it was shown that the application inhibited the microbial conversion of volatile fatty acids to CH_4, which caused the pH to drop during storage, resulting in a reduction of NH_3 emissions.

Kurzfassung

Der Agrarsektor befindet sich in einem tiefgreifenden Transformationsprozess, um internationalen und nationalen Vorschriften und Gesetzen gerecht zu werden. Diese werden von den politischen Entscheidungsträgern erlassen, um die enormen Gasemissionen des Sektors zu begrenzen. Dadurch soll die globale Erwärmung gebremst und die Auswirkungen des Klimawandels verringert werden. In diesem Prozess ist eine Verbesserung des Flüssigmistmanagements, d.h. die Lagerung und Ausbringung von Flüssigmist, von besonderer Bedeutung, da dieser Sektor eine der Hauptquellen für die Emission von Treibhausgasen wie Methan (CH_4) und Distickstoffoxid (N_2O) sowie des umweltschädlichen Gases Ammoniak (NH_3) ist. Daher werden in dieser Dissertation zwei Flüssigmistbehandlungsstrategien untersucht, die auf der Anwendung alkalischer Additive basieren, mit dem Ziel, diese Emissionen zu verringern.

In der **ersten Studie** wird ein neues mathematisches Werkzeug vorgestellt, das die Visualisierung der wichtigsten Puffersysteme in Flüssigmist ermöglicht. Die gewonnenen Erkenntnisse tragen zur Optimierung von Emissionsminderungsstrategien bei, die auf einer pH-Anpassung durch den Einsatz alkalischer Additive beruhen. Dieses Werkzeug wurde anhand von mehr als 300 Titrationen von Flüssigmist, die bei verschiedenen Temperaturen und über unterschiedliche Zeiträume gelagert wurde, erfolgreich verifiziert. Die gesammelten Daten zeigten, dass Puffersysteme während der Lagerung Schwankungen unterliegen, die von der Temperatur beeinflusst werden. Diese werden durch dynamische Bildungs- und Abbauprozesse verursacht, die miteinander verknüpft sind und somit ein komplexes System bilden.

In der **zweiten Studie** dieser Dissertation wird die erste vorgestellte Behandlungsstrategie, die auf dem Einsatz von Calciumhydroxid ($Ca(OH)_2$) als Additiv zur Erhöhung des pH-Wertes von Flüssigmist beruht, umgesetzt und bewertet. Die Ergebnisse der Studie zeigten, dass bei einer Lagerungsdauer von 8 Wochen und einem pH-Wert von 10 die Emissionen von CH_4 und CO_2 um 99 % und die von N_2O um 60 % reduziert wurden, während eine weitere Erhöhung keine größere Reduzierung bewirkte. Darüber hinaus werden die Rahmenbedingungen skizziert, die für eine erfolgreiche Umsetzung dieser Strategie eingehalten werden müssen.

In der **dritten Studie** wird das zweite Konzept vorgestellt, bei dem Calciumcyanamid ($CaCN_2$) als Additiv verwendet wird, um die Gasemissionen während einer Langzeitlagerung von 26 Wochen zu reduzieren. Es konnte nachgewiesen werden, dass $CaCN_2$ die CH_4 Emissionen schnell und langanhaltend unterdrückt, was zu einem Reduktionspotenzial der Treibhausgasemissionen von bis zu 99 % über den gesamten Lagerungszeitraum führte. Darüber hinaus konnte gezeigt werden, dass die Anwendung die mikrobielle Umwandlung von flüchtigen Fettsäuren in CH_4 hemmte, wodurch der pH-Wert während der Lagerung sank, was zu einer Verringerung der NH_3-Emissionen führte.

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List of Abbreviations

(aq)	aqueous solution
a	year
ALDH2	aldehyde dehydrogenase 2
ANOVA	analysis of variance
BLE	Bundesanstalt für Landwirtschaft und Ernährung
BMEL	Bundesministerium für Ernährung und Landwirtschaft
BMJ	Bundesministerium der Justiz
$C_2H_4O_2$	acetic acid
$C_3H_6O_2$	propionic acid
$C_4H_8O_2$	butyric acid
$Ca(OH)_2$	calcium hydroxide
$CaCN_2$	calcium cyanamide
CaO	calcium oxide
CBC	current buffer capacity
CC300	300 mg kg^{-1} pure cyanamide
CC500	500 mg kg^{-1} pure cyanamide
CH_3COO^-	acetate
CH_3COOH	acetic acid
CH_4	methane
Co	control
CO_2	carbon dioxide
CO_3^{2-}	carbonate ion
COP 26	26th Conference of Parties, UN Climate Change Conference
DC	dairy cattle/cow
DCD	dicyandiamide
EC	European Commission
ECD	electron capture detector
ECHA	European Chemicals Agency
EEA	European Environment Agency
eq.	equivalent
ESR	Effort Sharing Regulation
FID	flame ionization detector
FP	fattening pig
GC	gas chromatograph

GHG(s)	greenhouse gas(es)
Gt	gigaton
H^+	proton
H_2	hydrogen
H_2CO_3	carbonic acid
$H_2PO_4^-$	dihydrogen phosphate
H_2S	hydrogen sulfide
H_2SO_4	sulfuric acid
H_3O^+	oxonium ion
HCO_3^-	hydrogen carbonate ion
HNO_3	nitric acid
HPO_4^{2-}	phosphate
HSD	Honestly Significance Difference
IPCC	Intergovernmental Panel on Climate Change
K_2O	potassium oxide
MgO	magnesium oxide
N	nitrogen
N_2	nitrogen gas
N_2O	nitrous oxide
NaOH	sodium hydroxide
NH_3	ammonia
NH_4^+	ammonium ion
NH_4-N	ammonium nitrogen
N-Lime	nitrogen lime
NO_2^-	nitrite
NO_3^-	nitrate
O_2	oxygen
OH^-	hydroxonium ion
P_2O_5	phosphate
pKa	acid dissociation constant
$PM_{2.5}$	particulate matter (< 2.5 µm)
ppb	parts per billion
ppm	parts per million
PTFE	polytetrafluoroethylene
REACH	registration, evaluation, authorization and restriction of chemicals
REP	repetition
S	sulfur

S	sow
SEM	standard errors of the mean
$t_{1/2}$	half-life
TAN	total ammonia(cal) nitrogen
TIC	total inorganic carbon
VFA	volatile fatty acids
VS	volatile solids

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1 General Introduction

The general introduction provides differentiated background information that can be supportive in gaining a better understanding of the scientific context on which the three studies presented in this dissertation are based. Furthermore, the central problem and objectives that served as motivation for these studies are outlined.

1.1 Scientific Background Information

1.1.1 Buffer Systems in Slurry

The basic operation of a buffer system is that it resists changes in pH when small amounts of acids or bases are added to the solution. Maintaining certain pH ranges is necessary for chemical and/or biological processes and reactions to occur and take place. This affects processes from the cellular level to entire ecosystems, such as water bodies, soils and the atmosphere (Powers et al., 2005; Proksch, 2018; Salis & Monduzzi, 2016; Stets et al., 2017; Wang et al., 2015). In addition, they have a key function in the gastrointestinal tract of humans and animals, as they promote the bacterial flora and thus make a significant contribution to the digestive process (Arnoldini et al., 2018; Counotte et al., 1979; Grant & Mertens, 1992; Ramos et al., 2021, 2022). After digestion, microorganisms capable of influencing these buffer systems by forming and degrading them are excreted and continue to do so during the storage of slurry (Cassity-Duffey et al., 2015; Georgacakis et al., 1982; Sommer & Husted, 1995). The ability of a buffer system to resist pH changes derives from the establishment of a dissociation equilibrium between a weak acid and its corresponding conjugate base or a weak base and its corresponding conjugate acid, the so-called conjugate acid-base pair. The conjugated acid-base pair can absorb and thus neutralize small amounts of oxonium (H_3O^+) or hydroxonium (OH^-) ions and thus neutralize them, thereby maintaining the pH value of the solution.

The acetic acid-acetate buffer is an example of a buffer system that substantially determines the buffer capacity of slurry (Christensen & Sommer, 2013). This buffer consists of the weak acid, the acetic acid (CH_3COOH) and its conjugate base, acetate (CH_3COO^-). When a strong acid is added to the buffer solution, the H_3O^+ content increases and the CH_3COO^- absorbs the proton (H^+) of the H_3O^+ and forms CH_3COOH and H_2O. On the other hand, if a base is added to the buffer solution, the H^+ of the CH_3COOH reacts with the base by taking up the OH^- ions and converting them into H_2O. This results in the formation of CH_3COO^- and the prevention of an increase in pH. In both cases, this leads to minor pH value changes, keeping the pH of the solution at a relatively stable level.

Figure 1.1 illustrates this process. In unbuffered solutions, the pH would change sharply when either acids or bases are added because the ions cannot be neutralized. The amount of acids or bases required to change the pH by one unit is termed buffer capacity and can be expressed as mol l^{-1} (Georgacakis et al., 1982; Moosbrugger et al., 1993; Urbansky & Schock, 2000). The concentrations of the individual buffers, the position of the individual buffers on the pH scale and the pH of the solution are used to determine the buffer intensity of a solution (Georgacakis et al., 1982; Urbansky & Schock, 2000).

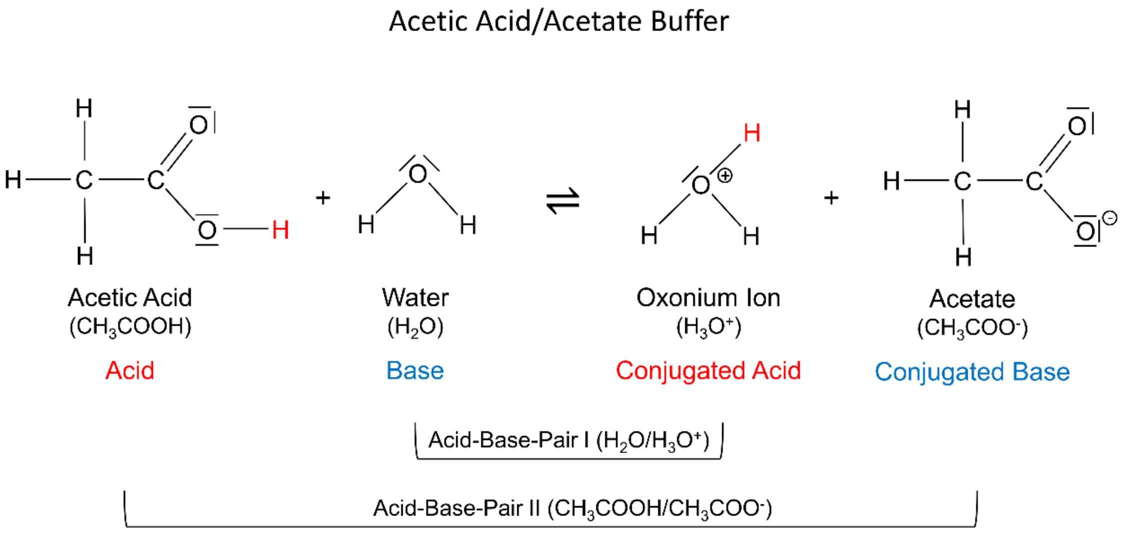

Figure 1.1: **Principle of buffer systems using the reaction equation of the acetic acid/acetate buffer.**

Buffers in slurry form a highly complex system consisting of four different main buffer systems that can be categorized as those operating mainly in the acidic milieu. These include the volatile fatty acids (VFA) buffer (includes CH_3COOH/CH_3COO^-, $pK_a = 4.72$) and the hydrogen carbonate buffer (H_2CO_3/HCO_3^-, $pK_a = 6.32$), as well as the two buffers operating mainly in the alkaline milieu, the ammonia buffer (NH_4^+/NH_3, $pK_a = 9.30$) and the carbonate ion buffer (HCO_3^-/CO_3^{2-}, $pK_a = 10.30$) (Christensen & Sommer, 2013; Sommer & Husted, 1995). In addition, buffers only function optimally within a certain pH range, which is determined by the acid dissociation constant (pKa value) of the respective buffer (Moosbrugger et al., 1993; Urbansky & Schock, 2000). In the case of the acetic acid-acetate buffer, the pKa value is 4.72, which means that the buffer works best at a pH value close to 4.72 (Christensen & Sommer, 2013). Further information about the exact position of the main buffer systems on the pH scale can be found in Figure 2.1. In literature, the hydrogen carbonate ion (HCO_3^-) and carbonate ion (CO_3^{2-}) buffers are often considered as a single buffer system, as they both originate from carbonic acid (H_2CO_3) and are therefore commonly termed the carbonic acid-bicarbonate buffer system

(Christensen & Sommer, 2013; Georgacakis et al., 1982). In this dissertation, however, they are considered separately due to their ability to buffer in different pH ranges.

Volatile fatty acids are intermediate products formed during the anaerobic microbial digestion of organic carbon in the animal's gastrointestinal tract and during the anaerobic storage of its excreta (Bergman, 1990; Gerardi, 2003; Sommer & Husted, 1995). In this context, VFA refers to a group of organic acids, the (volatile) fatty acids consisting of a C_2 to C_5 body, with only the fatty acids up to the C_4 body, namely acetic ($C_2H_4O_2$), propionic ($C_3H_6O_2$) and butyric acids ($C_4H_8O_2$), having a relevant share in slurry (Cooper & Cornforth, 1978; Popovic & Jensen, 2012; Sommer & Husted, 1995). The totality of these VFA represents the VFA buffer system, whose buffering ability is based on the equilibrium between the weak volatile fatty acids (such as acetic acid, propionic acid, and butyric acid) and their corresponding conjugate bases (acetate, propionate, and butyrate) (Sommer & Husted, 1995). However, the high accumulation of these VFA can lead to a drop in the pH of slurry and may become toxic to some bacterial species, thereby affecting the microbial activity in the slurry (Gerardi, 2003; Wang et al., 2009). Nevertheless, the degradation process of VFA occurs either anaerobically, producing CH_4 and CO_2 (Figure 1.2) or aerobically, producing CO_2 and H_2O. The CO_2 produced by both pathways either outgasses or dissolves in the slurry and contributes to the formation of both carbonate buffers, thus preventing strong acidification of the slurry caused by an overaccumulation of VFA (Cooper & Cornforth, 1978; Møller et al., 2004; Sommer & Husted, 1995).

The chemical basis for the formation of the carbonic acid-bicarbonate buffer arises from the (I) dissociation equilibrium of CO_2 and H_2CO_3 in water and the separation of H^+ from H_2CO_3, which occurs in two dissociation steps and results in the formation of the (II) HCO_3^- buffer and the (III) CO_3^{2-} buffer (Andersen, 2002). Both together are referred to as bicarbonate. In addition, the total amount of CO_2, H_2CO_3, HCO_3^- and CO_3^{2-} is often indicated as total inorganic carbon (TIC) (Christensen & Sommer, 2013).

(I) $CO_2 + H_2O \rightleftharpoons H_2CO_3$

(II) $H_2CO_3 + H_2O \rightleftharpoons H_3O^+ + HCO_3^-$

(III) $HCO_3^- + H_2O \rightleftharpoons H_3O^+ + CO_3^{2-}$

The CO_2 that forms the two buffer systems originates not only from the already described aerobic and anaerobic digestion of organic carbon but also from the enzymatically assisted hydrolysis of urea to CO_2 (Sigurdarson et al., 2018). Besides forming CO_2, this hydrolysis also generates NH_3, which enhances the concentration of NH_3 in the slurry and thereby increases the NH_3 buffer capacity (Georgacakis et al., 1982; Møller et al., 2004; Sigurdarson et al., 2018).

The mode of operation of the ammonia buffer is based on the establishment of a dissociation equilibrium in a liquid solution between the weak base NH_3 and its corresponding weak acid, ammonium (NH_4^+). The total amount of both compounds present in a solution, such as slurry, can be described as total ammoniacal nitrogen (TAN) (Christensen & Sommer, 2013). This buffer as well as the carbonate ion buffer counteract a decline in pH, as both compounds act as bases, keeping the pH of common slurry types within a range of usually 7.0–8.0 (Christensen & Sommer, 2013; Georgacakis et al., 1982). The hydrolysis of urea is the main pathway for the formation of the ammonia buffer, although other pathways contribute to the formation of NH_3, which are described in Chapter 1.1.2 in more detail.

In addition to these main buffer systems, slurry also contains other buffer systems that are less important in terms of their buffer capacity. These include surface-bound carboxylic groups such as those found in humic substances (pKa = 6.0), phosphate groups (pKa = 7.2) and amino groups (pKa = 9.4), which contribute to stabilizing the pH value in the slurry (Christensen et al., 2009; Sommer & Husted, 1995).

Nevertheless, these buffer systems form different capacities, may influence each other through their formation and degradation and thereby influencing the pH of the slurry (Christensen et al., 2009; Georgacakis et al., 1982; Sommer & Husted, 1995).

1.1.2 Gaseous Emissions related to Slurry Management

Slurry management, also known as manure management, involves the storage and application of slurry and is a large contributor to gaseous emissions in the agricultural sector (Behera et al., 2013; Chadwick et al., 2011). It releases millions of tons of CO_2 equivalents into the atmosphere each year worldwide (United Nations, 2021). These emissions primarily include climate-relevant trace gases, such as carbon dioxide, methane, and nitrous oxide, which are better known as greenhouse gases (GHGs) and the environmentally harmful trace gas ammonia (Behera et al., 2013; Philippe & Nicks, 2015). Therefore, the following chapter classifies the environmental relevance of these gases in an international context and outlines their occurrence in the agricultural sector, with a special focus on slurry management.

Carbon Dioxide

Carbon dioxide is by far the most predominant anthropogenic GHG in the world, with estimated emissions of 40.0 ± 2.9 Gt CO_2 in 2021. This gas is mainly produced during the combustion of fossil resources, such as coal, natural gas and oil (Friedlingstein et al., 2022). However, CO_2 from animal husbandry and slurry management is generally not included in the balance of GHGs, as they are considered climate neutral because the CO_2 exhaled by animals or produced during slurry storage is reabsorbed by the photosynthetic activity of the agriculturally grown forage crops (Philippe & Nicks, 2015). Nevertheless, in addition to animal respiration, CO_2 is formed during slurry storage in three different processes: (I) formation of CO_2 via the catalytically assisted degradation of urea to NH_3 and CO_2 by the enzyme urease (see Chapter 1.1.2, subchapter Ammonia), (II) anaerobic microbial digestion of organic matter, which produces both CO_2 and CH_4 (see Chapter 1.1.2, subchapter Methane and Figure 1.2) and (III) aerobic microbial degradation of organic matter by microorganisms in the near-surface areas of slurry storage facilities where oxygen (O_2) is present (Møller et al., 2004; Philippe & Nicks, 2015; Sigurdarson et al., 2018).

Methane

Methane is a powerful GHG, as it is after CO_2 the second most emitted GHG in the world at a quantity of 366 million t a^{-1} for the decade 2008–2017 (Saunois et al., 2020). In addition, it is 28 times more potent in terms of its ability to increase global temperature than CO_2 (IPCC, 2014). The CH_4 content in the earth's atmosphere has more than doubled compared to pre-industrial times (~ 722 ppb) with a concentration of ~ 1,911 ppb in 2022 (Lan et al., 2023; United Nations, 2021). The agricultural sector plays a crucial role in the concentration increase, as 32% of global anthropogenic CH_4 emissions are attributable to the fermentation of ruminants and the storage of organic fertilizers, such as slurry (IPCC, 2014).

The process of anaerobic fermentation involves the production of CH_4 and CO_2 via a cascade of microbial (domains, *Bacteria* and *Archaea*) degradation processes of organic matter in the absence of O_2 (Demirel & Scherer, 2008). This cascade can be divided into four steps (Figure 1.2):

> **(Step 1)** The first step, which is called hydrolysis, is carried out by bacteria belonging to the phlya, *Firmicutes* and *Bacteroidetes* (Nguyen et al., 2019). During hydrolysis, complex insoluble polymers, such as proteins and carbohydrates, are degraded into soluble oligomers and smaller monomers, such as simple sugars, amino acids, and long-chain fatty acids (van Hullebusch et al., 2019). This process is facilitated via the release of exoenzymes by the mentioned bacteria in the presence of H_2O (Gerardi, 2003; Nguyen et al., 2019).
>
> **(Step 2)** In the second step, namely acidogenesis (acidification step), these soluble monomers and oligomers formed during hydrolysis are further microbially (phyla, *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria*) degraded to lower molecular weight compounds, such as short-chain fatty acids, CO_2, hydrogen (H_2), and CH_3COO^- (Klass, 1984; Nguyen et al., 2019). In stored slurry, the most common short-chain fatty acids, which are also known as VFA, are acetic,

propionic and butyric acids (Conn et al., 2007; Cooper & Cornforth, 1978; Popovic & Jensen, 2012). However, high concentrations of propionic acid can cause irreversible damage to the growth of methanogenic bacteria and thereby inhibit CH_4 formation (Wang et al., 2009).

(Step 3) In the third step of the anaerobic fermentation, known as acetogenesis (acetic acid formation), VFA are further broken down into their simplest microbially degradable form, CH_3COOH/CH_3COO^-, by acetogens of the genus *Syntrophomonas* (species, e.g., *Syntrophobacter wolinii* and *Syntrophomonas wolfei*) (Nguyen et al., 2019; van Hullebusch et al., 2019). In addition, this degradation process forms CO_2 and H_2, both of which may also be converted into CH_3COO^- (van Hullebusch et al., 2019).

(Step 4) In the final step of anaerobic fermentation, called methanogenesis, CH_4 is produced by methanogenic microorganisms via two pathways, which are distinguished according to the substrate utilization of the microorganisms (Lyu et al., 2018). The first process is called acetoclastic methanogenesis (family, e.g., *Methansarcinaceae* and *Methanosaetaceae*) and requires CH_3COO^- as a substrate for microbial metabolism ($CH_3COO^- + H^+ \rightarrow CH_4 + CO_2$) (Demirel & Scherer, 2008). This pathway is responsible for about $^2/_3$ of the total methanogenesis rate (Meegoda et al., 2018). The other pathway is called hydrogenotrophic methanogenesis (family, e.g., *Methanobacteriaceae*, *Methanococcaceae* and *Methanomicrobiaceae*) and employs H_2 and CO_2 as substrates ($CO_2 + 4H_2 \rightarrow CH_4 + H_2O$). accounting for the remaining $^1/_3$ (Demirel & Scherer, 2008; Klass, 1984; Meegoda et al., 2018). The total gas mixture formed during methanogenesis usually consists of 50–75% CH_4 and 25–50% CO_2 as well as 2% of other gases, such as nitrogen (N_2), hydrogen sulfide (H_2S) and NH_3 (Da Costa Gomez, 2013). The lower share of CO_2 produced arises from the fact that hydrogenotrophic methanogenesis, unlike acetoclastic methanogenesis, does not produce CO_2. Consequently, more CH_4 is produced during fermentation than CO_2.

The production of biogas in a so-called anaerobic digester is based on the four steps described. The physical, chemical and biological parameters influencing these steps are optimized in a digester to increase the share of CH_4 in the biogas, ensuring a high-energy value of the biogas produced (Bhajani, 2022). In contrast, interrupting these biochemical processes and thus reducing or inhibiting CH_4 production are desirable aims during slurry storage to limit the unwanted release of GHG emissions.

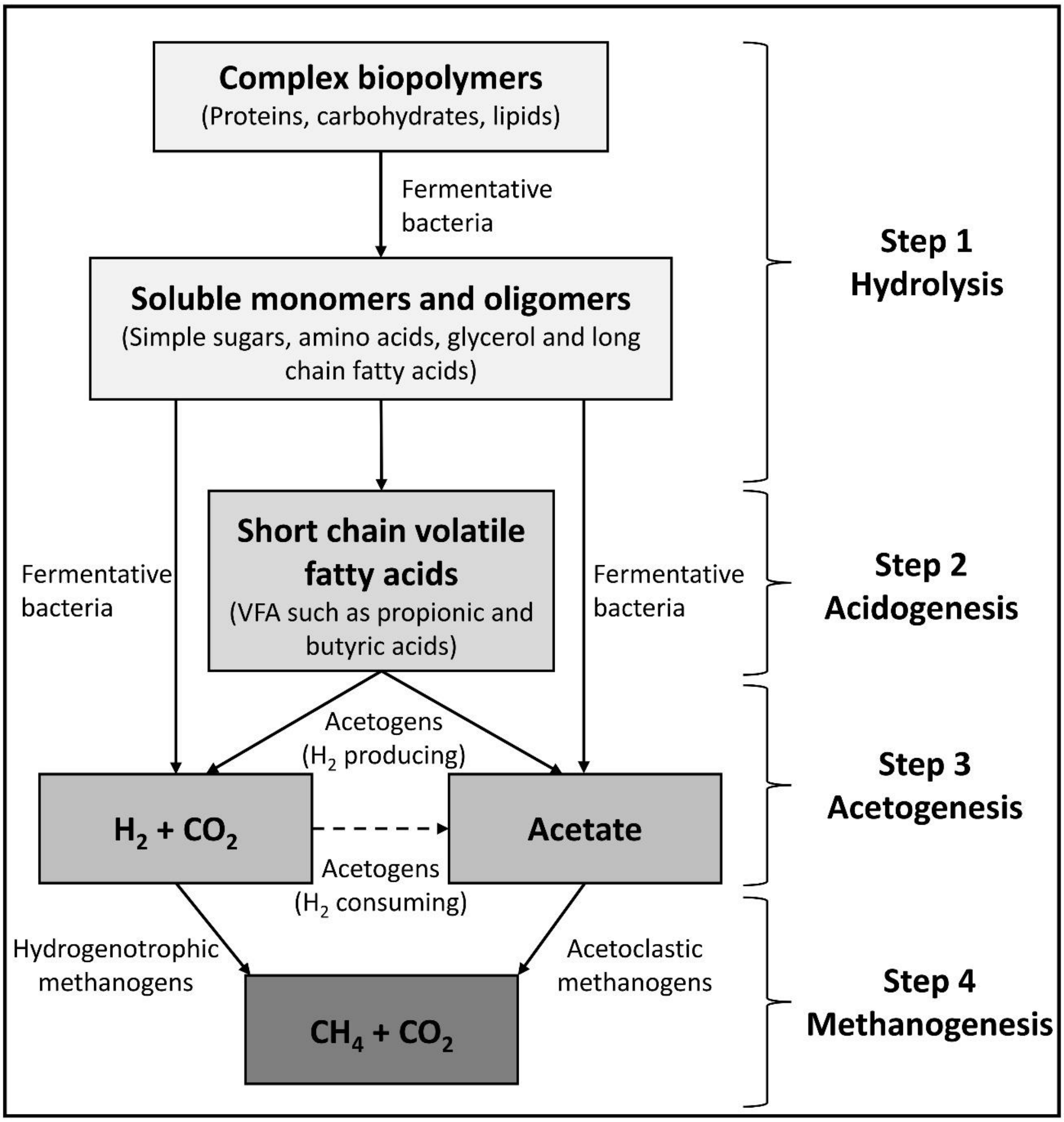

Figure 1.2: **Schematic representation of the anaerobic degradation of organic matter divided into four steps: (1) Hydrolysis, (2) Acidogenesis, (3) Acetogenesis, and (4) Methanogenesis (adapted from van Hullebusch et al., 2019).**

Nitrous Oxide

Nitrous oxide is a GHG that is about 265 times more potent than CO_2 and its concentration in the Earth's atmosphere is increasing rapidly due to anthropogenic activities (IPCC, 2014). At the end of the pre-industrial period, the concentration was about 270 ppb, while in 2022 it reached a level of 335 ppb, representing an overall increase of about 24% (Lan et al., 2023; MacFarling Meure et al., 2006). Nevertheless, the growth rate has accelerated in recent years and has exceeded 1 ppb a^{-1} since 2020 (Lan et al., 2023). In addition, global anthropogenic N_2O emissions have increased by 30% over the last four decades to 7.3 million t N a^{-1}, which represents the average N_2O emissions in the period 2007–2016 (Tian et al., 2020). These emissions are mainly caused by manure deposition on arable land and during manure management, which accounts for about 21% of total anthropogenic N_2O emissions (Tian et al., 2020).

Nitrous oxide is formed primarily as a result of incomplete nitrification and denitrification processes by microorganisms converting NH_3 to elemental nitrogen (N_2) (Figure 1.3). During the process of nitrification, NH_4^+ is oxidized to nitrate (NO_3^-) by obligate aerobic bacteria, producing nitrite (NO_2^-) as an intermediate compound. This process produces N_2O as a by-product, especially when nitrite accumulates and oxygen is lacking (Philippe & Nicks, 2015). Denitrification occurs under (near) anaerobic conditions by a broad spectrum of facultative aerobic heterotrophic bacteria, archaea and fungi (de Klein et al., 2008). In order to maintain their metabolism under anaerobic conditions, these microorganisms use enzymes to remove oxygen from oxygen-rich compounds, such as NO_3^- (Megonigal et al., 2003). In this process, N_2 is ultimately produced through the formation of intermediate products such as NO_2^-, nitric oxide (NO) and N_2O. In contrast to the separate processes of nitrification and denitrification, nitrifier denitrification involves a combination of both processes (de Klein et al., 2008). This pathway contains a sequence of reactions carried out by a single group of microorganisms known as autotrophic NH_3-oxidizers. These microorganisms oxidize NH_4^+ to NO_2^- and then reduce NO_2^- to N_2O and N_2 (Wrage et al., 2001). However, all three processes may proceed simultaneously if the environmental factors are suitable (de Klein et al., 2008).

After the application of nitrogen to agricultural soils, these reactions and the resulting N_2O emissions are favored by numerous factors, such as a high nitrogen input, the presence of readily available carbon sources, a finer-textured soil than sandy soils with > 60% water-filled pores and a temperature between 25 and 35 °C (Wang et al., 2021). In slurry storage, these factors also play a central role (apart from the soil parameters), with the dominant factor being the formation of a slurry crust or the usage of a slurry cover that provides aerobic and anaerobic conditions at the surface, allowing nitrification, denitrification and nitrifier nitrification to take place (Amon et al., 2006; Sommer et al., 2000). In addition, to improve the estimation of N_2O emissions from different slurry management systems and to simplify the impact of these factors, the Intergovernmental Panel on Climate Change (IPCC) has established N_2O emission factors. These are defined as the amount of N_2O-N per kg N excreted per animal, which in the case of slurry storage in tanks or open ponds (with crust) is 0.5%, below settled floors 0.2% and for field application of slurry 2% (IPCC, 2019).

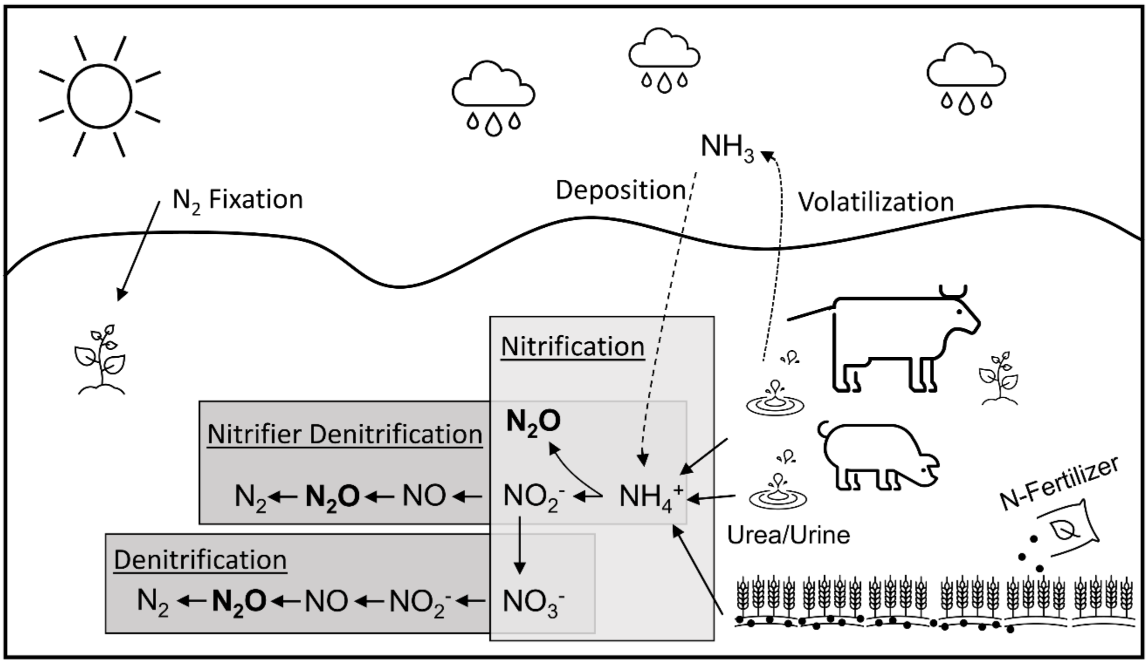

Figure 1.3: **Schematic illustration of the formation of N_2O emissions through nitrification and denitrification arising during slurry management and the application of nitrogen-containing fertilizers (adapted from de Klein et al., 2008; Wrage et al., 2001).**

Ammonia

In the EU-28, roughly 3.5 million t of NH_3 were emitted in 2019, representing a decrease of - 2.2% compared to the previous year and a decrease of - 28.1% compared to the last two decades (EEA, 2021). Nevertheless, the agricultural sector is responsible for 94% of these emissions, with slurry management and the application of inorganic and organic fertilizers being the main sources (EEA, 2021). Therefore, the hotspots of NH_3 emissions in the EU are directly related to regions that are primarily characterized by intensive livestock production, which include Belgium, the Netherlands, Lower Saxony (Germany), Lombardy (Italy) and Brittany (France) (Oenema et al., 2007; Scarlat et al., 2018). The main source of ammonia emissions associated with livestock farming are surfaces contaminated with feces, as the surface-to-volume ratio is higher compared to slurry storage facilities (Webb et al., 2014). Outside Europe, emission hotspots are mainly located in Central Asia and the Indo-Gangetic Plain (Pakistan, northern India, Nepal, and Bangladesh), but these are primarily driven by heavy fertilizer use (Clarisse et al., 2009).

In slurry, the main production pathway of NH_3 is based on the hydrolysis of urea into NH_3 and CO_2. This process is catalyzed by the enzyme urease, reducing the half-life ($t_{1/2}$) of urea from ~ 40 a to ~ 20 ms (Sigurdarson et al., 2018). In addition, the aerobic degradation of uric acid and the excretion of undigested proteins, which are mineralized to NH_3 during storage, can make a significant contribution to the formation of NH_3 (Arogo et al., 2006). In an aqueous solution, NH_3 is in a dissociation equilibrium between the non-volatile ionized NH_4^+ and the volatile non-ionized NH_3 (Figure 1.4) (Christensen & Sommer, 2013; Fricke et al., 2007). The formation of NH_3 and its emission are strongly dependent on the temperature and pH of the slurry. As the equilibrium shifts towards NH_3 with rising temperature and pH, the NH_3 concentration in the slurry increases, leading to higher emissions (Figure 1.5) (Arogo et al., 2006; Fricke et al., 2007; Mohammed-Nour et al., 2019).

Although NH_3 is not considered a GHG due to its inability to absorb thermal radiation, it is classified as an environmentally harmful gas because it can cause severe environmental damage if released into the atmosphere (Behera et al., 2013). High exposure to NH_3 concentrations can cause direct respiratory diseases in animals and humans (Drummond et al., 1980; Wyer et al., 2022). Furthermore, NH_3 reacts with atmospheric acids, such as nitric acid (HNO_3) and sulfuric acid (H_2SO_4), to form particulate matter ($PM_{2.5}$), which also impacts animal and human health negatively (Krupa, 2003; Wyer et al., 2022). The contribution of livestock to atmospheric $PM_{2.5}$ concentrations can be as high as 20% if weather conditions are appropriate (Hristov, 2011). However, these issues are prevalent indoors or in the near surroundings of barns, as NH_3 volatilization losses are high (Behera et al., 2013). Moreover, NH_3 emissions can lead to a reduction of nitrogen from organic or inorganic fertilizers. This reduces their fertilizing value and can lead to an uncontrolled and undesired input of nitrogen into ecosystems, resulting in the acidification of soils and eutrophication of water bodies (Arogo et al., 2006; Behera et al., 2013). Furthermore, NH_3 can directly harm the vegetation, causing leaf necrosis in severe cases (Krupa, 2003).

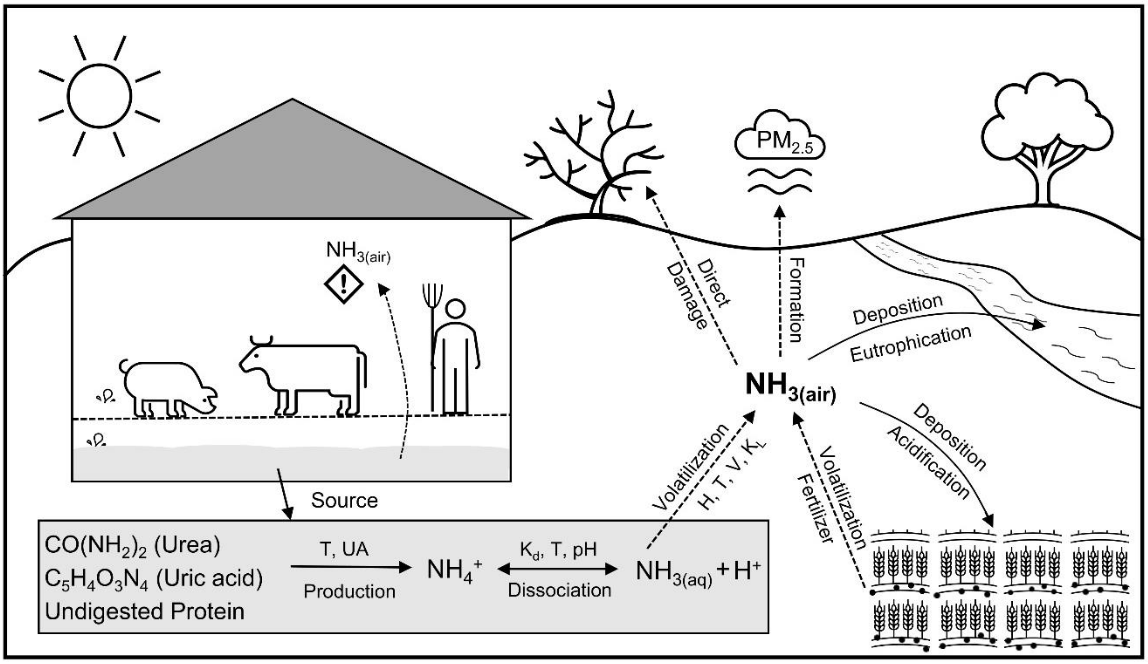

Figure 1.4: **The environmental impact of ammonia emission resulting from slurry management and the application of organic and inorganic fertilizers, as well as the illustration of the production and volatilization equilibrium of ammonia (Influencing factors; H – Henry's law constant, K_d – dissociation constant, K_L – mass transfer coefficient, pH, T – temperature, UA – urease activity, V – wind speed) (Illustration of equilibrium is adapted from Arogo et al., 2006).**

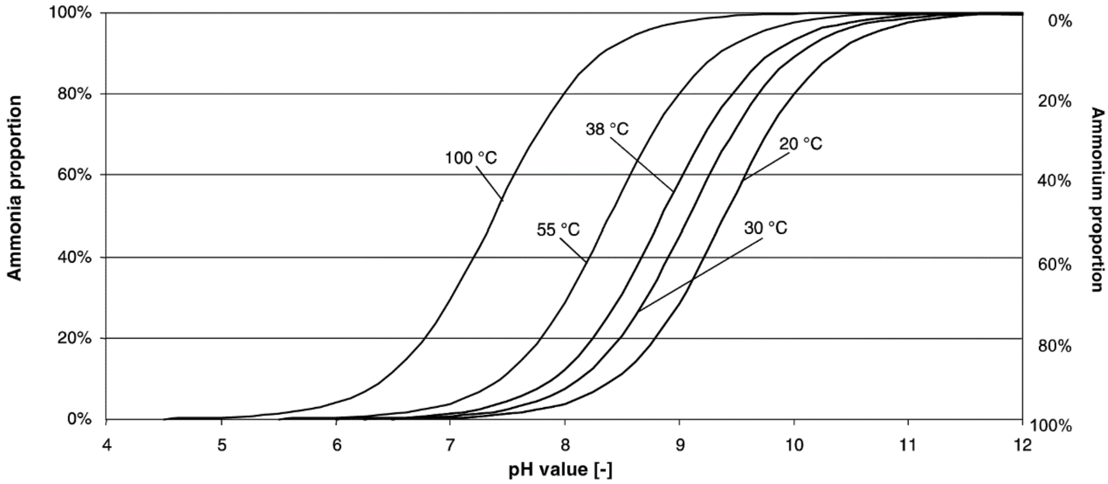

Figure 1.5: **Influence of pH and temperature on the dissociation equilibrium between ammonia and ammonium (Fricke et al., 2007).**

1.1.3 Calcium Cyanamide and its Relevance in Agriculture

Calcium cyanamide ($CaCN_2$), often referred to as nitrogen lime (N-Lime), is a nitrogen fertilizer that has been used in agriculture for more than 100 years. Besides its fertilizing effect, it serves as a liming agent for soil pH regulation, has a nitrification-inhibiting effect and acts as a pesticide against many soil-borne pathogens and germinating weeds (Güthner & Mertschenk, 2006; Klasse, 1996; Shi et al., 2009). The substance was first synthesized in the second half of the 19th century and the first commercial production process was patented in 1895 by two German chemists, Adolph Frank (1834–1916) and Nikodem Caro (1871–1935) (Güthner & Mertschenk, 2006). The production process is based on the reaction of elemental nitrogen (N_2) with calcium carbide (CaC_2) at about 1,100 °C, producing $CaCN_2$ (Güthner & Mertschenk, 2006). Today, commercially available $CaCN_2$-containing products have approximately the following composition: calcium cyanamide 43%, calcium hydroxide 16.5%, graphite 10%, calcium carbonate 10%, magnesium carbonate 8%, total N 18%, cyanamide-N 15%, and nitrate-N 0.1% (Güthner & Mertschenk, 2006). Calcium cyanamide was produced at a rate of 130,000 t a^{-1} during the years 2010–2014, most of which is primarily used in Europe and Asia as a slow-release nitrogen fertilizer (ECHA, 2018).

In moist soil, $CaCN_2$ is hydrolyzed to calcium hydroxide ($Ca(OH)_2$) and free cyanamide (H_2CN_2) as illustrated in Figure 1.6 (Smock, 1935). The resulting $Ca(OH)_2$, together with calcium and magnesium carbonates typically contained in $CaCN_2$ products, protect soils from over-acidification through its alkaline action, helping to regulate soil pH when applied as a fertilizer (Güthner & Mertschenk, 2006; Suzuki et al., 2021). Cyanamide, the second hydrolysis product, is first converted by soil microbes to urea and then further transformed into ammonium, which in turn is oxidized to nitrate via nitrification (Smock, 1935). The ammonium and nitrate formed are the main factors responsible for the high nitrogen fertilizing effect of $CaCN_2$. In addition to the direct degradation process of cyanamide, a second known degradation pathway has been identified, which leads to the formation of dicyandiamide (DCD) (Smock, 1935). The study by Zacherl & Amberger (1984) revealed that the first step of nitrification, the metabolic breakdown processes of NH_4^+ to NO_2^- by *Nitrosomonas europaea*, was specifically and periodically inhibited by DCD, leading to the nitrification-inhibiting effect, which may last up to several weeks (Figure 1.6) (Guiraud et al., 1989). Furthermore, it was shown that not only nitrification but also denitrification was inhibited (Yamamoto et al., 2014). This effect causes a reduced formation of N_2O in the soil, as nitrification and denitrification (Figure 1.3) are hindered (Ikezawa et al., 2022; Suzuki et al., 2021; Yamamoto et al., 2013, 2014). Apart from reducing N_2O emissions, the study by Schütz et al. (1989) indicated that adding $CaCN_2$ as a fertilizer in paddy rice fields reduced peak CH_4 emissions arising from the submerged fields. However, this effect has been described but not yet thoroughly investigated, therefore there is no scientific characterization of it.

Besides the emission-reducing effect, $CaCN_2$ has the additional advantage of being an effective pesticide primarily against soil-borne pathogens, such as *Plasmodiophora brassicae*, causing clubroot disease in many *Brassica* species (Dixon, 2016), *Fusarium solani*, causing root rot in cucumbers (Bourbos et al., 1997) and *Sclerotinia sclerotiorum*, a fungus, causing stem rot in many horticultural crops, such as peas, carrots and potatoes (Jones & Gray, 1973). Furthermore, $CaCN_2$ is used in weed control strategies because it reduces both the germination and relative growth rate of weeds (Leytur et al., 2018).

Since the mid-2010s, $CaCN_2$ and its approval as a fertilizer have increasingly become the focus of scientific and political discussions. This was triggered by a re-assessment of the compound $CaCN_2$ to determine if it poses an unacceptable risk to human health and/or the environment (ECHA, 2018). The assessment was primarily conducted by the European Chemicals Agency (ECHA), an EU agency that regulates the technical, scientific, and administrative aspects of the registration, evaluation, authorization and restriction of chemicals (REACH-Regulation). The ECHA assessment report stated that negative risks could not be excluded (ECHA, 2018). Based on this report, the European Commission, as a legislative body, can formulate a draft law banning the use of calcium cyanamide as a fertilizer, which must subsequently be confirmed by the majority of the European Parliament and the Council. Therefore, the future of $CaCN_2$ as a fertilizer remains uncertain.

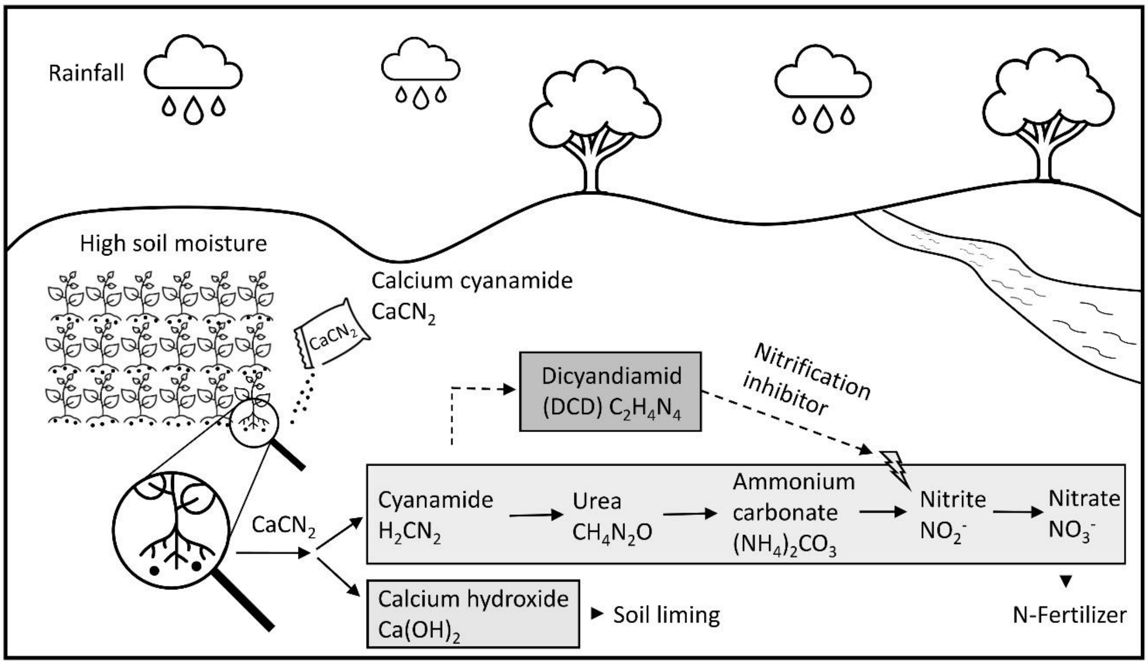

Figure 1.6: **Degradation pathways of calcium cyanamide in the soil (adapted from Smock, 1935; Zacherl & Amberger, 1984).**

1.2 Problem Statement

On November 15, 2022, the world's population surpassed 8 billion, more than tripling from the mid-20th century (1950: ~ 2.5 billion), with an increase of 1 billion people since 2010 and 2 billion since 1998 (United Nations, 2022). Although world population growth will slow down during the next few decades due to declining fertility rates, humanity is still expected to reach 8.5 billion in 2030 and 9.7 billion in 2050 (United Nations, 2022).

Therefore, the challenge of feeding the growing world population is great. Besides the increasing population, global consumption patterns have shifted and will continue to shift towards a more meat-based diet (Sans & Combris, 2015; Whitnall & Pitts, 2019). The demand-driven growth is primarily met by increasing the livestock population but also by the intensification of the husbandry systems (Thornton, 2010). However, both are associated with an increase in the amount of slurry produced, which can be economically advantageous in intensive livestock production systems as it enables viable slurry management systems that are based on the collection and directed application of slurry as an organic fertilizer. Globalization and the industrialization of agriculture have led to the emergence of livestock hotspots, as further described in Chapter 1.1.2. In these regions, the amount of slurry produced exceeds the area of land on which it is allowed to be applied, resulting in severe overproduction (Clarisse et al., 2009; Oenema et al., 2007; Scarlat et al., 2018). In addition, laws regulating application rates are often tightened by policymakers to minimize the negative effects of over-fertilization. In the European Union, for example, large areas have been declared "nitrate vulnerable zones" under the EU Nitrate Directive (Council Directive 91/676/EEC). In these areas, the use of organic fertilizer, such as slurry, is restricted to a nitrogen limit of 170 kg N ha^{-1} $year^{-1}$. Consequently, a larger amount of slurry often has to be stored for a longer period to accommodate the overproduction.

However, storage of slurry releases massive amounts of environmentally harmful gases, accounting for a large proportion of global emissions of CH_4, N_2O and NH_3, as explained in detail in Chapter 1.1.2, making slurry not only a valuable fertilizer but also a substance of concern (Misselbrook et al., 2016). Seeking to mitigate the negative effects of climate change and limit global warming to < 1.5 °C, if possible, the EU has made a legal commitment to reduce total GHG emissions to at least 55% below 1990 levels by 2030 with the enactment of the European Climate Law (EU, 2021b). This represents a tightening of the original target by a further 15%, as the first version proposed a reduction of only 40%. According to the Effort Sharing Regulation (ESR), GHG emissions from agriculture must be cut by at least 30% from 2005 levels by 2030 (EU, 2018). In addition, emissions of certain gases, such as NH_3 and CH_4, are regulated and limited in separate agreements. For example, the new NEC Directive (EU) 2016/2284 requires EU member states to reduce total EU emissions of NH_3 by 29% from 2005 levels by 2030. Furthermore, over 100 countries, including the United States and the European Union, which account for 70% of the global economy, signed the Global Methane Commitment at the UN Climate Change Conference (COP 26) in Glasgow in November 2021, agreeing to reduce global CH_4 emissions in all sectors by at least 30% by 2030 compared to 2020 levels (EC & USA, 2021).

These laws and regulations require the development and implementation of efficient slurry management strategies and technologies that can reduce emissions released during storage and application significantly so that these climate and environmental goals can be met. Therefore, this dissertation investigates and evaluates two different slurry treatment strategies based on the application of alkaline additives, with the aim of reducing these emissions.

1.3 Motivation and Objectives

One of the key challenges facing humanity in the 21st century is the mitigation of the advancing climate change. In this context, global decarbonization by the establishment and, in particular, achievement of net-zero GHG emission targets, as set out in the European Green Deal, is proving to be a pioneering but also a decisive step for our future (Black et al., 2021; EU, 2021a). The successful and sustainable pursuit of the net-zero GHG emission targets requires the establishment of a broad scientific basis for the development of effective emission reduction technologies (EU, 2018). This not only involves improving existing technologies but also exploring completely new and less researched technologies to identify the most efficient and feasible options for reducing GHG emissions. Therefore, this dissertation contributes to the acquisition of more in-depth and sophisticated scientific knowledge by presenting three studies that aim to reduce emissions attributable to the agricultural sector of slurry management. The insights acquired from Study 1 can be utilized to enhance the efficiency of existing and new slurry management strategies to reduce emissions. Additionally, the other two studies investigate and assess two new and innovative strategies that utilize alkaline slurry additives to reduce emissions.

The use of alkaline slurry additives to mitigate emissions is still a relatively unexplored slurry treatment concept. However, for these technologies, the amount of alkaline additives required to adjust a target pH needed to reduce these emissions effectively is primarily determined by the capacity of the main buffer systems in slurry. However, the current understanding of the composition and dynamics, e.g., microbial formation and degradation, of these buffer systems remains limited. Moreover, relevant studies are lacking that prove if buffers can be considered individually or if they influence each other, forming an interacting and complex system. Enhanced insight into these aspects could greatly contribute to the optimization of slurry treatments using alkaline additives, as it would allow a faster, more precise, and more efficient timing of the pH adjustment, helping to reduce the use of resources. Additionally, no operational mathematical tools are available to calculate and visualize these slurry buffer systems in detail. This would help to assess how factors such as temperature and time of storage affect the formation of buffer systems, so that deeper insights can be gained.

The second study presented in this dissertation investigates the alkalization of slurry using alkaline additives, such as $Ca(OH)_2$, to reduce GHG and NH_3 emissions as well as to recover valuable plant nutrients, such as nitrogen. However, as this is a new and poorly explored area of research, the study aims to determine the specific emissions reduction potential achievable by this approach, as well as to identify the pH range at which this reduction potential is maximized. In addition, the study outlines the technical requirements that must be met for the successful implementation of this technology.

Lastly, a novel approach based on the use of $CaCN_2$ as an alkaline slurry additive to reduce climate and environmentally relevant trace gases, such as CH_4, CO_2, N_2O and NH_3, was investigated and evaluated. In contrast to the application of $Ca(OH)_2$, $CaCN_2$ was not employed to increase the slurry pH intentionally. Therefore, a further aim of this study was to gain better knowledge about the underlying mechanism of action of $CaCN_2$.

In addition, it remains uncertain which concentration application ratios of $CaCN_2$ are optimal to maximize the emissions reduction potential in slurry from fattening pigs and dairy cattle at minimal application rates. Furthermore, the time required for the effect to occur is not yet known and needs to be determined in the study. This slurry treatment strategy is particularly notable for its ease of operation, requiring only the addition and stirring of $CaCN_2$ into the slurry. This enables a quick and easy implementation of this strategy without requiring major technical changes to the slurry storage facilities, thus avoiding high financial costs for the implementation and operation of this treatment strategy. The investigation and evaluation of this approach are therefore of great interest not only to farmers but also to policymakers.

The stated objectives of this dissertation initiated the formulation and addressing of the following three research questions:

I. How can Buffer Systems and their Capacities in Slurry be visualized?

II. What Influence do Time and Temperature of Storage have on the Dynamics of Buffer Systems in Slurry?

III. How and to what extent can the Application of Alkaline Additives in Slurry mitigate Climate and Environmentally relevant Trace Gases?

1.4 Dissertation Outline

The dissertation is divided into five scientific chapters, the content of which is generally focused on answering the research questions posed in Chapter 1.3 and is briefly described below.

1.4.1 Chapter 1 – General Introduction

The first chapter provides scientific background information that is supportive of achieving a comprehensive understanding of the following three individual research studies. In addition, Chapter 1 outlines the overall problem statements and motivations that justify the need and relevance of the research studies presented. Furthermore, the objectives of this dissertation are outlined.

1.4.2 Chapter 2 – Study 1, Research Article

Study 1 was published by MDPI in the journal Animals (EISSN: 2076–2615) and was published on May 21, 2020, under the following title:

Dynamics of Different Buffer Systems in Slurries Based on Time and Temperature of Storage and Their Visualization by a New Mathematical Tool

In Study 1, a new mathematical tool was presented that can be a powerful instrument to reveal the dynamics between the different main buffer systems in slurry by providing a detailed visualization of their buffer capacity at a specific pH value. The accuracy and reliability of this tool were validated by using various verification strategies, including the data output of over 300 titrations. These titrations were carried out by using sow, fattening pig and dairy cow slurry, with the pH being shifted to the acidic and alkaline milieu to cover the full buffer spectrum of these systems. Furthermore, the study focused on the change in the buffer capacity of the individual buffers with respect to time and temperature of storage. Therefore, the samples were stored for 12 weeks under either cold or warm conditions and were titrated during that period at regular intervals. Using this tool, it has been proven that the degradation of buffer systems operating in an acidic milieu provides the basis for the formation of buffer systems that can function in both an acidic and a basic milieu. These results provide a better understanding of the degradation processes, demonstrating that buffers cannot be considered individually but must be interpreted as complex and interacting systems. In addition, the combination of the knowledge gained, and the utilization of the mathematical tool enables the timing of the addition of alkaline additives in slurry to be optimized so that a desired target pH value can be set in a way that preserves resources and costs.

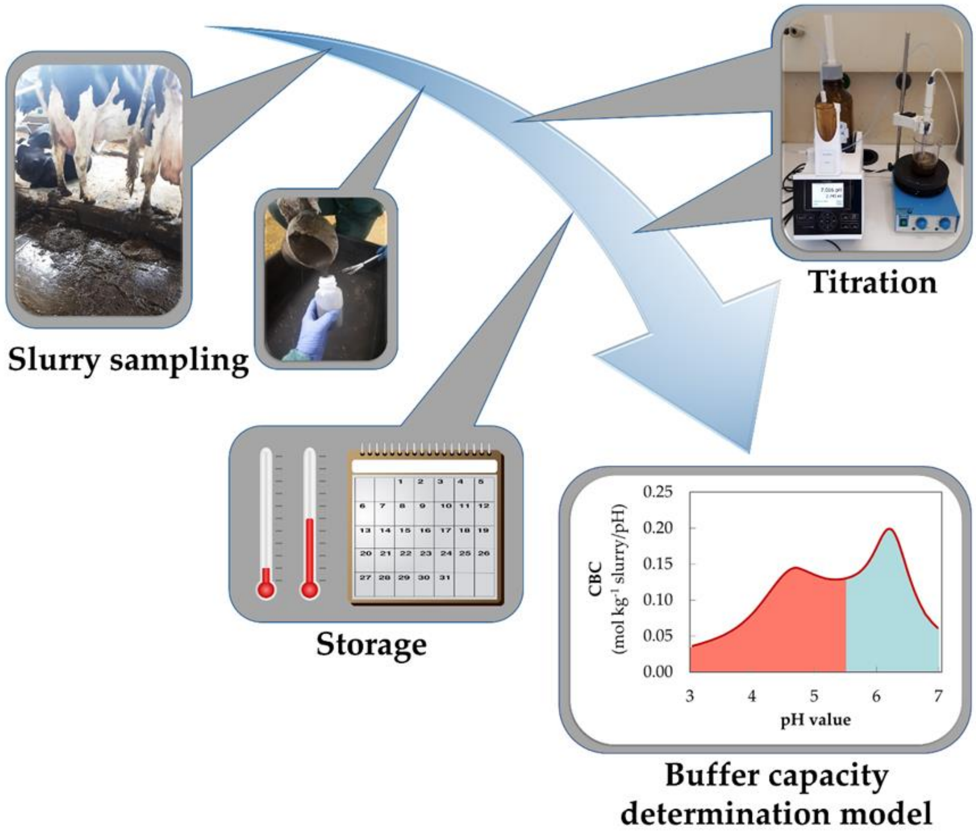

Figure 1.7: **Graphical Abstract of Study 1 (Overmeyer et al., 2020).**

1.4.3 Chapter 3 – Study 2, Conference Article

Study 2 was submitted as a conference article for the 15th Conference – Bau, Technik und Umwelt in der landwirtschaftlichen Nutztierhaltung (BTU-Tagung), which was held in Soest, Germany, September 13–15, 2022. The study was published under the following title:

Alkalization of Slurry, a Sustainable and Climate Friendly Concept

The second study follows up on the research conducted in Study 1 and provides background information on the technical prerequisites, requirements and procedures that need to be addressed before the concept of slurry alkalization can be successfully implemented. In addition, special attention was given to evaluating the effect of alkalized slurry on the formation of GHGs, such as CH_4, CO_2, and N_2O, during a storage period of eight weeks. The study results revealed that the alkalization of slurry to a pH of 10 by using $Ca(OH)_2$ significantly reduced the emissions of these GHGs. Increasing the pH further to 11 or 12 does not enhance the emission reduction effect, making it a strong candidate strategy to effectively reduce these emissions and thus may help to slow global warming.

1.4.4 Chapter 4 – Study 3, Research Article

Study 3 was published by Elsevier in the journal Waste Management (EISSN: 1879–2456) on March 2, 2023, under the following title:

Calcium cyanamide reduces methane and other trace gases during long-term storage of dairy cattle and fattening pig slurry

The third study presents a second slurry treatment concept based on the use of $CaCN_2$ as an alkaline slurry additive to reduce gaseous emissions. The objective of this study was to obtain a fundamental assessment of the emission reduction potential of $CaCN_2$ during the long-term storage of dairy cattle and fattening pig slurry. Therefore, both slurries were treated with either 300 mg kg^{-1} or 500 mg kg^{-1} cyanamide formulated in a low-nitrate $CaCN_2$ product called Eminex® (Alzchem Group AG, 83308 Trostberg, Germany). The slurry was stripped with nitrogen gas (N_2) to remove dissolved gases and was then stored for 26 weeks, during which CH_4, CO_2, and N_2O concentrations were measured. The gaseous emissions from stripping and storage were collected to determine the volume and total concentration of CH_4, CO_2, N_2O and NH_3, allowing the calculation of total GHG emissions. In addition, the experimental set-up was adapted from Study 2, with modifications and optimizations made to allow a more detailed scientific interpretation of the results. These experimental results obtained showed that CH_4 production was inhibited within 45 min after application and lasted in all variants for 26 weeks and more, except for the fattening pig slurry treated with 300 mg kg^{-1}. In this variant, the effect failed after 12 weeks. Nevertheless, total GHG emissions were reduced by 81% to 99%. The underlying mechanism of $CaCN_2$ is not based on a direct alkalization of slurry but rather on the inhibition of the microbial degradation of VFA and their conversion to CH_4 during methanogenesis. Consequently, the VFA concentration in the slurry increased, causing the pH to fall into an acidic milieu and thus leading to reduced ammonia emissions. This multi-layered emission-reducing effect, together with the easy handling of $CaCN_{2,}$ which consists of simply stirring it into the slurry storage facilities, make $CaCN_2$ based products a pioneering slurry additive that meets the requirements of the market and policymakers.

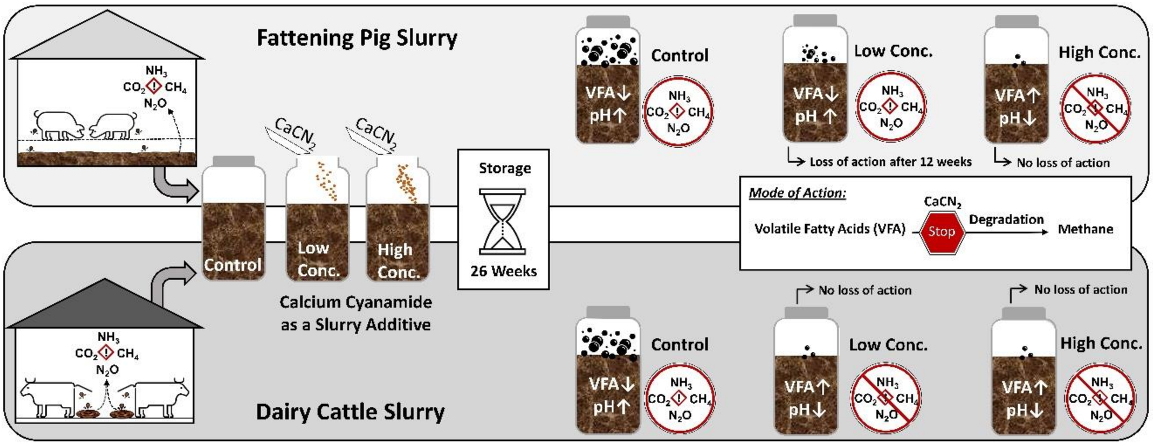

Figure 1.8: **Graphical Abstract of Study 3 (Holtkamp et al., 2023).**

1.4.5 Chapter 5 – Discussion and Conclusions

In the last chapter, the three scientific questions posed are discussed in detail. For this purpose, the results from the individual studies are placed in the general scientific state of the literature, so that the discussion and answer to the questions can be as substantial and differentiated as possible. Furthermore, conclusions are drawn from the studies presented that are useful in answering the questions. In addition, opportunities but also limitations of the studies are presented and approaches to solutions are described that could be achieved through further research.

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2 Study 1

Dynamics of Different Buffer Systems in Slurries Based on Time and Temperature of Storage and Their Visualization by a New Mathematical Tool

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Simple Summary

Efficient slurry management is a key strategy to reduce the release of environmentally harmful gases produced by farm animals. Slurry treatments such as acidification and alkalization have proven to be promising solutions to reduce these emissions. In this context, it is crucial to understand how buffer capacities behave and may influence each other during storage under the influence of different temperatures. To realize this, we have developed and successfully verified a new mathematical tool. It allows an exact calculation and detailed visualization of the most important buffer systems found in the analyzed slurries. This knowledge can be used to optimize slurry treatments, as it allows faster, more precise and efficient timing of pH adjustment, thus, reducing the use of resources.

Abstract

Slurry treatments such as acidification and alkalization have proven to be promising solutions to reduce gaseous emission produced by farm animals. The optimization of these technologies requires detailed knowledge of how and to what extent the buffer capacities in slurries will change during storage under the influence of different temperatures, as this may save resources needed to adjust a targeted pH value. Fresh slurries from dairy cows, fattening pigs and sows were collected and stored for 12 weeks under either cold (4.7 ± 1.1 °C) or warm (23.6 ± 2.1 °C) conditions to perform titrations in acidic and alkaline milieu at regular intervals. Based on these results, we successfully verified a new mathematical tool that we have developed to be able to calculate and visualize the most important buffer systems found in the analyzed slurries. Our experimental results showed a strong correlation between the degradation of the volatile fatty acid (VFA) buffer and the emergence of the carbonate buffers, i.e., the HCO_3^- and the CO_3^{2-} buffer. Furthermore, a drop in the pH value caused by enhanced microbial production of VFAs can be mitigated by the presence of the NH_3 buffer. In conclusion, we demonstrated that the buffers cannot be considered individually but must be interpreted as a complex and interacting system.

2.1 Introduction

The massive release of greenhouse gases (GHGs) into the atmosphere by anthropogenic activities drives and aggravates climate change, leading to an increase in global average temperatures, changes in precipitation patterns and melting of the ice sheets, resulting in a rise in sea levels (Committee on Extreme Weather Events and

Climate Change Attribution et al., 2016). However, to mitigate these negative effects arising from GHG emissions, the EU has committed itself to reduce GHG emissions to at least 40% below 1990 levels by 2030 (European Commission, 2014).

Improved manure management might have a high potential to achieve this goal, because in the EU in 2017, around 8% of total methane (CH_4) and nitrous oxide (N_2O) emissions and the biggest share of the total ammonia (NH_3) emissions were caused by direct or indirect effects of slurry storage and application (European Commission, DG Climate Action European Environment Agency, 2019; Tista et al., 2019). Ammonia emissions from the storage and spreading of digestate from the anaerobic digestion of energy crops are becoming increasingly important, as they were responsible for 10% of total NH_3 emissions in Germany in 2017 (Rösemann et al., 2019). Methane is a harmful GHG due to its 25 times higher global warming potential than CO_2 and its long residence time of 12 years in the atmosphere (IPCC, 2018). Ammonia is considered as an indirect greenhouse gas, as it does not directly promote the greenhouse effect, but can be naturally converted to the climate-damaging gas nitrous oxide in the soil (Denmead et al., 2008). Besides, ammonia has many other negative characteristics, for instance, it reacts in the atmosphere with acidic compounds to form particulate matter ($PM_{2.5}$) that is harmful to humans and animals. Furthermore, ammonia emissions may cause soil acidification and eutrophication of terrestrial and aquatic ecosystems by deposition (Sigurdarson et al., 2018). Regions such as North-West France, Lombardy in Italy, the Netherlands, Denmark and Lower Saxony in Germany often suffer from high ammonia emissions due to a high density of livestock farms, which produce and store large quantities of slurry (Scarlat et al., 2018; Clarisse et al., 2009; Oenema et al., 2007). Most of these areas have been declared ‘nitrate vulnerable zones’, i.e., the EU Nitrates Directive applies, which sets a nitrogen limit for livestock slurry of $170\ \mathrm{kg\ N\ ha^{-1} year^{-1}}$ (Council of the European Communities, 1991). As a result, the amount of slurry produced often exceeds the available area on which it is allowed to use slurry as an organic fertilizer. The consequence in these regions is a massive overproduction of slurry, which in combination with inappropriate slurry management concepts can lead to a failure of the environmental objectives set by the EU.

Promising approaches to avoid these problems are innovative slurry treatment technologies such as the acidification or alkalization of slurry. The equilibrium between $NH_4^+ \rightleftharpoons NH_3 + H^+$ is strongly pH dependent and shifts with decreasing pH value from the volatile non-ionized form NH_3 (pKs = 9.25) towards the nonvolatile ionized form NH_4^+ (Conn et al., 2007; Arogo et al., 2003). Slurry which has been acidified to a pH of 5.5 may cut emissions of NH_3 by more than 75% and emissions of CH_4 by 94% (Misselbrook et al., 2016). Besides that, a 95% reduction in ammonia emissions were also found in acidified digestate (Sánchez-Rodríguez et al., 2018). In Denmark, the acidification of slurry is already a well-established method to lower emissions during the storage and application of slurry (Kaupenjohann et al., 2019; Jacobsen, 2017). The alkalization of slurry is another method used to reduce emissions, but it is based on the addition of alkaline additives, that cause the precipitation of phosphorus and an increase in pH, which shifts the equilibrium towards the volatile form NH_3 (Cornel & Schaum, 2009; Arogo et al., 2003). A so-called stripping technology enables the removal of the growing share of NH_3 in the slurry, which is then captured and concentrated by absorption with concentrated sulphuric acid to produce ammonium-based mineral fertilizers (Mohammed-Nour et al., 2019; Laureni et al., 2013). It has been shown that an ammonia recovery rate of more than 90% is possible in cattle slurry at a pH of 12 (Mohammed-Nour et al., 2019). The adjustment of a targeted pH value can be very challenging, as the pH value of stored slurry may fluctuate widely over time due to an increase or decrease in the buffer capacity of the four main buffer systems within slurry, which are volatile fatty acid (VFA) buffer (predominantly CH_3COOH/CH_3COO^-), carbonic acid-bicarbonate buffers (H_2CO_3/ HCO_3^- and HCO_3^-/CO_3^{2-}, hereafter only HCO_3^- and CO_3^{2-} buffer) and ammonia buffer (NH_4^+/NH_3) (shown in Figure 2.1) (Patni & Jui, 1985; Georgacakis et al., 1982). Hence, the amount of acid/base must be constantly adapted to overcome the buffer capacities. These fluctuations are mainly influenced by changes in the VFA and ammonia buffer capacity (Paul & Beauchamp, 1989). The formation and degradation of the VFA buffer system depends on the ratio of anaerobic VFA-producing microorganisms that generate VFA via the decomposition of organic matter within the animal slurry and the aerobic VFA-consuming microorganisms (Christensen & Sommer, 2013). During storage, this ratio can change considerably, which may lead to altered VFA concentrations and thus to an increase or decrease in the pH value (Patni & Jui, 1985). Aeration of slurry can accelerate the decomposition process of VFA by oxidation (Paul & Beauchamp, 1989). Furthermore, the ammonia buffer system, which is mainly formed by the decomposition of urea into ammonia, could counteract the acidification of the slurry (Sigurdarson et al., 2018; Patni & Jui, 1985). Both processes may cause a shift of the pH value to the alkaline state, which changes the $NH_4^+ \rightleftharpoons NH_3 + H^+$ equilibrium in favor of NH_3 and thus promotes losses of NH_3 by volatilization (Paul & Beauchamp, 1989). In addition, the decomposition of VFAs and urea produces carbonate, which can act as a buffer system in alkaline and acidic milieu and has the ability to regulate NH_3 volatilization losses (Sigurdarson et al., 2018; Gerardi, 2003; Vandré & Clemens, 1996). These changes in the buffer systems were expected to occur mainly in fresh slurry, whereas in older slurry or digestate microbial degradation processes are almost complete.

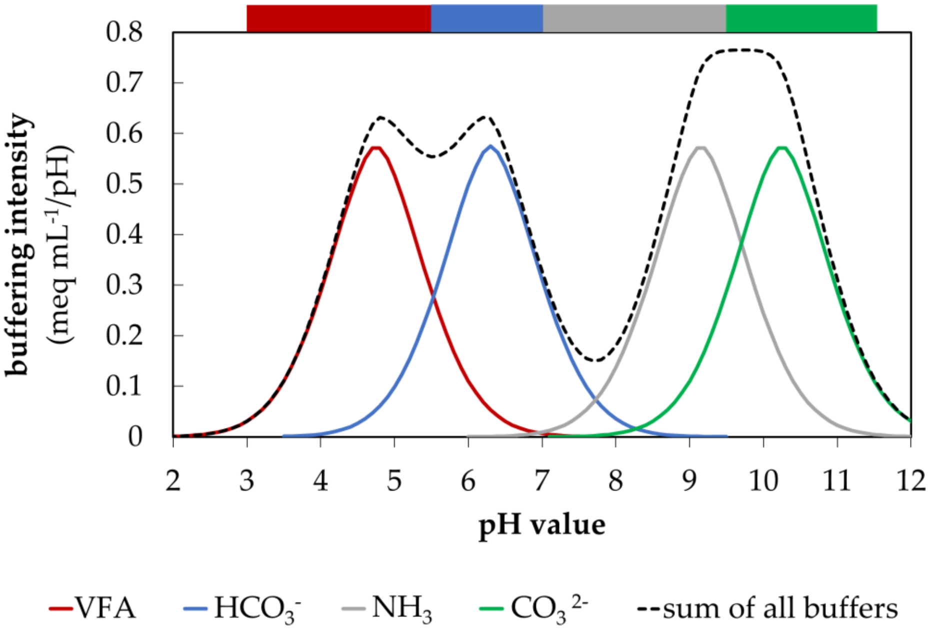

Figure 2.1: **Dynamics in buffering intensity with pH for different buffers commonly found in anaerobic digesters; vertical bars indicate the boundaries between the buffer areas (modified according to Georgacakis et al., 1982).**

In order to get a better understanding of the dynamic of these different buffer systems, (I) we have designed a new mathematical tool, which can be used for a detailed description and visualization of buffer capacity curves. (II) The tool was verified by comparing the generated values with the total inorganic carbon (TIC) and total ammonia nitrogen (TAN) contents as well as with the strength of the four main buffer systems found in the slurries that were identified based on over 300 titrations. (III) Furthermore, with the help of our mathematical tool and the titrations themselves, we aimed to reveal the dynamics of the individual buffer capacities based on storage time and temperature. Thus, a more precise understanding of the microbial degradation processes in slurry can be obtained. In addition, the model enables a better prediction of the amount of acid/base required to adjust a targeted pH value, allowing this to be done faster, more precisely and at the optimal time during storage. This may help to reduce the running costs of acidification and alkalization technologies by saving resources and time.

2.2 Materials and Methods

2.2.1 Slurry Sampling

Three fresh slurries (from fattening pigs, sows and dairy cows) not older than three days, were used for this investigation. The samplings occurred in the summer of 2019.

Two days before sampling the slurry pits of the fattening pigs (bodyweight 30–75 kg) were emptied to a small technical residual amount. The slurry sampling was done by using a sub-surface scraper system. The dairy cow slurry was taken from the walking alley of the cubicle barn. Here the slurry was collected for one hour before it was shoved off by a flap scraper. The feces and urine of high-bearing sows were collected separately for two days and then mixed.

Approx. 50 L of each slurry type was collected and divided into sample bottles necessary for the following laboratory tests (see Section 2.2.2).

In order not to change the microbial activity in fresh slurry the time between sampling and first analysis in the laboratory (hereafter: week 0) was kept as short as possible – between 4 (sow slurry) to 17 (dairy cow slurry) hours. Additionally, no cooling of the slurry was therefore required.

2.2.2 Storage

The influence of storage for a period of 12 weeks after removal from the slurry pits should be determined. Therefore, the titration investigations were carried out at week 0, 1, 2, 4, 6 and 12. Each slurry sample was stored in a separate 250 mL sample bottle (height: 119 mm, inner diameter: 63 mm, Low Density Polyethylene) to ensure that the samples were undisturbed during the complete storage period. The samples were stored under aerobic conditions as the lids were laid on the sample bottles (not screwed on tightly) allowing gas exchange but reducing strong evaporation losses.

In addition, the influence of the storage temperature on the buffer capacity of the slurry was investigated by comparing storage at cold (4.7 ± 1.1 °C) and warm conditions (23.6 ± 2.1 °C). Each variant consisted of three replications.

2.2.3 Analyses of the Ingredients

In weeks 0 and 8, slurry samples were analyzed by an external laboratory (AGROLAB Agrar und Umwelt GmbH, Sarstedt, Germany). The analyses included the determination of physico-chemical parameters (dry residue), macronutrients (total nitrogen (N), ammonium-nitrogen (NH_4-N), phosphate (as P_2O_5), potassium (as K_2O), magnesium (as MgO), calcium (as CaO), sulfur (S)), micronutrients (copper (Cu), zinc (Zn)) and the main volatile fatty acids (acetic acid, propionic acid, butyric acid, iso-butyric acid, valeric acid, iso-valeric acid, n caproic acid). The acetic acid equivalent was calculated from the volatile fatty acids.

Additionally, the total ammonia nitrogen (TAN = $NH_3 + NH_4^+$) was determined by the Quantofix-N-Volumeter (Tunney & Bertrand, 1989; Klasse, 1988; Klasse & Werner, 1987). As a reagent, the mixture of sodium hypochlorite and sodium hydroxide described by

Klasse (1988) was used. The measurement of the bicarbonate buffer expressed in total inorganic carbon (TIC = CO_2 + HCO_3^- + CO_3^{2-}) was carried out according to the methods of Clemens & Seufert (2007) and Hecht (2008).

2.2.4 Titration

The first slurry sample was titrated with 0.5 M HCl from the initial pH to 2.5, followed by a titration with 0.5 M NaOH from pH 2.5 to 12. The second sample was titrated from the initial pH to 12 by adding 0.5 M NaOH. A titrator (‘TitroLine 7000’, SI Analytics®, Mainz, Germany) with an ‘InLab Max Pro-ISM’ pH sensor (Mettler Toledo, Ohio, USA) was used for determination of pH value and temperature. For the titration process, 50 g slurry was diluted with 50 g deionized water, allowing the sample to be moved sufficiently with a magnetic stirrer. The buffer effect of distilled water was not considered due to its low ion content.

All titrations were performed via dynamic titration with a maximum step size of 0.5 mL. During the titration process, the current pH value, the amount of titrant and the temperature were automatically recorded. The pH sensor was calibrated according to manufacturer instructions. All titration experiments were performed at 24.7 ± 2.6 °C.

2.2.5 Calculations of the Titrations and New Determination Model for Buffer Capacity

The buffer capacity was determined from the amount of titrant (HCl or NaOH) during titration. The current buffer capacity (CBC) is the amount of acid or base required to change the pH by one unit at a specific pH value. The new mathematical tool (called: determination model for buffer capacity) to calculate this CBC proceeds in several steps (Figure 2.2).

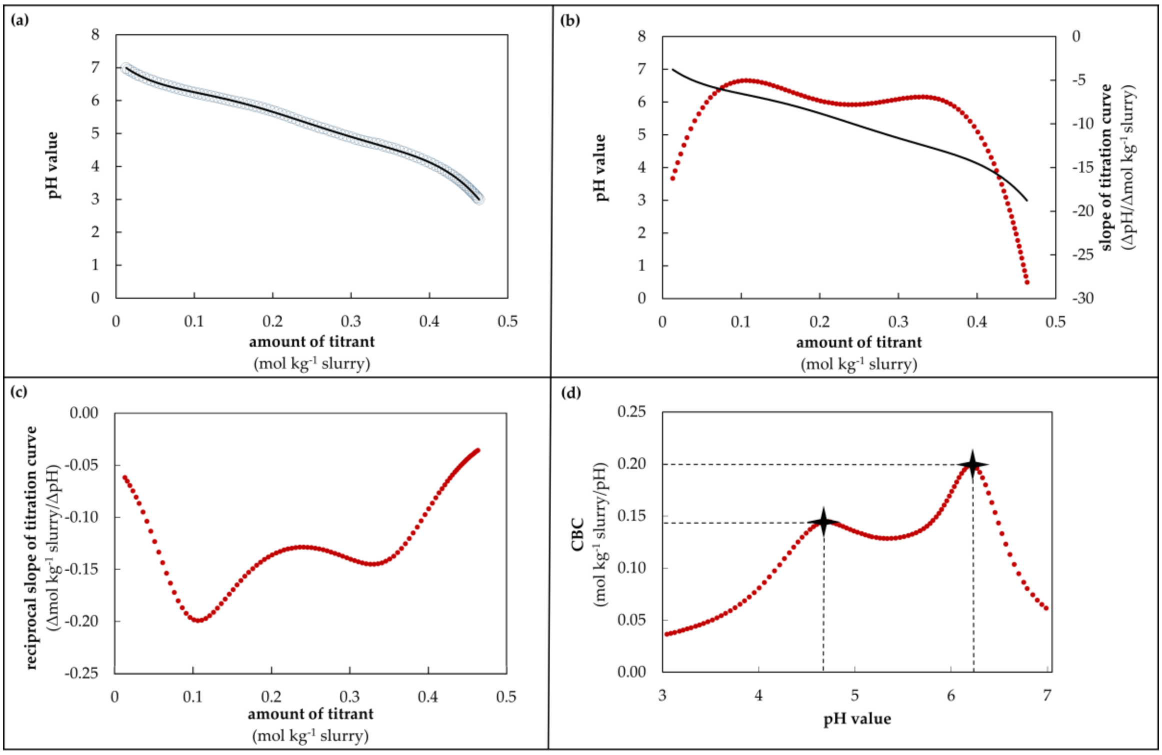

Figure 2.2: **Procedure of the determination model for buffer capacity; (a) pH value in dependency of the amount of titrant and polynomial regression line; (b) polynomial regression line and derived titration curve which stands for the slope of titration curve; (c) reciprocal derived titration curve in dependency of amount of titrant; (d) current buffer capacity in dependency of pH value.**

Firstly, the amount of titrant (mol kg^{-1} slurry) and pH value were plotted (Figure 2.2a). Then, the regression line (polynomial 6th degree) was calculated and mapped. In this equation, the amount of titrant is defined as the argument and the pH value is the value of the function (a, black line) (called: titration curve). The slope of the titration curve is equal to the first derivation of this curve. Therefore, the equation of the titration curve was derived (Figure 2.2b, red dots). The slope of the titration curve in dependency of the amount of titrant is outputted as ΔpH/Δmol kg^{-1} slurry. To determine the CBC, the reciprocal slope of the titration curve was formed (Figure 2.2c). The CBC refers to the amount of titrant per kg slurry needed to change the pH value by one unit. For visualization, the CBC (mol kg^{-1} slurry/pH) was plotted in dependency of the pH value (Figure 2.2d). The resulting graph represents the buffer capacity curve. The calculated maximum CBC (max. CBC) and the pH value of the maximum CBC are also exemplarily shown in Figure 2.2d (symbol). Furthermore, Equation (1) shows the calculation of the CBC for acidification and alkalization in a short form.

$$\text{CBC} = \left|\frac{1}{\text{slope in the respective pH value}}\right| \quad (1)$$

In order to determine the polynomial regression line needed to calculate exact CBC values, only specific value ranges of the titration data were chosen. The titration data during acidification from pH 7.0 to 3.0 and during alkalization from pH 7.8 to 11.5 were used. If the alkaline pH range began with a value higher than 7.8, the nearest value to 7.8 was chosen as the starting point. For the alkalization after acidification (pH 2.5 to 12.0), titration values from 7.0 to 11.5 were chosen and analyzed. The CBC during acidification is shown in mol H^+ kg^{-1} slurry/pH, whereas for alkalization the CBC is given in mol OH^- kg^{-1} slurry/pH. In the following study, only mol kg^{-1} slurry/pH is used.

The calculation of the CBC using the model for determining the buffer capacity was carried out with the R Studio software (Version 1.0.153). However, the calculation of the regression line can also be done with other mathematical software, e.g., Microsoft Excel or Mathematica (Wolfram Research).

2.2.6 Statistical Analysis

Erroneous titrations influencing the CBC and the position of the buffers were excluded. These titrations could be easily identified as the titration curves showed strong irregularities caused by an accumulation of organic material on the pH sensor or by retention of the titrant when entering the solution due to excessive foaming.

Statistical analysis were done using IBM® SPSS® Statistics, Version 25. The values represent mean values and are given with standard errors of mean (SEM) either in brackets or in vertical bars, except for temperature (mean value ± standard deviation). The number of considered values is indicated with n. Correlation analyses were performed using the Pearson correlation coefficient (r) at a significance level of $p < 0.05$. The graphical presentation of the correlation was performed by creating a linear regression line. One way analysis of variance (ANOVA) was performed at a significance level of 0.05 to describe the differences in pH values and the amount of acid used for the different weeks. Subsequently, the Tukey's Honestly Significance Difference (HSD) was used, if appropriate. In the absence of variance homogeneity, the Games-Howell test was chosen with significance level of 0.05 to indicate statistical significance.

2.3 Results

2.3.1 Slurry Characterization

The three types of slurry were analyzed in terms of their physico-chemical parameters, nutrient and volatile fatty acid contents (Table 2.1). The dry residue content decreases for all slurries (except coldly stored dairy cow slurry) over the storage period. The decrease is higher in warmly stored slurry. Dry residue is lowest in sow slurry compared to the other types of slurry. This is also indicated by the low P_2O_5, K_2O and VFA contents in the sow slurry. Instead, the NH_4-N and TAN contents in this slurry are higher. After eight weeks of storage, the NH_4-N contents were similar to week 0 irrespective of the storage temperature and slurry type. The highest acetic acid equivalent and thus the highest VFA concentration were detected in the fattening pig slurry. Acetic acid accounts for the greatest share of the acetic acid equivalent in all slurries. During storage, the variations in VFA and TIC contents showed no clear pattern between all three types of slurry. Further results of the slurry characteristics are shown in the Appendix (Table 2.2).

Table 2.1: Characteristics of fattening pig, dairy cow and sow slurry (fresh material) in week 0 and 8 depending on cold (4.7 ± 1.1 °C) and warm storage conditions (23.6 ± 2.1 °C).

		Fattening Pig			Dairy Cow			Sow		
Week and Storage Conditions **Ingredients** [1]		**0**	**8 Cold**	**8 Warm**	**0**	**8 Cold**	**8 Warm**	**0**	**8 Cold**	**8 Warm**
Dry residue	%	8.30	8.27	7.23	9.80	10.03	8.90	2.50	2.37	2.20
N	$kg\ m^{-3}$	4.81	4.92	5.02	4.20	4.22	4.11	5.37	5.41	5.03
NH_4-N	$kg\ m^{-3}$	2.88	2.99	3.17	2.42	2.37	2.51	4.97	4.20	4.37
P_2O_5	$kg\ m^{-3}$	2.56	2.94	3.36	1.16	1.53	1.58	0.92	0.87	0.93
K_2O	$kg\ m^{-3}$	4.14	4.72	5.03	4.14	5.31	5.36	1.70	1.72	1.78
Acetic acid	$g\ kg^{-1}$	7.00	5.83	2.67	6.20	5.83	6.87	2.70	2.77	2.50
Propionic acid	$g\ kg^{-1}$	1.60	1.53	2.17	1.20	1.17	1.97	0.14	0.15	0.29
Acetic acid equivalent [2]	$g\ kg^{-1}$	9.30	8.10	4.90	7.70	7.27	9.03	2.90	3.00	2.93
TAN	$kg\ N\ m^{-3}$	2.50	2.35	3.33	2.48	2.31	2.96	4.80	4.88	4.71
TIC	$kg\ C\ m^{-3}$	1.48	1.48	1.99	1.43	1.31	1.33	1.06	1.45	1.89

[1] Physico-chemical parameters, macronutrients and volatile fatty acids analyzed by an external independent laboratory; total ammonia nitrogen (TAN) and total inorganic carbon (TIC) were determined by own analysis;

[2] Acetic acid equivalents were calculated from the acetic acid, propionic acid, butyric acid, iso-butyric acid, valeric acid, iso-valeric acid and n caproic acid.

2.3.2 Verification of the Determination Model for Buffer Capacity

For the evaluation of the titrations, a model to determine the buffer capacity was developed, which allows the determination and visualization of buffer capacities based on titration curves. The verification of this new mathematical tool should reveal if the calculated values (maximum CBC, pH value of the maximum CBC) are reliable. Therefore, the pH of the maximum CBC calculated with the new mathematical tool was compared with the slurry temperature at the time of measurement. In addition, the calculated CBCs were compared with the TIC, TAN and amount of acid used for a defined pH range.

The first step, in verifying the model used to determine the buffer capacity was to correlate the sample temperatures with the pH values corresponding to the maximum CBCs of the HCO_3^- buffer in dairy cow slurry during acidification (Figure 2.3a) and of the NH_3 buffer in sow slurry during alkalization (Figure 2.3b). Both, the carbonate and the ammonia buffer are sensitive to temperature changes, thus the pH value for maximum CBC increases at lower temperatures and decreases at higher temperatures. Nevertheless, the ammonia buffer reacted more homogeneously to temperature changes, while the carbonate showed stronger fluctuations. This effect was checked for significance by using a correlation analysis that revealed for the carbonate buffer $r = -0.64$ ($p < 0.05$) and for ammonia buffer $r = -0.99$ ($p < 0.001$).

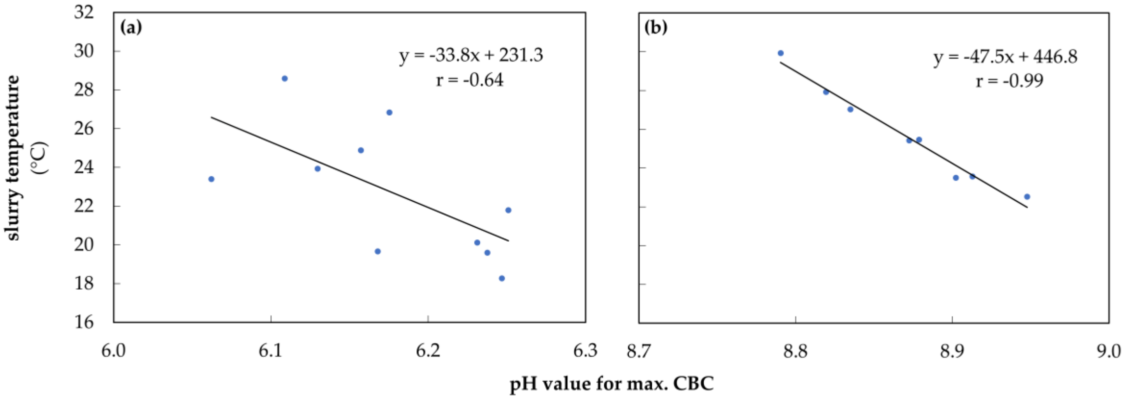

Figure 2.3: **(a) Temperature of dairy cow slurry depending on the pH value for maximum CBC in HCO_3^- buffer during acidification; (b) Temperature of sow slurry depending on the pH value for maximum CBC in NH_3 buffer during alkalization from pH 2.5 to 12.0.**

The measured TIC contents and the maximum CBC values in the pH range between 6.0 and 6.5 (HCO_3^- buffer) varied greatly in the analyzed slurries (Figure 2.4a). The maximum CBC differed between 0.06 and 0.27 mol kg^{-1} slurry/pH. The TIC content was between 1.04 and 2.44 kg C m^{-3}. In week 0, the sow slurry had low TIC levels, which were also indicated by the low maximum CBC values (red dots). Besides that, a strong correlation between these parameters was observed ($r = 0.86$, $p < 0.01$).

After the acidification of slurry to pH 2.5, titrations from pH 2.5 to 11.5 were performed, allowing the maximum CBC of the ammonia buffer to be shown with less influence of the carbonate buffer because the previous acidification eliminated the potentially interfering carbonate buffer (Figure 2.4b). Furthermore, in week 0, the sow slurry was found to be exceptional, as the maximum CBC values were lower than that of the other samples, although the TAN contents were within the same range. The correlation between the TAN content and the maximum CBC of the ammonia buffer was strong with $r = 0.85$ ($p < 0.01$) (excluding sow slurry in week 0).

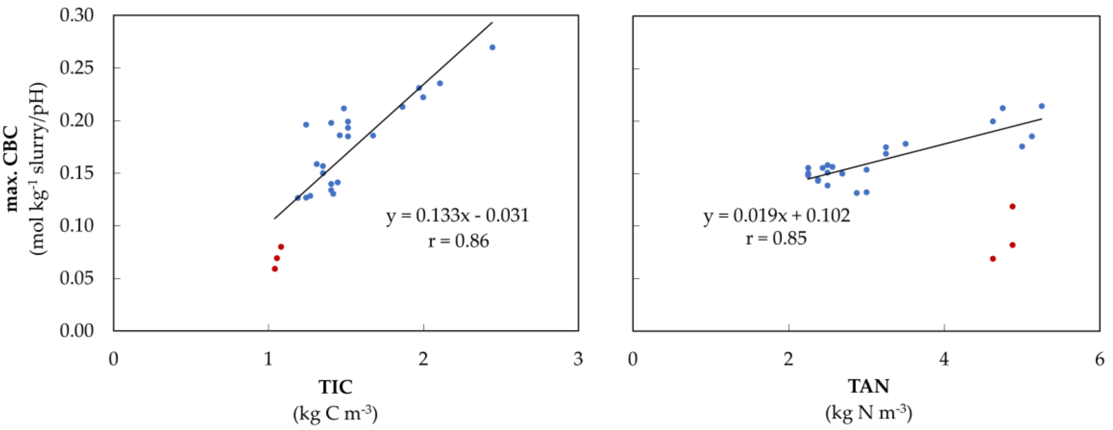

Figure 2.4: **(a) Maximum CBC during acidification in HCO_3^- buffer depending on the TIC content of the different slurries (sow slurry in red dots) in week 0 and 8 (*n* = 26); (b) Maximum CBC during titration from pH value 2.5 to 11.5 in the NH_3 buffer depending on the TAN content of the different slurries in week 0 and 8, sow slurry of week 0 (red dots) was excluded from the calculation of the regression line here (*n* = 23).**

In order to reduce the pH value from 7.0 to 5.5, between 0.07 to 0.28 mol HCl kg^{-1} slurry was used. The maximum CBC in this range was between 0.06 and 0.30 mol kg^{-1} slurry/pH (Figure 2.5).

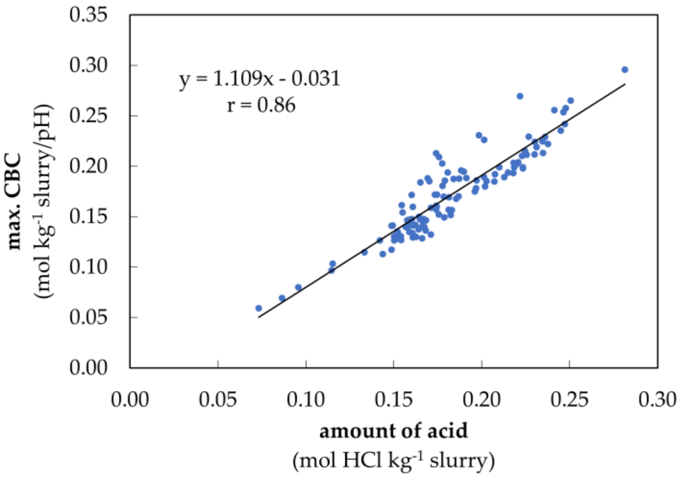

Figure 2.5: **Maximum CBC during acidification in the HCO_3^- buffer depending on the amount of acid from pH value 7.0 to 5.5 of the fattening pig, dairy cows and sow slurry (*n* = 115).**

Moreover, it could be shown that the amount of acid in the pH range 7.0 to 5.5 is directly proportional to the maximum CBC measured within the HCO_3^- buffer range. This results in a strong correlation value of r = 0.93 ($p < 0.001$).

In order to compare the amount of acid in the pH range of 5.5 to 3.0 with the maximum CBC of the VFA buffer, only the maximum CBC of dairy cow slurry could be used. A correlation of r = 0.92 ($p < 0.001$) between the amount of acid and the maximum CBC was observed (Figure 2.9). For the other two types of slurry, the maximum CBC of the VFA buffer could not always be differentiated from the carbonate buffer. This becomes clear by the visualization of the buffer curves during the storage period in warmly stored fattening pig slurry in week 0, 6 and 12 (Figure 2.6).

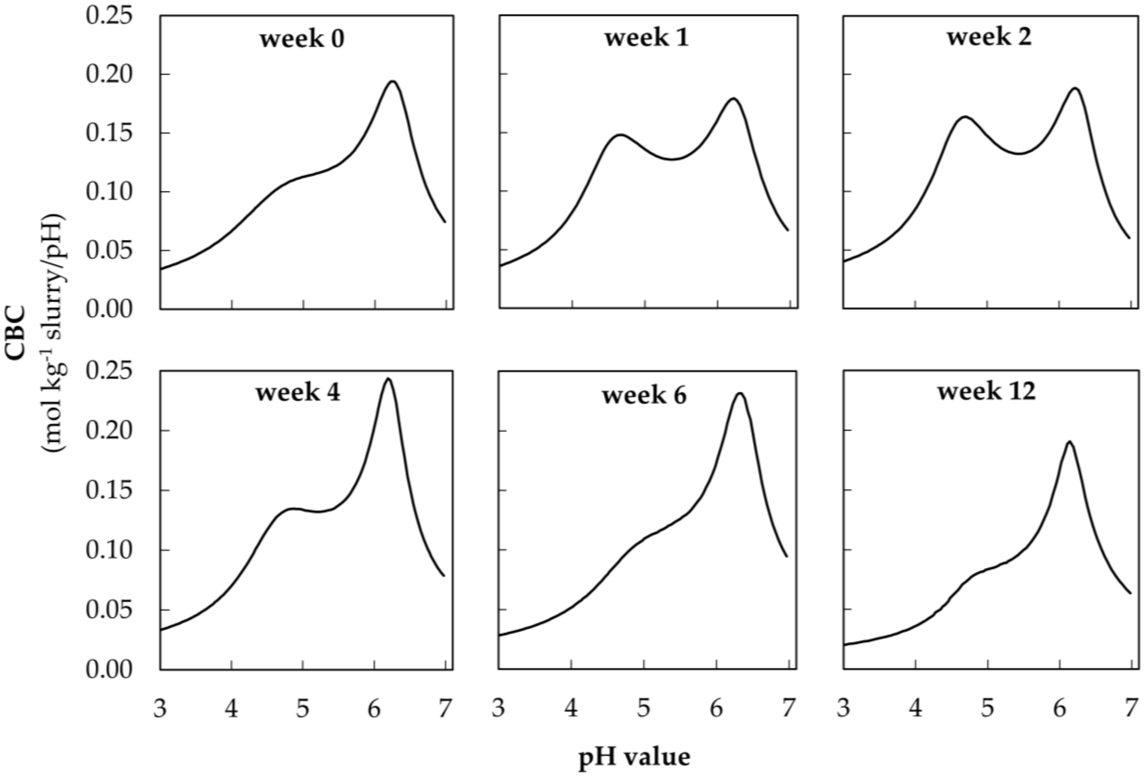

Figure 2.6: **Visualization of CBC depending on pH value in fattening pig slurry stored under warm conditions (23.6 ± 2.1 °C) from week 0 to 12.**

2.3.3 Visualization of CBC during Acidification

The visualization of the buffer curves allows a graphical representation of the change in each buffer system over 12 weeks of storage. These buffer curves are shown as an example of the acidification of warmly stored fattening pig slurry (Figure 2.6).

In the pH range between 4.0 and 4.5, the peaks indicate the maximum of the VFA buffer, which has been defined as the maximum CBC value in this pH range. In this interval, the maximum CBC increased from week 0 up to 0.16 (0.01) mol kg^{-1} slurry/pH in week 2. Then, the VFA buffer was reduced during storage until week 12. The maximum CBC could not be exactly quantified in weeks 0, 6, and 12, because there was no local maximum (see Section 2.3.2). In the pH range between 6.0 and 6.5 (HCO_3^- buffer) the CBC increased from 0.19 (0.01) to 0.24 (0.01) mol kg^{-1} slurry/pH after week 2 and finally decreased again to 0.19 (0.02) mol kg^{-1} slurry/pH until week 12.

2.3.4 Initial pH Value and Amount of Acid for Titration during Storage at Different Temperatures

As shown in Figures 2.5 and 2.6, the maximum CBC correlates very closely with the amount of acid required to adjust the pH values in the respective pH ranges in which the buffers are located. Since the maximum CBC could not always be determined with the mathematical tool, the dynamics of the VFA and the carbonate buffer were determined in Figure 2.7 based on the amount of acid in the pH ranges 5.5 to 3.0 (red) and 7.0 to 5.5 (blue), respectively. The grey column below the black line represents the amount of acid needed to lower the initial pH value to 7.0. This was done to minimize the influence of the changing initial pH and the ammonia buffer on the amount of acid used in the acidic milieu. Additionally, the initial pH values are plotted in this figure. The measured parameters are shown for all three types of slurry and both storage temperatures. The individual values and significant differences are shown in Table 2.3.

The dynamics of the VFA and carbonate buffer during the warm storage of the fattening pig slurry as already described in Figure 2.6 in Section 2.3.3 can also be seen for the amount of acid which is shown in Figure 2.7 (‘warm’, ‘fattening pig’). The amount of acid from 5.5 to 3.0 (VFA buffer) increased considerably from week 0 to 2, while the amount of acid between 7.0 and 5.5 (HCO_3^- buffer) remained constant during this period. Additionally, Figure 2.8b shows in detail the dynamics and interaction of these buffers with the CO_3^{2-} buffer. Thus, it could be seen that during the first week the amount of base from 9.5 to 11.5 (CO_3^{2-} buffer) remained constant as well. In the following four weeks, a close interaction between the VFA buffer and the carbonate buffer became clear, as the VFA buffer capacity rapidly decreased and the carbonate buffer rose to a peak value for HCO_3^- and CO_3^{2-} concentrations.

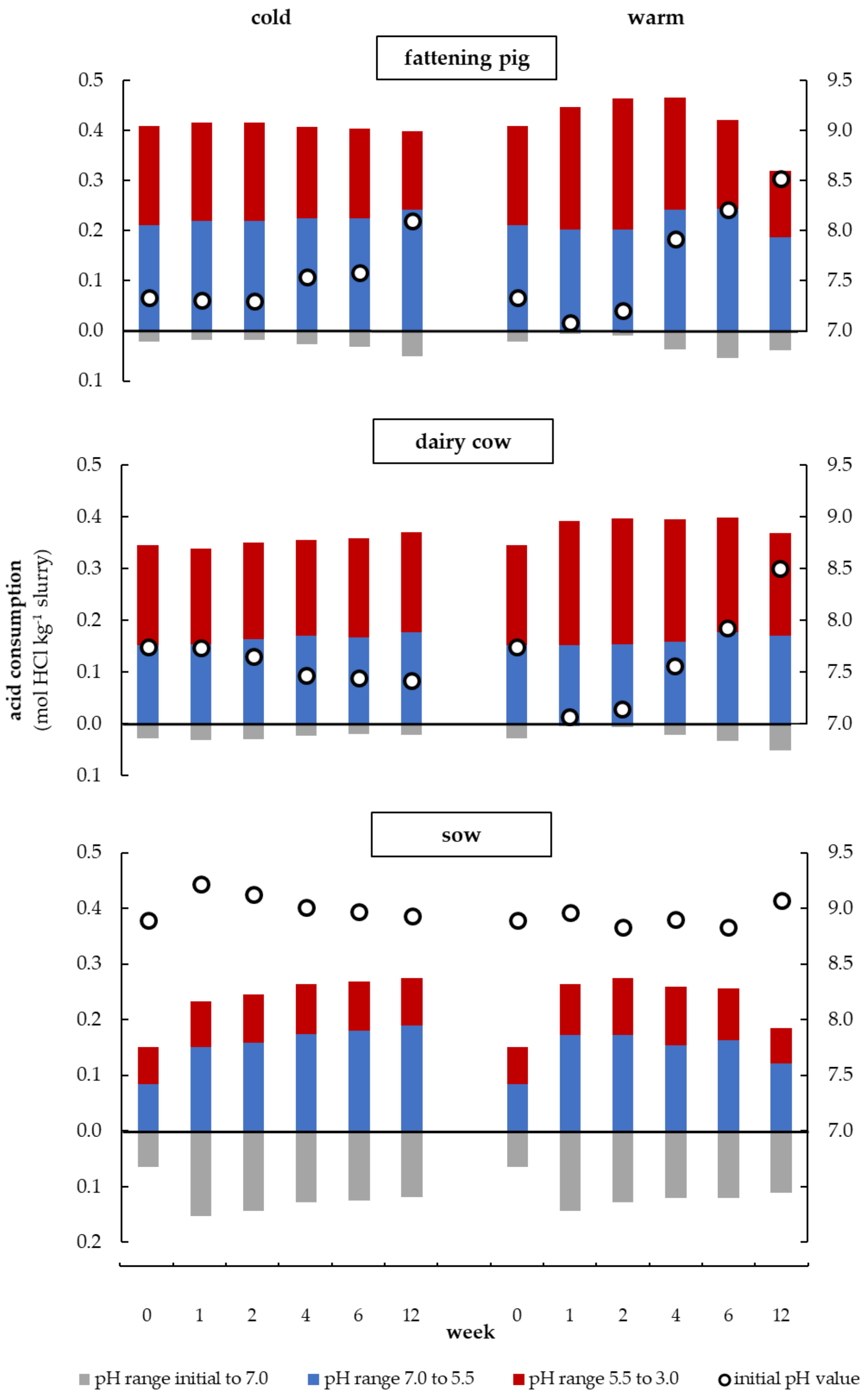

Figure 2.7: **Initial pH value of fattening pig, dairy cow and sow slurry as well as the amount of acid under cold (4.7 ± 1.1 °C) and warm (23.6 ± 2.1 °C) storage conditions in the pH range initial to 7.0, 7.0 to 5.5 and 5.5 to 3.0 over a storage period of 12 weeks; for better visualization, the amount of acid in the pH range initial to 7.0 is shown below the black line.**

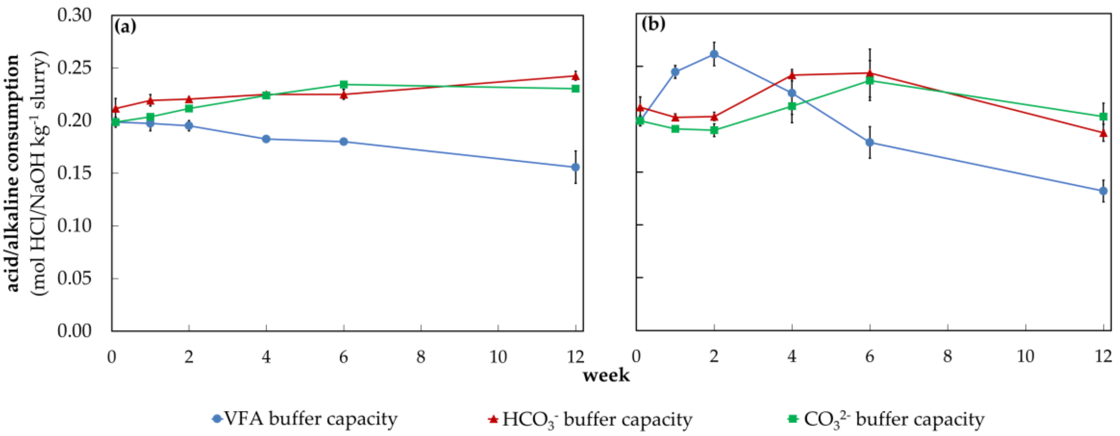

Figure 2.8: **Evolution of the acid/base amount for (a) coldly stored (4.7 ± 1.1 °C) and (b) warmly stored (23.6 ± 2.1 °C) fattening pig slurry shown over 12 weeks to visualize the dynamics of the VFA (pH range 5.5 to 3.0), HCO_3^- (pH range 7.0 to 5.5) and CO_3^{2-} (pH range 9.5 to 11.5) buffer systems, vertical bars represent standard errors (*n* = 3).**

Hereafter, the VFA and the carbonate buffer slowly but steadily decreased (Figure 2.7, 'warm', 'fattening pig'). Besides that, the two carbonate buffers showed only minor differences in their curve progressions and so did they in their buffer capacities dynamics during the entire storage period (Figure 2.8b). The initial pH value of the warmly stored fattening pig slurry developed contrarily to the VFA buffer. This pH value initially decreased from week 0 to 1 and then increased during the entire storage period (Figure 2.7, 'warm', 'fattening pig'). Moreover, there is a significant dependency on the initial pH value of the VFA buffer ($r = 0.91$, $p < 0.001$). The carbonate buffer has a lower influence on the initial pH value ($r = 0.41$, $p < 0.001$) compared to the VFA buffer (see Figure 2.10). The warmly stored dairy cow slurry showed similar dynamics of the VFA buffer, the carbonate buffer, and the initial pH value as those found in the fattening pig slurry (Figure 2.7, 'warm', 'dairy cow'). However, in the case of dairy cow slurry, the VFA buffer was reduced from week 6 instead of week 4 (in fattening pig slurry).

Both, the coldly stored fattening pig and the dairy cow slurry showed a clear delay in the development of the buffers. In addition, the VFA buffer in the coldly stored fattening pig slurry did not show any fluctuations but instead a linear degradation (Figure 2.7, 'cold', 'fattening pig' and Figure 2.8a). Furthermore, analogies between VFA buffer degradation and carbonate buffer formation were also observed in coldly stored fattening pig slurry, as the carbonate puffer increased in a similar ratio to what the VFA buffer decreased. It is noticeable that the initial pH value of the fattening pig slurry tends to increase, whereas in the dairy cow slurry it tends to decrease.

In the sow slurry, the lowest VFA buffer capacities and the highest initial pH values and thus the highest amount of acid in the pH range initial to 7.0 (Figure 2.7, 'sow', grey columns) were found compared to the two other types of slurry. In the first week of storage, the coldly stored sow slurry showed a significant increase in the pH value, whereas the warm conditions had no significant influence on the pH value. At the same time, however, there was a much stronger increase in the VFA buffer in the warmly stored slurry than in the coldly stored slurry. Regardless of the storage temperature, the HCO_3^- buffer approximately doubled its capacity during this period ($p < 0.05$).

2.4 Discussion

2.4.1 Verification of the Determination Model for Buffer Capacity

In order to demonstrate that the new mathematical tool can be used to calculate and visualize buffer capacity curves and their dynamics based on time and temperature of storage, the tool was verified by employing the results of seven different correlations. It was found that the model used for determining the buffer capacities in slurries is capable of detecting and plotting temperature-induced shifts of buffer range in both, the acidic and alkaline milieu (Figure 2.3). The carbonate buffer reacted with higher fluctuations to temperature changes than the NH_3 buffer, which showed a very homogeneous response. This was probably due to the excessive foam formation during the acidification process and the high sensitivity of the carbonate buffer to an enhanced ion input (H^+) (Millero et al., 2006). The maximum CBC value also correlated significantly with the TIC and TAN values found in the slurry (Figure 2.4). Thus, with the help of our mathematical tool, conclusions can be drawn about the amount of carbonate and nitrogen in the slurry. Furthermore, the maximum CBC values of the carbonate buffer (Figure 2.5) and the VFA buffer (Figure 2.9) showed a significant correlation with the amount of acid required to overcome these buffers. In conclusion, the mentioned results show a reliable correlation between the data calculated with our mathematical tool and the data which we have obtained from actual measurements. The CBC can quantify the ‘ability’ of the weak acid/base at a defined pH value to resist a change in pH when strong acid or base is added, as is also shown in the study of Moosbrugger et al. (1993).

However, the tool also has its limits, especially when the individual buffers overlap in their pH value ranges and are therefore no longer differentiable. This frequently was the case between NH_3 and CO_3^{2-} buffers. Hence, buffer capacity analyses in alkaline milieu were found to be particularly difficult. In the case of larger titration errors (see Section 2.2.6), the newly described model could not completely interpolate the data. Foam formation during acidification, especially with dairy cow slurry, complicated the titrations. This is due to rapid CO_2 releases through the addition of strong acids (Vandré & Clemens, 1996). It can be described as a mechanical buffering of slurry in comparison to, e.g., water.

In order to be able to compare the capacity of the individual buffers between the weeks not only visually but also statistically, the maximum CBC must be clearly identifiable. For example, this was possible for the VFA buffer only in the case of dairy cow slurry (Figure 2.9).

Georgacakis et al. (1982) titrated different swine digesters with acid and lye. The titration curves were subjected to regression analysis so that this could be expressed as a general mathematical model (polynomial 3rd degree). After the derivation of this function, the buffer capacity was displayed. However, the authors restricted their study to the pH range 6.5 to 9.5 (Georgacakis et al., 1982). Other authors have shown the buffer capacity by expressing the inverse slope of the titration curve in the dependency of pH (Husted et al., 1991). They visualized the buffer curve without performing an additional calculation of the maximum buffer capacity. It is also uncertain in which steps the respective gradient was calculated (Husted et al., 1991).

An advantage of the new model in this study is the calculation of the buffer capacity based on the titration curve which can be obtained without the need for more specific measurements as it was the case in other studies (Lahav & Morgan, 2004). Christensen et al. (2009) mentioned that they plotted the buffer capacity as a function of the pH value (only visualization). The focus of their study was on specific buffers (e.g., phosphate groups). Therefore, the carbonate was expelled by prior acidification and could not be considered in the presentation of the buffer capacity (Christensen et al., 2009). The carbonate buffer must be included in the acidification and alkalization of slurry. Our determination model for buffer capacity allows a detailed representation of the buffer curves over the whole pH range.

In a study to determine the pH buffer capacity in poultry litter, the titration curve was represented by a linear equation and a sigmoidal curve. After forming the reciprocal of the slope, the sigmoidal curve allowed to plot the pH buffer capacity as a function of pH in the range from 6.5 to 9.5 (Cassity-Duffey et al., 2015). Costello & Sullivan (2014) also calculated the buffer capacity of compost by the negative reciprocal of the slope. However, they used the slope of a linear regression, which represented the titration curve after adding different amounts of acid (Costello & Sullivan, 2014). Therefore, the authors could only show a general but no corresponding buffer capacity for the current pH value.

The area under the curve between two pH values can be used to determine the amount of H^+ ions that need to be added or removed to change the pH value (Moosbrugger et al., 1993). This could be enabled by further calculations with our model.

2.4.2 Slurry Characterization, Initial pH Value and Amount of Acid during Storage at Different Temperatures

2.4.2.1 Slurry Characterization

In fattening pig slurry, a higher VFA content was found than in dairy cow slurry. The predominant VFA in animal slurries is acetic acid (Table 2.1). This is similar to other studies (Popovic & Jensen, 2012; Conn et al., 2005; Cooper & Cornforth, 1978). Sommer & Sherlock (1996) also reported that acetic acid represents more than two-thirds of the fatty acids. In contrast, Miller & Varel (2003, 2001) found almost twice as many VFA in the cattle slurry compared to the fattening pig slurry. The microbial degradation of the acids does not change the ratio (Sommer & Sherlock, 1996). In this analysis, acetic acid is also the predominant fatty acid after a storage period of eight weeks (Table 2.1). Popovic & Jensen (2012) reported that the VFA for pig slurry will decrease over the storage period (43 weeks). This is similar to our study. Nevertheless, no consistent tendency can be observed for the sow and dairy cow slurry. This might be explained by the slower degradation processes of the VFA in the dairy cow and sow slurry. Most of the VFAs in fattening pig slurry produced in the first weeks were degraded during storage, whereas a higher amount of VFA was still present in the other two types of slurry at the end of storage. Thus, an analysis of the VFA concentration at only two times cannot show the entire dynamics of the VFA buffer.

The dry residue is highest in the dairy cow slurry compared to the other two slurry types (Table 2.1). Cooper & Cornforth (1978) also noted this observation. During storage, the dry residue of almost all slurries was reduced. These dry residue losses were also observed by other authors during the storage of solid manure (Petersen et al., 1998), raw slurry and its liquid fraction irrespective of storage temperature (Popovic & Jensen, 2012). In our study, the dry residue losses are higher in warmly stored slurry (confer Popovic & Jensen, 2012). They are due to the decomposition of organic matter, resulting in the transformation of carbon into methane and carbon dioxide (Møller et al., 2002). At the end of the storage period, a decrease in the carbonate buffer was also observed in warmly stored slurries (Figures 2.6 – 2.8). The resulting emissions depend on several factors such as storage temperature or the presence of an adapted microbial community in pre-stored slurry (Sommer et al., 2017).

The reduction in NH_4-N and N contents during storage, which was also presented by the authors Popovic & Jensen (2012), could not be confirmed by this study (Table 2.1). The shorter storage period (8 weeks compared to 43 weeks) could probably be a reason.

2.4.2.2 *Initial pH Value*

The pH value of fresh slurry is around 7.5 (Christensen & Sommer, 2013). This could be confirmed for the fattening pig and dairy cow slurry, whereas the fresh sow slurry had an initial pH value of 8.9, which could be due to the farm-specific feeding. After 12 weeks, the pH value of almost all slurries had increased compared to the start pH value, especially during warm storage. Popovic & Jensen (2012) observed a continuous pH increase in pig slurry during storage (Figure 2.7).

The pH value of slurry is strongly dependent on HCO_3^-/CO_3^{2-} and NH_4^+/NH_3 buffer systems and the amount of VFA (Patni & Jui, 1985; Georgacakis et al., 1982). Sommer & Husted (1995b) also reported a strong influence of the VFA content on the pH value of slurry. As the concentration of VFA increases (Figure 2.7, week 2), the importance of the carbonate buffer system decreases. The pH value is then mainly determined by VFA and ammonia concentration (Georgacakis et al., 1982). However, other authors declare that bicarbonate is the most important pH buffering compound in slurry (Vandré & Clemens, 1996). In this study, it was found that the VFA buffer mainly influenced the pH value (Figures 2.7 and 2.10). The study of Sommer & Husted (1995a) explained that the pH value increases if the VFA content decreases at constant TIC concentration, whereas the pH value decreases if the VFA content remains constant but the TIC concentration increases due to enhanced CO_2 production. The microbial oxidation of VFA can lead to an increase in the pH value because these acids have been metabolized (Paul & Beauchamp, 1989; Cooper & Cornforth, 1978; Stevens & Cornforth, 1974). This can be confirmed in the present study when considering the VFA progression and the initial pH starting from week 4 (fattening pig) and week 6 (dairy cow and sow) in Figure 2.7. VFAs are metabolized under aerobic conditions resulting in the production of CO_2. The CO_2 can be absorbed by the solution in various degrees (Sommer & Husted, 1995b) which contributes to the pool of TIC (Christensen & Sommer, 2013). Besides that, low ionic concentrations lead to an increase in the pKs value of the carbonate buffer (Millero et al., 2006). Possibly the different ionic strength of the different slurries influences the initial pH value. This could explain the low correlation between the carbonate buffer and the initial pH value (Figure 2.10).

The slurry pH of dairy cow (Paul & Beauchamp, 1989) and pig slurry (Conn et al., 2005) was reported to be significantly correlated with $VFA/NH_4^+ + NH_3$. Eriksen et al. (2014) demonstrated that an increase in pH value during the first week of storage was caused by the mineralization of organically bounded N (Sommer et al., 2007). This pH increase could only be observed for the very fresh sow slurry (cold: +0.33 and warm: +0.08 pH units from week 0 to week 1) (Figure 2.7, ‘sow’) because it still contains a high content of urea (see

Section 2.4.3). The pH value of slurry from dairy cattle fed with a low nitrogen diet decreases from 8.15 (day 0) to 5.94 (day 56) (Aguerre et al., 2012). In comparison to this study, it was found that the pH value during warm storage dropped sharply in the first week (but only to pH 7.07) and increased continuously again until week 12. On the other hand, the coldly stored dairy cow slurry showed a continuous decrease in the pH value (but not as strong as reported by Aguerre et al. (2012)) (Figure 2.7, ‘dairy cow’).

The pH value of the warmly stored sow slurry is higher in week 12 than in week 2 (+0.24), although the total amount of acid required to obtain a pH of 3 is much lower in week 12 (−0.11 mol HCl kg^{-1} slurry) (Figure 2.7, ‘warm’, ‘sow’). This indicates a lower buffer capacity in week 12. The same tendency can be observed for all warmly stored slurries by comparing week 6 and 12. Consequently, high initial pH values do not necessarily require high amounts of acid to adjust a targeted low pH value, e.g., 5.5. It is the strength of the buffer capacity that mainly influences this amount.

2.4.2.3 Amount of Acid in the VFA Buffer Range

During the first two weeks of storage under warm conditions, the amount of acid of all types of slurry increased in pH range 5.5 to 3.0 and thus the buffer capacity of the VFA buffer (Figure 2.7, red columns). This can be explained by the degradation of cellulose, hemicellulose, and lipids by microorganisms that generate acetic, propionic and butyric acid as degradation products (Hobson et al., 1974). The higher the concentration of easily fermentable carbohydrates, cellulose, and hemicellulose and the lower the lignin concentration in the feed is, the higher the concentration of VFA in the slurry (Canh et al., 1998). The formation of organic acids under anaerobic conditions reduces the pH value (Christensen & Sommer, 2013). Most of the VFA are formed during anaerobic storage of fattening pig slurry in the first weeks after excretion (Miller & Varel, 2003). Therefore, the pH value is initially reduced in fresh slurry (Christensen & Sommer, 2013), similar to the results of our analysis (fattening pigs: +0.05 mol HCl kg^{-1} slurry and −0.26 pH units respectively dairy cow: +0.05 mol HCl kg^{-1} slurry and −0.67 pH units from week 0 to week 1). The amount of acid used in pH range 5.5 to 3.0 decreased with the length of the storage period in all warmly stored types of slurry, which indicates a reduction in the VFA buffer. The higher the storage temperature, the more the VFA content is reduced compared to coldly stored slurry (McGill & Jackson, 1977). Degradation processes take place even at a temperature of 0 °C (McGill & Jackson, 1977). Hence, the degradation of VFA in this study can also be assumed for the cold storage conditions (Figure 2.7, ‘cold’, ‘fattening pig’). Whereas other authors described a lower activity of the bacteria at cold conditions, so the VFA persists (Cooper & Cornforth, 1978). According to Sommer &

Sherlock (1996), the duration until the start of microbial degradation of VFA increases with decreasing temperature.

2.4.2.4 Dynamics of the Carbonate Buffer and its Impacts on the Alkaline Milieu

In the anaerobic microbial transformation of organic matter, parts of the carbon hydrates are converted into VFA or directly into CO_2 (aq) (Christensen & Sommer, 2013). In a second step, VFA consuming aerobic bacteria decompose the emerged VFA into methane and CO_2, which are then emitted during storage through naturally occurring volatilization processes (Christensen & Sommer, 2013; Dinuccio et al., 2008). In Section 2.4.2.3 we mentioned in more detail that the storage temperature has a considerable influence on the described carbon turnover, as the microbial conversion occurs more quickly at higher temperatures (McGill & Jackson, 1977). In this study, warm storage conditions caused stronger fluctuations and a faster decrease in the VFA and HCO_3^- buffer compared to coldly stored slurry (Figures 2.7 and 2.8), which emphasizes the mentioned temperature effects on slurry. In addition, the saturation volume of these gases in water decreases with increasing temperature, allowing less gas to be dissolved in the liquid phase of the slurry. Hence, faster and more intensive volatilization losses of CO_2 occur at higher temperatures (Dinuccio et al., 2008). These losses of CO_2 reduced the carbonate buffer capacity (Sommer & Sherlock, 1996), which could explain the decrease in carbonate buffer capacity from week 0 to 2 and 6 to 12 in warmly stored slurry (Figure 2.8b). However, in the coldly stored slurry, the carbonate buffer increased continuously until week 12, indicating that this loss can be neglected at cold storage conditions (Figure 2.8a). Furthermore, we were able to show that the CO_2 produced by the microbial decomposition of VFAs does not immediately emit, but rather functions as HCO_3^- and CO_3^{2-} buffer in the acidic or alkaline milieu. This was particularly shown by the fact that the rapid degradation of VFA in the warmly stored fattening pig slurry caused a rapid increase in both carbonate buffer concentrations (Figure 2.8b). Coldly stored slurry showed a similar pattern, in which continuous VFA degradation caused a continuous increase in the carbonate buffer (Figure 2.8a). Regardless of the storage temperature, both carbonate buffer curves showed minor differences, which confirms that the CO_2 produced by VFA degradation can act as HCO_3^- and CO_3^{2-} buffer.

This leads to two particular findings: Firstly, the degradation of the VFA buffer does not contribute to a general reduction in the total buffer capacity in the acidic milieu, since the HCO_3^- buffer itself is formed during the degradation of the VFA buffer and therefore increases in a similar proportion at which the VFA buffer degrades. Secondly, the degradation of the VFA buffer has a direct effect on the total buffer capacity in the alkaline milieu, due to the ability of the generated CO_2 to act as CO_3^{2-} buffer in the alkaline milieu.

This leads to the paradox that despite the decomposition of VFAs and the resulting increase in pH, the amount of base needed to alkalize the slurry increases.

2.4.3 Degradation of Urea in Slurry

High TAN contents were detected in the sow slurry, week 0, which, however, showed low maximum CBC values of the ammonium buffer (Figure 2.4b). This contradicts the significant positive correlation between the TAN contents and the maximum CBC values of the ammonium buffer obtained for the remaining slurry types. The deviation observed in the sow slurry could most likely be attributed to the high urine content (dry residue = 2.5%) and the short storage time of fewer than four hours between the collection and the first titration of the slurry. Therefore, a large proportion of the urea in the urine has probably not yet been degraded, as this may take up to 20 h (Dai & Karring, 2014). However, urea has no buffer capacity, but its N content is detectable as TAN by its reaction with hypochlorite in the Quantofix-N-Volumeter, leading to the observed erroneous values shown in Figure 2.4b. The fattening pig and dairy cow slurry had to undergo a longer storage time, which negated this effect due to over-advanced degradation processes of urea (Figure 2.4b). The sharp increase in the HCO_3^- buffer capacity of 49% in the first week in warmly stored sow slurry supports the mentioned hypothesis (Figure 2.7, ‘sow’) because urea decomposes into ammonia and carbonic acids and thus has a direct influence on the NH_3 and HCO_3^-/CO_3^{2-} buffer formation (Sigurdarson et al., 2018). In addition, the low CO_2 and low maximum CBC values in the sow slurry (week 0) indicated that the urea in the slurry has not yet been fully degraded (Figure 2.4a).

As already described in Section 2.3.4, these buffer systems and the VFA buffer have a decisive influence on the pH value in slurries. High concentrations of NH_3 in the slurry arising from the decomposition of urea may cause the pH value of slurry to rise. However, this effect only occurred at cold storage conditions, whereas the pH of warmly stored slurry remained unaffected (Figure 2.7, ‘sow’, week 1). This could be explained by the fact that warm storage caused a 12% increase in the VFA buffer compared to coldly stored slurry. The VFA buffer in the slurry stored under cold conditions was therefore not sufficient to compensate the increasing NH_3 buffer and thus to counteract a rise in the pH value.

As a result, slurry acidification treatments that have been carried out without long storage periods may require considerably less acid to adjust to a target pH value than slurry in which urea has already been completely degraded. However, this does not provide any information on the pH stability of a target pH value and thus on the amount of acid needed to maintain it. The alkalization technologies employ a different approach, as they are aimed at removing high quantities of nitrogen in the form of NH_3 from the slurry via stripping processes. That means, the storage period of the slurry must be based on the time it takes for the complete degradation of urea into CO_2 and NH_3. Furthermore, we found that buffers present in the acidic and alkaline milieu can compensate each other.

2.5 Conclusion

The new mathematical tool introduced in this study to determine the dynamics of buffer capacities in slurry has proven its effectiveness, as it is capable of calculating and simultaneously visualizing individual buffer capacity curves. Based on the long storage period of 12 weeks, the warm and cold storage conditions and the small step intervals in which the slurry was analyzed, we were able to determine and visualize the dynamics of the buffer capacities in great detail. The experimental results showed that greater changes in VFA and HCO_3^- buffer concentrations occurred over the whole storage period when the slurry was stored in warm conditions. On the other hand, less strong but constant increases were found in cold storage conditions. Furthermore, a strong dependency between the buffers present in the acidic and alkaline milieu was observed during storage, indicating that buffers cannot be considered individually, but must be interpreted as a complex and interacting system. Based on these results, we can provide a recommendation for acidification technologies, stating that immediate acidification of slurry has a positive effect on the amount of acid used to adjust a targeted pH, because microbial conversions of organic matter that increase the buffer capacity may not yet have taken place. However, alkalization technologies are based on different approaches. For phosphorus precipitation, alkalization at a very early stage is recommended to minimize the consumption of bases. To remove as much nitrogen as possible from the slurry in the form of NH_3 so that it can be recovered in an acid reserve, the alkalization should only be carried out after the urea has been completely degraded, even though this significantly increases the consumption of bases.

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2.6 Appendix

Table 2.2: Additional characteristics of fattening pig, dairy cow and sow slurry (fresh material) in week 0 and 8 depending on cold (4.7 ± 1.1 °C) and warm storage conditions (23.6 ± 2.1 °C).

		Fattening Pig			**Dairy Cow**			**Sow**		
Week and Storage Conditions **Ingredients** [1]		**0**	**8 Cold**	**8 Warm**	**0**	**8 Cold**	**8 Warm**	**0**	**8 Cold**	**8 Warm**
MgO	kg m^{-3}	2.01	2.22	2.51	0.81	1.03	1.02	0.62	0.61	0.64
CaO	kg m^{-3}	3.63	3.72	4.30	3.13	4.03	3.96	0.89	0.77	0.86
S	kg m^{-3}	0.55	0.65	0.69	0.53	0.67	0.60	0.35	0.36	0.35
Cu	g m^{-3}	15.00	16.10	18.47	3.50	4.48	4.47	2.87	2.75	2.93
Zn	g m^{-3}	80.40	90.87	102.40	17.40	22.10	21.97	16.20	15.47	16.47
Butyric acid	g kg^{-1}	0.82	0.77	< 0.05	0.51	0.50	0.37	0.09	0.09	0.11
Iso-butyric acid	g kg^{-1}	0.35	0.35	0.38	0.12	0.14	0.24	< 0.05	0.06	0.12
Valeric acid	g kg^{-1}	0.06	0.05	0.05	0.05	< 0.05	0.10	< 0.05	< 0.05	< 0.05
Iso-valeric acid	g kg^{-1}	0.38	0.38	0.37	0.08	0.09	0.16	0.05	0.06	0.10
n-caproic acid	g kg^{-1}	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

[1] macronutrients, micronutrients and volatile fatty acidy analyzed by external independent laboratory.

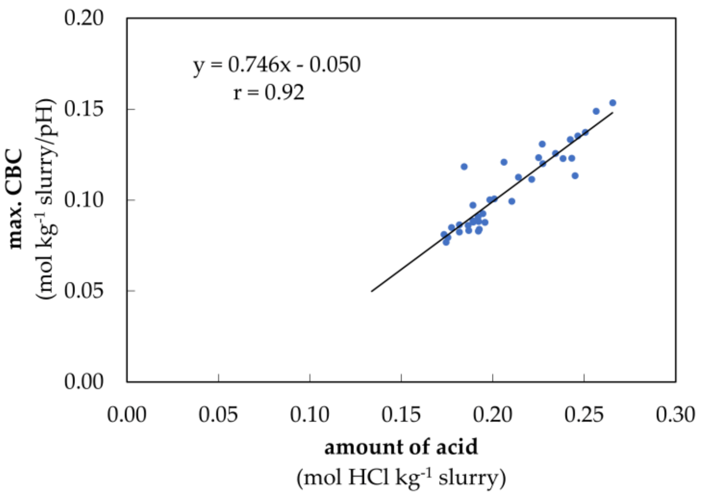

Figure 2.9: **Maximum CBC during acidification in the VFA buffer depending on the amount of acid from pH value 5.5 to 3.0 of the dairy cow slurry (*n* = 36).**

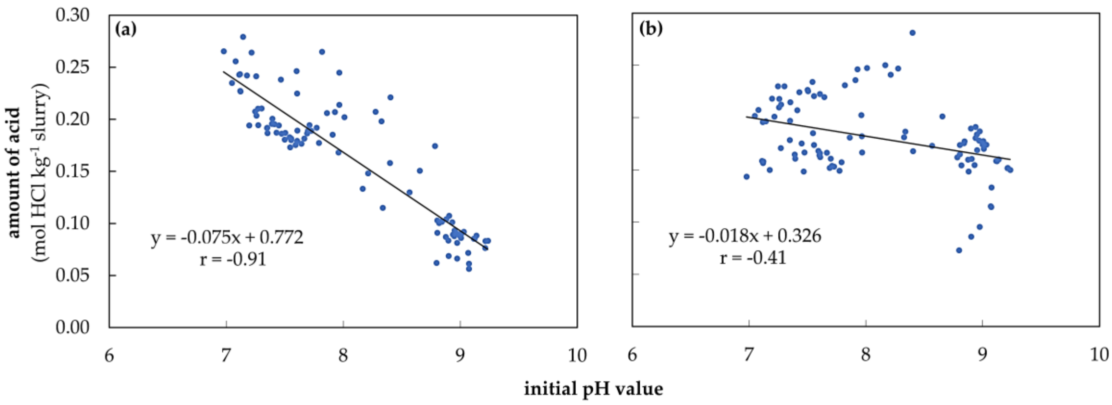

Figure 2.10: **Amount of acid in pH range 5.5 to 3.0 (VFA buffer) (a) and in pH range 7.0 to 5.5 (HCO_3^- buffer) (b) in dependency of initial pH value (*n* = 105).**

Table 2.3: **Initial pH value of fattening pig, dairy cow and sow slurry as well as amount of acid under cold (4.7 ± 1.1 °C) and warm (23.6 ± 2.1 °C) storage conditions in the pH range initial pH value to 7.0, 7.0 to 5.5 and 5.5 to 3.0 over storage period of 12 weeks (means (SEM)), same letters within rows and omitted letters indicate no significant differences among the weeks.**

Slurry Characteristics			**Week**					
Fattening Pig			**0**	**1**	**2**	**4**	**6**	**12**
initial pH value		cold	7.34 (0.05) ^{ab}	7.31 (0.02) ^{a}	7.29 (0.07) ^{a}	7.54 (0.03) ^{ab}	7.58 (0.03) ^{b}	8.09 (0.09) ^{c}
		warm	7.34 (0.05) ^{a}	7.08 (0.02) ^{a}	7.20 (0.03) ^{a}	7.92 (0.06) ^{b}	8.21 (0.13) ^{bc}	8.52 (0.09) ^{c}
initial to 7.0		cold	0.021 (0.004) ^{ab}	0.017 (0.001) ^{a}	0.018 (0.002) ^{a}	0.027 (0.001) ^{ab}	0.032 (0.001) ^{b}	0.051 (0.005) ^{c}
		warm	0.021 (0.004) ^{ab}	0.005 (0.001) ^{a}	0.009 (0.002) ^{a}	0.037 (0.003) ^{bc}	0.053 (0.007) ^{c}	0.038 (0.003) ^{bc}
7.0 to 5.5	mol HCl kg^{-1} slurry	cold	0.211 (0.010) ^{a}	0.219 (0.005) ^{ab}	0.220 (0.002) ^{ab}	0.225 (0.005) ^{ab}	0.225 (0.005) ^{ab}	0.243 (0.004) ^{b}
		warm	0.211 (0.010) ^{ab}	0.202 (0.003) ^{ab}	0.203 (0.004) ^{ab}	0.242 (0.005) ^{b}	0.244 (0.023) ^{b}	0.182 (0.008) ^{a}
5.5 to 3.0		*cold*	0.198 (0.005)	0.197 (0.007)	0.195 (0.005)	0.182 (0.002)	0.180 (0.002)	0.156 (0.015)
		warm	0.198 (0.005) ^{bc}	0.245 (0.006) ^{cd}	0.262 (0.011) ^{d}	0.225 (0.020) ^{bcd}	0.178 (0.015) ^{ab}	0.132 (0.010) ^{a}
Dairy cow								
initial pH value		cold	7.74 (0.03) ^{b}	7.74 (0.03) ^{b}	7.66 (0.03) ^{ab}	7.47 (0.07) ^{ab}	7.45 (0.05) ^{a}	7.42 (0.08) ^{a}
		warm	7.74 (0.03) ^{bc}	7.07 (0.05) ^{a}	7.14 (0.03) ^{a}	7.56 (0.05) ^{b}	7.93 (0.03) c	8.50 (0.14) ^{bc}
initial to 7.0		cold	0.029 (0.001)	0.031 (0.001)	0.030 (0.003)	0.020 (0.002)	0.021 (0.004)	0.021 (0.004)
		warm	0.029 (0.001) ^{c}	0.004 (0.002) ^{a}	0.007 (0.001) ^{ab}	0.022 (0.002) ^{bc}	0.032 (0.001) ^{bc}	0.052 (0.006) ^{bc}
7.0 to 5.5	mol HCl kg^{-1} slurry	cold	0.152 (0.002) ^{a}	0.154 (0.002) ^{a}	0.163 (0.002) ^{ab}	0.171 (0.004) ^{b}	0.167 (0.004) ^{ab}	0.177 (0.004) ^{b}
		warm	0.152 (0.002) ^{ab}	0.153 (0.005) ^{a}	0.154 (0.004) ^{ab}	0.160 (0.005) ^{ab}	0.177 (0.005) ^{b}	0.170 (0.006) ^{ab}
5.5 to 3.0		cold	0.193 (0.001)	0.184 (0.004)	0.187 (0.003)	0.185 (0.005)	0.193 (0.006)	0.193 (0.011)
		warm	0.193 (0.001)	0.240 (0.013)	0.243 (0.000)	0.237 (0.006)	0.222 (0.012)	0.198 (0.014)
Sow								
initial pH value		cold	8.89 (0.05) ^{a}	9.22 (0.01) ^{d}	9.12 (0.01) ^{cd}	9.01 (0.01) ^{bc}	8.97 (0.02) ^{ab}	8.94 (0.02) ^{ab}
		warm	8.89 (0.05) ^{ab}	8.97 (0.02) ^{bc}	8.83 (0.01) ^{a}	8.90 (0.01) ^{ab}	8.83 (0.02) ^{a}	9.07 (0.00) ^{c}
initial to 7.0		cold	0.064 (0.009) ^{a}	0.152 (0.002) ^{d}	0.143 (0.001) ^{cd}	0.128 (0.001) ^{bc}	0.125 (0.002) ^{bc}	0.118 (0.002) ^{b}
		warm	0.064 (0.009) ^{a}	0.144 (0.002) ^{c}	0.127 (0.005) ^{bc}	0.120 (0.006) ^{bc}	0.120 (0.006) ^{bc}	0.110 (0.006) ^{b}
7.0 to 5.5	mol HCl kg^{-1} slurry	cold	0.085 (0.007) ^{a}	0.151 (0.001) ^{b}	0.159 (0.001) ^{c}	0.174 (0.000) ^{d}	0.180 (0.002) ^{de}	0.189 (0.001) ^{e}
		warm	0.085 (0.007) ^{a}	0.173 (0.004) ^{c}	0.173 (0.004) ^{c}	0.155 (0.003) ^{c}	0.163 (0.006) ^{c}	0.121 (0.006) ^{b}
5.5 to 3.0		cold	0.066 (0.002) ^{a}	0.081 (0.002) ^{b}	0.086 (0.001) ^{bc}	0.090 (0.001) ^{c}	0.088 (0.001) ^{bc}	0.085 (0.002) ^{bc}
		warm	0.066 (0.002) ^{a}	0.091 (0.001) ^{b}	0.102 (0.000) ^{bc}	0.104 (0.002) ^{c}	0.093 (0.004) ^{bc}	0.063 (0.005) ^{a}

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3 Study 2

Alkalisierung von Flüssigmist, ein nachhaltiges und klimafreundliches Konzept

Alkalization of Slurry, a Sustainable and Climate Friendly Concept

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Felix Holtkamp contributed the most important intellectual input to this study.

Zusammenfassung

Flüssigmist ist zum Problemstoff geworden, da durch Überproduktion die Umwelt und das Klima geschädigt werden. Diese Studie untersucht deshalb Teilaspekte eines innovativen Konzepts, welches durch die Alkalisierung von Flüssigmist Emissionen mindert und Nährstoffe durch Fällungsprozesse trennt.

Dazu sollen Pufferkapazitäten in Flüssigmist charakterisiert werden, um den Einsatz von Laugen oder alkalischen wirkenden Additiven zu optimieren. Es konnte herausgefunden werden, dass Puffer, die im sauren Milieu agieren, einem mikrobiellen Zersetzungsprozess unterliegen, welcher paradoxerweise zu einer Zunahme des Laugenverbrauchs im alkalischen Milieu führt. Folglich müssen Puffer als komplexe und interagierende Systeme betrachtet werden. Ferner wurde der Einfluss einer pH-Werterhöhung auf die Emissionsrate von Gasen, die während der Lagerung von Flüssigmist entstehen, untersucht. Unsere Ergebnisse zeigten, dass sich eine Alkalisierung auf pH 10 positiv auf Klima und Umwelt auswirken könnte, da die Emissionen von Methan (CH_4) und Kohlenstoffdioxid (CO_2) um ca. 99 % und die von Lachgas (N_2O) um ca. 60 % reduziert wurden.

Summary

Slurry has become a problematic substance, as overproduction causes severe damage to the environment and climate. Therefore, this study investigates aspects of an innovative concept based on the alkalization of slurry which reduces emissions and separates nutrients via precipitation processes.

This involves the characterization of buffer capacities in slurry with the aim of optimizing the use of bases or alkaline-acting additives. It was found that buffers functioning in the acidic milieu are subject to a microbial decomposition process, which paradoxically can lead to an increase in the consumption of bases in the alkaline milieu. Consequently, buffers must be considered as complex and interacting systems. Furthermore, the influence of an increase in pH on the emission rate of gases produced during slurry storage was investigated. Our results showed that an alkalinization to pH 10 could have a positive effect on climate and environment, as the emissions of methane (CH_4) and carbon dioxide (CO_2) were reduced by about 99% and those of nitrous oxide (N_2O) by about 60%.

3.1 Einleitung und Zielsetzung

Im ASAP-Verbundprojekt befassen sich die Universität Bonn, die Hochschule Magdeburg und die Fa. Soepenberg mit Umweltproblemen des Wirtschaftsdünger-Managements. Ziel ist die Emissionsminderung und die Nährstoffabtrennung durch Fällungsprozesse nach einer Alkalisierung von Flüssigmist.

Unsere Aufgabe in dem Projekt ist es, die Pufferkapazitäten von Flüssigmist im alkalischen Milieu zu beschreiben, um den Einsatz von Laugen und Additiven zu optimieren. Die Zugabe von Calciumadditiven ermöglicht die Ausfällung und Abtrennung von Ca-Phosphaten, um sie als separate Dünger einsetzen zu können. Durch die pH-Wertanhebung kann Ammoniak-Stickstoff ausgetrieben und als schwefelsaure Düngerlösung genutzt werden. Mit der Alkalisierung geht auch eine Hygienisierung des Flüssigmists einher, wodurch mikrobielle Umsetzungsprozesse reduziert werden und ein überbetrieblicher Einsatz separierter Fraktionen erleichtert wird. Ferner untersuchten wir den Einfluss einer pH-Wertanhebung auf die Emissionen der klimarelevanten Gase CH_4, CO_2, N_2O während einer anschließenden Lagerung.

Die Ziele dieses Projektes sind, (I) den Alkalisierungsprozess in Flüssigmist zu charakterisieren, um diesen zu optimieren, (II) die Wirksamkeit von Calciumadditiven als Phosphatfällmittel zu überprüfen, (III) den Wirkungsgrad eines Ammoniakstrippungsprozesses zu evaluieren und (IV) eine potenzielle Emissionsreduktion von klimarelevanten Gasen durch Alkalisierung während einer Langzeitlagerung von Flüssigmist abzuschätzen.

3.2 Material und Methoden

Ziel (I): Es wurden Titrationsversuche mit frischem und separiertem Milchvieh-, Mastschweine- und Sauenflüssigmist unter kalter und warmer Lagerung über einem Zeitraum von 12 Wochen durchgeführt. Mit Hilfe eines Titrators wurden die Flüssigmistproben bis auf pH 2,5 angesäuert und bis auf pH 12 alkalisiert.

Die mathematischen Berechnungen zur Visualisierung der aus den Titrationen resultierenden Pufferkurven wurden durchgeführt mit einem selbst entwickelten mathematischen Werkzeug, welches mit der Datenverarbeitungssoftware R Studio oder Excel betrieben werden kann. Eine Beschreibung der Vorgehensweise ist in Overmeyer et al. (2020) zu finden.

Ziele (II–IV): Es wurden Strippungs- und Ausgasungsversuche mit separierten Flüssigmistproben durchgeführt, die zuvor mit verschiedenen Laugen und Additiven alkalisiert worden waren. Bevor die Proben mit Stickstoffgas gestrippt wurden, erfolgte eine mit Calciumhydroxid induzierte Phosphatfällung. Das anfallende Gas wurde mit Ammoniakteströhrchen analysiert. Die gestrippten Proben lagerten 8 Wochen lang in verdunkelten Glasflaschen. Das ausströmende Gas wurde aufgefangen und quantifiziert. Jede Woche wurden Gasproben entnommen und die CH_4, CO_2 und N_2O Konzentrationen mit Hilfe eines Gaschromatographen bestimmt.

3.3 Ergebnisse und Diskussion

Die Puffersysteme in Flüssigmist sind verantwortlich für die Menge an Säure oder Lauge, die zur Änderung des pH-Wertes notwendig sind. So wird beispielsweise zur Alkalisierung von Flüssigmist auf einen pH-Wert von 10 rund 1000-mal mehr $Ca(OH)_2$ benötigt als bei reinem Wasser. Die wesentlichen Puffersysteme sind im sauren Bereich der Fettsäure- (VFA) und der Kohlensäurepuffer (HCO_3^-) und im alkalischen Bereich der Karbonat- (CO_3^{2-}) und der Ammoniakpuffer. Die Puffersysteme unterliegen während der Lagerung eigenen Dynamiken und Wechselbeziehungen, die im Wesentlichen von mikrobiellen Umsetzungsprozessen verursacht werden. So kann ein Abbau von Fettsäuren paradoxerweise über die Bildung von CO_2 und die Erhöhung des Karbonatpuffers zu einem erhöhten Laugenbedarf bei der Alkalisierung führen (Figure 3.1). Diese mikrobiellen Prozesse sind jedoch stark temperaturabhängig. So finden bei warmer Lagerung verstärkt Umbauprozesse statt, die sich im Vergleich zur Kühllagerung als Zu- und Abnahmen der Fettsäure-, Kohlensäure- und Karbonatpuffer bemerkbar machen (Overmeyer et al. 2020). In Lagerungsversuchen konnten wir zeigen, dass nach der Zugabe von $Ca(OH)_2$ die Neubildung von Methan in dem Flüssigmist nahezu vollständig unterbunden wurde (Figure 3.2). Bereits eine Alkalisierung auf pH 10 reichte aus, um die CH_4 und CO_2 Emissionen um ca. 99 % und die N_2O Emissionen um ca. 60 % zu reduzieren. Eine weitere pH-Wertanhebung brachte diesbezüglich kaum noch Vorteile.

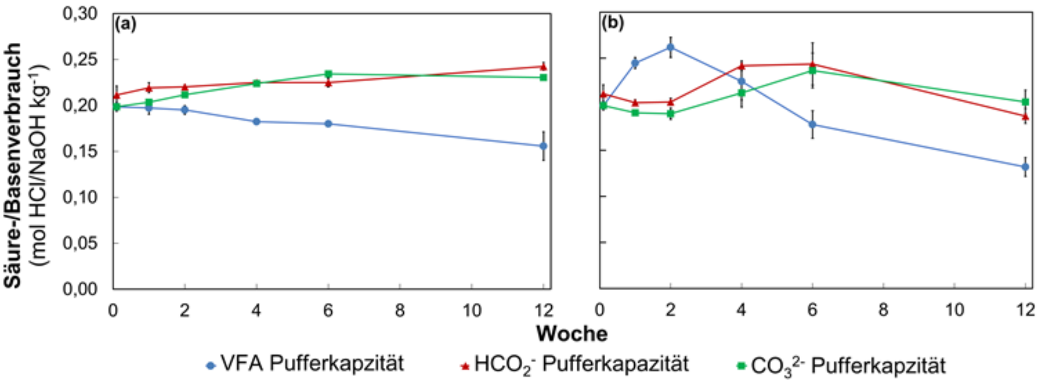

Figure 3.1: Development of the buffer systems VFA (pH range 5.5 to 3.0), HCO_3^- (pH range 7.0 to 5.5) and CO_3^{2-} (pH range 9.5 to 11.5) in (a) cold and (b) warm stored fattening pig slurry over 12 weeks; vertical bars represent standard errors (n = 3) (Overmeyer et al., 2020).

Abbildung 3.1: Entwicklung der Puffersysteme VFA (pH-Bereich 5,5 bis 3,0), HCO_3^- (pH-Bereich 7,0 bis 5,5) und CO_3^{2-} (pH-Bereich 9,5 bis 11,5) in (a) kalt und (b) warm gelagertem Mastschweineflüssigmist über 12 Wochen; vertikale Balken stellen den Standardfehler dar (n = 3) (Overmeyer et al., 2020).

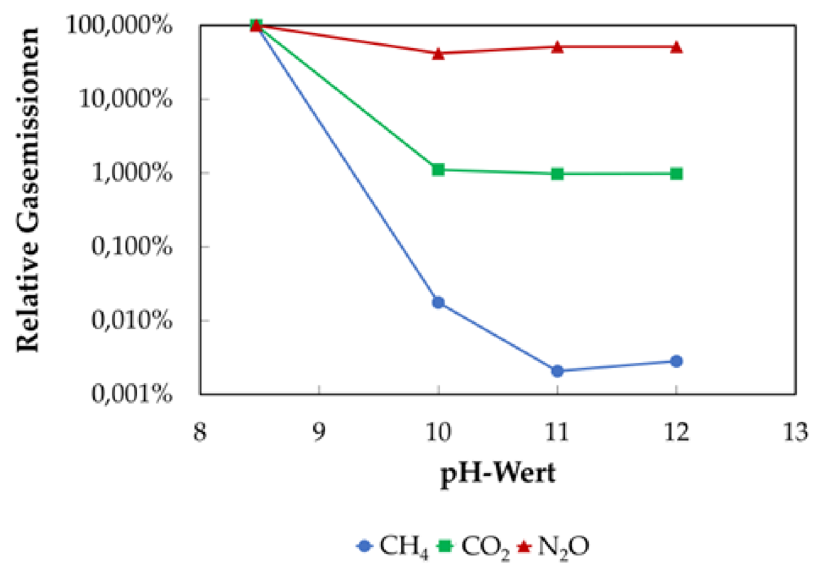

Figure 3.2: Relative gas emissions from fattening pig slurry samples stored for 8 weeks, which were previously untreated or alkalized with $Ca(OH)_2$ to pH 10, 11, and 12.

Abbildung 3.2: Relative Gasemissionen aus 8 Wochen lang gelagerten Mastschweineflüssigmistproben, welche zuvor unbehandelt oder mit $Ca(OH)_2$ auf pH 10, 11 und 12 alkalisiert wurden.

3.4 Schlussfolgerung

Die Alkalisierung von Flüssigmist zeigt, dass Puffersysteme eigenen Dynamiken und Wechselbeziehungen unterliegen und sich gegenseitig beeinflussen, was den Laugenbedarf während des Alkalisierungsprozesses erhöht. Aufgrund dieser Erkenntnisse kann dieser Prozess optimiert und damit effizienter und kostengünstiger gestaltet werden. Ferner erweist sich die Flüssigmistbehandlung mit Calciumadditiven als effektive Strategie zur Reduzierung von Emissionen, die während der Lagerung entstehen. Hierbei stellte sich schon ein alkalischer pH-Wert von 10 als höchst effektiv heraus.

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4 Study 3

Calcium cyanamide reduces methane and other trace gases during long-term storage of dairy cattle and fattening pig slurry

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Abstract

Calcium cyanamide ($CaCN_2$) has been used in agriculture for more than a century as a nitrogen fertilizer with nitrification inhibiting and pest-controlling characteristics. However, in this study, a completely new application area was investigated, as $CaCN_2$ was used as a slurry additive to evaluate its effect on the emission of ammonia and greenhouse gases (GHG) consisting of methane, carbon dioxide, and nitrous oxide. Efficiently reducing these emissions is a key challenge facing the agriculture sector, as stored slurry is a major contributor to global GHG and ammonia emissions. Therefore, dairy cattle and fattening pig slurry was treated with either 300 mg kg^{-1} or 500 mg kg^{-1} cyanamide formulated in a low-nitrate $CaCN_2$ product (Eminex®). The slurry was stripped with nitrogen gas to remove dissolved gases and then stored for 26 weeks, during which gas volume and concentration were measured. Suppression of methane production by $CaCN_2$ began within 45 min after application and persisted until the storage end in all variants, except in the fattening pig slurry treated with 300 mg kg^{-1}, in which the effect faded after 12 weeks, indicating that the effect is reversible. Furthermore, total GHG emissions decreased by 99% for dairy cattle treated with 300 and 500 mg kg^{-1} and by 81% and 99% for fattening pig, respectively. The underlying mechanism is related to $CaCN_2$-induced inhibition of microbial degradation of volatile fatty acids (VFA) and its conversion to methane during methanogenesis. This increases the VFA concentration in the slurry, lowering its pH and thereby reducing ammonia emissions.

4.1 Introduction

Since the pre-industrial era, the average methane (CH_4) concentration has increased by 162% from ~ 722 ppb to a level of ~ 1,895 ppb in 2021, making CH_4 after carbon dioxide (CO_2) the second largest contributor to anthropogenic global warming (Lan et al., 2022; United Nations, 2021) However, CH_4 is a far more aggressive greenhouse gas (GHG) than CO_2, as it contributes to the greenhouse effect about 28 times more (IPCC, 2014). Agriculture plays a crucial role in increasing atmospheric CH_4 levels, as 32% and therefore the largest share of global anthropogenic CH_4 emissions is attributable to ruminant fermentation and the storage of organic fertilizer such as slurry (United Nations, 2021). The potential for CH_4 formation to occur is high wherever organic material is stored under anaerobic conditions, such as in landfills, digestate, and slurry storage facilities (Amon et al., 2006; Czepiel et al., 1996; le Mer & Roger, 2001). During this formation, organic material is degraded by microorganisms to VFA such as acetic and propionic acids, which are metabolized by methanogenic bacteria (*archaea*) resulting in the formation of CH_4 and CO_2 (Dalby et al., 2021).

Besides CH_4, agriculture releases other harmful gaseous emissions, of which the environmentally relevant ammonia (NH_3) is particularly prominent, as agriculture alone accounts for 94% of total NH_3 emissions in Europe with slurry storage and spreading being the main sources (European Environment Agency et al., 2021). NH_3 is formed mainly via the urease-catalyzed hydrolysis of urea, but can also be formed by the decomposition of other organic compounds (Sigurdarson et al., 2018). These emissions have a wide range of negative characteristics, as they can harm the health of humans and animals, causes acidification of soils and eutrophication of aquatic ecosystems and can form particulate matter ($PM_{2.5}$) in the atmosphere (Drummond et al., 1980; Krupa, 2003; Xu et al., 2018).

Furthermore, during microbial degradation of NH_3, nitrous oxide (N_2O), a greenhouse gas, 265 times more potent than CO_2 can be produced, making NH_3 indirectly harmful to the climate (IPCC, 2014). Under aerobic conditions, ammonium (NH_4^+) is oxidized via nitrification to nitrate (NO_3^-) and nitrite (NO_2^-), with N_2O being formed in an intermediate step. Once oxygen is depleted and anaerobic conditions prevail, microorganisms begin to metabolize oxygen from oxygen-rich compounds such as NO_3^- and NO_2^- and reduce them to elemental nitrogen (N_2), producing N_2O and nitric oxide (NO) as intermediates in a process known as denitrification (Petersen et al., 2013; Philippe & Nicks, 2015). In agriculture, N_2O emissions mainly occur during the application of slurry, but emissions also arise in slurry storage facilities or in the barn (Clemens et al., 2006; Schmithausen et al., 2018). According to the study by Kupper et al. (2020) pig slurry stored in tanks releases on average, about 15% of total ammoniacal nitrogen as NH_3 emissions, 0.1% of nitrogen as N_2O, and 4.7% and 9.2% of volatile solids (VS) as CH_4 and CO_2 emissions, respectively.

The ammonia and greenhouse gas emissions released during the storage and application of slurry must be reduced to protect the environment and the climate. The new NEC Directive (EU) 2016/2284 obliges EU member states to reduce the overall EU emissions of NH_3 by 19% compared to 2005 levels by 2030. Furthermore, at the 2021 UN Climate Change Conference (COP 26) over 100 countries, including the USA and the EU, signed the Global Methane Pledge, committing to reduce global CH_4 emissions by at least 30 percent from 2020 levels by 2030 (European-Commission, 2021).

To meet these objectives, various techniques are being investigated and have already been implemented to reduce NH_3 and GHG emissions released during the storage and application of slurry. These include covering slurry storage facilities and low-loss near-ground slurry application and incorporation. However, these mitigation strategies primarily reduce NH_3 emissions, have little impact on CH_4 emissions and may even increase N_2O

emissions (Amon et al., 2006; Kupper et al., 2020; Wulf et al., 2002a, 2002b). Therefore, technologies such as cooling, acidification, and aeration of slurry have been developed and tested showing high potential to reduce NH_3 and CH_4 emissions (Kupper et al., 2020; Mostafa et al., 2019; Myczko et al., 2007; Petersen et al., 2012). However, a major drawback of the above-mentioned techniques is the requirement of a fundamental technical modification of the storage facilities, which can lead to substantial financial costs during implementation and operation. Therefore, the treatment of slurry with calcium cyanamide ($CaCN_2$) could be a promising approach to avoid emission-related on the one hand and technical-financial problems on the other, as the additive can be easily applied by mixing it into the slurry and thereby reducing treatment costs keeping them in the low single-digit Euro range per cubic meter of slurry.

In agriculture, $CaCN_2$ has been widely used for more than 100 years as a nitrogen fertilizer with soil liming, nitrification inhibiting, and pest-controlling characteristics (Güthner & Mertschenk, 2006; Klasse, 1996; Shi et al., 2009). In addition, the application of $CaCN_2$ as a fertilizer has an emission-reducing effect on N_2O due to its nitrification-inhibiting ability in the soil and was found to reduce CH_4 emissions right after its application in paddy rice fields, but it was not further described (Schütz et al., 1989; Suzuki et al., 2021). However, very little is known about this effect, as it is rarely described in scientific literature.

In order to assess the impact of $CaCN_2$ in slurry and to gain deeper insights into the emission-reducing effect, we stored glass bottles filled with either fattening pig or dairy cattle slurry for 26 weeks and treated them with two different concentrations of $CaCN_2$. Throughout this storage period, gas measurements were performed to gain a complete and accurate record of the concentration changes of CH_4, CO_2, N_2O, and NH_3. Therefore, the main objectives of this study were (I) to assess the emission-reducing effect of $CaCN_2$ on CH_4, CO_2, N_2O, and NH_3 emissions during long-term storage of slurry; (II) to evaluate the rapidity and permanence of the effect of $CaCN_2$ at different concentrations; (III) to determine if $CaCN_2$ causes changes in physico-chemical parameters and thus affects the slurry characteristics.

4.2 Material and methods

4.2.1 Slurry sampling and treatment

Experiments were carried out by using slurry from fattening pigs (FP) and dairy cattle (DC) obtained from the campus Frankenforst, 53639 Königswinter, Germany owned by the University of Bonn. Sampling of both slurries was done 5 d before the start of the experiments and was taken directly from either the slurry channel of the pig barn or from a slurry pre-pit into which flap scrapers transported the fresh slurry from the walkway of the cattle barn. Both slurries were homogenized before removal and stored at 4.5 °C for 5 d for the FP and 6 d for the DC until the start of the experiments.

The emission-reducing effect of $CaCN_2$ was investigated by treating the slurry with 2 different concentrations of Eminex® (Alzchem Group AG, 83308 Trostberg, Germany), a low-nitrate formulated calcium cyanamide (CC) product. An untreated raw slurry served as a control variant (Co). The calcium cyanamide variants were treated with 1.24 g (CC300) or with 2.07 g (CC500) of Eminex® per kg of slurry to obtain a concentration of approximately 300 and 500 mg kg^{-1} pure cyanamide for the CC300 and CC500 treatments, respectively. In addition, Eminex® has the following main ingredients: calcium cyanamide 43%, calcium hydroxide 16.5%, graphite 10%, calcium carbonate 10%, magnesium carbonate 8%, total N 18%, cyanamide-N 15%, and nitrate-N 0.1%.

4.2.2 Experimental set-up and procedure

The experimental set-up we used to answer the research questions posed in the introduction included the following basic steps and is illustrated in Figure 4.1:

(I) Pretreatment: 1 l laboratory glass bottles were filled with 500 g of untreated FP or DC slurry to prepare the control variant. The $CaCN_2$ variants were treated according to the indicated quantities of Eminex® and were then homogenized for 15 min using a magnetic stirrer. Four analytical replicates were made for each variant.

(II) Stripping: After pretreatment, the filled glass bottles were immediately hermetically sealed with a lid. Two polytetrafluoroethylene (PTFE) tubes per lid were used for either the inlet of pure N_2 gas or the outgoing resulting gas mixture. The length of the inlet tubes in the bottle was adjusted so that they were completely covered with slurry but leaving enough space at the end for an air stone to be attached. The air stone formed fine pore bubbles enhancing the stripping of dissolved gases. The actual stripping process, i.e. the gasification of the slurry samples to flush out dissolved gases, was carried out using a volumetric pump (Gas Sampler GS212, SARSTEDT AG & Co.KG, 51588 Nürmbrecht, Germany). This pump simultaneously delivered 0.25 L N_2 min^{-1} to each of the 4 glass bottles of the same treatment variant via the inlet tube, which was also connected to a flow meter that allowed the gas flow to be adjusted. The stripping process lasted 30 minutes and was done on a horizontal shaker at 100 rpm to assist the process. The outflowing gas mixture was collected in gas bags and its volume was measured. For concentration determination, gas samples were collected at 2-minute intervals using evacuated 20-mL headspace vials and sampling cannulas. For this purpose, a sampling port was attached to the outlet tube, which was sealed with a membrane. Leakage detection tests were carried out to ensure complete hermetic sealing of the experimental setup.

(III) Storage: After the stripping process was completed, the bottles were connected to new gas bags and separated from the rest of the experimental setup. The bottles were covered with aluminum foil and stored in the dark for 26 weeks at 20.2 ± 1.1 °C. This period was chosen in line with the statutory minimum storage volume of slurry storage facilities specified in the German Fertilizer Ordinance, which must be designed for a period of 26 weeks of slurry production. Furthermore, gas samples were taken from all bottles at 7-day intervals via headspace vials to monitor changes in CH_4, CO_2, and N_2O gas concentrations.

(IV) End: At the end of the 26-weeks storage period, the gas bags were removed from the system to take a final vial sample and to measure the NH_3 concentration together with the total volume.

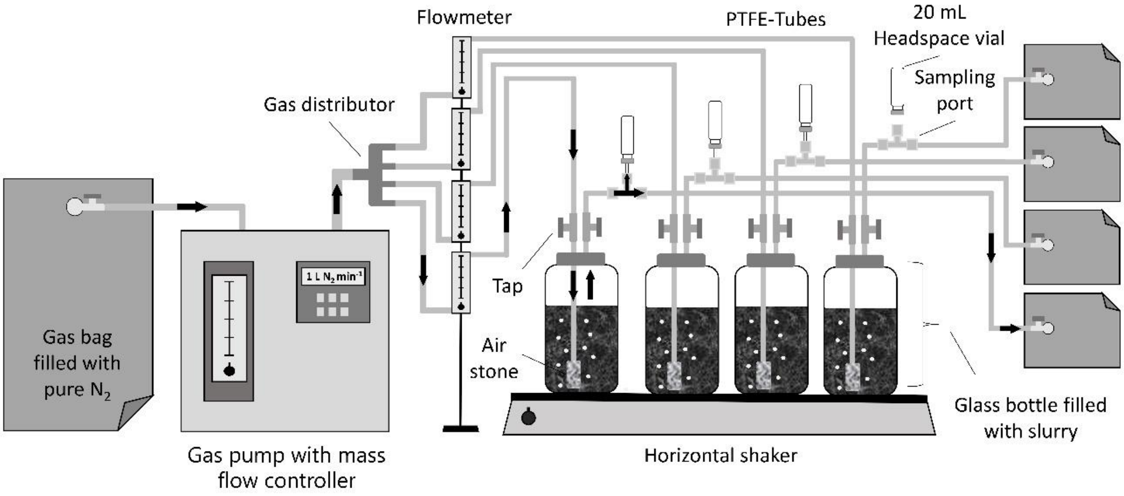

Figure 4.1: **Experimental set-up used for a stripping process to remove dissolved gases from slurry, which was either untreated or previously treated with two different concentrations of calcium cyanamide. The flow direction of the nitrogen gas used for stripping is indicated by black arrows on one sample as an example. After stripping, the glass bottles together with the outlet tubes and gas bags were stored for 26 weeks.**

4.2.3 Slurry characterization

Slurry samples were characterized before and after storage by an external laboratory (LUFA-NRW, 48147 Münster, Germany) according to the specification of the VDLUFA method book (VDLUFA, 2000). Before storage, samples for each slurry type and after storage a pooled sample consisting of all replicates of one variant were analyzed. The analyses included the determination of dry residue, volatile solids (VS), the macronutrients such as total N, ammonium nitrogen (NH_4-N), phosphate (as P_2O_5), potassium (as K_2O), magnesium (as MgO), calcium (as CaO), and sulfur (S), the micronutrients such as copper

(Cu) and zinc (Zn), and the major volatile fatty acids such as acetic acid, propionic acid, butyric acid, iso-butyric acid, valeric acid, iso-valeric acid, caproic acid, and iso-caproic acid. Slurry pH was measured before and after adding and stirring the additive into the slurry and at the end of storage.

Table 4.1: Characteristics of dairy cattle and fattening pig slurry (fresh material) at week 0 and 26 that were untreated (Co) or treated with 300 mg kg^{-1} (CC300) or 500 mg kg^{-1} (CC500) of calcium cyanamide.

		Dairy Cattle				**Fattening Pig**			
Treatment and Weeks of Storage		**Co**		**CC300**	**CC500**	**Co**		**CC300**	**CC500**
		0	**26**	**26**	**26**	**0**	**26**	**26**	**26**
Ingredients									
Dry residue	%	7.20	5.00	6.90	6.70	6.60	4.90	5.90	6.30
VS	%	5.86	3.80	5.47	5.24	5.36	3.56	4.44	4.88
C/N ratio	-	17.00	10.00	14.00	14.00	8.00	5.00	6.00	7.00
N	kg m^{-3}	1.96	2.06	2.24	2.23	3.80	3.96	4.18	4.18
NH_4-N	kg m^{-3}	0.76	0.84	1.05	1.11	2.42	2.97	2.88	3.02
P_2O_5	kg m^{-3}	1.12	1.06	0.93	1.50	2.55	2.64	2.52	2.49
CaO	kg m^{-3}	1.33	1.26	1.71	2.93	1.78	1.69	2.08	2.53
Propionic acid	g kg^{-1}	1.10	<0.05	1.90	1.70	1.70	<0.05	<0.05	3.00
Acetic acid	g kg^{-1}	3.70	0.08	5.00	6.10	5.30	<0.05	0.30	6.80
Acetic acid equiv[1]	g kg^{-1}	5.18	0.08	7.46	8.21	8.06	-	0.30	11.09
pH	-	6.84	7.26	6,98[2]/6.57	7,11[2]/6.61	6.75	7.50	7,31[2]/7.51	7,55[2]/6.95

[1] Acetic acid equivalents were calculated from the acetic acid, propionic acid, butyric acid, iso-butyric acid, valeric acid, iso-valeric acid, caproic acid and iso-caproic acid.

[2] The pH shows the value measured in week 0 after stirring in calcium cyanamide.

4.2.4 Analysis of gas concentrations

(I) Chromatographic analysis: Gas samples were collected using 20-mL headspace vials that had been previously evacuated to a pressure of < 5 mbar. These were analyzed to determine the concentrations of CH_4, CO_2, and N_2O using a gas chromatograph (GC) (8610 C, SRI Instruments, Torrance, CA, USA) equipped with a flame ionization detector (FID) for CH_4 and an electron capture detector (ECD) for CO_2 and N_2O. The column oven was heated at 40 °C and the ECD operated at 320 °C. In addition, pure nitrogen was used as a carrier gas at 35 mL min^{-1} and as a make-up gas at 6 mL min^{-1} for the ECD. The exact methodology and the GC settings are described in Wulf et al. (2002b). Furthermore, several samples had to be diluted to different degrees allowing accurate measurements to be made. If appropriate, the mean value of the individual measurements was taken when the same sample was analyzed multiple times.

(II) Analysis of ammonia concentrations: Concentrations were measured directly from the gas bags after stripping and after storage using Dräger short-term tubes (Drägerwerk AG & Co. KGaA, 23558 Lübeck, Germany) of varying sensitivity. Before each measurement, the gas bags were preheated to 30 °C to avoid condensation of water in the gas bag and thus prevent the gaseous ammonia from dissolving in the condensation water.

4.2.5 Calculation of total gas emission

The total gas emissions were calculated using the ideal gas law, allowing the calculation of the amount of substance (n), which combined with the molar mass of the gas (M_{gas}) that is being calculated, gives the total mass of the gas (m) emitted by the slurry and thus represents the total gas emissions. The volume (V_{total}) equals the total gas volume measured after the end of storage and the concentration (c_{gas}) equals the gas concentration of the respective gas. The multiplication of both corresponds to the amount of substance. Furthermore, the following parameters were used to calculate the total gas emissions: The absolute pressure (p), the ideal gas constant (R) and the absolute temperature of the gas (T).

$$m = M_{gas} * \frac{p * \left(c_{gas} * V_{total}\right)}{R * T}$$

Emissions of each CH_4 and N_2O were converted to CO_2 equivalents (eq) using gas conversion factors defined by the Intergovernmental Panel on Climate Change (IPCC), called global warming potential (GWP) and then summed with CO_2 emissions to obtain total GHG emissions using a factor of 28 for CH_4 and 265 for N_2O (IPCC, 2014).

4.2.6 Statistical analysis

Statistical analysis was performed using IBM®SPSS®Statistics, version 27. In the figures, the mean values with the associated standard errors of the mean (SEM) are shown as vertical bars. One-way analysis of variance (ANOVA) was performed to determine differences in gaseous emissions. Values within individual groups were tested for normal distribution using the Shapiro-Wilk test. Total CH_4, CO_2, N_2O, and GHG emissions were subjected to a Box-Cox power transformation, as normal distribution was not given. The ANOVA was continued with the Tukey's Honestly Significance Difference (HSD). In the absence of variance homogeneity, the Games-Howell test was chosen instead with a significance level of both tests of 0.05 to indicate statistical significance. The statistical results of the ANOVA performed on the Box-Cox transformed data were plotted on the original data set. Furthermore, one replicate from each of the following treatments: DC/Co, DC/CC500, FP/Co, and FP/CC300 was excluded from all gas analyses after stripping, as anaerobic conditions could not be completely guaranteed.

4.3 Results

4.3.1 Slurry characterization

During the 26-weeks storage period, dry residue, VS, and C/N ratio decreased in the control variants of both slurry types (Table 4.1). The application of $CaCN_2$ alleviated this decrease. Total nitrogen and NH_4-N concentrations were higher in the FP slurry compared to the DC and increased with storage and with the application of $CaCN_2$. In addition, storage was observed to reduce all measured VFA concentrations, with total VFA concentrations expressed in acetic acid equivalents decreasing by more than 98% for DC/Co slurry and below the detection limit for FP/Co slurry. This decline was also observed in the FP/CC300 variant, but not for the DC/CC300 and for both CC500 variants showing increased concentration values and exceeded the initial value measured in week 0. The pH responded inversely to this development as it increased at the end of storage and decreased as a result of the treatment with $CaCN_2$, except in the FP/CC300 variant. In addition, the full analytical profile is included in Table 4.2.

4.3.2 Greenhouse gas emissions during stripping

The outgassing curves generated by the stripping process followed the same general pattern for all variants regardless of the gases measured and the type of slurry used: they increased rapidly, peaked at about 2 minutes and then decreased (Figure 4.2). The peak value in the control variants of both slurry types exceeded 45,000 ppm for CO_2 (Figure 4.2c,d), while the value for CH_4 peaked slightly above 2,000 ppm (Figure 4.2a,b). The decrease in CH_4 concentration was much more pronounced in the FP compared to the DC slurry. However, the $CaCN_2$-treated samples showed a much flatter curve and a far less pronounced peak, regardless of the gas measured and type of slurry. In addition, differences between CC300 and CC500 treatments were only found in FP slurry (Figure 4.2c,d), but not in the DC slurry (Figure 4.2a,c). After 26 min of stripping, CH_4 concentrations in the control variants maintained stable levels of about 270 ppm and 170 ppm for DC and FP, respectively (Figure 4.2a,b). In contrast, $CaCN_2$ treatment reduced CH_4 concentrations to near zero as they decreased on average to < 52 ppm ($p < 0.001$) and 31 ppm ($p < 0.01$) with no significant differences between the treated variants. CO_2 concentrations leveled off at about 15,000 ppm and 13,000 ppm for DC and FP, respectively and were further reduced to below 7,500 and 7,400 by the $CaCN_2$ treatment, resulting in relatively lower reduction rates compared to CH_4 (Figure 4.2c,d). Throughout the stripping process N_2O concentrations in the DC slurry were only slightly above the GC detection limit and did not exceed a concentration of 4 ppm (Figure 4.7a). Whereas the FP slurry treated with CC500 and CC300 showed peak values of 33 ppm and 13 ppm, respectively, but decreased sharply as stripping proceed showing values below 4 ppm at minute 30 and thus were no longer significantly different ($p > 0.05$) from the concentrations found in the DC slurry (Figure 4.7b).

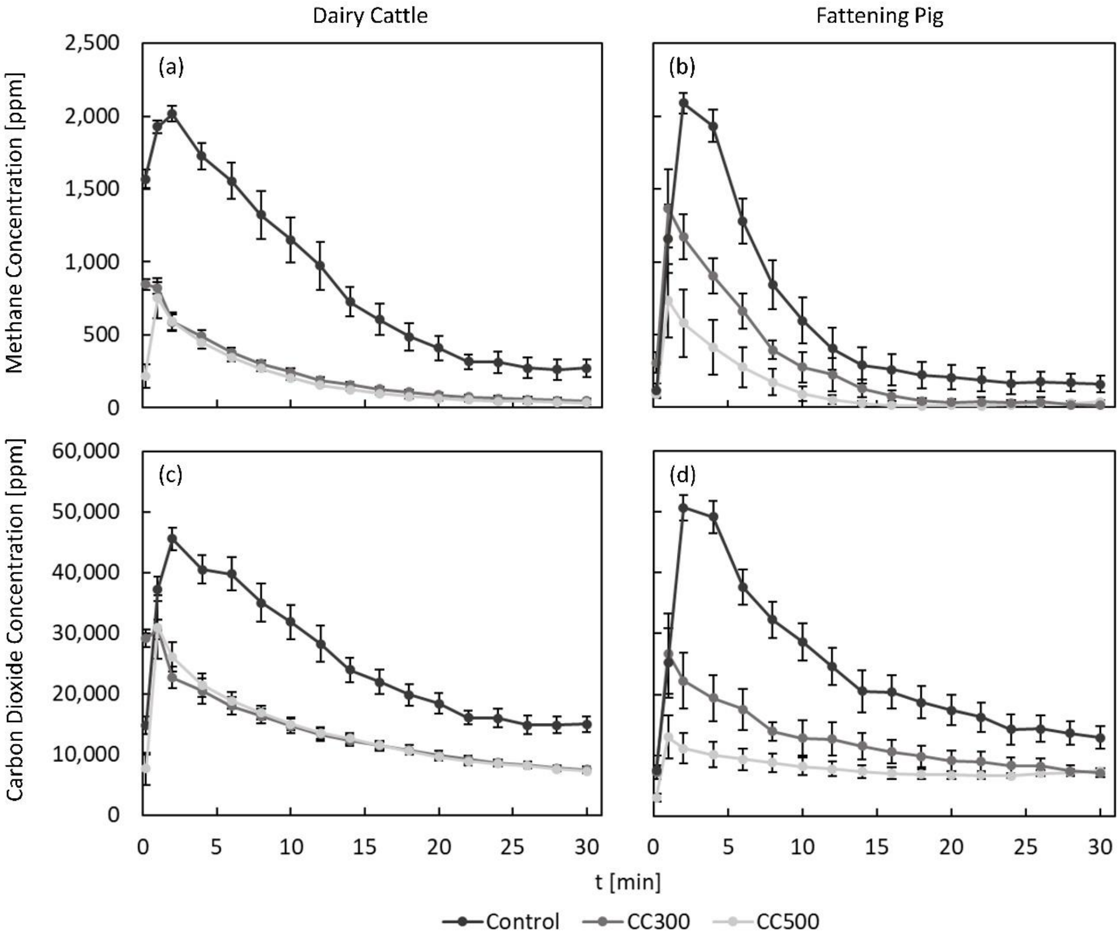

Figure 4.2: **Outgassing curves representing the mean methane and carbon dioxide concentrations [ppm] generated by a stripping process using 0.25 L N_2 min^{-1} for 30 min per sample to eliminate dissolved gases from dairy cattle and fattening pig slurry. Slurry was untreated (Control) or treated with either 300 (CC300) or 500 (CC500) mg kg^{-1} cyanamide equivalents. Vertical bars represent the mean values ± standard errors (n = 4).**

4.3.3 Ammonia emission during stripping and storage

The amount of NH_3 removed from the slurry during the stripping process was overall much lower in the DC slurry compared to the FP slurry (Figure 4.3, Week 0). Moreover, the variants of the DC slurry neither showed significant differences nor followed a trend. However, in the FP slurry the CC300 and CC500 treatments induced an enhancement of more than 50% and 200%, respectively, compared to the control variants. Statistical analysis showed that the only significant difference was found between the CC500 variant and the other two. During storage, the treatment of $CaCN_2$ in DC and FP slurry caused a reduction of approximately 79% and 69%, respectively. However, this reduction could not be verified by the statistical analysis conducted (Figure 4.3, Week 26).

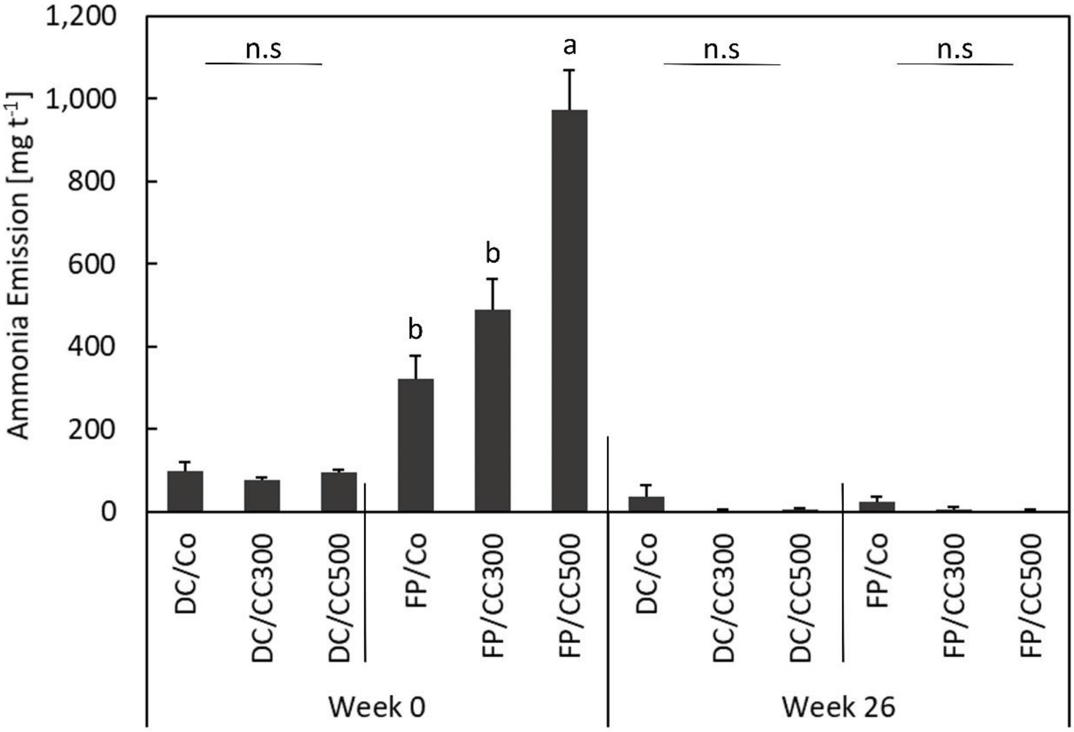

Figure 4.3: **Ammonia emissions [mg t^{-1}] emitted at the beginning of the storage during a stripping process using 0.25 L N_2 min^{-1} for 30 min per sample (Week 0), and ammonia emission emitted during a 26-week storage period (Week 26) of dairy cattle (DC) and fattening pig (FP) slurry. Slurry was untreated (Co) or treated with either 300 (CC300) or 500 (CC500) mg kg^{-1} cyanamide equivalents. Vertical bars represent the mean values ± standard errors (n = 4 in Week 0, n ≥ 3 in Week 26). Different letters above bars indicate differences in significance ($p < 0.05$, n.s = not significant).**

4.3.4 Changes in concentration of greenhouse gases during storage

Over a period of 26 weeks, CH_4, CO_2 and N_2O concentrations were measured weekly from a hermetically sealed storage system consisting of a gas bag connected to a glass bottle. The DC control variants showed a lagged increase in the mean CH_4 concentration of about 5 weeks, starting from the first week of storage (Figure 4.4a). In contrast, the mean concentration of the FP control variants increased rapidly by around 471,000 ppm over the same period peaking at a value of 496,000 ppm at week 7 (Figure 4.4d). That was 6 weeks earlier compared to the DC slurry, which peaked at 297,000 ppm in week 13. Thereafter, the concentration steadily decreased, reaching 100,000 ppm at week 26. In the FP slurry, the concentration decreased much more rapidly, by an average of 193,000 ppm in 2 weeks, but remained stable thereafter, averaging 225,000 ppm in the last week.

The $CaCN_2$ treatment significantly ($p < 0.001$) suppressed CH_4 production up to week 12 regardless of the treatment concentration and slurry type (Figure 4.4b,c,e,f, and Figure 4.9). The emission-reducing effect persisted throughout the entire storage period for the DC/CC300, CC500, and FP/CC500 variants because no high CH_4 releases were detected. However, by the end of storage (week 26), significant differences between treated and untreated samples could no longer be detected (Figure 4.9). This long-lasting suppressive effect of $CaCN_2$ proved to be limited in the FP/CC300 variant because a comparable increase in CH_4 concentration was observed from week 12 onwards as it has been observed in the control variant from week 0 (Figure 4.4e).

In addition, neither the delayed CH_4 increase found in the DC/Co variant nor a pronounced peak was observed for the CO_2 concentration regardless of the slurry type and treatment (Figure 4.5). Instead, the CO_2 concentration remained at a relatively constant level and did not differ greatly during storage for all treatments and slurry types. Considering the development of the CO_2 concentration, it is evident that the emission-reducing effect of $CaCN_2$ was weaker compared to CH_4. Slight reductions were achieved only in DC/CC300 and DC/CC500, with the latter being more pronounced (Figure 4.5b,c). However, the FP slurry appeared to be almost unaffected by the treatment, although a small increase in CO_2 concentration was found at the storage end in the FP/CC300 variant (Figure 4.5e). In addition, the formation of N_2O did not occur during storage (Figure 4.8).

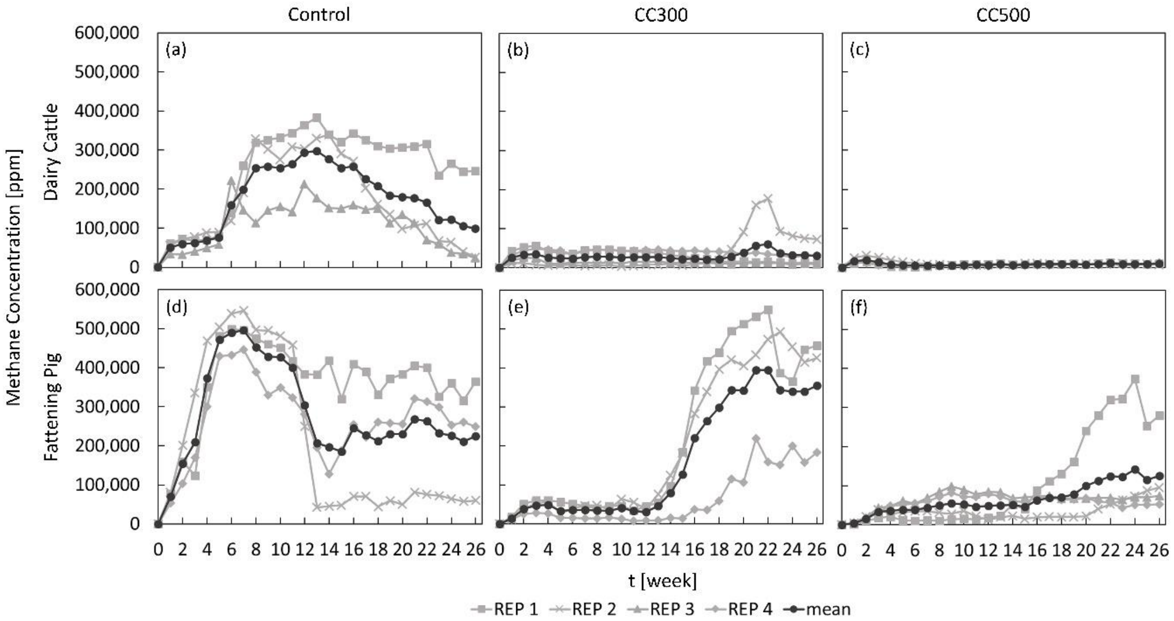

Figure 4.4: Methane concentrations [ppm] emitted during a 26-week storage period at 20.2 ± 1.1 °C of dairy cattle and fattening pig slurry. Slurry was untreated (Control) and treated with either 300 (CC300) or 500 (CC500) mg kg^{-1} cyanamide equivalents. Mean values are indicated in dark gray and the individual repetitions (REP) in light gray (n ≥ 3).

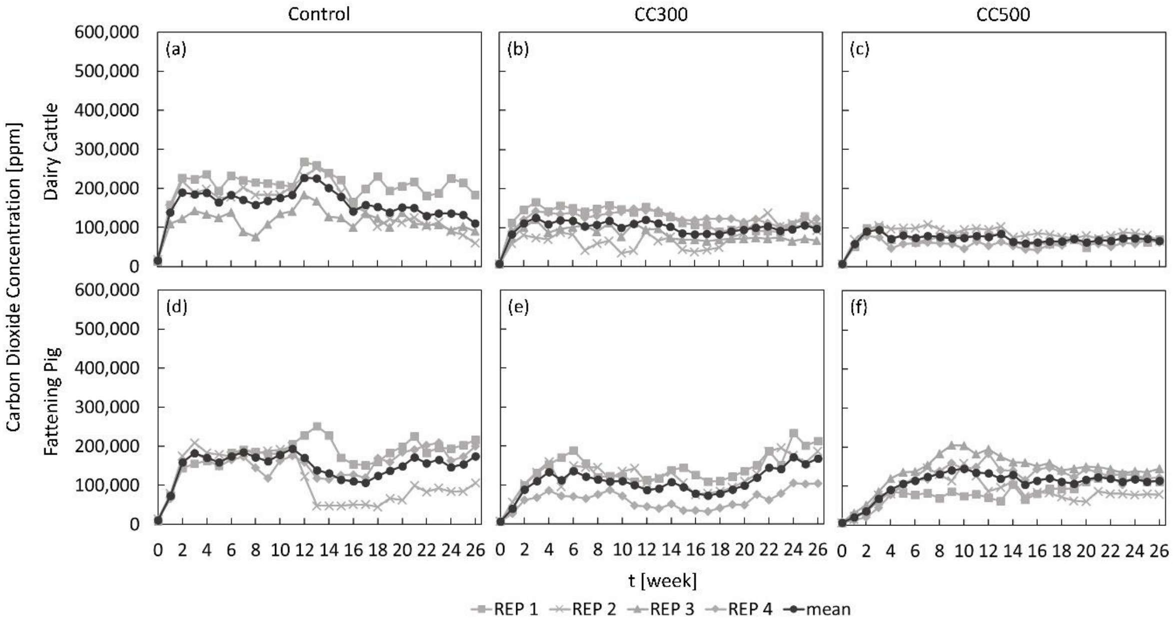

Figure 4.5: Carbon dioxide concentrations [ppm] emitted during a 26-week storage period at 20.2 ± 1.1 °C of dairy cattle and fattening pig slurry. Slurry was untreated (control) and treated with either 300 (CC300) or 500 (CC500) mg kg^{-1} calcium cyanamide. Mean values are indicated in dark gray and the individual repetitions (REP) in light gray (n ≥ 3).

4.3.5 Total greenhouse gas emissions

The application of $CaCN_2$ as an emission-reducing slurry additive showed that at both high and low application concentrations in DC slurry and FP slurry, total CH_4 and CO_2 emissions were significantly lowered by up to 99% (Figure 4.6a,b). An exception to this rate represents the FP/CC300 treatment, which reduced CH_4 and CO_2 emissions by 81% and 86%, respectively. In contrast to the high CH_4 and CO_2 emissions, the control variants of both slurry types emitted very limited N_2O levels (Figure 4.6c). Despite the overall low N_2O levels, both $CaCN_2$ treatments resulted in a reduction of about 80% and least 60% in the FP slurry and DC slurry, respectively. The total GHG emissions include CH_4, CO_2, and N_2O emissions as a sum adjusted to the respective GWP, which was 265 for N_2O and 28 for CH_4. Thus, a calculated mean of 20.2 kg and 50.4 kg CO_2 eq t^{-1} slurry was emitted from DC and FP slurry, respectively during the 26-week storage period. Considering that about 95% of the CO_2-eq calculated in the experiment originated from CH_4, 0.003-0.35% from N_2O, and the rest from CO_2, similar reduction rates to those found for CH_4 can be found for total GHG emissions. Therefore, $CaCN_2$ significantly reduced emissions by more than 99% for DC/CC300 and DC/CC500, respectively and by 81% and 99% for FP/CC300 and FP/CC500, respectively.

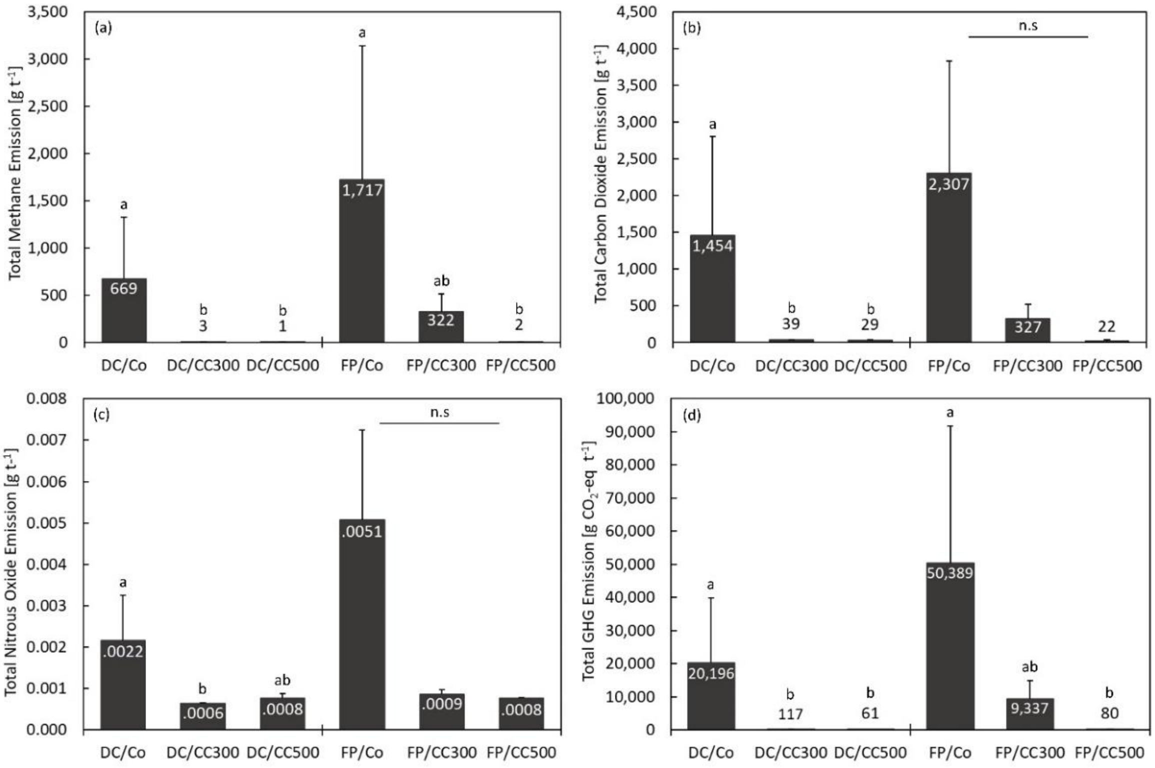

Figure 4.6: **Total mean methane (a), carbon dioxide (b), nitrous oxide (c), and greenhouse gas (d) emission [g t^{-1}] measured after a 26-week storage period at 20.2 ± 1.1 °C of dairy cattle (DC) and fattening pig (FP) slurry. Slurry was untreated (Co) and treated with either 300 (CC300) or 500 (CC500) mg kg^{-1} cyanamide equivalents. Calculation of the total greenhouse gas was based on the factor 265 for nitrous oxide and the factor 28 for methane. Vertical bars represent the mean values ± standard errors ($n \geq 3$). Different letters above bars indicate differences in significance ($p < 0.05$, n.s = not significant).**

4.4 Discussion

4.4.1 Assessment of the onset of action of calcium cyanamide considering the stripping process

The stripping procedure performed prior to the 26-weeks of storage was conducted to remove all dissolved GHGs from the slurry. This ensured that gas concentrations measured during and after storage could be attributed exclusively to newly formed gas production rates and is therefore not envisaged in the practical application of $CaCN_2$. In scientific research and practice, stripping of slurry is mainly used for removing nitrogen in the form of NH_3 from liquids such as digestates, wastewaters, and slurry (Kinidi et al., 2018; Laureni et al., 2013; Liao et al., 1995; Zhang et al., 2012). We consider a substantial impact of this pretreatment on gas formation to be very limited, as the study of Clemens et al. (2006) showed similar GHG emission during anaerobic storage of slurry and we demonstrated below that CH_4 production rates were comparable to those from non-stripped samples in the literature.

However, the gas concentrations measured during stripping indicated that CH_4, CO_2, and N_2O were removed in all cases to a high degree, whereas CO_2 removal was least effective, most likely because CO_2 establishes an equilibrium with carbonic acid (H_2CO_3) in aqueous solution (Andersen, 2002). Furthermore, H_2CO_3 is in equilibrium with its deprotonated forms of hydrogen carbonate (HCO_3^-) and carbonate ions (CO_3^{2-}), which is mainly influenced by the pH (Andersen, 2002; Georgacakis et al., 1982). If CO_2 is removed, the equilibrium re-establishes as both carbonate ions are being protonated into the form of H_2CO_3 and thus into CO_2. Hence, CO_2 can be replenished over a longer period causing slower removal rates. In addition, CO_2 can be dissolved in water to a much higher degree than CH_4 (Sander, 2015).

Furthermore, the data set generated during stripping was utilized to determine the time span from incorporation to the onset of the emission-reducing effect of $CaCN_2$. This assumption was based on the observation that both $CaCN_2$-treated slurry types showed no nominal CH_4 concentrations towards the end of stripping, while the control samples were still emitting CH_4 (Figure 4.2a,b). Therefore, the onset of action of $CaCN_2$ was assumed to start within 45 min, covering the time of incorporation and the stripping process. In addition, the stripping process allowed us to visualize and to mathematically calculate the current methane production rate in the FP slurry. This was accomplished by estimating the mean CH_4 concentration emitted by the FP/Co variants during the last 4 minutes of stripping (Figure 4.2a). This period was chosen as the concentration showed stable values, indicating that the current methane production rate must have been reached. The mean concentration and the known volumetric flow rate at which the samples were stripped allowed us to apply the formula of the ideal gas law to calculate the current methane production rate, which is expressed in mg CH_4 kg^{-1} VS h^{-1} and was 67.62 for the control as well as 9.42 and 10.9 for CC300 and CC500, respectively. Petersen et al. (2016) conducted a study to determine methane production rates using 20 pig manures from 6 farms revealing an average rate of 81.97 mg CH_4 kg^{-1} VS h^{-1}. This confirms the assumption that the stripping curve of sample FP/Co in minutes 26-30 visualized the current methane production and that $CaCN_2$ exerts its reducing effect within 45 minutes.

4.4.2 Effects of calcium cyanamide on ammonia emissions and the pH of slurry

4.4.2.1 During stripping (Week 0)

The application of the $CaCN_2$ product had an alkalizing effect on both slurries, because it contains high shares of calcium hydroxide, calcium carbonate, and magnesium carbonate. As a result, the pH increases after stirring from 6.75 to 7.31 and 7.55 for FP/CC300 and FP/CC500, respectively, as well as from 6.84 to 6.98 and 7.11 for DC/CC300 and DC/CC500, respectively. The intensity of the rise may vary as the pH in slurry can be strongly influenced by many factors such as the type of slurry, temperature, buffer capacity, time of storage and organic matter content (Overmeyer et al., 2020). Liao et al. (1995) demonstrated that at a constant stripping rate, the temperature and pH of the slurry are significantly responsible for high NH_3 recovery rates. Since both temperature and stripping rate were controlled factors in our experiment, the pH increase after stirring in $CaCN_2$ in the FP slurry, was determined to be the cause of the significant increase in NH_3 emissions (Table 4.1). This pH increase shifts the equilibrium between $NH_4^+ \rightleftharpoons NH_3 + H^+$ towards the volatile and non-ionized form NH_3 and enhances the emission rates (Arogo et al., 2006). In the DC slurry, NH_3 rates did not differ due to a relatively small increase in pH of 0.14 and 0.27 units for the CC300 and CC500 variants, respectively. Comparing both slurry types, significantly less NH_3 was removed in the DC slurry because the higher viscosity and organic matter content inhibited the formation of fine pore bubbles during stripping, reducing the overall stripping performance (Figure 4.3). This assumption was also confirmed by the slower decrease in CH_4 and CO_2 concentrations, as illustrated by the concentration curves generated during stripping (Figure 4.2). The relationship between high organic matter contents and low NH_3 removal rates was also observed in the study by Laureni et al. (2013) and was explained by the ability of organic matter to bind cations such as NH_4^+ and thereby lowers the amount of NH_3 that can be removed. Furthermore, the FP slurry contained 3.18 times more NH_4-N on average compared to the DC slurry (0.76 vs. 2.42 kg m^{-3}) and had a higher pH as well as a lower organic matter content. We assume that the combination of better strippability, higher NH_4-N content and higher pH in the FP slurry were the most dominant factors contributing to the higher NH_3 effluent rates, as the differences in organic matter content were relatively small. However, the impact of the individual factors cannot be fully clarified. Higher N_2-flow rates would have been necessary to enhance the stripping efficiency in the DC slurry, which would entail the risk of severe foaming in the slurry, especially at alkaline pH, causing severe interference with the stripping process (Liao et al., 1995).

4.4.2.2 During storage (Week 26)

The $CaCN_2$-treatment reversed the alkalization effect during the 26-weeks, as the pH in nearly all treated samples was within the acidic range at the end of storage. This induced pH decline re-shifted the ammonium-ammonia equilibrium from NH_3 towards the non-volatile and ionized form NH_4^+ (Arogo et al., 2006). This principle is utilized in the technology of slurry acidification, which is already widely employed in practice and is based on reducing the pH to typically 5.5 with sulfuric acid to cut NH_3 and CH_4 emissions (Fangueiro et al., 2015; Kupper et al., 2020). A review study by Kupper et al. (2020) summarized that acidification caused average NH_3 reduction potentials of 71% for cattle slurry and 77% for pig slurry. The application of low pH slurry is considered to have no negative impact on the fertilizer value, making it suitable for disposal on the field as an organic fertilizer (Fangueiro et al., 2015). In our study, the NH_3 reduction potential was within 69-79% regardless of treatment concentration or slurry type. Furthermore, an acidic slurry pH enhances animal welfare due to lower NH_3 concentrations in the air (Jones et al., 1996). At this point, we would like to mention that for a more precise estimation and statistical verification of this potential, further experiments are needed.

However, the addition of $CaCN_2$ appeared to inhibit the microbial degradation of VFA during storage, resulting in a pronounced VFAs accumulation (Table 4.1). In slurry, VFA are one of the predominant buffering systems responsible for a natural and self-induced decrease in pH and are therefore considered to be the cause of the lower pH measured in the samples containing high VFA concentrations (Georgacakis et al., 1982; Overmeyer et al., 2020; Sommer and Husted, 1995). Further, in our previous study Overmeyer et al. (2020) were able to visualize and record the formation of VFAs in slurry, which showed a peak value after 1-2 weeks indicating that a pH drop in the recent study might have also occurred during that period. Additional information about the mode of action is presented in 4.4.3.1.

In summary, the experimental results suggest that increased NH_3 might have occurred during these first weeks of storage due to the addition of $CaCN_2$ and its associated increase in pH. As storage progressed, VFA were formed and its degradation was inhibited by $CaCN_2$, which led to the acidification of slurry and probably dropped the pH right after the first weeks of storage, as a reduction in total NH_3 emissions was found at the end of storage.

4.4.3 Greenhouse gas concentration and total emissions during storage

4.4.3.1 Methane emission

During storage, the DC slurry showed a 5-week lag phase for the CH_4 production to occur, which then increased rapidly and reached a maximum value at week 13 (Figure 4.4a). The study by Cárdenas et al. (2021) found similar results as the lag phase lasted 14 weeks while reaching a maximum production rate after 21 weeks in DC slurry stored at 20 °C The study by Massé et al. (2003) explained this lag phase by the fermentation process of cellulosic material in the rumen of dairy cows, which results in feces containing a lower percentage of readily available organic carbon, delaying methane production at the beginning of storage. In our study, as well as in others, no lag phase was observed in FP slurry as it generally contains a higher share of readily available organic carbon (Martinez et al., 2003; Massé et al., 2003).

Furthermore, after about 12 weeks of storage, we noticed a decrease in CH_4 concentration, for which two processes could be responsible. The first refers to a shift of the emitted CH_4:CO_2 ratio as storage proceeds towards microbial processes other than methanogenesis, whose product does not contain CH_4. The second process refers to the ability of CO_2 to dissolve in the slurry as H_2CO_3, HCO_3^-, and CO_3^{2-}, which are collectively referred to as carbonate buffer (Andersen, 2002; Dalby et al., 2021). The study by Overmeyer et al. (2020) revealed that this buffer degrades with increasing storage time, resulting in enhanced CO_2 emissions. Both processes would cause delayed CO_2 effluxes, which increases the total gas volume and causes the observed dilution of CH_4 concentration to occur at later storage period. These assumptions are supported by relatively low increase of CO_2 in both control variants at the beginning of storage compared to CH_4 and the high CO_2 share of the total gas emission, which was 57% for FP and 68% for DC slurry, being higher than the average of 25-50% reported for anaerobic slurry storage (de Klein et al., 2008). In addition, we can exclude the following assumptions: diffusion through the material of the storage system, because this is prevented by the material selection; and the presence of methane-oxidizing bacteria, because our samples were stored anaerobically (Kalyuzhnaya et al., 2019). The sudden and steep drop in the FP/Co REP2 sample could have been caused by a momentary loss of gas during a necessary repositioning of the bottle, although a reduced gas volume at the end of storage was not evident (Figure 4.4d).

However, we intended to determine and assess the effect of $CaCN_2$ on slurry. Therefore, we consider this experimental approach to be sufficient to achieve this objective, as we could clearly demonstrate that $CaCN_2$ significantly inhibited CH_4 production in the first 12 storage weeks in all variants (Figure 4.4). After that period, CH_4

production started in the FP/CC300 variant, indicating that the emission-reducing effect of $CaCN_2$ is reversible, but still caused an 81% reduction in total CH_4 emission. In all other variants, production rates remained suppressed for more than 26 weeks, although no significant differences were found at week 26 compared to the control variants (Figure 4.9). This might be explained by the decreasing concentrations found in the control variants. Nevertheless, the treatment led to a total CH_4 emissions reduction of more than 99%.

VFA analyses of both $CaCN_2$-treated slurries revealed an increased accumulation of nearly all C_2-C_6 fatty acids (especially acetic acid and propionic acid) after storage and while $CaCN_2$ was still effective. This inhibition of VFA degradation and resulting accumulation of VFA was associated with a decrease in CH_4 production, with $CaCN_2$-treated slurries emitting significantly less than the control or the FP/CC300 variant. Thus, it can be assumed that this inhibition may also have interfered with the degradation of acetic acid/acetate to CH_4 during methanogenesis (Dalby et al., 2021). We consider a complete sanitizing of the microorganisms as an explanation for this assumption as unlikely, since CO_2 is still produced at the beginning of storage. Furthermore, the rapid suppression of CH_4 production within 45 min after application indicates that the blocking of VFA degradation involved a rapid mechanism of action, suggesting the involvement of enzymatic processes. Cyanamide is described in literature as a potent inhibitor of the enzyme aldehyde dehydrogenase (ALDH2), which is present in many organisms, including the human body, where it is responsible for the degradation of ethanol and acetaldehyde to acetate, the most prominent VFA in our study (Güthner & Mertschenk, 2006; Loomis & Brien, 1983; Peachey et al., 1981). We hypothesize that this function of cyanamide also interferes with exoenzymes produced and released by microorganisms, which play a crucial role in degrading VFA into acetate and converting it into CH_4 for maintaining microbial metabolism. In the long term, this might also lead to a suppression of the proliferation of VFA degrading and methanogenic bacteria, as their metabolism would be impeded. However, further studies are required to explain the mechanism of action of cyanamide in detail. Furthermore, in the study by Berg and Pazsiczki (2006) acidified slurry was shown to reduce CH_4 emissions at pH below 6.0. In our study, such low pH-values were not detected, making it unlikely that the induced pH decrease contributes to the overall CH_4 reduction. In addition, we proved that $CaCN_2$ is a fast-acting additive starting to inhibit CH_4 production before the pH dropped. Furthermore, high propionic acid concentrations can irreversibly damage the growth of methanogenic bacteria and thus reduce the total emission (Wang et al., 2009). Nevertheless, this most likely was not the case in our study, because we could prove that the effect of $CaCN_2$ is reversible.

4.4.3.2 Carbon dioxide emission

The addition of $CaCN_2$ reduced the total CO_2 emission by 81-99%. In slurry, the main sources of CO_2 generation are the decomposition of organic matter under aerobic conditions and the fermentation of organic matter under anaerobic conditions that produce CH_4 besides CO_2 (Philippe & Nicks, 2015). Despite the complete removal of oxygen and the inhibition of methanogenesis, the CO_2 concentration increased at the beginning of storage in all variants. This indicates that other anaerobic microbial processes are still ongoing, e.g. carbon fermentation processes, which remain unaffected by the inhibition of methanogenesis (Dalby et al., 2021; Klass, 1984). This is an evidence that $CaCN_2$ does not have a complete sanitizing effect on the whole microbiome in the slurry. No sanitizing effect was also observed during mesophilic storage of $CaCN_2$-treated slurry in the study by Simujide et al. (2013). Furthermore, the onset of CH_4 production and the slightly delayed increase of CO_2 concentration in sample FP/CC300 visualize the formation of both gases during methanogenesis (Figure 4.5e). We consider an increase of the CO_2 concentration at the beginning of the storage due to the catalytic conversion of urea by urease to NH_3 and CO_2 as being unlikely because the time interval between sampling and start of the experiment was sufficient for this reaction to be completed (Overmeyer et al., 2020; Philippe & Nicks, 2015). However, the effect of CO_2 emission reduction is limited to the storing process. Once the slurry is applied to the fields, the emission-reducing effect is revoked as aerobic conditions prevail and the organic components begin to decompose, resulting in CO_2 emissions.

4.4.3.3 Nitrous oxide emission

Nitrous oxide emissions averaged 0.022 g t^{-1} for DC/Co and 0.051 g t^{-1} for FP/Co, which were very low compared to other studies because of the strictly anaerobic storage conditions (Amon et al., 2006; Kupper et al., 2020; Philippe & Nicks, 2015). However, we detected a very slightly elevated N_2O concentration during stripping and at the beginning of storage in the treated FP slurry (Figure 4.7b), with total N_2O emissions after stripping corresponding to the amount of nitrate-N (0.1%) added in the form of the additive. In preliminary experiments, we utilized a formulation with 1.8% nitrate-N instead of 0.1%, which led to an increase in N_2O concentration of over 300 ppm during the first week of storage. Thus, these findings indicate that the small amount of nitrate-N contained in the additive is rapidly and fully converted to N_2O. The absence of this trend in the DC slurry might be attributable to the lower strippability of the slurry. However, no further N_2O formation was detected during the whole storage period indicating that N_2O emissions can be exclusively attributed to the nitrate-N added and that no N_2O originated from the conversion of $NH_{3\,(aq)}$ through nitrifier nitrification or anamox processes (Philippe & Nicks, 2015).

4.5 Conclusion

Although the importance of optimizing manure management strategies to mitigate the advancing climate change has become widely recognized, many of these strategies fail to reduce both NH_3 and CH_4 emissions without incurring high financial costs to implement and operate these strategies. The use of $CaCN_2$ containing products as a slurry additive has the potential to fill this gap, as it is inexpensive to apply by simply stirring it into the slurry and suppresses CH_4 formation within 45 min after its application and lasted for 12 to 26 weeks and more, depending on the dosage and thereby reducing total GHG emissions by 81% to 99%. The experimental results indicate that the mechanism of action is based on the inhibition of microbial degradation of VFA and its conversion to CH_4, causing CH_4 production to collapse. This led to an over-accumulation of VFA in the slurry, resulting in its acidification. As a consequence, NH_3 emission decreased during storage, which, however, could not be statistically verified. Research should therefore be continued not only to further clarify the effects on NH_3 emissions, but also to reveal the exact mechanism of action, to uncover the response of microbial diversity to $CaCN_2$ and to demonstrate its efficacy beyond the laboratory scale in practical trials with various substrates such as digestate, wastewater and slurry. In this context, the timing and dosage of application should be investigated to make this process as effective and resource-efficient as possible.

CRediT authorship contribution statement: Felix Holtkamp: Conceptualization; Data Curation; Formal analysis; Funding acquisition; Investigation; Methodology; Software; Validation; Visualization; Writing – original draft. Manfred Trimborn: Conceptualization; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Writing – review &editing. Joachim Clemens: Conceptualization; Methodology; Resources; Validation; Visualization; Writing – review & editing.

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4.6 Appendix

Table 4.2: **Characteristics of dairy cattle and fattening pig slurry (fresh material) at week 0 and 26 that were untreated (Co) or treated with 300 mg kg^{-1} (CC300) or 500 mg kg^{-1} (CC500) of cyanamide equivalents.**

		Dairy Cattle				**Fattening Pig**			
Treatment and Weeks of Storage		**Co**		**CC300**	**CC500**	**Co**		**CC300**	**CC500**
		0	**26**	**26**	**26**	**0**	**26**	**26**	**26**
Ingredients									
K_2O	kg m^{-3}	3.04	2.83	2.72	2.77	2.26	2.10	2.08	2.10
MgO	kg m^{-3}	0.56	0.53	0.50	0.51	1.51	1.49	1.48	1.47
S	kg m^{-3}	0.26	0.20	0.21	0.22	0.45	0.43	0.46	0.44
Cu	g m^{-3}	2.27	2.23	2.21	2.22	9.60	9.74	9.60	9.45
Zn	g m^{-3}	12.70	12.20	11.70	11.60	58.70	57.00	57.60	56.30
Iso-caproic acid	g kg^{-1}	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Caproic acid	g kg^{-1}	<0.05	<0.05	0.06	<0.05	0.26	<0.05	<0.05	0.22
Iso-valeric	g kg^{-1}	0.15	<0.05	0.22	0.19	0.22	<0.05	<0.05	0.81
Valeric acid	g kg^{-1}	0.12	<0.05	0.12	0.10	<0.05	<0.05	<0.05	0.20
Iso-butyric acid	g kg^{-1}	0.11	<0.05	0.27	0.30	1.100	<0.05	<0.05	0.59
Butyric acid	g kg^{-1}	0.52	<0.05	0.74	0.53	0.55	<0.05	<0.05	1.10

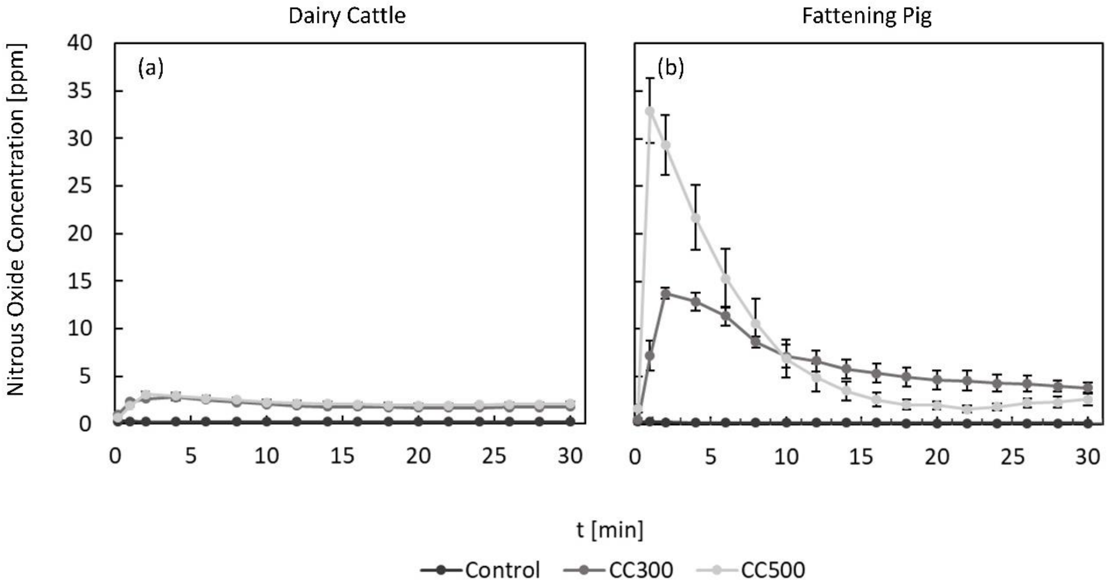

Figure 4.7: **Outgassing curves representing the mean nitrous oxide concentrations [ppm] generated by a stripping process using 0.25 L N_2 min^{-1} for 30 min per sample to eliminate dissolved gases from dairy cattle and fattening pig slurry. Slurry was untreated (Control) or treated with either 300 (CC300) or 500 (CC500) mg kg^{-1} cyanamide equivalents. Vertical bars represent the mean values ± standard errors (n = 4).**

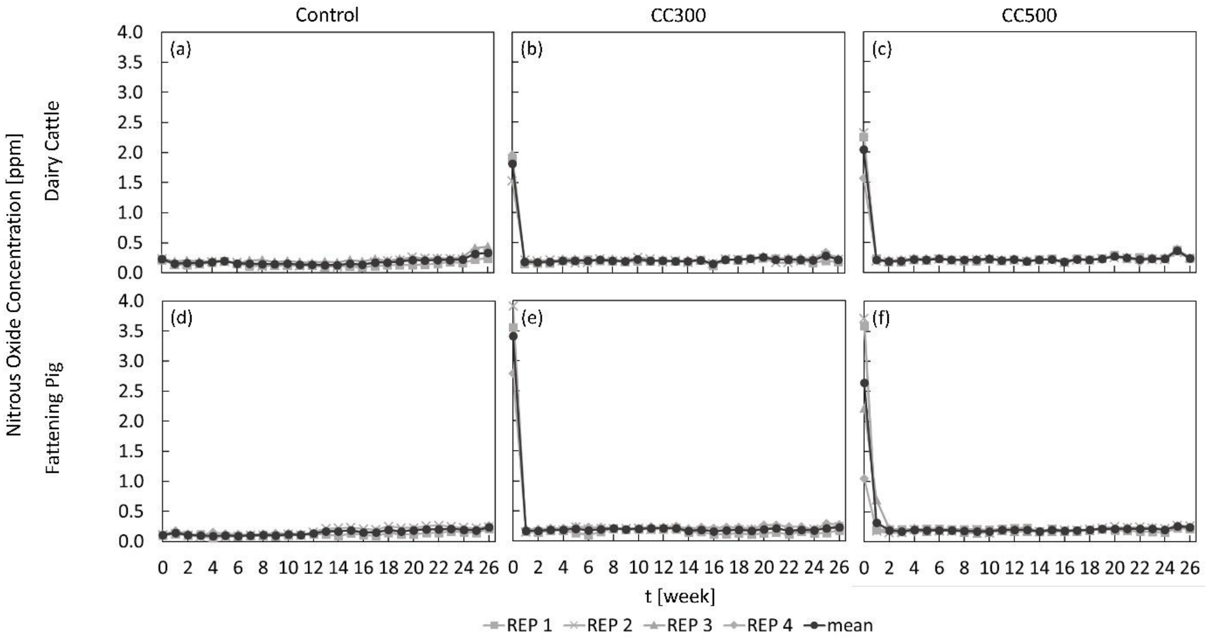

Figure 4.8: **Nitrous oxide concentrations [ppm] emitted during a 26-week storage period at 20.2 ± 1.1 °C of dairy cattle and fattening pig slurry. Slurry was untreated (Control) or treated with either 300 (CC300) or 500 (CC500) mg kg^{-1} cyanamide equivalents. Mean values are indicated in dark gray and the individual repetitions (REP) in light gray (n ≥ 3).**

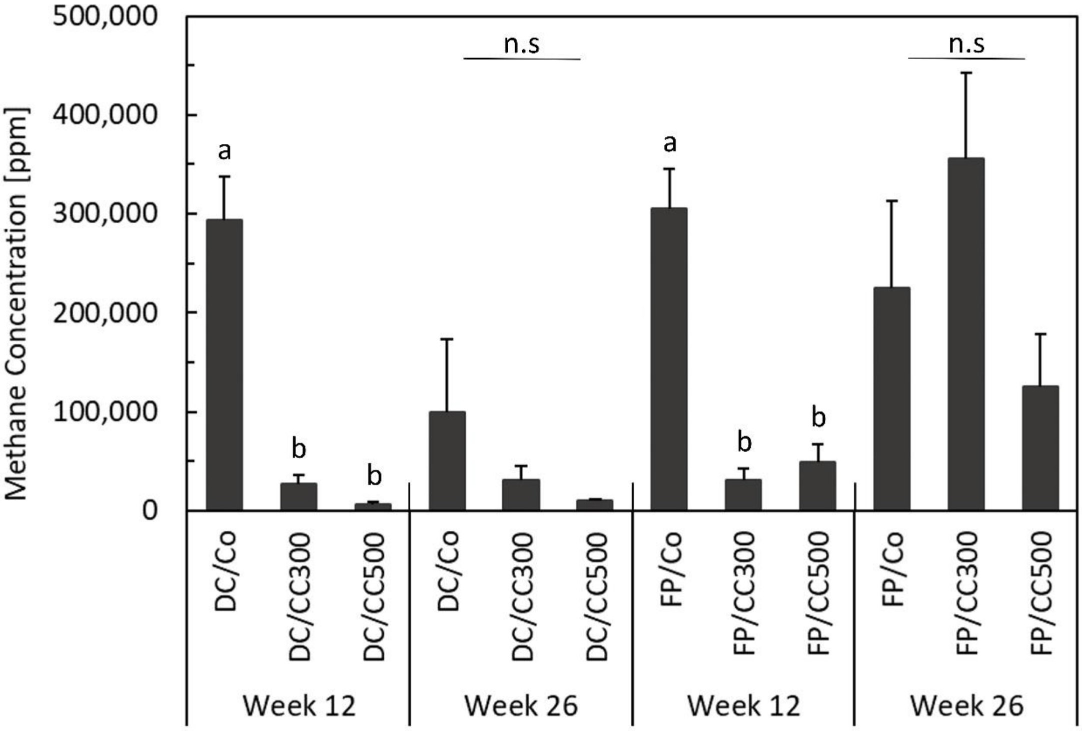

Figure 4.9: **Mean methane concentrations [ppm] measures at storage week 12 and 26 during a 26-week storage period (week 26) of dairy cattle (DC) and fattening pig (FP) slurry. Slurry was untreated (Co) or treated with either 300 (CC300) or 500 (CC500) mg kg^{-1} cyanamide equivalents. Vertical bars represent the mean values ± standard errors (n ≥ 3). Different letters above bars indicate differences in significance ($p < 0.05$, n.s = not significant).**

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5 Discussion and Conclusions

The three research questions raised in this dissertation are thoroughly discussed by interpreting the research results of the individual studies and explaining their significance in the context of the current state of knowledge in the scientific literature. Furthermore, in this section, both the opportunities and challenges arising from the study results are highlighted and conclusions are drawn, which can be helpful to develop new or improve existing slurry management strategies that are aimed at reducing gaseous emissions from slurry.

5.1 Discussion of Research Question posed

5.1.1 (I) How can Buffer Systems and their Capacities in Slurry be visualized?

The simplest method for visualizing buffer capacities in slurry is to graph titration curves by plotting the pH value against the amount of titrant. The respective slopes of these curves at a given pH value allow conclusions to be drawn about the capacities of the corresponding buffer systems. However, a precise differentiation of the individual buffers and their capacities is only possible to a very limited extent (Husted et al., 1991). This method is used today primarily to identify the total buffer capacity of slurry (Cassity-Duffey et al., 2015) and to determine the effectiveness of individual acids or bases in achieving specific target pH values (Rodrigues et al., 2021). Nevertheless, the data obtained from titrations serve as a basis for more complicated mathematical models, allowing a greater differentiated consideration of the individual systems. Therefore, other approaches are based on calculating the reciprocal of the slope of linear regression lines passing through titration points or on plotting the inverse slopes of entire titration curves (Costello & Sullivan, 2014; Husted et al., 1991). Both approaches enable the visualization of buffer capacities at specific pH values, providing a more accurate determination of buffer systems and their capacities, especially when compared to traditional titration curves. However, they are not capable of visualizing the interdependence and dynamics between different buffer systems during slurry storage. The study of Georgacakis et al. (1982) subjected titration data to regression analysis so that they could be expressed as 3rd degree polynomial functions, allowing them to identify extrema by their derivations and thus visualize individual buffer systems and their capacity in detail (Figure 2.1). Based on this model, the authors were able to identify interactions between the different main buffer systems. They described and demonstrated these interactions by manipulating buffer systems in digesters by adding urea or glucose to pig slurry in order to draw conclusions about the effect of buffer systems on the pH stability of digesters. In summary, it can be concluded that studies addressing the visualization and the associated possibility of a detailed characterization of buffer systems in slurry and the changes they undergo during storage are lacking.

Moreover, there has been a lack of methods and techniques that are capable of detecting and determining changes in buffer capacities that occur during the storage of slurry. Therefore, the mathematical tool presented in Study 1 was developed to fill this gap. However, this tool is largely based on the above-mentioned model developed and described in the study of Georgacakis et al. (1982). The tool can be understood as an extended and improved version of it, allowing visualization of buffer systems and their capacity, as well as their buffer ranges, with greater complexity than previous models. The new mathematical tool used in Study 1 allowed an accurate visualization of the formation and degradation dynamics of the primary buffer systems that occurred during eight weeks of slurry storage, as shown in Figure 2.6. Additionally, this mathematical tool demonstrated the impact of different storage temperatures on these processes (Figure 2.8). Based on the results obtained, it can be stated that buffer systems in slurry cannot be considered individually, as they are subject to considerable changes during storage, so they must be interpreted as complex and interacting systems. However, these interactions are explained and discussed in detail in the second scientific question posed. Furthermore, according to the best of our knowledge, the mathematical tool presented is the first one that has undergone an intensive scientific validation process, not only demonstrating the mentioned capabilities and accuracy of the tool but also identifying its limitations.

The main limitation of the tool is the inability to individually visualize buffer systems with strongly overlapping buffer ranges as the buffer curves of individual buffers may merge. This was particularly prevalent in the alkaline milieu between the NH_3 and CO_3^{2-} buffer (dotted line in Figure 2.1). However, the aforementioned issue was resolved by acidifying the slurry prior to the alkalization process, which eliminated both carbonate buffers (HCO_3^- and CO_3^{2-}) from the slurry in the form of outgassing CO_2 (Vandré & Clemens, 1996). This method has the advantage that the NH_3 buffer can be considered individually without the interference of the CO_3^{2-} buffer. Nevertheless, the strong CO_2 outgassing induced by the acidification process caused the slurry to foam, which could lead to titration errors and thus jeopardize the accurate calculation of the buffer capacities present in the acidic milieu. This could be avoided by performing smaller titration steps, which reduced foam formation and also allowed a clearer visualization of the VFA and HCO_3^- buffer. Furthermore, the study conducted by Christensen et al. (2009) not only removed both carbonate buffers by acidification but also separated the slurry into different fractions needed to visualize and calculate less strong buffer systems, such as carboxylic acids, phosphate groups and amino groups. The new mathematical tool presented was not able to visualize these systems. However, the experimental set-up from Study 1 was not designed and optimized for visualizing these weak buffer systems. Combining the use of the new mathematical tool with modifications to the experimental set-up in order to detect weak buffer systems may significantly contribute to gain deeper insights into the dynamics of these systems.

The mathematical tool presented in Study 1 has proven to be extremely effective in calculating and visualizing these buffer systems and their capacities, allowing a very complex and accurate description of their dynamics. The main limitation of this tool is the inadequate consideration of overlapping buffer systems and weak buffer systems that are overshadowed by the dominant main systems. Future research should focus on improving the tool to account for individual consideration of these systems, as this would represent a major advancement in comprehending the complexity of buffer systems. Furthermore, assessing the applicability of this mathematical tool to describe buffer systems in other environments, such as soils or aquatic ecosystems, would be a valuable contribution to a broader understanding of such systems and their underlying dynamics.

5.1.2 (II) What Influence do Time and Temperature of Storage have on the Dynamics of Buffer Systems in Slurry?

In order to fully discuss the effect of time and temperature on buffer systems in slurry thoroughly, it is relevant to consider that the formation of buffer systems is largely dependent on the slurry characteristics, such as the content of VFA, VS, dry matter and TAN. The composition of these parameters in the slurry is primarily determined by the nutrient profile of the feed consumed and the efficiency of the animals' digestive tract to breakdown and absorb these nutrients (Bergman, 1990; Christensen & Sommer, 2013; Velthof et al., 2000). In some cases, slurry also contains feed and bedding residues, as well as cleaning water or water from drinkers, which either increases or decreases the dry matter content of the slurry and thus can affect the formation of buffer systems (Pain & Menzi, 2011; Triolo et al., 2013).

Answering the research question posed, therefore, requires a differentiated approach that takes different types of slurry into account. Hence, Study 1 adopted three different types of slurry from fattening pigs, dairy cows, and sows as the primary experimental basis for discussion to answer this research question. Generally, one of the most important changes that occurs during slurry storage is the degradation of organic compounds by microorganisms (Velthof et al., 2000). These degradation processes lead to the formation of the four main buffer systems present in slurry.

In the absence of O_2, microorganisms degrade complex carbon-containing organic compounds into CH_4 and CO_2, producing VFA as degradation intermediates (as seen in

Figure 1.2) (van Hullebusch et al., 2019). In Study 1, on the one hand, this degradation process could be observed directly, as the dry matter content, which usually consists of the mentioned complex organic compounds (Chávez-Fuentes et al., 2017; Fangueiro et al., 2013), decreased over a period of eight weeks in all slurry samples when they were stored warmly. This is in line with the results reported by other authors (Petersen et al., 1998; Popovic & Jensen, 2012). On the other hand, in Study 1, this degradation process could also be indirectly observed via the emergence of the VFA buffer system and the intensification of its capacity during the first two to three weeks of storage, as illustrated in Figure 2.6, Figure 2.7 and Figure 2.8. The VFA buffer in the fattening pig slurry built up and degraded more rapidly compared to that in the dairy cow slurry, which exhibited less fluctuation and maintained higher consistency over the entire storage period. This may be attributable to the higher occurrence of readily available carbon sources and the lower content of slowly available carbon sources, such as lignin, in pig slurry compared to cattle slurry. (Massé et al., 2003; Triolo et al., 2013). As a result, the decomposition processes are promoted and the formation of VFA is increased. In addition, the temperature of storage has a great influence on the emergence and degradation of the VFA buffer, as microbial processes are enhanced by higher and slowed down by lower temperatures (Cooper & Cornforth, 1978; McGill & Jackson, 1977; Popovic & Jensen, 2012; Sommer & Sherlock, 1996). This was also observed in Study 1, as the fluctuations of the VFA buffer during slurry storage were more pronounced at high storage temperatures (23.6 ± 2.1 °C, warm storage) compared to low storage temperatures (4.7 ± 1.1 °C, cold storage).

Furthermore, as the degradation process of slurry continues, it may undergo an acidification process due to the production and accumulation of VFA, which leads to a decrease in pH (Christensen & Sommer, 2013; Overmeyer et al., 2020; Paul & Beauchamp, 1989). Acidification of the stored slurry was also observed in Study 1, with the pH value decreasing in the first few weeks of storage (Figure 2.7). However, the degree of acidification varied depending on the storage temperature and type of slurry. In particular, the warm-stored pig and dairy cow slurry showed greater pH fluctuations than the cold-stored slurry. Acidification variability may be attributed to slower rates of decomposition of organic compounds to VFA occurring at low temperatures, as stated previously. Therefore, the buffer capacity of the VFA buffer in slurry stored at low storage temperatures was found to be relatively smaller and more consistent, resulting in a lower or no pH drop as opposed to warm storage conditions.

The results of the analysis of the VFA concentration (Table 2.1) and those of the VFA buffer system (Figure 2.7) demonstrated that the decline of the VFA buffer progressed considerably faster in the pig slurry compared to the dairy cow slurry. The decreasing VFA concentration and the associated decline in the VFA buffer capacity can be attributed to the microbial conversion of VFA into CH_4 and CO_2 during methanogenesis, as explained in detail in Chapter 1.1.2. Methane formation typically starts earlier and occurs at higher rates in pig slurry than in cattle slurry, which may be associated with the higher levels of VFA in fresh pig slurry compared to cattle slurry (Cooper and Cornforth, 1978; Holtkamp et al., 2023; Overmeyer et al., 2020). As a result, less VFA is available for microbial conversion to CH_4 and CO_2 in the later stages of storage, which is evident in the lower VFA concentrations detected in the fattening pig slurry at week 8 compared to the dairy cow slurry (Table 2.1). In contrast, the VFA buffer in the cow slurry remained relatively constant, probably due to the lower proportion of readily available carbons. Hence, the production of VFA and its conversion to CH_4 were delayed, which is a phenomenon (Figure 4.4) that is commonly referred to as lag phase and can last up to several weeks (Hilgert et al., 2022; Holtkamp et al., 2023; Sommer et al., 2007, 2000).

Besides CH_4, the final microbial degradation step of VFA, known as methanogenesis, also produces CO_2, which undergoes an equilibrium reaction with H_2CO_3, as described in Chapter 1.1.1, leading to the emergence of both carbonate buffers, the HCO_3^- and CO_3^{2-} buffer (Christensen & Sommer, 2013; Overmeyer et al., 2020). In Study 1, this was especially visualized in Figure 2.6 and Figure 2.8. The correlation between the VFA and HCO_3^- buffers was also observed and described in the study by Georgacakis et al. (1982). However, the temporal aspect of this correlation between the VFA and HCO_3^-, which would have revealed the interdependence of the two buffers during slurry storage, was not considered. Therefore, Study 1 graphically illustrates and thereby reveals that the degradation of the VFA buffer not only affects the buffer capacity in the acidic milieu, as the VFA degradation leads to the emergence of the HCO_3^- buffer. It also directly affects the total buffer capacity in the alkaline milieu, as the CO_2 formed by the breakdown of VFA may act as the CO_3^{2-} buffer in the alkaline milieu. Furthermore, both the resulting CO_3^{2-} and the decreasing VFA concentration in the slurry might induce a re-increase of the slurry pH, thereby mitigating any potential excessive acidification process (Cooper & Cornforth, 1978; Georgacakis et al., 1982; Patni & Jui, 1985). In Study 1, it was observed that despite the decomposition of VFA and the resulting pH increase, the requirement for the amount of bases to alkalize the slurry increased due to the increasing CO_3^{2-} buffer capacity. In contrast to the build-up of both carbonate buffers, a reduction of their capacity starting from week 6 was particularly visible in warm-stored fattening pig and sow slurry, as illustrated in Figure 2.7 and Figure 2.8. This phenomenon can be attributed to the emission of CO_2 occurring during slurry storage (Vandré & Clemens, 1996). When slurry is stored anaerobically, CO_2 primarily arises from methanogenesis (Philippe & Nicks, 2015). It can be hypothesized that in later storage periods, the loss of CO_2 due to naturally occurring emissions could not be resupplied by the methanogenesis rate due to the decreasing VFA concentration, which might have slowed down the CO_2 production rates. In contrast to warm storage conditions, both carbonate buffers remained stable or even increased during cold storage conditions, as illustrated in Figure 2.7 and Figure 2.8. On the one hand, this might be attributed to the higher solubility of CO_2 in colder liquids, which enhances the potential buffering capacity of both carbonate buffers (Figure 2.7) (Wiebe & Gaddy, 1940). In addition, the slurry still contained high VFA levels at the end of the storage period, which may indicate the presence of a lag phase, as mentioned above. On the other hand, the higher VFA buffer capacities found in the cold-stored slurry suggest that the methanogenesis rate and the associated CO_2 production have not yet been reduced and are therefore still sufficient to compensate for the CO_2 losses caused by emissions (Figure 2.7). This latter explanation could also explain the lack of decrease in the HCO_3^- buffer observed in the warm-stored dairy cow slurry. In addition, by the end of the storage period, the slurry still contained high VFA levels, which might indicate the presence of a lag phase, as mentioned earlier.

Another important degradation process that occurs during slurry storage is the breakdown of nitrogenous compounds, which greatly affects the buffer capacity and pH of slurry as it leads to the formation of the NH_3 buffer (Christensen and Sommer, 2013; Georgacakis et al., 1982; Sommer & Husted, 1995). Nitrogen is primarily excreted through urine in the form of urea and through undigested proteins in feces (Arogo et al., 2006; Sigurdarson et al., 2018). The decomposition of urea is catalyzed by urease, an enzyme found in feces that can rapidly degrade urea to NH_3 and CO_2, which increases the TAN and TIC concentrations in slurry at the same time (Christensen & Sommer, 2013; Sigurdarson et al., 2018). This catalytic-driven process is usually completed in slurry within 24 h (Dai & Karring, 2014; Moraes et al., 2017). In contrast, the mineralization of undigested protein to NH_3 takes considerably longer, as it is based on microbial degradation processes (Arogo et al., 2006). However, both processes are enhanced by warm ambient conditions (Bussink & Oenema, 1998; Hao et al., 2019; Moraes et al., 2017). A differentiated consideration of these conversion processes on the build-up and degradation of the NH_3 buffer during the storage of slurry could not be carried out in any of the presented studies. The considerable overlap of the pH buffer ranges between the NH_3 and CO_3^{2-} buffer is the underlying reason for this issue. However, it is explained in more detail in the discussion of the first research question. Another complicating factor is that the catalytic-driven breakdown of urea produces 0.5 mol of CO_3^{2-} per mol of NH_3, which increases the capacity of both buffers, making the distinction even more difficult

(Sommer & Husted, 1995). Nevertheless, it can be assumed that the capacity of NH_3 buffer is higher in fattening pig slurry than in cattle slurry, as pigs consume more proteins and therefore excrete more nitrogen. This is evidenced by the generally higher TAN content found in pig slurry compared to cattle slurry (Velthof et al., 2015). In Study 1, this was also the case, as the fattening pig and sow slurry contained high levels of NH_4-N. Furthermore, it can be assumed that the NH_3 buffer emerges quickly in fresh slurry, as the catalytic-driven hydrolysis of urea is a very fast and efficient process (Dai & Karring, 2014; Sigurdarson et al., 2018). Nevertheless, in Study 1 it could be shown that in fresh sow slurry, which was less than 12 h old, the degradation of urea was not yet complete (Figure 2.4). However, after the first week of storage, the experimental results indicated that the HCO_3^- buffer had considerably increased, suggesting that the degradation of urea had been completed during this time (Figure 2.7). Furthermore, this indirectly implies that the formation of the NH_3 buffer must also have taken place during this period, which is evidence that the capacity of the NH_3 buffer in slurry intensifies rapidly in fresh slurry.

This discussion, together with the research findings presented in Study 1 showed that these buffer systems in slurry cannot be considered individually, as they change and influence each other significantly during storage. Therefore, buffer systems in slurry must be interpreted as complex and interacting systems. Although the study presented has comprehensively illustrated the development of buffer systems during storage, further research should include the use of an experimental set-up specifically designed for a better individual consideration of the NH_3 buffer. This would provide deeper insights into the characteristics of this buffer and how its capacity changes during storage, thus providing a better scientific basis for optimizing slurry treatment strategies.

5.1.3 (III) How and to what extent can the Application of Alkaline Additives in Slurry mitigate Climate and Environmentally relevant Trace Gases?

The experiments carried out in Study 2 and 3, together with their scientific results, serve to characterize and evaluate two different slurry treatment strategies. These are based on the application of alkaline slurry additives with the aim of reducing climate and environmentally relevant trace gas emissions formed during the storage of slurry. However, both slurry treatment strategies differ fundamentally from each other and will be discussed separately in a differentiated and well-founded manner to answer the scientific question posed.

Study 2: Study 2 assesses the first slurry treatment strategy, which is based on a targeted alkalization process utilizing alkaline additives, such as calcium oxide (CaO), magnesium oxide (MgO) or $Ca(OH)_2$. The aim of this strategy is to increase the pH value of slurry to a degree that is inhospitable to most microbes. This prevents GHG emissions, such as CH_4, CO_2 and N_2O that result from microbial degradation processes, as described in Chapter 1.1.2. The experimental results of Study 2 demonstrated that increasing the slurry pH to at least 10 by adding $Ca(OH)_2$ effectively suppressed the formation of CH_4 in fattening pig slurry (Figure 3.2). Moreover, raising the pH to 10 was enough to reduce CH_4 and CO_2 emissions by approximately 99% and N_2O emissions by around 60% during a storage period of eight weeks. Further increasing the pH value to 11 or 12 did not increase the reduction of these emissions. According to our knowledge, we are the first to have established an emission reduction potential for the storage of alkalized slurry. Therefore, no other comparative data is available in scientific literature. However, the study by Rodrigues et al. (2021) revealed that sanitization of pig and dairy cattle slurry can be achieved starting at a pH of 9.5. Furthermore, pH values above 8 have a restrictive effect on the fermentation process in anaerobic digesters and are rather toxic for methane-forming microorganisms, which may cause a complete failure of the process (Gerardi, 2003). It can therefore be assumed that achieving a pH value between 8 and 9.5 may be sufficient to reduce emissions effectively.

Nevertheless, a pH increase is associated with the drawback of highly increasing NH_3 emissions from slurry, as illustrated in Figure 1.5 and described in several other studies (Arogo et al., 2006; Fangueiro et al., 2015; Fricke et al., 2007). However, the alkalization of slurry opens up the possibility of removing TAN in the form of NH_3 from slurry. The experimental concept of slurry alkalization utilizes this possibility, as it aims to even enhance NH_3 removal efficiency by employing stripping technologies. In order to prevent the release of NH_3 into the environment, both the alkalization and stripping processes have to be carried out within an enclosed reactor. The outgassing NH_3 can be converted into the valuable nitrogen fertilizer ammonium sulfate by absorbing and concentrating NH_3 with concentrated sulfuric acid (Laureni et al., 2013; Liao et al., 1995; Mohammed-Nour et al., 2019). Eliminating NH_3 extensively is crucial to avoid environmental damage, especially damage to crops, which occurs when slurry containing high levels of dissolved NH_3 is applied to the fields as a fertilizer (Arogo et al., 2006; Krupa, 2003; Kupper et al., 2020). Moreover, achieving a nitrogen removal rate that reduces the nitrogen content in the dry matter of slurry to below 1.5% could circumvent the German fertilizer regulations, allowing higher slurry application rates regardless of the season, thereby mitigating problems related to slurry overproduction (BMJ, 2017).

However, this slurry treatment strategy is still in the early stages of development and has many challenges to overcome before it can become established. The primary challenge that must be addressed is the reduction of TAN in slurry to minimize the overall N content, which can only be achieved through an extremely efficient stripping process.

Therefore, experiments were conducted to evaluate the effectiveness of N removal via stripping processes. The results revealed that a maximum N removal rate of ~ 10% was achieved at a pH of 12 and a stripping rate of 0.25 L N_2 min $^{-1}$ for 30 min and 500 ml of fattening pig slurry (for details regarding the experimental set-up, see Figure 4.1). At a pH of 10, which already significantly reduced GHG emissions, a removal rate of ~ 6% was achieved. In the study by Mohammed-Nour et al. (2019), a removal rate of > 90% was reached at a pH value of 12, albeit with considerable energy input, as the slurry had to be heated to 95 °C. This would immensely increase the cost of the stripping procedure, making the financial feasibility of the slurry treatment strategy highly doubtful. Additionally, to obtain optimal stripping rates, it is essential to separate the slurry into its solid and liquid phase. This causes the removal of TAN to become more efficient as the viscosity is reduced, but this may cause additional expenses. Designing the most efficient stripping process possible is therefore an indispensable criterion that must be met to make this technology financially viable and feasible. Consequently, research should be conducted that aims to maximize the TAN removal efficiency while minimizing the resource input, helping to keep costs as low as possible.

Nevertheless, the experimental results obtained in Study 1 were used to identify the optimal timing of an alkalization process in order to minimize the use of alkaline additives, which contributes to limit the operating expenses of this strategy. The study revealed that warmly stored fattening pig and dairy cow slurry was subjected to an acidification process caused by the accumulation of VFA, which led to a drop in pH within the first three weeks of storage. Consequently, a higher amount of additive to alkalize the slurry is required. As storage progresses, the VFA buffer degrades, leading to an increase in pH. However, the CO_2 produced by the degradation of VFA may act as a CO_3^{2-} buffer in the alkaline milieu, thereby increasing the overall buffer capacity of the slurry. This leads to a paradoxical situation where, despite the degradation of VFA and the subsequent increase in pH, more bases are needed to alkalize the slurry. Hence, during warm storage conditions, such as during the summer months, it is recommended to alkalize the slurry as soon as possible before the VFA buffer emerges. However, the storage period of the slurry must be long enough to ensure complete degradation of urea to NH_3 in order to achieve a high N removal rate. The degradation of urea is usually completed within 24 h in slurry (Dai & Karring, 2014; Moraes et al., 2017). Furthermore, no relevant changes in pH were observed in the coldly stored slurry. Therefore, the timing of alkalization during the winter months, when the temperature of the stored slurry is low, is less critical for resource conservation. Nevertheless, a minimum storage time of 24 h should be respected to ensure complete urea decomposition.

In conclusion, the findings revealed that reducing GHG emissions via the alkalization of slurry is highly efficient and results in a long-lasting effect, which can be achieved by reaching a pH value of 10 or even lower. However, the success of this slurry treatment strategy depends critically on enhancing the efficiency of the stripping process, which must be achieved before further research should be conducted to develop this technology.

Study 3: The second slurry treatment strategy presented in Study 3 of this dissertation investigates the use of $CaCN_2$ as an alkaline slurry additive to reduce gaseous emissions, with a particular focus on reducing CH_4 emissions. In agriculture, $CaCN_2$ has been a widely used nitrogen fertilizer for over a century, known for its soil alkalizing, nitrification-inhibiting and pest-controlling characteristics (Güthner & Mertschenk, 2006; Klasse, 1996; Shi et al., 2009). However, little is known about its effect on gaseous emissions. Therefore, Study 3 is a novel study that investigates the effects of $CaCN_2$ on CH_4, CO_2, N_2O and NH_3 emissions during 26 weeks of storage of fattening pig (FP) and dairy cattle (DC) slurry. In order to evaluate the effectiveness of $CaCN_2$, both slurry types were treated with either 300 mg kg^{-1} (CC300) or 500 mg kg^{-1} (CC500) of pure cyanamide formulated in a low-nitrate $CaCN_2$ product (Eminex®), which equals 1,24 g and 2,04 g Eminex® per kg of slurry, respectively. The treatment with $CaCN_2$ only requires a device that stirs the slurry so that $CaCN_2$ can be incorporated thoroughly. This is a considerable advantage over other slurry treatment strategies, such as slurry acidification, aeration, cooling and the already discussed alkalization, which usually require sustainable and often expensive technical changes to the storage facilities (Kupper et al., 2020; Mostafa et al., 2019; Myczko et al., 2007; Petersen et al., 2012).

Unlike Study 2, the mechanism of action of $CaCN_2$ does not rely on the alkalization of slurry because the pH increase is negligible compared to the increase required in Study 2 to efficiently reduce emissions. Nonetheless, in response to the treatment, the pH rose from 6.75 to 7.31 and 7.55 for FP/CC300 and FP/CC500, respectively, as well as from 6.84 to 6.98 and 7.11 for DC/CC300 and DC/CC500, respectively (Table 4.1). Yet, they were strong enough to significantly increase the NH_3 emissions resulting from a stripping process performed prior to the storage period (Figure 4.3). The stripping process was carried out to remove dissolved gases from the slurry so that only newly formed gases were measured during the subsequent storage period (Figure 4.1). However, this effect was observed only in the fattening pig slurry, since the pH increase was more pronounced, the slurry contained higher levels of NH_4-N and was better strippable due to its lower viscosity compared to the cattle slurry (Table 4.1). During the 26-week storage period, the $CaCN_2$ treatment induced a reversal of the alkalization effect as it caused an acidification process. This resulted in a pH drop into the acidic milieu in almost all treated samples.

However, these pH changes were not severe enough to affect methanogenesis negatively, as the study by Berg & Pazsiczki (2006) found that acidified slurry reduced CH_4 emissions starting at a pH of 6 and below. In Study 3, such low pH values were not detected (Table 4.1), making it unlikely that the induced pH reduction contributes to the overall CH_4 reduction. Nevertheless, the decrease in pH caused a shift in the ammonium-ammonia equilibrium, favoring the ionized and non-volatile form NH_4^+ over NH_3 (Arogo et al., 2006; Fricke et al., 2007). This led to a non-significant NH_3 reduction potential of 69 to 79%, regardless of the treatment concentration or the slurry type (Figure 4.3). The reason for the underlying acidification process is that $CaCN_2$ inhibits the microbial degradation of VFA during storage, leading to a pronounced accumulation of VFA (Table 4.1). High concentrations of VFA are capable of significantly lowering the pH value in slurry (Christensen & Sommer, 2013; Overmeyer et al., 2020; Sommer & Husted, 1995). It was assumed that this drop in pH might have occurred within the first two weeks of storage, as the experimental results of Study 1 revealed that during this period, the formation of VFA and its associated drop in pH had taken place.

In conclusion, the experimental results indicate that, on the one hand, increased NH_3 emissions are likely to occur during the first weeks of storage, as the addition of $CaCN_2$ leads to an increase in pH. On the other hand, it was revealed that $CaCN_2$ induced an over-accumulation of VFA, which led to an acidification of the slurry during storage and reduced NH_3 emissions when considering the whole storage period.

Furthermore, it was demonstrated that the use of $CaCN_2$ suppressed the formation of CH_4 within 45 minutes after its application (Figure 4.2) and that this effect persisted until the end of the 26-week storage period in all variants, except for the fattening pig slurry treated with 300 mg kg^{-1}. In this variant, the CH_4 formation started after 12 weeks of storage, indicating that this effect is reversible (Figure 4.4). In addition, the CO_2 formation during storage was reduced by the treatment, but not completely suppressed (Figure 4.5). Nevertheless, the total GHG emissions measured in CO_2-eq. and consisting of CH_4, CO_2 and N_2O emissions decreased by 99% for dairy cattle treated with 300 and 500 mg kg^{-1}, and by 81% and 99% for fattening pigs, respectively (Figure 4.6). The reversibility of the effect and the incomplete suppression of the CO_2 formation suggest that the emission-reducing effect of $CaCN_2$ is not based on a complete sanitization of the slurry, but rather on blocking targeted biological degradation processes during the anaerobe fermentation of organic matter (Klass, 1984; van Hullebusch et al., 2019). This was also supported by the study of Simujide et al. (2013), in which no sanitizing effect was observed during mesophilic storage of $CaCN_2$-treated slurry. The experimental results rather suggest that the microbial degradation of VFA during anaerobic fermentation is inhibited by the application of $CaCN_2$, as the concentration of almost all measured C_2–C_6 fatty acids (especially acetic acid and propionic acid) was elevated compared to the control and fattening pig slurry treated with 300 mg kg^{-1} (Table 4.1 and Table 4.2). This implies that $CaCN_2$ must have also interfered with the degradation of acetic acid/acetate to CH_4 and CO_2 during methanogenesis, which also explains the observed massive reduction of both gases during storage. This emission-reducing effect was also reported in a study earlier conducted by Schütz et al. (1989), which found that fertilizing paddy rice fields with $CaCN_2$ reduced CH_4 emission peaks that usually occur shortly after fertilizing with N-based fertilizers. However, this effect was not further discussed. Nevertheless, studies have shown that cyanamide is a potent inhibitor of the enzyme aldehyde dehydrogenase (ALDH2), which is responsible in many organisms, including the human body, for the degradation of acetaldehyde to acetate , an important step in the breakdown of ethanol (Güthner & Mertschenk, 2006; Loomis & Brien, 1983; Peachey et al., 1981). Therefore, it was assumed that $CaCN_2$ may be involved in blocking enzymes produced and used by microorganisms to degrade acetate to CH_4 and CO_2. Although Study 3 revealed that the production of CH_4 was inhibited effectively, the study was insufficient to provide conclusive explanations on the underlying mechanism of action. To clarify this aspect, specific studies need to be designed and conducted with the precise aim of answering this question.

Besides CH_4 and CO_2, the change in the N_2O concentration during storage and the total N_2O emissions after storage were measured. It was found that the NO_3^- contained in the $CaCN_2$ product used was converted to N_2O, as evidenced by increased emissions during stripping and at the beginning of storage (Figure 4.7 and Figure 4.8). However, the concentration of NO_3^--N in the product amounted to only 0.1%, which makes the enhancement of the N_2O emission negligible in comparison to the reduction of CH_4 and CO_2. Moreover, high additional emissions were not recorded during storage. However, this may also be due to the anaerobic storage condition, which inhibits the formation of N_2O (Philippe & Nicks, 2015). Nevertheless, the study conducted by Zacherl & Amberger (1984) found that DCD, a by-product of the degradation of $CaCN_2$, periodically inhibits the first step of nitrification, the breakdown processes of NH_4^+ to NO_2^-. This results in a nitrification-inhibiting effect that can last for weeks (Guiraud et al., 1989; Zacherl & Amberger, 1984). Studies showed that this effect is responsible for reduced N_2O formation in the soil when $CaCN_2$ is applied as a fertilizer, as nitrification and all subsequent N_2O-forming processes (Figure 1.3) are hindered (Ikezawa et al., 2022; Suzuki et al., 2021; Yamamoto et al., 2014, 2013). Whether the field application of slurry treated with $CaCN_2$ has a similar effect on the N_2O emission remains to be clarified by further research.

The use of $CaCN_2$ as a slurry additive is a very efficient slurry treatment technology, which has a significant advantage over most other strategies because of its high emission reduction potential of up to 99% of total GHG emissions and its easy handling. However, its potential at farm-level remains uncertain. Therefore, future studies should determine the reduction potential achievable at farm-level, taking into account different types of slurry and storage facilities. In addition, the exact mechanism of action of $CaCN_2$ and its impact on the microbiome in slurry are largely unknown. Thus, it will be crucial that future research investigates the precise mechanism of action and its impact on the microbiome to rule out the possibility of negative environmental effects when $CaCN_2$ is used as a slurry additive.

5.2 General Conclusion

This dissertation investigates and evaluates the use of alkaline additives to reduce gaseous emissions from slurry. Following this overall task, three individual scientific studies were presented and discussed within the framework of this dissertation. The first study served to determine the dynamics of buffer capacities in slurry. The other two studies present two novel slurry treatment strategies that have the potential to reduce climate and environmentally relevant trace gases almost completely. The implementation of these strategies may help reduce the carbon footprint of agriculture, thereby slowing down the progress of global warming.

The new mathematical tool discussed in this dissertation has proven to be successful, as it is able to calculate individual buffer capacity curves and, at the same time, can visualize them in more detail than previous methods could. The results revealed that the VFA buffer concentrations fluctuated considerably during warm slurry storage conditions. This had a direct impact on the pH value of the slurry, as increasing concentrations caused the pH to drop. Consequently, this increases the amount of alkaline slurry additives needed to raise the pH value. Less pronounced or no fluctuations were observed during cold slurry storage conditions. Furthermore, it was shown that the degradation of VFA buffer, which acts in an acidic milieu, increases the pH value but does not decrease the amount of alkaline additives needed for an alkalization process of slurry. The reason for this is that the degradation of the VFA buffer leads to the formation of the CO_3^{2-} buffer, which enhances the buffer capacity in the alkaline milieu. These results demonstrate that buffer systems must be interpreted as complex and interacting systems. The utilization of this mathematical tool has provided a more in-depth understanding of the characteristics of buffer systems. Interesting future research that can be derived from the use of this tool should consider its applicability to describe and investigate buffer systems in other environments, such as soils or aquatic ecosystems. This could be a useful step towards gaining deeper insights into these systems and their dynamics. However, the tool also has several limitations, such as the inability to visualize the NH_3 buffer due to its overlap with the CO_3^{2-} buffer. In addition, weak buffer systems cannot be visualized as they are overshadowed by the more dominant main systems. The optimization of the tool to remedy these limitations would certainly be an interesting field of research for future studies.

The first slurry treatment strategy is based on the concept of using alkaline additives to increase the slurry pH to reduce the emissions of GHGs. The results of the study showed that storing slurry for eight weeks at a pH of 10 reduces CH_4 and CO_2 emissions by 99% and those of N_2O by 60%, while a further pH increase did not cause higher reduction rates. However, the success of this strategy depends on achieving high N removal rates to minimize the total N content in the slurry. According to the current state of knowledge, this can only be achieved with considerable energy input. However, this calls into question the financial feasibility of this strategy. Therefore, further studies should primarily focus on the development of an efficient N removal strategy. Nevertheless, the optimal time for slurry alkalization could be identified using the results generated by the new mathematical tool. Alkalization should be carried out after complete urea decomposition but before the formation of the VFA buffer to conserve resources and achieve high N removal rates.

The second slurry treatment strategy presented involves the use of $CaCN_2$ as a slurry additive to reduce gaseous emissions. Application of $CaCN_2$ has been shown to suppress CH_4 formation within 45 min after its application and to sustain this suppression for 12 to 26 weeks, depending on the dosage, resulting in an 81 to 99% reduction in total GHG emissions. The experimental results suggest that the mechanism of action is based on the inhibition of microbial degradation of VFA and its conversion to CH_4, causing CH_4 production to collapse. This led to an over-accumulation of VFA in the slurry, resulting in its acidification. Consequently, NH_3 emissions decreased during storage, although this could not be statistically verified. Moreover, the application of $CaCN_2$ as a slurry additive has the advantage over most other treatment strategies used to reduce emissions that the emission-reducing effect sets in rapidly and is permanent. Furthermore, this strategy does not incur high financial costs as it can be implemented and operated at low expenses. Since this promising strategy is a completely new approach, further research is needed to investigate the utilization of $CaCN_2$ as a slurry additive in more detail. Therefore, future studies should firstly verify the reduction potential of $CaCN_2$ on NH_3 emissions, secondly fully elucidate the exact mechanism of action, thirdly investigate its impact on the microbiome present in slurry, fourthly evaluate its application in practical farm-level trials with different substrates and slurry storage facilities and lastly investigate the timing and dosage of application to make this process as effective and resource-efficient as possible.

5.3 References

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