AIR POLLUTION FROM ANIMAL AND MUNICIPAL WASTEWATER: ASSESSMENT OF PRODUCTION AND RELEASE OF NOXIOUS GASES

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AIR POLLUTION FROM ANIMAL AND MUNICIPAL WASTEWATER: ASSESSMENT OF PRODUCTION AND RELEASE OF NOXIOUS GASES

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Preface

This thesis is submitted as a partial fulfillment of the requirements for the Doctor of Philosophy (PhD) degree in Energy and Environmentally Efficient Technologies at the Faculty of Engineering, University of Southern Denmark.

The researches were carried out at Biomass Lab, Department of Chemical Engineering, Biotechnology and Environmental Technology, University of Southern Denmark. The work was part of the StaldTek project- “Bedre arbejdsmiljø, øget dyrevelfærd og reducerede miljøpåvirkninger i svineproduktionen ved indførelse af sensor- og robotteknologi i bygninger, inventar og udstyr” (StaldTek – Improved working environment, animal welfare and reduced environmental impact in pig production by introduction of sensors- and robot technologies in pig buildings), funded by the Danish Agency for Science, Technology and Innovation. Part of the fund was also provided by the Department of Chemical Engineering, Biotechnology and Environmental Technology, University of Southern Denmark.

This thesis is based on the work presented in two published research articles and two manuscripts to academic journals. They are entitled:


During the PhD study, I spent four months in Purdue Agricultural Air Quality Laboratory (PAAOL), Department of Agricultural and Biological Engineering, Purdue University, USA. I have acquired skills on online measurement techniques, data analysis, and data quality control. I attended three international conferences on subjects directly related to my PhD study and made two oral and one poster presentations. Additionally, I was involved in supervising MSc students on experiments, and preparing practical exercises for BSc at Department of Chemical Engineering, Biotechnology and Environmental Technology, University of Southern Denmark.

In addition, the following journal paper and conference proceeding are produced during the PhD study:


Acknowledgements

At the point of completing my Ph.D. thesis, I would like to take this opportunity to express my sincere thanks to all those who have helped me in various aspects during the study.

First and foremost I would like to express my sincere gratitude to Dr. Victoria Blanes-Vidal, my principal supervisor. Her profound knowledge, rigorous academic standards and professional personal integrity motivated me to be a better scientist and to perfect my character accomplishment. I will never have enough words to express my thankfulness and appreciation to her, for giving me the opportunity to work on this project, and for her proficient support, moral and psychological guidance, helpful advices on my writing, and great efforts on revising my papers. I am also extremely thankful to Henrik Karring, my co-supervisor, who gave me much help in my research work. I benefited from many discussions and knowledge exchanges with him on the urease activity determination, which has expanded my scientific curiosity. His comments and suggestions on the publication that we prepared together were always elaborate and instructive.

Particular appreciation also goes to Dr. Jiqin Ni at Purdue University not only for the host of my research exchange stay, but also for the inspiring suggestions on, encouragement for, and great patience with my draft revisions and questions, the immeasurable intellectual input and lively discussion throughout this project. I am also grateful cordially to Dr. Guoqiang Zhang, for his support on providing the experimental sample, and for reviewing the paper that we worked together.

My sincere thanks also go to the researchers, technicians and staff of the Dep. Chemical Eng., Biotechnology and Environmental Technology of the University of Southern Denmark for making my stay there rewarding. I value all the friends I have made in Denmark. Your friendship supports me to go through all phases of the study.

I, especially, would like to give my special thanks to my parents, Ying He (何英) and Changde Dai (代昌德), whose love and support guide me to the final destination. I also dedicate the dissertation to my husband Yulin Xiao (肖玉林) and my son Musheng Xiao (肖牧昇), they are always been there for me.
Abstract

Airborne contaminants and odor from animal manure and municipal wastewater can affect human physical and psychological health, and the environment. The estimation of gas emission rates and development of technologies to reduce the release of noxious gases from wastewater is limited by current knowledge on the production pathways of gases and the release mechanisms from various sources. The overall objective of this PhD project was to assess the production and release of noxious gases from animal manure and municipal wastewater by giving emphasis on the effects of waste management (such as, surface disturbances during storage, acidification and aeration), the hydrolysis of urea by bacteria, the waste types and wastes physicochemical characteristics.

Animal wastewater stored in under-floor deep pit is characterized by the frequent occurrence of surface liquid disturbances caused by the urine and feces that fall into the pit, and alter the chemical equilibrium of the liquid surface. A laboratory study was conducted in manure reactors with simulated in-barn storage conditions for determining the \( \text{NH}_3 \), \( \text{H}_2\text{S} \), and \( \text{CO}_2 \) emissions as affected by liquid surface disturbances.

To extend knowledge about quantification and release behavior of pollutant gases from various waste sources, \( \text{NH}_3 \), \( \text{CO}_2 \), \( \text{H}_2\text{S} \), and \( \text{SO}_2 \) emissions during storage of five types of wastewater (i.e., swine manure, dairy manure, beef manure, layer hen manure and municipal wastewater) were studied and compared.

Ammonia is a gas pollutant generated from animal manure (mixture of urine and feces) by hydrolysis of urinary urea catalyzed by microbial urease present in feces. To better understand the enzymatic process of ammonia formation in manure, experiments based on Michaelis-Menten kinetics were conducted to obtain accurate estimates of the kinetic parameters of urease activity of feces and manure from pig and cattle, and to investigate the effects of pH on animal fecal urease by individual ammonium generation rate determination at five pH levels.

Investigating the gas production and release mechanisms is important not only for estimating better gas emissions from wastewater, but also for improving gas emission abatement technologies, such as slurry acidification. Experiments of slurry aeration and acidification were conducted in animal wastewater reactors which acted as dynamic flux chambers. Ammonia, hydrogen sulfide, and carbon dioxide emissions during the storage were measured and their relations to the chemical compositions of the slurry were analyzed.
The results of this PhD study suggest that future estimation of gas emissions should consider transient-state conditions, especially in the case of H₂S, as occupational exposures and the associated health risks will be highly underestimated if the evaluation of exposures to H₂S is based on emissions from slurries stored under undisturbed conditions. The convective mass transfer governed NH₃ release, while bubble-release was dominant in the releases of CO₂, H₂S, and SO₂. The physicochemical characteristics of different types of wastes (e.g., the total nitrogen, total ammoniacal nitrogen, dry matter, and pH) had great influence on the releases of NH₃, CO₂, H₂S, and SO₂. The investigation of kinetic parameter showed that the maximum urease activity for pig feces is at around pH 7, while that for the cattle feces is around pH 8, indicating that the predominant fecal ureolytic bacteria species differ between the animal species. The study on urease activity determination in animal feces contributed to a better understanding of the urea hydrolysis process in manure, and provides the basis for further studies of enzymatic degradation process in manure, and the obtained enzyme-kinetic parameters can be utilized in prediction modeling of ammonia production rates and thus ammonia release from animal productions. The results of the acidification study showed that slurry acidification can reduce ammonia emissions by 50-77% and has no significant effect on CO₂ and H₂S emissions during treatment and subsequent storage.
Resumé


Spildevand fra husdyrsproduktion opbevares gyllekanaler under staldgulvet og er kendeteget ved hyppige forstyrrelser af overfladevandet forårsaget af urin og afføring, som falde i graven/kanalerne, og ændre den kemiske balance i væskeoverfladen. Et laboratorieforsøg blev gennemført i reaktorer med husdyrgødning under betingelser svarende til dem i en dyrestald. Der blev i denne forbindelse målt udledning af NH₃, H₂S og CO₂-, som påvirkes af væskeoverfladen forstyrrelser.

For at kvantificere og få en bedre forståelse af frigivelsen af forurenende gasser fra forskellige affaldskilder, blev emissionen af NH₃, CO₂, H₂S og SO₂-emissioner under opbevaring af fem typer af spildevand (dvs. gødning fra svin, gødning fra malkekvæg, gødning fra kødkvæg, gødning fra æglæggende høns og kommunalt spildevand ) undersøgt og sammenlignet.


Undersøgelser af gasproduktionen og frigivelsesmekanismer er vigtige ikke kun for at estimere bedre gasmissioner fra spildevand, men også for at forbedre teknologier til emissionsreduktion, såsom gylleforsuring. Forsøg med belufning og forsuring af gylle blev udført i reaktorer med husdyrsspildevand, som fungerede som dynamiske flux-kamre. Ammoniak, hydrogensulfid, og kuldioxidemissionerne blev målt under lagring, og deres
relationer til de kemiske sammensætninger af gyllen blev analyseret.

Resultaterne af dette ph.d.-studie tyder på, at den fremtidige vurdering af drivhusgasser bør baseres på ”transient-state” betingelser, især i tilfælde af H\textsubscript{2}S, idet den erhvervsmæssige eksponering og de tilknyttede sundhedsrisici vil være meget undervurderet, hvis evalueringen af H\textsubscript{2}S er baseret på emissioner fra uberørte slam. Den konvektive massetransport regulerede NH\textsubscript{3} udslip, mens bobler var dominerende i udslip af CO\textsubscript{2}, H\textsubscript{2}S, og SO\textsubscript{2}. De fysisk-kemiske egenskaber ved forskellige typer af affald (f.eks den samlede mængde kvælstof, total ammoniumkvælstof, tørstof og pH) havde stor indflydelse på udsippet af NH\textsubscript{3}, CO\textsubscript{2}, H\textsubscript{2}S, og SO\textsubscript{2}. Undersøgelsen af de kinetiske parametre viste, at afføring fra grise har den maksimale urease-aktivitet ved omkring pH 7, mens det for afføring fra kvæg er omkring pH 8. Dette antyder, at de fremherskende fækale ureolytic bakteriearter er forskellige mellem dyrearterne. Undersøgelsen af urease-aktiviteten i dyrs afføring har bidraget til en bedre forståelse af hydrolyse-processen for urinstof i gyllen, og danner grundlag for yderligere undersøgelser af den enzymatiske nedbrydningsproces i husdyrgødning. De opnåede enzym-kinetiske parametre kan anvendes i forudsigelsesmodellering af ammoniakproduktion og dermed ammoniak fordampning fra dyreprodukter. Resultaterne fra undersøgelsen af forsuret gyllen viste, at gylleforsuring kan reducere ammoniakfordampningen med 50-77%, mens det ikke har en signifikant effekt på CO\textsubscript{2} og H\textsubscript{2}S-emissioner under behandling og efterfølgende opbevaring.
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1. Introduction

Animal production is a source of odor and airborne contaminants that cause annoyance on human physical and psychological health, and environmental issues (O’Neill and Phillips, 1992; Blanes-Vidal et al., 2009b; Blanes-Vidal et al., 2012b; Blanes-Vidal, 2015). The major aerial pollutants released from animal wastes are acidifying gases (e.g., ammonia, NH$_3$), greenhouse gases (e.g., carbon dioxide, CO$_2$; methane, CH$_4$) and odorous gases (e.g., hydrogen sulfide, H$_2$S). Quantifying the releases of gas pollutants, assessing the effect of gases release to ecosystems and evaluating the health risks posed by noxious gases are necessary since they contribute to the development of techniques on farm operation and gas emission mitigation.

The main factors that cause variations on pollutant gas releases from animal wastewater are the waste types and source, housing types and manure management, and treatment (Anderson et al., 2003; Sommer et al., 2006). The first paper of this thesis (Paper I) evaluated the air pollutant emissions from animal wastewater with frequently surface disturbance, which normally happens when animal excretion drops into deep pit. The second paper obtained the knowledge on emissions of NH$_3$, CO$_2$, H$_2$S and SO$_2$ from five types of wastewaters in a laboratory test conducted using dynamic manure reactors, and presented the relationship between gaseous emissions and the chemical characteristics (Paper II). A better understanding of ammonia production mechanisms have been achieved by determining urease activity from cattle and pig feces in relation to pH (Paper III). The effect of lowering manure pH (e.g., acidification by strong acids) on NH$_3$ emission mitigation has been known for many years, but its implications on the emission of other compounds, such as CO$_2$ and H$_2$S, has not been fully documented in the literature. Paper IV studied the emissions of NH$_3$, CO$_2$ and H$_2$S during and after slurry acidification treatment, and evaluated the effect of pH, mixing and aeration on gas release.

1.1. Objectives

The objectives of the present PhD were: (i) to study the effects of waste management (such as, surface disturbances during storage, acidification and aeration) on gaseous emissions from wastes, (ii) to study the hydrolysis of urea by bacteria in the slurry and identify important factors affecting NH$_3$ production, and (iii) to study gaseous
emissions from various types of animal manure and municipal wastewater in relation to the physicochemical characteristics of wastes.

The PhD-thesis is trying to answer the following questions:
1. How are gaseous emissions affected by waste surface disturbances?
2. How are the enzyme activity affected by manure types and pH?
3. How are gaseous emissions affected by the aeration and acidification processes of animal manure?
4. How the waste physicochemical characteristics affect the gaseous release from various waste types?

1.2. Outline of this thesis

The present PhD-thesis is composed of two parts. Firstly, an overview of the fundamental knowledge regarding gaseous emission from animal waste is provided, mainly focusing on the production of gases and release mechanisms, and the manure management related to gas pollutants abatement technologies. The second part consists of four experimental studies presented as four scientific papers (Paper I – IV, listed in Preface). The four papers can be found in the appendix with full length. First, the effect of slurry surface disturbance caused by animal excretion on emission of ammonia, carbon dioxide, and hydrogen sulfide was studied (Paper I). A second investigation was carried to measure gas pollutants from four animal manure (swine manure, dairy manure, beef manure and layer hen manure) and municipal wastewater (Paper II). To further understand the production of ammonia from animal manure, a determination of urease activity in faeces and fresh manure was conducted (Paper III). Finally, paper IV investigated the emission of ammonia, carbon dioxide and hydrogen sulfide during and after slurry acidification treatment, and evaluated the effect of pH, mixing and aeration on gas release.

2. A general view of gases emitted from animal wastes

Animal wastes, named slurry or manure in most publications, are important source of atmospheric pollutants. More than hundreds of compounds have been identified in animal production facilities, including nitrogen containing compounds, sulfur compounds, carboxylic acids, amines, amides, alcohols, aldehydes, aromatics, ethers, esters, halogenated hydrocarbons, hydrocarbons, ketones, phenols and indoles as
classified by the chemical characteristics (O’Neill and Phillips, 1992; Schiffman et al., 2001; Blanes-Vidal et al., 2009a). Among these compounds, ammonia (NH₃), hydrogen sulfide (H₂S), carbon dioxide (CO₂) and methane (CH₄) are considered to be the major gases emitted from animal wastes to the atmosphere (Table 1) (Muehling, 1970; Sommer et al., 2007). Exposure to these compounds can result in health problems both for animals and humans (e.g., bronchitis, asthma and chronic obstructive pulmonary disease) (Schiffman and Williams, 2005; Werth et al., 2014). With the development of intensive swine production in U.S., Europe and some Asian countries, mechanically ventilated swine buildings equipped with slatted floor and a slurry pit are widely utilized in commercial farms. Cattle houses often have natural ventilation and floor systems of either under-floor pit for manure connection or solid floor with bedding material and automated scrapers (Sommer et al., 2006). In these swine and cattle production facilities, the main sources of gas pollutants are faeces and urine excreted on slats and concrete surfaces, and slurry (i.e., mixture of faeces and urine) in the under-floor pit (Cortus et al., 2010). Besides, the other major sources of gas pollutants are manure storage/treatment and land application. Generally, the most important factors that influence gas pollutant emissions from animal production systems are: (1) animal species, (2) animal mass and phase of production, (3) house type and management, (4) manure storage and treatment, (5) land application, (6) feed composition, and (7) environmental conditions (Figure 1) (Sommer et al., 2006; Leneman et al., 1998). The increased clustering of livestock production and growth of concentrated animal feeding operations can cause excess animal manure production and gas pollutants emissions, which led to growing environmental and public health problems. This will be discussed in the following sections.
<table>
<thead>
<tr>
<th>Gas</th>
<th>Color</th>
<th>Density (^{[a]})</th>
<th>Solubility</th>
<th>Odor</th>
<th>Origin Source</th>
<th>Reported Concentration in Animal Houses</th>
<th>Recommended Concentration for Workers and Animals, ppm</th>
<th>Conditions for Production (P) or Consumption (C)</th>
<th>Some Important General Reactions or equilibria (^{[e]})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia (NH(_3))</td>
<td>No</td>
<td>0.77</td>
<td>0.77 to 0.77</td>
<td>Highly soluble</td>
<td>Manure</td>
<td>15 - 35 ppm; 1 - 100 ppm</td>
<td>50</td>
<td>NA(^{[f]})</td>
<td>P: Anaerobic OM → NH(_3) (slow) Urea → NH(_4^+) NH(_3) ↔ NH(_4^+), pKa ≈ 9.2</td>
</tr>
<tr>
<td>Hydrogen sulfide (H(_2)S)</td>
<td>No</td>
<td>1.54</td>
<td>0.0001 to 0.01</td>
<td>Offensive</td>
<td>Manure</td>
<td>0.09 ppm; 2 mg/m(^3); 0.09 - 0.28 ppm</td>
<td>10</td>
<td>0.1-0.2</td>
<td>P: Anaerobic OM → H(_2)S SO(_4^{2-}) + OM → H(_2)S H(_2)S → HS(^-), pKa ≈ 7.0</td>
</tr>
<tr>
<td>Carbon dioxide (CO(_2))</td>
<td>No</td>
<td>1.98</td>
<td>3.1×10(^{-2}) to 4.5×10(^{-2})</td>
<td>Slightly soluble</td>
<td>Manure</td>
<td>500-3000 ppm; 350-450 ppm</td>
<td>5000</td>
<td>-3000</td>
<td>P: Anaerobic or aerobic OM → CO(_2) CO(_2) ↔ H(_2)CO(_3), HCO(_3^-)</td>
</tr>
<tr>
<td>Methane (CH(_4))</td>
<td>No</td>
<td>0.72</td>
<td>9.7×10(^{-3}) to 9.2×10(^{-3})</td>
<td>Very slightly soluble</td>
<td>Manure</td>
<td>0.004 mg/m(^3); 2.2 ppm</td>
<td>NA(^{[f]})</td>
<td>NA(^{[f]})</td>
<td>P: Anaerobic OM → acetate → CH(_4) CO(_2) → CH(_4)</td>
</tr>
</tbody>
</table>

\(^{[a]}\) At 0 °C, kg/m\(^3\). Density of air: 1.29 at 0 °C.

\(^{[b]}\) Henry's Law constant for solubility in water at 298.15 K, mol kg\(^{-1}\) bar\(^{-1}\).

\(^{[c]}\) MAK, maximum concentration in working location (for man, 8 hours/day).

\(^{[d]}\) MIK, maximum concentration in animal location (for mammals, 24 hours/day).

\(^{[e]}\) OM = Organic Matter; Org-C = Organic Carbon; Org-S = Organic sulfur.

\(^{[f]}\) NA, not available.
2.1. Ammonia emissions from livestock production and related issues

2.1.1. Environmental impacts and health concern

Ammonia is a colorless alkaline gaseous compound that is highly soluble in water and is able to react with oxides of nitrogen to form ammonium nitrate, and further constitute particulate matter which will contribute to acidification of ecosystem and health implications.

Livestock production has been identified as the largest source of NH₃ emissions, accounting for 54000 kt of the 21600 kt NH₃-N arising globally (Table 2) (Olivier et al., 1998; Atia et al., 2014). Studies have also reported that animal buildings, stored animal manure and manure application contributes to 55% of the global NH₃ emissions, 80% of the total NH₃ emissions in Europe (Webb et al., 2005). The estimated NH₃ emissions
from UK agriculture for 2009 was 231.8 kt NH₃, representing a 6.2 kt increase from the previously estimate for 1997 (Misselbrook et al., 2000; Misselbrook et al., 2010). The total French NH₃ emissions from livestock production were estimated at 382 kt N in 2003 (Gac et al., 2007). Hyde et al. (2003) estimated total emissions from Irish agriculture were 89.9 and 91.8 kt NH₃-N for 1991 and 2010, respectively. The total Danish emissions for 2007 was 65 kt TAN (NH₄⁺-N), with agriculture accounting for nearly 97% (Gyldenkærne and Mikkelsen, 2007; Jacobsen, 2011). While, emissions from animal manure accounted for 76% of agricultural emissions in Denmark (Hutchings et al., 2001). In Switzerland, the total NH₃ emissions was 44.6 kt N in 2000, with agriculture contributing 93%. Emissions from livestock production and manure management accounted for 88% of Swiss agriculture NH₃ emissions (Reidy et al., 2008).

According to Gao et al. (2013), in 2009, NH₃ emissions from pigs, layers, beef and dairy cattle and broiler production systems in China were 1230, 520, 240, 210 and 90 kt, respectively. As reported by U.S. Environmental Protection Agency (USEPA, 2014), livestock production is the largest NH₃ emission source category in the United States, with waste from livestock responsible for about 3530 kt of ammonia in 2011. Bittman et al. (2014) reported that the total annual Canadian NH₃-N ammonia emission has been estimated as 482 kt in 2001-2003, 80% of these ammonium nitrogen is from agricultural activities. Approximately 82% of the total agricultural ammonia is from livestock production in Canada. A large amount of NH₃ emitted from livestock production deposits into water and soil, which causes significant effect on the environment. Besides, ammonia is considered as an important substance that causes health hazard due to its corrosion to eyes, skin, and lungs. The normal concentration of NH₃ can reach 100 ppm in some cases according to Table 1. With a low odor threshold (20 ppm), ammonia can be noticed with pungent smell in animal houses. The U.S. National Institute for Occupational Safety and Health (NIOSH) recommends a maximum exposure limit (REL) of NH₃ concentration of 25 ppm. High concentrations (above recommended thresholds) of NH₃ inside animal buildings pose serious potential health risk (e.g., chronic respiratory diseases such as asthma and bronchitis) to human and animals. An exposure to NH₃ concentration of 300 ppm (parts per million) is immediately dangerous to human/animal life and health (U.S.DepartmentofLabor, 2014a).
2.1.2. Ammonia emission factors and rates

Some of the main factors that can affect NH₃ emissions from animal wastes are: 1) the type, quantity and weight/age of animals; 2) housing type and management; 3) manure storage facilities and treatment; 4) land application techniques; 5) N excretion rates per animals; 6) wastewater pH (Figure 1) (Muck, 1982; Aarnink et al., 1997; Groot Koerkamp et al., 1998; Arogo et al., 2003; Ivanova-Peneva et al., 2008; Dai and Blanes-Vidal, 2013). The housing system can be defined as a combination of the typical housing system for the specific animal type, the waste treatment and/or removal system, and the storage system. Groot Koerkamp et al (1998) compared ammonia emissions from various housing systems for cattle, pigs and poultry in England, The Netherlands, Denmark and Germany. The results revealed that emissions from each animal category in different countries varied, and emissions from various animal categories within the same country were also different. The laboratory study of the present PhD thesis aiming at investigating the gas release from five types of wastes during storage (Paper II) also indicated a variation in the daily mean NH₃, CO₂, H₂S and SO₂ emissions for the different wastes.

Ammonia is a weakly basic compound emitted from wastewater, thereby its volatilization is largely pH dependent. Other factors that affect NH₃ emissions are waste composition (e.g., nitrogen content), waste temperature, air temperature and air velocity according to Arogo et al. (1999a). Bajwa et al. (2006) reported that NH₃ flux increases exponentially with lagoon wastewater pH and temperature, while a linear increase was
observed when the total ammoniacal nitrogen (TAN) increased. This result has also been confirmed by Paper II.

Table 3. Ammonia emissions rates from animal waste storage.

<table>
<thead>
<tr>
<th>Storage type</th>
<th>Storage time</th>
<th>Emissions (g m⁻² day⁻¹)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stored swine manure</td>
<td>112d</td>
<td>4.35</td>
<td>Hobbs et al., 1999</td>
</tr>
<tr>
<td>Stored original swine manure with DM = 6.71%</td>
<td>30d</td>
<td>6.72</td>
<td>Ni et al., 2010</td>
</tr>
<tr>
<td>Stored diluted swine manure with DM = 3.73%</td>
<td>30d</td>
<td>4.78</td>
<td>Ni et al., 2010</td>
</tr>
<tr>
<td>Stored swine manure without any treatment</td>
<td>155d</td>
<td>6.51</td>
<td>Dai and Blanes-Vidal, 2013</td>
</tr>
<tr>
<td>Stored swine manure acidified to pH=5.5</td>
<td>155d</td>
<td>0.71-0.79</td>
<td>Dai and Blanes-Vidal, 2013</td>
</tr>
<tr>
<td>Stored swine manure acidified to pH=6.0</td>
<td>155d</td>
<td>0.96-1.08</td>
<td>Dai and Blanes-Vidal, 2013</td>
</tr>
<tr>
<td>Stored swine manure acidified to pH=6.5</td>
<td>155d</td>
<td>1.08-1.15</td>
<td>Dai and Blanes-Vidal, 2013</td>
</tr>
<tr>
<td>Deep pit, pull plug</td>
<td>August and September 1997</td>
<td>57</td>
<td>Zahn et al., 2001</td>
</tr>
<tr>
<td>Earthen, concrete-lined, steel tank</td>
<td>1997</td>
<td>144</td>
<td>Zahn et al., 2001</td>
</tr>
<tr>
<td>Lagoon without photosynthetic bloom</td>
<td>94</td>
<td></td>
<td>Zahn et al., 2001</td>
</tr>
<tr>
<td>Lagoon with photosynthetic bloom</td>
<td>77</td>
<td></td>
<td>Zahn et al., 2001</td>
</tr>
<tr>
<td>Dairy manure stored in 200-L barrels</td>
<td>77d</td>
<td>2.2-3.6</td>
<td>Aguerre et al., 2012</td>
</tr>
</tbody>
</table>

As presented in Table 3, NH₃ emission rates reveal differences between laboratory studies and the field measurement. Therefore, when estimating NH₃ emission factors, laboratory conditions may not fully represent field conditions.

2.2. Carbon dioxide from animal husbandry and related issues

Carbon dioxide (CO₂) is an inodorous and colourless gas that is slightly soluble in water. There are two sources of CO₂ in animal houses: animal exhalation and release from manure. The normal concentrations of CO₂ in animal houses range from 350 ppm to 4350 ppm. The National Institute for Occupational Safety and Health (NIOSH) recommended maximum concentration for worker respiration exposure is about 5000 ppm, while for animals exposure, the limit is 3000 ppm (Table 1).
2.2.1. Exhalation by animals

Animal exhalation is considered to be the major source of CO\textsubscript{2} production, especially for herbivore livestock (i.e., cattle). The factors determining CO\textsubscript{2} from animal exhalation are animal species, the body weight (BW) (substitute for the age and rearing stages), the production level (e.g., milk production for dairy), and the feed compositions (Philippe and Nicks, 2014). Philippe and Nicks (2014) proposed the following equation for estimating CO\textsubscript{2} emission (E-CO\textsubscript{2}) from fattening pigs exhalation (Eqn. (1)).

\[
E\text{-}CO_{2,\text{pig}} = 0.136 \times BW^{0.573}
\]

Where E-CO\textsubscript{2, pig} is the CO\textsubscript{2} emission rate, kg day\textsuperscript{-1}; BW is the pig body weight, kg.

2.2.2. Animal manure source

Emissions of CO\textsubscript{2} from animal manure were believed to be negligible or to account for less than 5% of the total production of CO\textsubscript{2} by some researchers (Anderson et al., 1987; Dong et al., 2007). However, according to Pedersen et al. (2008), CO\textsubscript{2} release from animal manure should be considered to account for 10% of respiratory CO\textsubscript{2}. Ni et al. (1999b) reported that CO\textsubscript{2} emission from manure was around 40% of the CO\textsubscript{2} exhalation rate based on measurements conducted in a commercial fattening house. Some studies have reported CO\textsubscript{2} emission rates from stored animal wastes, indicating that manure disturbance or treatment have significant influence on its release behaviors (Table 4).

<table>
<thead>
<tr>
<th>Storage type</th>
<th>Storage time</th>
<th>Emissions (g m\textsuperscript{-2} day\textsuperscript{-1})</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stored swine manure</td>
<td>112d</td>
<td>626</td>
<td>Hobbs et al., 1999</td>
</tr>
<tr>
<td>Dairy manure stored in 200-L barrels</td>
<td>77d</td>
<td>345-388</td>
<td>Aguerre et al., 2012</td>
</tr>
<tr>
<td>Stored original swine manure with DM=6.71%</td>
<td>30d</td>
<td>223</td>
<td>Ni et al., 2010</td>
</tr>
<tr>
<td>Stored diluted swine manure with DM=3.73%</td>
<td>30d</td>
<td>126</td>
<td>Ni et al., 2010</td>
</tr>
<tr>
<td>Stored swine manure without any treatment</td>
<td>155d</td>
<td>206</td>
<td>Dai and Blanes-Vidal, 2013</td>
</tr>
<tr>
<td>Stored swine manure acidified to pH=5.5</td>
<td>155d</td>
<td>1571</td>
<td>Dai and Blanes-Vidal, 2013</td>
</tr>
<tr>
<td>Stored swine manure acidified to pH=6.0</td>
<td>155d</td>
<td>1600-1701</td>
<td>Dai and Blanes-Vidal, 2013</td>
</tr>
<tr>
<td>Stored swine manure acidified to pH=6.5</td>
<td>155d</td>
<td>1240-1453</td>
<td>Dai and Blanes-Vidal, 2013</td>
</tr>
</tbody>
</table>
2.3. Volatile sulfur compounds released from animal wastes and related issues

Volatile sulfur compounds (VSCs) are a major class of chemicals associated with odor from livestock production (Trabue et al., 2008). The emission of VSCs from animal wastes gives rise to several health and environmental problems. These compounds usually give off unpleasant smells with trace levels due to their low odor thresholds, which can create objectionable situations for farm workers and residents who live close to emitting sources (e.g., animal farms and waste treatment plants). Furthermore, concentrations of VSCs as low as parts per billion by volume (ppbv) levels can be deleterious to human health, causing nausea, headaches, eye irritation, respiratory symptoms, and neuropsychological symptoms (Andersson et al., 2004). Volatile sulfur compounds comprised hydrogen sulfide (H$_2$S), methanethiol (CH$_3$S), carbon disulfide (CS$_2$), dimethyl sulfide (DMS), dimethyl disulfide (DMDS), and dimethyl trisulfide (DMTS) (Gutarowska et al., 2014). Banwart and Bremner (1975) identified that the major VSC from animal waste was H$_2$S. Trabue et al. (2008) confirmed that H$_2$S was the dominant odorous VSC detected at all swine facilities. Blanes-Vidal et al. (2009a) concluded that concentration of H$_2$S accounted for 68% of the variation in odor concentrations (OC) above the stirred slurry samples.

2.3.1. Hydrogen sulfide emissions

Hydrogen sulfide is soluble in water and heavier than air (Table 1). It is a poisonous gas that can cause death of animals and human (Zaba et al., 2011). Hydrogen sulfide is produced when sulfate and sulfur-containing organic compounds in the animal waste are catabolized by sulfate-reducing bacteria (SRB) under anaerobic conditions (Arogo et al., 2000). In animal waste, sulfate is normally from the water supply which is used for animal drinking and facility cleaning. Arogo et al. (2000) cited AWWA (1989) who reported that the sulfate concentration in water supplies in the United States varied from 0 to 770 mg/L. The organic sulfur concentration in the animal waste depends on the feed composition and animal metabolism (Arogo et al., 2000).

The odor detection threshold of H$_2$S is 0.005 ppm, and its smell is usually described as “rotten eggs”. The recommended maximum concentration for animals and farm operators is 10 ppm (Table 1) (Muehling, 1970). According to U.S. Department of labor (2014b), prolonged exposure to H$_2$S concentration of 20 ppm may cause possible
fatigue, loss of appetite, headache, irritability, poor memory and dizziness; while exposure to 1000 ppm will cause instant death (Table 5).

Table 5. Short-term (also called acute) symptoms and effects of exposure to H₂S (U.S. Department of labor, 2014b).

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Symptoms/Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00011-0.00033</td>
<td>Background concentrations</td>
</tr>
<tr>
<td>0.01-1.5</td>
<td>Odor threshold (when rotten egg smell is first noticeable to some). Odor becomes more offensive at 3-5 ppm. Above 30 ppm, odor described as sweet or sickeningly sweet.</td>
</tr>
<tr>
<td>2-5</td>
<td>Prolonged exposure may cause nausea, tearing of the eyes, headaches or insomnia. Airway problems (bronchial constriction) in some asthma patients.</td>
</tr>
<tr>
<td>20</td>
<td>Possible fatigue, loss of appetite, headache, irritability, poor memory, dizziness.</td>
</tr>
<tr>
<td>50-100</td>
<td>Slight conjunctivitis (&quot;gas eye&quot;) and respiratory tract irritation after 1 hour. May cause digestive upset and loss of appetite.</td>
</tr>
<tr>
<td>100</td>
<td>Coughing, eye irritation, loss of smell after 2-15 minutes (olfactory fatigue). Altered breathing, drowsiness after 15-30 minutes. Throat irritation after 1 hour. Gradual increase in severity of symptoms over several hours. Death may occur after 48 hours.</td>
</tr>
<tr>
<td>100-150</td>
<td>Loss of smell (olfactory fatigue or paralysis).</td>
</tr>
<tr>
<td>200-300</td>
<td>Marked conjunctivitis and respiratory tract irritation after 1 hour. Pulmonary edema may occur from prolonged exposure.</td>
</tr>
<tr>
<td>500-700</td>
<td>Staggering, collapse in 5 minutes. Serious damage to the eyes in 30 minutes. Death after 30-60 minutes.</td>
</tr>
<tr>
<td>700-1000</td>
<td>Rapid unconsciousness, &quot;knockdown&quot; or immediate collapse within 1 to 2 breaths, respiratory arrest, death within minutes.</td>
</tr>
<tr>
<td>1000-2000</td>
<td>Nearly instant death</td>
</tr>
</tbody>
</table>

The main sources of H₂S are floors with slat or bedding materials, indoor slurry pits, outdoor storage tanks and treatment plants (Figure 1) (Chénard et al., 2003). As reported by Hobbs et al. (1999), sulfide emissions from finishing swine slurry during 112 days storage were estimated over 393 kt per annum. Generally, H₂S concentration in animal buildings is under 5 ppm as reported by different studies (Table 6) (Avery et al., 1975; Ni et al., 2002; Zhao et al., 2005; Heber et al., 2006; Zhao et al., 2007; Ni et al., 2012; Rumsey et al., 2014). However, Patni and Clarke (1991) and Blanes-Vidal et al. (2012a) found that mixing of manure caused an increase of H₂S concentration up to 150-205 ppm close to the surface, which is over the limit of occupational exposures and would result in health risks for humans and animals (Table 6 and Table 7).
### Table 6. Hydrogen sulfide concentrations from animal waste storage.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sources and notes</th>
<th>Periods</th>
<th>Hydrogen sulfide concentration (ppm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Range</td>
<td></td>
</tr>
<tr>
<td>Farrowing sows</td>
<td>Fresh manure</td>
<td></td>
<td>205.4</td>
<td>(Blanes-Vidal et al., 2009b)</td>
</tr>
<tr>
<td>Farrowing sows</td>
<td>Stored manure</td>
<td>60d</td>
<td>83.9</td>
<td>(Blanes-Vidal et al., 2009b)</td>
</tr>
<tr>
<td>Finishing pigs</td>
<td>Fresh manure</td>
<td></td>
<td>148.7</td>
<td>(Blanes-Vidal et al., 2009b)</td>
</tr>
<tr>
<td>Finishing swines</td>
<td>Stored manure</td>
<td>60d</td>
<td>23.9</td>
<td>(Blanes-Vidal et al., 2009b)</td>
</tr>
<tr>
<td>Weaning pigs</td>
<td>Fresh manure</td>
<td></td>
<td>24.7</td>
<td>(Blanes-Vidal et al., 2009b)</td>
</tr>
<tr>
<td>Weaning pigs</td>
<td>Stored manure</td>
<td>60d</td>
<td>24.1</td>
<td>(Blanes-Vidal et al., 2009b)</td>
</tr>
<tr>
<td>Swine</td>
<td>Deep pit</td>
<td></td>
<td>0.163-1.96</td>
<td>(Clanton and Schmidt, 2000)</td>
</tr>
<tr>
<td>Swine and dairy</td>
<td>Stored manure without addition</td>
<td>63d</td>
<td>0.233</td>
<td>0.004-1.3</td>
</tr>
<tr>
<td>Swine and dairy</td>
<td>Stored manure with two additions weekly</td>
<td>56d</td>
<td>0.889</td>
<td>0.007-2.82</td>
</tr>
<tr>
<td>Swine and dairy</td>
<td>Field study</td>
<td></td>
<td>0.296</td>
<td>0.004-1.96</td>
</tr>
<tr>
<td>Swine</td>
<td>Stored original manure with DM = 6.71%</td>
<td>30d</td>
<td>0.1949</td>
<td>0.072-0.2716</td>
</tr>
<tr>
<td>Swine</td>
<td>Stored diluted manure with DM = 3.73%</td>
<td>30d</td>
<td>0.1415</td>
<td>0.0915-0.1733</td>
</tr>
<tr>
<td>Swine</td>
<td>Housing</td>
<td></td>
<td>0.624</td>
<td>0.120-2.174</td>
</tr>
</tbody>
</table>

### Table 7. Hydrogen sulfide emissions rates from animal waste storage.

<table>
<thead>
<tr>
<th>Storage type</th>
<th>Storage time</th>
<th>Emissions (g m⁻² day⁻¹)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep pit, pull plug</td>
<td>August and September 1997</td>
<td>0.32</td>
<td>Zahn et al., 2001</td>
</tr>
<tr>
<td>Earthen, concrete-lined, steel tank</td>
<td></td>
<td>0.95</td>
<td>Zahn et al., 2001</td>
</tr>
<tr>
<td>Lagoon without photosynthetic bloom</td>
<td></td>
<td>0.28</td>
<td>Zahn et al., 2001</td>
</tr>
<tr>
<td>Lagoon with photosynthetic bloom</td>
<td></td>
<td>0.21</td>
<td>Zahn et al., 2001</td>
</tr>
<tr>
<td>Stored swine manure</td>
<td>112d</td>
<td>66.6</td>
<td>Hobbs et al., 1999</td>
</tr>
<tr>
<td>Stored original swine manure with DM = 6.71% (30d)Swine</td>
<td>30d</td>
<td>0.03</td>
<td>Ni et al., 2010</td>
</tr>
<tr>
<td>Stored diluted swine manure with DM = 3.73% (30d)Swine</td>
<td>30d</td>
<td>0.02</td>
<td>Ni et al., 2010</td>
</tr>
<tr>
<td>Stored swine manure without any treatment</td>
<td>155d</td>
<td>4</td>
<td>Dai and Blanes-Vidal, 2013</td>
</tr>
<tr>
<td>Stored swine manure acidified to pH=5.5</td>
<td>155d</td>
<td>98-156</td>
<td>Dai and Blanes-Vidal, 2013</td>
</tr>
<tr>
<td>Stored swine manure acidified to pH=6.0</td>
<td>155d</td>
<td>346-386</td>
<td>Dai and Blanes-Vidal, 2013</td>
</tr>
<tr>
<td>Stored swine manure acidified to pH=6.5</td>
<td>155d</td>
<td>225-281</td>
<td>Dai and Blanes-Vidal, 2013</td>
</tr>
</tbody>
</table>
3. Manure biochemistry reactions related to gases production

3.1. Degradation of nitrogenous components in animal wastewaster

Ammonia is a main product from the degradation of nitrogenous compounds. The biochemical degradation processes of NH$_3$ production can be simplified as three reactions: (1) urea hydrolysis, (2) uric acid decomposition, and (3) undigested protein mineralization.

Urea hydrolysis:

\[
\text{CO(NH}_2\text{)}_2 + \text{H}_2\text{O} \xrightarrow{\text{urease}} \text{CO}_2 + 2\text{NH}_3
\]  

(2)

Aerobic decomposition of uric acid:

\[
\text{C}_5\text{H}_4\text{O}_3\text{N}_4 + 1.5\text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{uricase}} 5\text{CO}_2 + 4\text{NH}_3
\]  

(3)

Mineralization:

undigested protein (bacterial degradation) $\rightarrow$ NH$_3$

(4)

The source of NH$_3$ emission from livestock production is total ammonium nitrogen (TAN) in the excreta. Approximately 50% of the N excreted by pigs, cattle, and sheep is in the urine, and 65%-85% of urine-N is in the form of urea (Sommer et al., 2006). Urea is rapidly hydrolyzed to ammonium carbonate ((NH$_4$)$_2$CO$_3$) by the catalysis of enzyme urease, thus providing the major source of NH$_3$ (Eqn. (2)). Urease is widespread in animal faeces and soil in cattle feedlots and exercise areas, thereby, hydrolysis of urea is usually initiated when urine and faeces get contacted on the slatted floor or pasture, and this process usually completes within 30 to 80 h (Paper III). The degradation process of urea (Eqn (2)) follows the law of Michaelis-Menten and is positively influenced by the urease activity, pH and temperature (Muck, 1982). The optimum pH for urease activity is around seven in pig feces and closer to eight in cattle feces (Paper III). A main source of TAN from poultry is faeces, which mainly contains uric acid and undigested proteins as N constituent. The aerobic decomposition of uric acid is catalyzed by enzyme uricase presented in microorganisms (Eqn (3)). The degradation of uric acid and proteins is positively affected by temperature, pH and moisture content. When the temperature is above 30°C, the organic matter degradation is known as composting under aerobic conditions. In this process, nitrogen in organic
compound can be released as ammonia. Under anaerobic conditions (e.g. in stored animal slurry), many gaseous components can be produced, e.g. ammonia (NH₃), methane (CH₄), carbon dioxide (CO₂), hydrogen sulfide (H₂S) and fatty acids (Sommer et al., 2007).

Painter (1970) reviewed the main biological processes involving the transformation of inorganic nitrogen, which is shown diagrammatically in Figure 2. There are three main processes. Firstly, the fixation (or called immobilization) of elementary nitrogen (e.g. N₂) leads transformation of inorganic N to ammonia production in aerobic or anaerobic conditions. Mineralization of organic nitrogen can increase total ammonium nitrogen (TAN) in animal wastewater, thereby increases production of ammonia (Eqn. (4)). As reported by Sommer (2006), the balance of immobilization and mineralization on TAN concentration in wastewater depended on the ratio of carbon and nitrogen content. Secondly, the process of nitrification results in converting of ammonia/ammonium to nitrite (NO₂⁻) and nitrate (NO₃⁻) (Eqn. (5)). Thirdly, assimilation synthesis of N in nitrate can produce NH₃, while dissimilation of O in nitrate can produce nitrite (NO₂⁻), nitric oxide (NO), nitrous oxide (N₂O) or dinitrogen (N₂) (Eqn. (6-8)). The process of gaseous nitrogen (i.e., NO, N₂O, N₂) production is called denitrification.

Figure 2. Biological transformation of nitrogen. (Updated from Painter, 1970)

Nitrification:

\[ \text{NH}_3 \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^- \rightarrow \text{N}_2\text{O} \]  

(5)

Denitrification:

\[ \text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2 \]  

(6)

Nitrifier denitrification:

\[ \text{NH}_3 \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2 \]  

(7)

Anamox:
3.2. Carbon dioxide generation pathway in animal wastewater

The production of CO\(_2\) from animal manure has to be accounted for its total emissions, although it is not the main source from building. There are three sources: (1) urea hydrolysis (Eqn. (2)); (2) the anaerobic degradation of organic matter; (3) the aerobic degradation of organic material (Møller et al., 2004; Philippe and Nicks, 2014). Møller et al. (2004) measured CO\(_2\) production in a laboratory facility, and concluded that anaerobic and aerobic degradation were considered to be equally important at 20 °C of manure temperature, while aerobic process played a major role in organic matter degradation when under 15 °C. Under anaerobic conditions, organic matters such as proteins and polysaccharides are able to be degraded by hydrolytic enzymes generated by fermentative microorganisms (Eqn. (9)). This process will produce CO\(_2\), together with CH\(_4\), fatty acids, alcohols, acetate, and hydrogen (H\(_2\)). The fatty acids and alcohols can be further degraded to CO\(_2\) and H\(_2\) by proton reducing acetogenic bacteria (Eqn. (10)).

\[
\text{Anaerobic degradation} \\
\text{organic matter (fermentation)} \xrightarrow{\text{hydrolytic enzymes}} \text{CO}_2 \\
\text{fatty acids (or alcohols)} \xrightarrow{\text{proton reducing acetogenic bacteria}} \text{CO}_2
\]

When oxygen is supplied, the principal origin of CO\(_2\) is aerobic production, which is accelerated by mesophilic/thermophilic microbial communities (Møller et al. 2004). The aerobic process can also occur in stored manure under steady conditions, and lead to converting of CH\(_4\) into CO\(_2\) by oxidation at the air-liquid interface, where atmosphere oxygen enters manure. On contrary, CO\(_2\) together with H\(_2\) can be converted to CH\(_4\) if H\(_2\)-utilizing methanogens function as methanogenic microorganisms.

3.3. Sulphur compound degradation in animal manure

In stored animal manure under anaerobic conditions, organic compounds containing sulfur are primarily transformed to intermediate sulfur-containing compound by mineralization, then generating H\(_2\)S. For example, the amino acid methionine (CH\(_3\)SCH\(_2\)CHNH\(_2\)COOH) is firstly hydrolyzed to methyl mercaptan (MM: CH\(_3\)SH) and then to methyl alcohol (CH\(_3\)OH) and H\(_2\)S (ASCE, 1989; Clanton and Schmidt, 2000):

\[
\text{CH}_3\text{SCH}_2\text{CHNH}_2\text{COOH} + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{SH} + \text{NH}_3 + \text{CH}_3\text{CH}_2\text{COCOOH}
\]
After being produced in the animal waste, H\(_2\)S moves by molecular diffusion (diffusivity in liquid: 10–5 cm\(^2\)/s). This process is promoted when wastewater is agitated, and the H\(_2\)S solubility decreases and its liquid diffusivity increases (Arogo et al., 1999b). Sulfides in the animal wastewater exist in three different forms: S\(_2^-\), HS\(^-\), and H\(_2\)S. The emission of H\(_2\)S and the form of sulfides presented in animal wastewater depends on its pH: when pH < 5, H\(_2\)S is the only sulfide form in solution, while when pH=7, equal proportions of H\(_2\)S and HS\(^-\) exist. At pH= 10, HS\(^-\) is the only sulfide form, and at pH=14, equal proportions of HS\(^-\) and S\(_2^-\) exist (Arogo et al., 1999b). Knowledge on the biochemical pathways of H\(_2\)S formation in animal wastewater under different storage conditions is still limited, therefore, additional research work is needed.

3.4. pH buffer system

Manure pH has been known as an important factor that affects gaseous emission. The dominating pH buffer components in animal wastewater are the total inorganic carbon (TIC) = CO\(_2\) + CO\(_3\)\(^{2-}\) + HCO\(^-\), total ammonium nitrogen (TAN) = NH\(_3\)+NH\(_4^+\) and volatile fatty acids (VFA) = C2-C5 acids. Hydrolysis of urea (Eqn.(2)) will increase pH, due to the production of NH\(_3\), NH\(_4^+\), CO\(_3\)\(^{2-}\) and HCO\(^-\) (NH\(_3\) and CO\(_3\)\(^{2-}\) are bases, and NH\(_3\)/NH\(_4^+\): pKa=9.48; CO\(_3\)\(^{2-}\) / HCO\(^-\)=10.4). At the emitting surface, release of CO\(_2\) will increase pH, while release of NH\(_3\) will decrease pH. Due to the lower solubility of CO\(_2\) than that of NH\(_3\), released of CO\(_2\) is more readily compared to NH\(_3\), therefore, the overall pH tends to increase in fresh manure, which will result in greater loss of total inorganic carbon (TIC) than total ammonium nitrogen (TAN), since CO\(_2\) is originated from TIC while NH\(_3\) is generated from TAN. However, in stored bulk liquid manure, more TIC is produced by anaerobic fermentation of organic matters, resulting in a slowly decrease of pH after it reaches the maximum after 20 h incubation (Paper III). According to Eqn. (5-8), nitrification and denitrification in the animal wastewater may also affect pH due to the equilibrium among nitrate (NO\(_3^-\)), nitrite (NO\(_2^-\)) and NH\(_3\), etc.

The surface of animal wastewater in contact with oxygen in air promotes the transformation of organic matter to CO\(_2\) though aerobic processes other than to VFA in bulk wastewater. A pH gradient from deeper layers to the top layers can be formed due to the emission of gases at the surface (Blanes-Vidal et al., 2009d; Blanes-Vidal et al., 2012a; Dai and Blanes-Vidal, 2013). As our studies presented in Paper I and unpublished data obtained in Paper IV, the pH in the top layers (0.5 cm below the surface) of settled
animal wastewater was 0.43 to 1.38 unit higher than the pH of bottom layers (6 cm below the surface).

The relationship between pH change and gas release has been discussed in the literature, for instance, higher pH favors emissions of basic gases (e.g., NH₃), but is unfavorable to emissions of acidic gases (e.g., CO₂, H₂S) and vice versa. A positive effect of the wastewater pH on the emission of NH₃ has been known and confirmed by many studies (Stevens et al., 1989; Dai and Blanes-Vidal, 2013; Sommer and Sherlock, 1996; Sommer and Husted, 1995; Sommer et al., 2006; Aguerre et al., 2012). The relationship between emissions of CO₂ from animal wastewater and physicochemical characteristics such as pH has been rarely reported, but our study showed a positive correlation ($r = 0.65-0.7$) between manure pH and CO₂ release (Paper III). According to Stevens et al. (1993), change in pH negatively affects release of H₂S from liquid manure, this has also been confirmed by Paper II in our study.

4. Mechanisms of gas release from animal wastewater

The whole process of gases emitted from animal wastewater can be defined as three successive steps, viz., production, release or volatilization and emission (Figure 3) (Braam and Swierstra, 1999; Ni, 1999; Blanes-Vidal et al., 2010). Organic materials from wastewater are firstly degraded by microbial enzymolysis and catalysis in the liquid phase. The products of microbial degradation exit in the liquid as ionized and unionized forms. The dissolved unionized forms of gases in the liquid across the air-liquid interface due to the different concentration gradients between liquid and air phase. Subsequently, gases in the air above the liquid are transported to atmosphere by ventilation. This process may be referred to as “emission” (Figure 3). To illustrate the process of gases release from liquid to air phase, two main mechanisms have been discussed in the literature: mass transfer process and gas bubble release process (Ni, 1999; Ni et al., 2009b; Blanes-Vidal and Nadimi, 2011; Saha et al., 2012; Arogo et al., 1999b; Arogo et al., 1999a; Vaddella et al., 2013).
Figure 3. Schematic overview of processes involved in gas release and emission from livestock houses.

4.1. Mass transfer process of gas release

Gases release process is essentially the transfer of the dissolved gases from wastewater (liquid manure) surface into the immediate free air stream above the liquid. The release rate of gases from liquid animal manure is influenced by the wastewater pH, chemical composition and temperature, and air temperature and velocity (Blanes-Vidal and Nadimi, 2011). Convective mass transfer release is mostly interpreted by the two-film theory and the boundary layer theory as reviewed particularly by Ni (1999).

According to the two-film theory, three steps are involved in the gases release. They are the diffusion mass transfer inside bulk liquid phase \( (K_D) \), the diffusion transfer across the two film as influenced by Henry’s constant \( (K_H) \) and the convective mass transfer in gaseous phase \( (K_C) \) (Figure 4). The concentration in aqueous phase (e.g., \([\text{NH}_3\text{(aq)}]\), \([\text{CO}_2\text{(aq)}]\) and \([\text{H}_2\text{S}\text{(aq)}]\)) is a function of the chemical composition of the solution (e.g., total ammoniacal nitrogen (TAN), total inorganic carbon (TIC) and total sulfide (TS)) and transformations within the wastewater that increase or decrease the concentration due to the equilibrium between unionized form and ion form. The rate of release is further governed by the concentration gradient and the difference in partial pressure between the emitting surface and the free atmosphere. The convective mass transfer plays a dominant role in \( \text{NH}_3 \) release and to some extent affects \( \text{H}_2\text{S} \) and \( \text{CO}_2 \) releases. Solubility of gases is important in the convective releases, as higher solubility is related to higher convection release. The two-film theory has been used in models of \( \text{NH}_3 \) emission from lagoon-atmosphere interface by Bajwa et al. (2006). Blunden et al. (2008) applied the two-film theory in modeling \( \text{H}_2\text{S} \) emission across the gas-liquid interface of an anaerobic swine wastewater storage treatment system, results of the model prediction
are largely dependent on the sulfide concentration, pH, and liquid temperature, however, the model need to be verified since it significantly over predicted the measured flux rates. Blanes-Vidal et al. (2010) developed a gas emission-pH (GE-pH) model to estimate the release of NH₃, CO₂, H₂S and HAc (acetic acid) from liquid swine manure stored in animal houses, outside storage tanks and lagoons, and the results suggested that modeling on gas emission with consideration of the pH could improve the predictive accuracy. Blanes-Vidal and Nadimi (2011) further developed a GE-pH-film model that improved the accuracy of estimated NH₃ release during the first 10 h after liquid disturbance, from an averaged error of 82% (gas emission model) to 25% (GE-pH-film model).

![Diagram of gas-liquid interface system](image.png)

Figure 4. Two-film theory of gas–liquid interface system

### 4.2. Gas-bubble release

A bubble release model was developed by Ni et al. (2009b) to explain the release behavior characteristics related to H₂S, SO₂, and CO₂ (Figure 5). A sudden increase in H₂S release (>100%) was detected when manure was disturbed by mixing or agitation, in comparison to previous release in deep-pit swine houses. This phenomenon of sudden release (also called “Burst - release”) was interpreted by “Bubble - release” as these bubbles likely contained concentrated gases (e.g., H₂S and CO₂). Under anaerobic conditions, various gases and volatile organic compounds are generated by microbial decomposition of biomass such as biogas (CH₄ as main gas component). These generated gases firstly dissolve in manure, and then form micro air bubbles when they reach their degree of saturation in solution. These micro air bubbles can be aggregated due to
heterogeneity of the wastewater and temperature difference, etc. The air bubbles gradually gain in size, and move upward with accelerated speed ($S_b$) by the effect of buoyant forces. Bubbles ascending from animal wastewater finally reach the surface and release to the bulk air phase. The rate of gas release is controlled by the bubble volume ($V_b$) and the gas concentrations ($C_{g,b}$) (Ni et al., 2009b). The disturbance of wastewater can accelerate the speed ($S_x$) of bubble release (Paper I). The instantaneous release behavior has been confirmed by Blanes-Vidal et al. (2012a), who reported that slurry mixing increased CO$_2$ and H$_2$S emissions by 1515% and 40471%, respectively.

Figure 5. Mechanism of Bubble-release (Ni et al., 2009b): $C_{g,\infty}$ = gas concentration in free air phase, $C_{g,0}$ = gas concentration in the free air phase above liquid surface, $C_{g,b}$ = gas concentration in bubbles, $q_r$ = flux of gas release, $V_b$ = volume of bubble, $S_b$ = speed of ascending bubble movement, and $S_x$ = speed of bubble movement relative to liquid caused by disturbance.

5. Waste management in relation to gas emission reduction technologies

Pollutant gases, such as ammonia, hydrogen sulfide, methane, and carbon dioxide, are produced inside livestock buildings, in open feedlots, in manure storage facilities, during manure handling and treatment, and manure application to land. In order to reduce the pollutants, many mitigation strategies have been developed to fulfill the requirement of government regulations and ecological environment sustainability. Ndegwa et al. (2008) and Philippe et al. (2011) reviewed ammonia emission mitigation techniques for animal houses. Atia et al. (2014) summarized that strategies for reducing
NH₃ and H₂S from animal husbandry could be divided into three main categories: suppression methods, inhibition methods, and capture and control methods. For each category, different control techniques are included. Chadwick et al. (2011) reviewed the potential mitigation methods for N₂O and CH₄ from the manure management. Choosing the appropriate technology for reducing the gas emissions should consider both the efficiency and the cost. The following sections will discuss and evaluate most commonly used technologies in practice.

5.1. Covers

Using manure storage covers is an efficient mitigation measure that can reduce emissions of NH₃, H₂S, and other odorants (Table 8). There are two main mechanisms for the gas emissions reduction: 1) covers acting as a physical barrier to limit the emissions; 2) covers creating a biologically active zone on the manure surface where the emitted gases will be decomposed by microorganisms, acting as a biofilter. Covers used in practice have been constituted by natural materials (e.g. natural crusts, straw, light expanded clay aggregates and peat), synthetic materials (e.g. plastic, geotextile and rubber), and composites of both. Blanes-Vidal et al. (2009c) tested the age, moisture content, and microbiological development of the straw cover affect the emissions of odor and odorants. They found that aged straw covers were able to reduce emissions of NH₃ by 99%, dimethyl sulfide by 81%, phenol by 82%, p-cresol by 95%, skatole by 98) and benzylalcohol by 97%, while emissions of odor, hydrogen sulfide, volatile fatty acids, dimethyl disulfide, and indole were not effectively influenced by covers. The results of this study suggested that the reduction in emissions of NH₃, dimethyl sulfide, p-cresol, and benzylalcohol appeared to be caused by the straw cover acting as both physical barriers and biofilter, while the main mechanism for odor and odorant emission reduction in straw covered slurry was associated with the cover acting as a physical barrier. VanderZaag et al. (2008) reported that nearly all types of cover could reduce NH₃ effectively, most cover types (except that oil cover used alone can produce offensive odor) were able to reduce odor and H₂S, however, aged covers had the potential to increase CH₄, CO₂, and N₂O emissions. VanderZaag et al. (2009) concluded that straw covers with two depth on the top had increased the emission of CO₂ and N₂O. However, in another study reported by VanderZaag et al. (2010), permeable synthetic cover (Biocap (TM)) was capable of reducing both CO₂ and N₂O emissions. The mechanisms of different covers in relation to gaseous emissions are not clear yet, while simultaneous assessments of the effects on those gases are lacking for all covers.
Table 8. Summary of research on covers and the observed effects on gas emission from stored liquid slurry (Review of papers published from 2007 to 2014, updated from VanderZaag et al. (2008))

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Description</th>
<th>Slurry type</th>
<th>T (°C)</th>
<th>DM</th>
<th>pH</th>
<th>Reps</th>
<th>Period</th>
<th>Cover Description</th>
<th>Percent Reduction (%)</th>
<th>Odor</th>
<th>H₂S</th>
<th>NH₃</th>
<th>CO₂</th>
<th>CH₄</th>
<th>N₂O</th>
<th>C or E</th>
</tr>
</thead>
<tbody>
<tr>
<td>VanderZaag et al., 2009</td>
<td>Six flux chambers (6.6 m² each) divided into three groups, including control, two thicknesses of straw covers</td>
<td>Dairy slurry</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Y</td>
<td>June and October 2007</td>
<td>Straw covers 15 cm</td>
<td>NS</td>
<td>NS</td>
<td>78</td>
<td>24</td>
<td></td>
<td>24</td>
<td></td>
<td>E</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Straw covers 30 cm</td>
<td>NS</td>
<td>NS</td>
<td>90</td>
<td>28</td>
<td></td>
<td>28</td>
<td></td>
<td>E</td>
</tr>
<tr>
<td>VanderZaag et al., 2010</td>
<td>Six flux chambers (1.3 m depth x 6.6 m² each) divided into two groups including control and treatment (tested for 165 d)</td>
<td>Dairy slurry</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Y</td>
<td>NA</td>
<td>Permeable synthetic cover (Biocap (TM)) Natural crust formed after 50 d of storage in control</td>
<td>NS</td>
<td>NS</td>
<td>90%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>E</td>
</tr>
<tr>
<td>(Sakamoto et al., 2008)</td>
<td>Laboratory trials were carried out for 13 days using a pilot scale device.</td>
<td>Digested slurry</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Y</td>
<td>NA</td>
<td>Superphosphate and silica (8% w/w)</td>
<td>NS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>E</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Calcium carbonate and silica (8% w/w)</td>
<td>NS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>E</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ammonium phosphate and ammonium sulfate, and silica (8% w/w)</td>
<td>NS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>E</td>
</tr>
<tr>
<td>(Parker, 2008)</td>
<td>Lagoon of dairy farm housed 3000 cows, wind tunnel were used to evaluate odorous emission after 30 months of covering lagoon</td>
<td>Dairy slurry</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>N</td>
<td>NA</td>
<td>Covering the treatment lagoon while adding additional aeration capacity</td>
<td>80</td>
<td>96</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>C</td>
</tr>
<tr>
<td>(Blanes-Vidal et al., 2009c)</td>
<td>Laboratory study on cover age, moisture content was conducted in 15 flux chambers (0.049 m³ with a diameter of 0.36 m) (9 weeks of storage)</td>
<td>Swine slurry</td>
<td>15±4</td>
<td>4.1</td>
<td>7.09</td>
<td>Y</td>
<td></td>
<td>50 to 77</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>E</td>
</tr>
<tr>
<td>(Hudson et al., 2008)</td>
<td>Polyethylene shade cloth and supported straw were assessed in terms of efficacy in reducing odour emission rates over a 40-month period.</td>
<td>Swine slurry</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>N</td>
<td>NA</td>
<td>Polypropylene-shade cloth Shade- cloth Straw</td>
<td>50-77</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>E</td>
</tr>
</tbody>
</table>

Up and down arrows indicate increase and decrease, respectively. Line indicates no effect. C: concentration, F: flux or emission. NS: not studied. NA: not available. Calculation based on C: concentration / E: emission.
5.2. **Manure additives**

Chemical methods using manure additives for digestive, acidifying, adsorbent and urease inhibition can slow down or inhibit the release of NH$_3$, H$_2$S, and other odorants from animal manure (McCorry and Hobbs, 2001). The following section will focus on the evaluation of manure acidification and urease inhibitor for gases abatement potentials.

### 5.2.1. **Lowering manure pH—acidification**

Reduction of pH through manure acidification is an effective way to reduce NH$_3$ emissions (Table 9). Stevens et al. (1989) found that lowering pig manure pH to 6.0 by addition of sulfuric acid decreased NH$_3$ emission by 82%, and lowering cattle manure pH to 5.5 decreased NH$_3$ emission by 95%. Petersen et al. (2012) tested the emission of NH$_3$ and CH$_4$ from fresh and aged cattle manure during 90 days of storage with treatment of acidifying manure pH to 5.5, the results showed that NH$_3$ emission was reduced by 95%, while CH$_4$ emission reduced by 67 to 87%. Lowering pH of manure by adding chemical acid can reduce some basic gases (e.g. NH$_3$), but may lead to generation of another pollutant such as H$_2$S. Dai and Blanes-Vidal (2013) studied three pH levels of acidification on the emissions of NH$_3$, CO$_2$, and H$_2$S, the results showed that acidification had no effect on emissions of CO$_2$ and H$_2$S, and the increase of the two gases were attributed to the agitation during addition of acid while mixing (Paper IV). However, Wang et al. (2014) reported that acidification of digested pig manure reduced emissions of CH$_4$ and NH$_3$, but increased H$_2$S dramatically. Although manure acidification have been accepted as the Best Available Technology (BAT) in Denmark (Kai et al., 2008), but there is still a lack of systematical assessment of acidification technologies.

Biological additives contain mixed cultures of enzymes or microorganisms that can alter the manure characteristics, thereby change the gas emissions. McCrory and Hobbs (2001) showed that using combined L. platatorium and glucose decreased the pH of pig manure from 8 to 6. Huang et al. (2006) reported that the combination of L. plantarum and soluble carbohydrates dramatically reduced manure pH, resulted in the reduction of NH$_3$ emissions by 34.6%-92.4%, but increased H$_2$S emission and NH$_4^+$-N.

### 5.2.2. **Microbial activity inhibition**

The use of manure additives to impede enzymatic activity (e.g. urease) and to kill the bacteria (e.g. sulfide-producing bacteria) can change the microbial environment, thereby
limit the gas emissions. Animal urine contains approximately 97% urea nitrogen, which is rapidly converted to ammonium/ammonia and carbonate/bicarbonate by enzyme urease shortly after it come into contact with feces when it is excreted (Paper III) (Watson, 2000). Urease inhibitors can reduce NH₃ emissions by up to 90% (Watson, 2000; Watson et al., 1994; Sanz-Cobena et al., 2011; Salazar et al., 2014; Ni et al., 2014; Saggar et al., 2013). Various urease inhibitors have been evaluated for their ability to reduce urea hydrolysis rate in animal manure. Among these, N-(n-butyl) thiophosphoric triamide (NBPT) and nitrification inhibitor dicyandiamide (DCD) are currently most promising and effective when applied with urea or urine. Varel et al. (Varel et al., 1999) reported that applying NBPT weekly to beef cattle feedlot pens could reduce production of ammonia, and lower the pH, but had no significant effect on the total volatile acids compared to untreated pens. Zaman and Blennerhassett (2010) investigated the effect of applying rate and their mixing proportion of two urease inhibitors NBPT and DCD to the grazed pastures, and the results suggested that applying Agrotain + DCD at a ratio of 1:7 (v/w) might provide the best option for both mitigating N losses and improving pasture production in intensively grazed systems. Saggar et al. (2013) concluded that an application rate of 0.025% w/w (NBPT per unit of N) is optimum for reducing NH₃ emissions (reduction of 11-93%) from temperate grasslands. The effect of applying urease inhibitor on other N losses such as gaseous emissions of N₂O, NO and NO₃⁻ leaching has also been evaluated by some researchers. Sanz-Cobena et al. (2012) reported that applying NBPT reduced N₂O emissions by 4-54%; while the combination of NBPT and DCD treatment reduced N₂O emission by 18-43%. And the study suggested that the main factors affecting the effectiveness of urease inhibitor in reducing N losses were the management practices, such as irrigation, and the climatic conditions. In conclusion, urease inhibitors can be used to control ammonia emissions from animal wastes, reduce environmental contamination, and produce a high efficiency fertilizer from manure.
### Table 9. Summary of research on manure acidification and the observed effects on gas emission from animal excreta.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Description</th>
<th>Source</th>
<th>T (°C)</th>
<th>Initial DM (%)</th>
<th>Reps</th>
<th>Periods</th>
<th>Acidification</th>
<th>Percent Reduction (%)</th>
<th>C or E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kai et al., 2008</td>
<td>A whole farm estimation, study was carried out in an animal house with 1200 pigs, two outside storage tanks, and land spread with acidified and non-acidified slurry.</td>
<td>Swine house</td>
<td>NA</td>
<td>Y</td>
<td>3.3</td>
<td>7.5</td>
<td>Field, 2002-2003, including three seasons</td>
<td>pH = 6.3; DM = 4.2%</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Stored slurry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Applied slurry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petersen et al., 2012</td>
<td>Investigation of the evolution of CH4 and from fresh and aged cattle slurry during 95d of storage as influenced by pH adjustment to 5.5 with sulfuric acid.</td>
<td>Cattle slurry</td>
<td>Room</td>
<td>NA</td>
<td></td>
<td>Y</td>
<td>95d</td>
<td>pH = 5.2 ± 0.2 and 6.3 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Dai and Blanes-Vidal,</td>
<td>Slurry was stored in 30L dynamic flux chambers for 155d. Slurry was acidified to pH = 6.0, pH = 5.8 and pH = 5.5 with sulfuric acid. Short-term aeration treatments were also studied.</td>
<td>Swine slurry</td>
<td>5.43</td>
<td>6.31</td>
<td>Y</td>
<td>155d</td>
<td>pH=5.5</td>
<td>NS</td>
<td>–</td>
</tr>
<tr>
<td>2013</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>77</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.65</td>
<td>6.26</td>
<td>Y</td>
<td></td>
<td>pH=5.8</td>
<td>–</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.46</td>
<td>6.38</td>
<td>Y</td>
<td></td>
<td>pH=6.0</td>
<td>–</td>
<td>50</td>
</tr>
<tr>
<td>Wang et al., 2014</td>
<td>Nine reactors (100L barrel put in a 150L barrel for slurry storage and air flux) were divided to one control and two treatment groups.</td>
<td>Digested swine slurry</td>
<td>3.88</td>
<td>7.46</td>
<td></td>
<td>95d</td>
<td>pH=5.5 (end of study pH=7.95)</td>
<td>NS</td>
<td>–11,324</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>pH=6.5 (end of study pH=8.00)</td>
<td>40.2</td>
<td>80.8</td>
</tr>
</tbody>
</table>

*% reduction is calculated in comparison with non-treatment control. Negative reduction indicated that treatment increased the gas concentration or emissions. Calculation based on C: concentration / E: emission*
5.3. Manure management in barns

5.3.1. Solid manure collection

In-barn manure separation can reduce the costs of storage, transport and manure application remarkably (Koger et al., 2014; Alonso et al., 2010). Peng (2014) investigated the waste management methods from 50 commercial pig farms in 11 provinces in China, and found that daily manually scraping of pig pens for collecting solid manure and floor water-line flushing for cleaning pens were commonly used in most farms. To reduce the labor cost and the volume of wastewater from animal husbandry, concrete slatted floors, automatic manure collection system and underground pipe (for wastewater collection and avoid mixing with rainwater) are becoming more accepted and utilized in new designed farms. These new systems can also help animal farms to meet the Chinese standards of gaseous emission. A new design of pig pens with 20° angle concrete floor under slatted floor for automatically separating urine and feces was reported by Ye et al. (2007). Alonso et al. (2010) tested the manure separation efficiency of two types of conveyor belts (flat belt and concave belt) installed under a partially slatted floor for fattening pigs. The flat and concave belts harvested the solid faction with a dry matter of 31.2% and 23.8%, respectively. Their results also showed that the flat belt was more efficient at 6° than other slope angles. Koger et al. (2014) reported that using a conveyor belt placed at a 4° angle beneath the slats could harvest urine and feces (49% dry matter) separately (Figure 6). They also concluded that belt system could be easily operated and reduce emissions of NH₃ and CH₄, and odor annoyance. Other benefit such as being conducive to animal and worker health, and improving development of environmental sustainability, can further improve the economic feasibility of using the in-barn manure separation system.

Figure 6. Belt manure separation system (from Koget et al., 2014)
5.3.2. Manure emptying and pen cleaning

Frequent removal of manure from animal buildings or pens can reduce ammonia, hydrogen sulfide and odor emissions effectively (Lim et al., 2004; Ivanova-Peneva et al., 2008). Braam et al. (1997) reported that raising the dairy manure scraping frequency from 12 to 96 times per day resulted in 5-26% of ammonia emission reduction. Heber et al. (2001) reported that pit emptying biweekly reduced H₂S emissions by 79% compared to emptying every six weeks.

Flushing is commonly used in practice for barn cleaning. Kroodsma et al. (1993) reported that floors flushed with water decreased NH₃ emissions by 14-70%, compared to slatted floors with or without manure scraper in dairy housing. Lim et al. (2004) reported that daily flushing led to odor emission reduction of 41% and 34% compared to emptying pit manure every 7 days and 14 days, respectively. Flushing could reduce gaseous emission from animal buildings, however, the amount of manure volume was nearly doubled by the flush water (Kroodsma et al., 1993), thereby, the costs of manure transportation and application are increased. Besides, storage of diluted manure had potential to increase the total release of NH₃, H₂S and SO₂ according to Ni et al. (2010), since dilution increased the manure volume, thereby increased the total gas emission surface area.

5.4. Liquid-solid separation

Liquid-solid separation of manure or slurry based on mechanical screening could remove approximately 80% of its total solid (TS) content and reduce the volume of solid fraction (Burton, 2007). The limitation of physical separation (e.g. screen) is that soluble nutrition remains in the water and the solid fraction has relatively low fertilizer efficiency. Adding a decanter centrifuge and pre-treatment of flocculation or raising the pH could increase the possibility of removing suspended matter and most phosphorous (Paz Pérez-Sangrador et al., 2012). However, wastewater residual nitrogen (as NH₃) and potassium still remained and could only be removed when the technology was coupled with membrane separation (Burton, 2007). Regarding the effect of manure separation on gaseous emissions, different results have been reported by several researchers (Table 10). Dinuccio et al. (2008) found that mechanical separation could not reduce emissions, but increased GHGs by up to 30% of CO₂ equivalents when compared to untreated manure during the 30 days storage of the liquid and solid factions from swine and cattle manures. Riaño and García-González (2014 In press) reported that using manure treatment of solid-liquid separation followed by coagulation-flocculation and nitrification-denitrification of the liquid fraction led to a reduction of CO₂ and CH₄ emissions by 72% and 69%, respectively, whereas N₂O emissions
were not significantly different between treatment and non-treatment. Fangueiro (2008) reported that manure separation by screw press significantly increased emissions of NH₃ (15-38%), CO₂ (634-650%) and N₂O (1216-1240%), but caused a decrease of CH₄ by 25-50%. Moset et al. (2010) found that stored raw manure had significantly higher emissions of CO₂ (18%), CH₄ (12%), N₂O (23%) compared to separated manure factions.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Description</th>
<th>Source</th>
<th>T (°C)</th>
<th>Reps</th>
<th>Periods</th>
<th>Separation</th>
<th>Percent Reduction (%)</th>
<th>Odor</th>
<th>NH₃</th>
<th>CO₂</th>
<th>CH₄</th>
<th>N₂O</th>
<th>C OR E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fangueiro et al., 2008</td>
<td>Study tested gaseous emission from four stored separated manure fractions and untreated manure. Plastic barrel of 125-L capacity was used for storage.</td>
<td>Dairy manure</td>
<td>&lt;15</td>
<td>Y</td>
<td>January and March 2006</td>
<td>Screw pressing</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>-650</td>
<td>&gt;25</td>
<td>-1239</td>
<td>E</td>
</tr>
<tr>
<td>Dinuccio et al., 2008</td>
<td>Dynamic flux chambers with volume of 1.5 L were used to store solid and liquid fractions from separated cattle and swine manure at two room temperatures. Four gases were measured during 30 days of storage.</td>
<td>Cattle manure</td>
<td>25</td>
<td>Y</td>
<td>30 d</td>
<td>Screw pressing</td>
<td>Combined separation</td>
<td>NS</td>
<td>NS</td>
<td>15</td>
<td>-634</td>
<td>50</td>
<td>-1216</td>
</tr>
<tr>
<td>Moset et al., 2010</td>
<td>100 L polyethylene vessels were used for storing raw manure and separated factions during 15 weeks of measurement.</td>
<td>Swine manure</td>
<td>25</td>
<td>Y</td>
<td>15 weeks</td>
<td>Mechanical separation</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>18</td>
<td>12</td>
<td>23</td>
<td>E</td>
</tr>
<tr>
<td>Riaño and García-González, 2014 In press</td>
<td>GHGs reduction were evaluated in a farrow-to-finish farm with about 300 sows, manure was treated by solid–liquid separation and coagulation-flocculation and nitrification-denitrification of the liquid fraction.</td>
<td>Swine manure</td>
<td>25</td>
<td>Y</td>
<td>15 weeks</td>
<td>Screw pressing</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>72</td>
<td>69</td>
<td>-</td>
<td>E</td>
</tr>
</tbody>
</table>

Percent reduction is calculated in comparison with non-treatment control. Negative reduction indicated that treatment increased the gas concentration or emissions. Calculation based on C: concentration / E: emission.
5.5. General discussion on gases reduction technologies

Animal facilities and wastes are important sources of noxious gaseous emissions. For decades, numerous investigations have been carried out in order to determine the available gaseous emission abatement technologies. In addition to the waste management measures mentioned above, housing and floor system modification (Philippe et al., 2012), diet manipulation on adjusting the feed component (e.g., fiber content, crude protein content, feed additives) (Canh et al., 1997), and air contaminant capture by biofiltration and bioscrubbing (Melse and van der Werf, 2005; Guieysse et al., 2008; Carew, 2010) can also control gaseous emission effectively.

The animal housing and floor facilities can affect the gaseous emission through the factors such as ventilation rates, floor types, floor surface characteristics, fouling area of floor, using of nature material litter, etc (Aarnink et al., 1997; Philippe et al., 2012; Braam et al., 1997; Braam and Swierstra, 1999). Aarnink et al. (1997) evaluated NH$_3$ emission from five types of slatted floor in fattening pig houses. The results showed that partially slatted floor made of metal slats with a triangular form in cross-section (1 cm wide slats with 1 cm wide gaps) emitted 27% less NH$_3$ than a concrete slatted floor (10 cm slats with 2.0 cm gaps), and partially covering the slatted floor with studs can prevent pigs laying on the floor thereby resulted in less NH$_3$ emissions than solid floor. Wang et al. (2011) reported a reduction in ammonia of 76% in a fermented deep litter in comparison with fully slatted floor units for fattening pigs. The current research focuses on optimizing the slatted floor parameters for the maximal solid manure collection and a better animal welfare in pig and cattle houses.

Studies in swine and poultry have shown that there is a potential for reducing NH$_3$ and H$_2$S from animal manure by diet manipulation (Sutton et al., 1999; Stevens et al., 1993; Nørgaard et al., 2010; Mathot et al., 2012; Liu and Zhou, 2014; Li et al., 2012; Le et al., 2009; Kreuzer et al., 2002; Kluge et al., 2010; Gralapp et al., 2002; Cole et al., 2005). However, additional research is required to evaluate the effectiveness of diet manipulation techniques since the results of these studies are inconsistent. Also additional research is needed to determine whether diet manipulation can adversely affect the animal health and productivity.

Introducing a single technology to regulate air pollutants in one stage of animal production may have limitations and will affect the emissions of downstream in the management chain of manure of the farm (Kai et al., 2008). Therefore, combination of waste treatment technologies has been proposed and should be evaluated with a whole-farm
perspective. The acidification followed by solid-liquid separation on the physical and chemical composition of swine manure has been assessed by Fangueiro et al. (2009). They concluded that the proposed treatment generated valuable slurry fractions which comply with plant nutrient requirements especially for nitrogen. Daumer (2010) evaluated acidification followed by a liquid-solid separation and precipitation of phosphorus from the liquid manure from environmental, technical and economic aspects. The product (superphosphate fertilizer) of this process could be competitive in efficient and environmentally friendly terms. The organic acids (e.g., formic and acetic acid) have been reduced by this combined acidification-separation process, however, gaseous emissions have not been appraised. Sørensen and Eriksen (2009) reported that combined acidification-aeration treatment increased mineral N fertilizer equivalence (MFE) of cattle manure N from 39 to 63% and that of pig manure N from 74 to 101% after surface banding, but had no obvious effect on subsequent mineral N release in soil, indicating that NH₃ release from acidified pig and cattle manure was low after applied to field. Pereira et al. (2010) assessed the combined treatment of separation followed by addition of nitrification inhibitors, showing that a single slurry separation had no effect on N emissions, while nitrification inhibitor had no significant effect on emissions of CH₄, CO₂ and N₂O, but decreased NO emissions remarkably, as a result, the combination of the process with nitrification inhibitor led to a small reduction in total N emissions.

6. General discussion and conclusion

In this section, the main findings from each experimental study of the present PhD-thesis are summarized and discussed and their implications for future research perspective are presented.


Air contaminant from animal manure affects the sustainability of the livestock production. The factors and rates of gaseous emission have been estimated in laboratory studies, measured in field conditions, and modeled using mathematic functions based on biological, chemical and physical mass transfer process by many researchers. Animal slurry stored in under-floor pit is characterized by the frequent occurrence of surface liquid disturbances caused by the urine and feces that fall into the pit, and alter the equilibrium of the slurry surface. Previous studies investigating the release of gases from slurry mostly
focused on the steady-state conditions (e.g., Arogo et al., 2000; Sommer et al., 2007). Recent studies have suggested that emissions from transient-state conditions could represent a significant part of the total emissions (Blanes-Vidal and Nadimi, 2011; Blanes-Vidal et al., 2012a). However, there are still research gaps in obtaining a reliable estimate of gaseous emission rate from animal production: (1) how does animal activity such as excretion affect gas generation and release from under-floor pit manure storage? (2) what is gas release behavior under transient-state conditions and the related gas release mechanisms? In order to answer the two specific questions, we evaluated the effects that the addition of wastewater (spatially and temporally distributed based on animal’s activity models) have on NH₃, CO₂ and H₂S emissions from simulated in-barn storage conditions in laboratory manure reactors (Paper I). The results showed that addition of manure caused a short-term variation in the emissions of NH₃, CO₂ and H₂S. Each of the addition caused NH₃ emissions following an increase-decrease-increase pattern, while CO₂ and H₂S followed an increase-decrease pattern according to Blanes-Vidal et al. (2012a), similar tendency was also observed in this study except the first immediate increase in NH₃ emission due to the lower frequency of measurements. The mechanisms of the gas release have been discussed in Section 4, being that convective mass transfer process plays a major role in release of NH₃ while bubble release is responsible for CO₂ and H₂S release. All three gases are affected by wastewater pH. Significantly higher emissions of CO₂ and H₂S were measured in manure addition chambers compared to controls during the days of addition performed, this suggested that future estimation of gaseous emission should consider transient-state conditions to avoid underestimation of the total emissions. This is of special importance in the case of H₂S, as occupational exposures and the associated health risks will be highly underestimated if the evaluation of exposures to H₂S is based on emissions from slurries stored under undisturbed conditions.

6.2. Paper II: Characteristics of pollutant gas releases from swine, dairy, beef, and layer manure, and municipal wastewater.

To extend knowledge about quantification and release behavior of gases from various waste sources, five types of wastewaters were tested in dynamic flux chambers with respect to NH₃, CO₂, H₂S, and SO₂ emissions during storage with weekly addition performed. This direct comparison of physicochemical characteristics of wastes and their gases release potentials is not only helpful for establishing environmental regulations, but also important for the development of gas emission reduction technologies. For different livestock species,
the production of manure and their physicochemical characteristic are different due to the feed intake and metabolism process. For instance, poultry manure usually has a high dry matter content due to that no urine is produced, whereas swine manure contains more nitrogen compared to other manure wastes due to the intake of fine feed with more crude protein. Physicochemical characteristics of different types of wastes (e.g., the total nitrogen, total ammoniacal nitrogen, dry matter, and pH) have great influence on the releases of NH₃, CO₂, H₂S, and SO₂. Previous studies have reported that the manure pH, temperature, ammoniacal nitrogen content, and ventilation rate were the main factors influencing NH₃ release (Rong et al., 2009; Chaoui et al., 2009; Saha et al., 2011). In the present study, NH₃ releases from different wastes were positively correlated with their initial TKN (correlation coefficient: r = 0.98, n = 8) and TAN (correlation coefficient: r = 0.90, n = 8). The factors that influence CO₂ release from manure have not been discussed individually, except the total pig weight, manure temperature, and ventilation rate under field conditions in the CO₂ release model developed by Ni et al. (1999a) and confirmed by Zong et al. (2014). In this study, a positive correlation between CO₂ releases and initial dry matter content of wastes was found (correlation coefficient: r = 0.99, n = 8). This was in agreement with the results reported by Fangueiro et al. (2008) and Mathot et al. (2012), who showed that the CO₂ emissions were generally higher in the solid fraction of separated manure than that in the liquid fraction and raw manure. Both H₂S and SO₂ releases were negatively correlated with the waste pH. However, little is known about the relationship between H₂S and SO₂ releases, which deserve further investigations. The two mechanisms (convective mass transfer release and bubble-release) described in section 4 are responsible for illustrating the gas release behavior. Different release mechanisms are responsible for different gases. As reported by Ni et al. (2009a), convective mass transfer governed NH₃ release, while bubble-release was dominant in the releases of CO₂, H₂S, and SO₂.

6.3. Paper III: Determination and Comparison of Urease Activity in Faeces and Fresh Manure from Pig and Cattle in Relation to Ammonia Production and pH Changes.

Ammonia formation in animal manure is a process of urea hydrolysis catalyzed by enzyme urease. To better understand the pathway of ammonia formation, we determined the urease activity in fresh feces and manure from pigs and cattle using Michaelis-Menten kinetic analysis. The results showed that both V_max and K_m value were more than two times higher for pig feces than for cattle feces, suggesting that a lower concentration of urea is required to saturate the urea hydrolysis capacity of cattle feces than pig feces. The differences between the fecal urease kinetic parameters of pig and cattle may indicate that their feces are
dominated by different ureolytic bacteria species. Compared to the study reported by Muck (1982) who used 1 h incubation time to determine kinetic parameters, 5 min reaction time used in this study should give more correct initial reaction velocity measurement, and thus $V_{\text{max}}$ and $K_m$ values according to the kinetic theory. The determination of initial velocity of TAN formation revealed that ammonia production rate in fresh pig manure was 4 times faster than that in fresh cattle manure. This may be explained by our observation that chemical compositions of samples from pig are higher than that from cattle. Urea hydrolysis rates at different pH levels measured in this study showed that the maximum urease activity for pig feces and cattle feces were at around pH 7 and pH 8, respectively. Thus, application of urease inhibitor or manure acidification to pH < 6 will lead to reduction of ammonia production and release. Manure acidification of both pig and cattle samples to pH < 6 caused a reduction of urease activity by 10-20% compared to the maximum urease activity at the optimal pH level, this may be due to that acidification alters the microbial metabolic process. Kinetic parameters of urease activity have been utilized in process modeling of ammonia production from animal houses (e.g., floor) and manure storage, the results of our study will be fill the research gap of urease activity determination for both pig and cattle, and will be useful in ammonia production prediction modeling in future studies.

6.4. Paper IV: Emissions of ammonia, carbon dioxide, and hydrogen sulfide from swine slurry during and after acidification treatment: Effect of pH, mixing and aeration

In-house slurry acidification has been identified as a promising technology for improving the environmental performance of manure management in pigs and dairy farms (Wesnæs et al., 2009) and has been approved as the Best Available Technology (BAT) in some EU countries (Kai et al., 2008). This technique, which consists to lower slurry pH through the addition of acid, primarily aims to reduce ammonia emissions from animal houses. Previous studies have reported that about 70-85% of the NH$_3$ release from swine slurry can be reduced by decreasing the slurry pH to 5.5 through the addition of sulfuric acid (H$_2$SO$_4$) (Stevens et al., 1989; Frost et al., 1990; Kai et al., 2008). Moreover, it does not only reduces NH$_3$ emissions in-house (i.e. at the point of intervention), but also throughout the whole manure continuum (storage, field application). Although effect of acidification on NH$_3$ emissions has been known for many years, its implications on the emission of other compounds, such as CO$_2$ and H$_2$S, has not been fully documented in the literature. Therefore, we assessed the implication of acidification on emission of NH$_3$, CO$_2$ and H$_2$S, and a precise knowledge of currently used acidification system in practice on its economic and
environmental impacts could help on its popularizing. Our results suggested that acidification could reduce ammonia emission during storage (compared to non-acidified slurry) by 50-77%, when slurry pH was lower than 6.0. Theoretically, the reduction of slurry pH will favor the emission of weak acidic gases such as CO₂ and H₂S, and addition of H₂SO₄ may result in an increase of H₂S as additional sulfate is added. However, our measurement showed that acidification had no significant effect on average CO₂ and H₂S emissions during storage after acidification compared to non-acidified slurry stored under the same conditions. Therefore, we recommend acidification as an available manure treatment technology for the purpose of reducing ammonia and for environmentally friendly releasing of CO₂ and H₂S.

6.5. Further research perspective

The preceding contents of this thesis have discussed different areas of gases production, release, emissions and mitigation technologies. More research work is needed to further promote the understanding of gaseous production and release from animal wastewater, and to develop suitable technologies for noxious gases mitigation. Based on this thesis, the following research perspectives can be addressed:

- Gaseous release behaviors under transient conditions (i.e., frequently manure addition) in a laboratory scale were studied in this thesis, and found to have significant effect on emissions of NH₃, CO₂ and H₂S. This needs to be further studied and verified in a farm scale facility.

- The present thesis still has limitations on assessing the micro-biological degradation pathway of nitrogen, carbon and sulfur compounds. The production, transport, release mechanisms of various gases from animal wastewater need to be further studied for a better understanding of their emission behaviors, thereby developing suitable reduction technologies.

- The determined urease activity in Paper III can be further applied in developing ammonia release models.

- Waste management and treatment technologies presented in this thesis should be evaluated comprehensively in relation to gaseous emission reduction, cost saving and environmental sustainability.

6.6. Conclusion

The conclusion of this PhD thesis can be summarized as follows:
1. Frequently disturbed manure by animal excretion altered the chemical equilibrium of the manure surface and caused instantaneous increase of CO$_2$ and H$_2$S emissions, which were then followed by a decrease, while short-term emission of NH$_3$ fluctuated in an increase-decrease-increase pattern.

2. Emissions of CO$_2$ and H$_2$S from manure disturbed by frequently manure additions were significantly higher than manure stored under steady conditions. Consequently, CO$_2$ and H$_2$S measurement based on undisturbed manure may underestimate the emissions from practical farm conditions. This is of special importance in the case of H$_2$S, as occupational exposures and the associated health risks will be highly underestimated if the evaluation of exposures to H$_2$S is based on emissions from slurries stored under undisturbed conditions.

3. Physicochemical characteristics (i.e., TN, TAN, dry matter, and pH) of different types of wastes had great influence on the NH$_3$, CO$_2$, H$_2$S, and SO$_2$ releases. The releases of NH$_3$ from various wastes were strongly affected by the concentrations of TN and TAN in the wastes. The CO$_2$ releases from different types of wastes were positively correlated with initial dry matter content of the wastes. The releases of H$_2$S and SO$_2$ from different types of wastes had high correlations with the waste pH.

4. The urease activity can be represented by kinetic parameters, $V_{\text{max}}$ and $K'_{m}$. Using 5 min reaction time to determine initial reaction velocities based on total ammoniacal nitrogen (TAN) concentrations, the determined $V_{\text{max}}$ values was 2.06±0.08 mmol urea/kg/min and 0.80±0.04 mmol urea/kg/min for pig feces and cattle feces, respectively. $K'_{m}$ was 32.59±5.65 mmol urea/l and 15.43±2.94 mmol urea/l for pig feces and cattle feces, respectively.

5. The optimal pH for urease activity in pig and cattle feces were around 7 and 8, respectively, these results suggested that changing the manure pH level can inhibit the urease activity, thereby inhibit ammonia formation.

6. The manure acidification treatment reduced NH$_3$ emissions during storage (compared to non-acidified slurry) by 50%, 62% and 77%, when slurry pH was decreased to 6.0, 5.8 and 5.5, respectively. Average CO$_2$ and H$_2$S emissions during storage of slurry after acidification were not significant different from non-acidified slurry stored under the same conditions.

7. Short-time aeration of animal wastewater had no effect on average NH$_3$, CO$_2$ and H$_2$S emissions during the storage after treatment.


**Appendix: Paper I - IV (full text)**


Indoor air pollution from stored animal wastewater: Assessment of the dynamic processes caused by surface disturbances

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Abstract

Animal wastewater is a source of environmental pollutants. Previous studies investigating the release of gases from wastewater stored in-barn have mostly focused on undisturbed conditions. However, when stored in-barn, the liquid surface is frequently disturbed by wastewater that falls into the pit and alters the equilibrium of pH at the surface and the gas emission patterns. We evaluated the effects that the addition of wastewater (spatially and temporally distributed based on animal’s activity models) have on NH₃, CO₂ and H₂S emissions. Wastewater additions broke the liquid surface chemical equilibrium, causing an increase-decrease-increase pattern of NH₃ emissions and an increase-decrease pattern of CO₂ and H₂S emissions. Average daily emissions of NH₃, CO₂ and H₂S were significantly increased during the days of wastewater disturbance. Emissions of CO₂ and H₂S from disturbed wastewater were significantly higher (by up to 128% and 4500%, respectively) compared to undisturbed wastewater. Future estimations of CO₂ and H₂S emissions should consider the effects that slurry disturbances have on these emissions. This is of special importance in the case of H₂S, as indoor exposures and the associated occupational health risks will be highly underestimated if the evaluation of H₂S exposures is based on emissions from wastewater stored under undisturbed conditions.

Key word: air quality, hydrogen sulfide, pH, swine, slurry, ammonia
1. Introduction

Ammonia (NH₃), carbon dioxide (CO₂) and hydrogen sulfide (H₂S) are primary gaseous contaminants emitted from animal waste. In Europe and US, NH₃ from animal husbandry contributes approximately by 80% to the total anthropogenic NH₃ release (Battye et al., 1994; Webb et al., 2005). Ammonia emitted to the atmosphere can cause eutrophication and acidification of water and soil systems. Recent studies have shown that outdoor NH₃ concentrations are related to odor annoyance (Blanes-Vidal et al., 2012a; 2012b) and health effects on local residents (Blanes-Vidal et al., 2014a; 2014b; Blanes-Vidal, 2015). Hydrogen sulphide is a well-known toxic gas, which can cause animal and human’s health problems, when high amounts are released during slurry operations and cumulated in the animal buildings (Chénard et al., 2003; Hoff et al., 2006). A recent study has estimated that animal barns contribute to about 98% of total North Carolina H₂S swine CAFO emissions, while H₂S swine CAFO emissions contribute to about 18% of North Carolina H₂S emissions (Rumsey et al., 2014 In press). Besides, H₂S has also been recognized as a major odorous gas due to its low odor detection limit (ranges from 0.0001to 0.02 ppm) (Blanes-Vidal et al., 2009). Carbon dioxide is a greenhouse gas released by animals and from animal wastes. Although animal production is not considered a major contributor to the greenhouse effect, determining CO₂ emissions is of interest because release (or volatilization) of CO₂ has a co-effect on NH₃ emissions (Ni et al., 2000) and CO₂ can also be used as a trace gas for determining ventilation rates (Blanes and Pedersen, 2005).

Gas release (or volatilization) is a process in which dissolved gases transfer from the liquid slurry to gas phase, across the gas-liquid interface due to diffusion processes as affected by concentration gradients, air velocity, and temperature of the liquid and air (Ni, 1999; Blanes-Vidal and Nadimi, 2011). The major gases released from animal wastewater (hereafter called, slurry) are weak acidic (e.g. CO₂, H₂S) or basic (e.g. NH₃), which are influenced by the slurry pH. In an animal wastewater
storage system, the concentrations of buffer components (i.e. HCO$_3^-$ and NH$_4^+$) controlling the pH in the surface layer of the wastewater change over time due to volatilization of gases (Sommer and Husted, 1995; Blanes-Vidal et al., 2010). Therefore, a pH gradient formed inside the slurry, which makes the pH at the top layers of settled slurry higher than the pH at deeper layers.

The main sources of gases pollutants from animal husbandry are: animal buildings, outdoor slurry storages and field application of animal wastes. Animal slurry stored under-floor pit is characterized by the frequent occurrence of surface liquid disturbances caused by the urine and feces that fall into the pit, and alter the equilibrium of the slurry surface. Previous studies investigating the release of gases from slurry mostly focused on the steady-state conditions (e.g., Arogo et al., 2000; Sommer et al., 2007). Recent studies have suggested that emissions from transient-state conditions (during the period of time in which the pH profile is formed) could represent an important part of the total emissions (Blanes-Vidal and Nadimi 2011; Blanes-Vidal et al., 2012c). However, information about gases emissions during and after slurry disturbances is very limited (Wang et al., 2006). Evaluation of the effect of these disturbances on the total emissions of NH$_3$, CO$_2$ and H$_2$S is needed for obtaining a reliable estimate of the total emissions from animal production.

The objective of this study was to evaluate the effects that the disturbances of the stored slurry (caused by intermittent slurry additions) may have on the emissions of NH$_3$, CO$_2$ and H$_2$S, both in total and on the gas emission patterns, and the potential health risks derived from these effects. Unlike previous studies, in which slurry additions were simulated as single events or studied at large time scales (time between disturbances of days or weeks) (Ni et al., 2009; Blanes-Vidal et al., 2012c); in this study we considered: (1) the frequent additions of slurry that occur daily under real conditions, (2) the random nature of the spatial distribution of these slurry additions, and (3) the temporal variation in slurry additions, as determined by daily variations in animals’ level of activity.
2. Material and Methods

2.1. Dynamic flux chambers

In the study, ten dynamic flux chambers with a volume of 30 L and a height of 51 cm were used (Figure 1). The airflow rate was 1.9 l/min, (corresponding to a slurry surface velocity of 0.023 m/s, Aarnink and Elzing, 1998), which was maintained by a critical orifice inserted in the outflow tube, between a filter and the air pump. Each chamber was airtight. Several ports were installed in the lid. These ports were used as air inlet and outlet, for headspace NH\textsubscript{3}, CO\textsubscript{2} and H\textsubscript{2}S concentration measurement, for pH measurements, and finally two ports were used for addition of fresh slurry. The inlet and outlet air ducts and the sampling points were located 3 cm below the lid. All the ports were closed by plugs except during slurry addition and pH measurement.

2.2. Experimental design and procedure

Two types of slurry were considered in this study, which were collected from under-floor deep pits of a fattening swine barn. Animals weight was of about 62 kg body weight on average (slurry type A) and under 30 kg body weight (slurry type B). Slurry was collected after 2 to 4 days of accumulation from last evacuation of slurry in the swine farm.

Four experiments (experiment A1, B1, A2 and B2) on the two types of slurry were carried out in this study. Each experiment consists of a series of actions executed during 8 consecutive days (Table 1). In each of the experiments, two of the ten dynamic flux chambers were considered as “control chambers” (containing undisturbed stored slurry), and the other eight chambers were “treatment chambers” (containing stored slurry that is disturbed by slurry additions). Treatment chambers were divided into two groups Group a and Group b (replicates) of four chambers each (Figure 1). Two days before starting the experiments, each flux chamber was filled with 15 L of homogenized swine slurry to a depth of 26 cm. The slurry surface of each treatment group corresponds to 0.24 m\textsuperscript{2} of under barn deep-pit slurry storage. The slurry production per day
corresponding to this area was calculated as 6.47 kg day$^{-1}$, from an animal body weight of 60 kg, a density of 0.55 m$^2$ animal$^{-1}$, a 1/3 of partial slatted floor and a slurry production of 84 g kg$^{-1}$ LW, as reported by ASABE (2005) and Barker and Overcash, (2007).

In order to calculate the total number of slurry additions per day, Eq. 1 (Cortus et al., 2005) was used, which describes the urination frequency for both male and female swine between 51 and 78 kg considering an average urination frequency of 0.62 U swine$^{-1}$ h$^{-1}$.

$$N_U = N_{swine} \cdot 0.62 \cdot \left( 1 - 0.58 \cdot \sin \left( \frac{2\pi}{24} \cdot (\text{time} + 6 - 2.5) \right) \right)$$

Where $N_U$ is the total number of slurry additions, $N_{swine}$ is the number of swine, and time is the number of hours after midnight.

According to the equation, a minimum urination activity occurs at 2:30 am and the variation in frequency over the 24-h period is ±0.58 U pig$^{-1}$ h$^{-1}$. Applying this equation for one swine and during 24-h period, it results in a total number of approximately 15 urinations per day and swine. The amount of slurry for each addition was 431 g.

According to Pleasants et al, (2007) an animal voids urine with a Poisson probability distribution, and each urine deposition covers a random area with a Gaussian probability density. In our study, Matlab software was used to randomly calculate the timing of the slurry additions on the slurry surface, using a Poisson random number generator. The Poisson number generator was used to simulate the number and timing of slurry additions for a large number of days (i.e. 100 days). Two days from a total of 100 days obtained from Matlab were randomly selected to determine the timing of slurry additions (Figure 1S). Each chamber was provided with two ports for slurry addition, which resulted in eight potential locations for slurry addition, per treatment group (i.e., 4
chambers/group). To simulate the spatial variability, the location of the slurry addition at each time was selected out of these eight locations, using random generated numbers (Figure 1S).

2.3. Measurements

2.3.1. NH$_3$ and CO$_2$ concentrations

Concentrations of NH$_3$ and CO$_2$ were measured during the measurement days described in Table 1, using an infrared 1412 photoacoustic multi-gas analyzer and a multiplexer 1309 (Innova Air tech Instruments A/S, Denmark), compensated for gas and water interferences. The detection limits for CO$_2$ and NH$_3$ are 1.5 ppm and 0.2 ppm, respectively (1 atm, 20ºC). Each set of NH$_3$ and CO$_2$ measurements takes 45 s, so gas emission measurements from each chamber were obtained every 9 minutes as the multiplexer had 12 measurement channels (one at each of ten chambers’ headspace and two at the inlet air) (Figure 1).

2.3.2. H$_2$S concentrations

Concentrations of H$_2$S were measured between 11:00 and 12:00 the day before the treatment (Day 3, represented as D-), between 11:00 and 12:00 the day after the treatment (Day 6, represented as D+), and three times on each treatment day (i.e., Day 4 and Day 5, represented as D1 and D2, respectively) (Table 1). Measurements were performed using a H$_2$S analyzer (Arizona instrument LLC, model Jerome 631-X, measurement range 0.001 – 50 ppm) and precision gas detector tubes (Kitagawa, Japan; ranges of 0.75 to 300 ppm and 0.005% to 0.16%) for the disturbed chambers during days of treatment. A Teflon tube was used to connect the analyzer to the inlet and outlet connections of the Jerome meter.

2.3.3. Slurry characteristics and pH

Slurry subsamples (0.5 L) from each chamber were collected on Day 0 and Day 7 after homogenization for analysis of dry matter (DM), total ammonium nitrogen (TAN), and total
nitrogen (TN). Slurry subsamples were evaporated to dryness with a constant weight in an oven at 105°C for 24 h, and the remaining mass was recorded as DM. TAN and TN were analyzed by the Kjeldahl method. The pH was measured with a pH meter (model PHM210, Meterlab Radiometer Analytical) at three depths (0.5 to 1 cm, 4 cm and 6 cm from the slurry surface) during the experiment days (Table 1).

2.4. Emission calculation

The gas emissions from slurry were calculated from Eq. 2:

\[
E = \frac{(C_o - C_i) \cdot Q}{S}
\]  (2)

where \(E\) is the gas release rate (mg m^{-2} s^{-1}), \(C_o\) and \(C_i\) are the gas concentrations (mg m^{-3}) at the outlet and inlet, respectively, \(Q\) is the airflow rate (m^{3} s^{-1}), and \(S\) is the emitting surface area (m^{2}).

2.5. Statistical Analysis

Data on CO₂, NH₃ and H₂S emissions were first calculated from each measured concentrations by using Eq. 2. As all chambers had equal surface area, gas emissions of each treatment group (in mg m^{-2} s^{-1}) were calculated as the average of the emissions from the four chambers’ of each treatment.

Statistical analyses were used to compare average emissions from disturbed and undisturbed slurries (treatment vs. control chambers) during the day before disturbance (D-), the days of slurry disturbance (D1 and D2) and the day after disturbance (D+), and to determine whether there were statistical differences between each of the days. Student t-tests were used to compare the difference of gas emission and slurry characteristics between treatments and controls. Changes on daily average gas emission obtained during the experiments were analyzed by Paired t-tests. All the statistical analyses were performed with SYSTAT 13 ® (Systat Software, Inc., Chicago) with a significance level of \(\alpha = 0.05\).
3. Results

3.1. Slurry characteristics

The average slurry dry matter (DM), total ammonium nitrogen (TAN) and total nitrogen (TN) of slurry type A and B before and after each treatment are shown in Table 2. The two types of slurries used in this study (type A and type B) had initial DM of 8.76±0.2 % and 0.85±0.09 %, TN of 6.19±0.19 g/kg and 0.87±0.02 g/kg, and TAN of 3.66±0.03 g/kg and 0.71±0.03 g/kg, respectively. The results showed that slurry type A had much higher values on the DM, TAN and TN than the type B, so some differences on gas emissions due to having different compositions, were expected. No differences between treatment and control regarding slurry characteristics were found in none of the experiments (Table 2). Regarding the comparison of compositions before and after treatment, no difference on DM and TN were found. TAN experienced a slight increase (5.4%) from before to after the treatment in A1 and a slight decrease (-5.5%) in A2.

3.2. Gas emissions

3.2.1. NH₃ emission

Average ammonia emissions from the four experiments (A1, A2, B1, B2) during the day before the additions were carried out (D–), during each of the two days of slurry additions (D1 and D2) and during the day after slurry additions (D+) are shown in Figure 2. In all experiments, average NH₃ emissions in the treatment group during the days of slurry addition (D1 and D2) were significantly higher compared to emissions the day before slurry addition (Table 3). Short-term effects of slurry disturbances are shown in Figure 2S, where the sequence of slurry additions caused a fluctuating NH₃ emission pattern. Right after each slurry addition, a sharp decrease of NH₃ occurred, followed by a gradual increase.
Emissions of NH$_3$ from slurry type A ranged from 1.28 to 3.87 g m$^{-2}$ day$^{-1}$ and were significantly higher than those from slurry type B, which ranged from 0.43 to 0.92 g m$^{-2}$ day$^{-1}$ (Figure 2). Regarding the comparison between control and treatment, the day before treatment (D$^{-}$), average NH$_3$ emissions were not significantly different between the treatment and control flux chambers in all experiments. Although in general terms, emissions from the treatment group appeared to be higher in the treatment groups compared to the control groups (solid line vs. broken line in Figure 2), statistical analysis showed that these differences were only significant in some cases. During the two days of slurry additions (D1 and D2), NH$_3$ emission in the treatment groups were between 21% and 43% higher than emissions in the control (undisturbed) chambers in experiment B1, and were 13% higher during D2 in experiment B2. During the subsequent storage day (D+), higher NH$_3$ emission in the treatment groups compared to controls were found in experiment A1 (14% higher) and B2 (21% higher) (Figure 2). Cumulative NH$_3$ emissions were calculated based on hourly average emissions, the results showed that no significant differences were found between treatment and control during the two treatment days, except in experiment B1 (32% higher in treatment than in control) (Figure 3S) (Table 4).

3.2.2. CO$_2$ emission

Figure 3 shows the average emissions of CO$_2$ from the treatment and control chambers during the four measurement days. The comparison between CO$_2$ emissions during the different days did not show clear patterns (e.g. an increase of CO$_2$ emission from the day before addition to the first day of slurry addition (D1) was observed in experiment A2, while a decrease of CO$_2$ emission was found in experiment B2). A more detailed observation of short-term effects (Figure 4S) showed that when the slurry addition was performed, CO$_2$ emissions immediately increased, and were followed by a decay during the next 2 to 30 min. As in the case of NH$_3$ emissions, this resulted in a fluctuation in the CO$_2$ emission patterns as caused by the intermittent slurry disturbances.
1 Similarly to NH$_3$, CO$_2$ emissions from slurry type A (which ranged from 49 to 94 g m$^{-2}$ day$^{-1}$), were
2 higher than those from slurry type B (range of 22 - 36 g m$^{-2}$ day$^{-1}$). During the treatment days (D1
3 and D2) and the day after treatment (D+), CO$_2$ emissions in the treatment chambers were between
4 12 % and 60% higher than those from the control chambers in experiment B1 and B2 (Figure 3).
5 Those differences were not observed in the case of slurry A. Regarding to cumulative CO$_2$
6 emissions, slurry additions in treatment chambers caused CO$_2$ emission to increase by 30-128%
7 compared to the controls during the two treatment days (Figure 3S) (Table 4).

8 3.2.3. H$_2$S emission

9 The daily averaged H$_2$S emissions in the treatment and control chambers during the four days of
10 experiment are shown in Figure 4. In all four experiments (A1, A2, B1, B2), average H$_2$S emissions
11 in the treatment group during the day before slurry additions (D–) were significantly lower
12 compared to emissions the days of slurry addition (D1 and D2) (Table 3). A significantly lower
13 emissions of H$_2$S were also found during the subsequent storage day after slurry addition (D+)
14 compared to emissions the days of slurry addition (D1 and D2) in experiment A2, B1 and B2 (Table
15 3).
16 During the day before slurry addition (D–), no significant differences were found between treatment
17 and control in none of the experiments. Regarding the comparison between treatment chambers and
18 control chambers during the treatment days (D1 and D2, when slurry additions were performed),
19 H$_2$S emissions in the treatment chambers were from 20% to 4445% higher than in the control
20 chambers. During the subsequent storage day (D+), significantly higher emissions of H$_2$S were
21 found only in B1 (96% higher).

22 3.3. Slurry surface pH profiles

23 The slurry pH was measured at three depths (0.5 cm, 4 cm, 6 cm) below the surface in all
24 experiments, and in general terms, the surface pH increased over time when the slurry was stored
undisturbed. Figure 5 shows these results for all experiments. Taking experiment A1 as an example, the surface pH (i.e. at 0.5 cm below the surface) on the day after treatment (D+) was 0.40-0.82 units higher than the pH on day 0, which was measured right after the slurry was mixed. A pH gradient from deeper to top layers was formed during the days of undisturbed storage, showing a pH at 0.5 cm below the surface of 0.43 ± 0.10 units higher than at deeper layers (6 cm). During the treatment days (D1 and D2), the pH gradients were broken by the addition of new slurry that affected the air-liquid interface. As a result the pH during D1 and D2 did not vary with depth. Besides, the pH at surface (0.5 cm) during D1 and D2 was 0.33 ± 0.21 units lower compared to the day before addition (D–).

4. Discussion

4.1. Mechanisms of NH$_3$, CO$_2$ and H$_2$S release from frequently disturbed animal wastewater

The addition of slurry caused a short-term change in the emissions of all three gases. Slurry disturbances were followed by a decrease in the emission of NH$_3$ (which increased gradually after that), and an increase in the emission of CO$_2$ and H$_2$S (followed by a decrease). The immediate increase was especially sharp in the case of H$_2$S.

The short-term emission patterns of NH$_3$, CO$_2$ and H$_2$S can be explained by the changes in surface pH and the release of gas bubbles containing CO$_2$ and H$_2$S. The release of NH$_3$ from liquid slurry (animal wastewater) is generally considered a process of convective mass transfer, in which the gaseous NH$_3$ at the liquid slurry surface is released into the free air stream, across an aqueous-gaseous interface, which is affected by gradients of free ammonia concentration, air velocity and temperature (Arogo et al., 1999; Ni, 1999, Ye et al., 2008). As NH$_3$ is a basic gas, a higher surface pH shifts the equilibrium of $\text{NH}_4^+ \leftrightarrow \text{NH}_3 + \text{H}^+$ to the right, thereby favoring the release of NH$_3$.

During slurry storage, a pH gradient in the slurry surface was formed, being higher in the slurry surface than in deeper layers. As our measurements showed, the pH gradient at the slurry surface
layers was broken when the slurry disturbance occurred, resulting in a decrease of pH in the surface, which can contribute to the decrease of NH$_3$ after slurry disturbance (Blanes-Vidal et al., 2012c). The lower pH after slurry addition favored the emission of acidic gases (i.e. CO$_2$ and H$_2$S). This, in turn, increases the pH, then accelerate the emission of basic gases (i.e. NH$_3$). The rate of release NH$_3$ increases gradually and then reaches a steady-state condition when a new equilibrium of surface pH is established. Previous studies have reported a transient period of 90-200 min before achieving the dynamic equilibrium of slurry pH and the more stable gases release (Ni et al., 2009; Blanes-Vidal and Nadimi, 2011; Blanes-Vidal et al., 2012c). In our study, the slurry addition intervals ranged from 60 min to 420 min based on the animal activity model. As a consequence, before the NH$_3$ release reached the steady-state emission, the equilibrium was disrupted again, resulting in a fluctuating pattern of NH$_3$ emissions (Figure 2S).

The mechanisms of CO$_2$ release are mainly related to the release of gas bubbles containing CO$_2$ and the existence of CO$_2$ dissolved in aqueous phase. Due to its low solubility (Henry’s constant of CO$_2$ at 20 °C = 1.63 dimensionless gas/liquid) and the bubbles release, most of the CO$_2$ generated and cumulated inside the slurry can be released more quickly than other gases with higher solubility (i.e. Henry’s constant of NH$_3$ at 20 °C = 5.4 x 10$^{-4}$ dimensionless gas/liquid) (Ni et al., 2000, 2009). In our experiment, immediate increases of CO$_2$ emissions were observed (Figure 4S), which were followed by a decay during the next 2 to 30 min, in agreement with Ni et al. (2000, 2009) and Blanes-Vidal et al. (2012c).

Previous studies have concluded that H$_2$S release from swine wastewater does not follow a predictable pattern (Chénard et al., 2003). Ni et al. (2009) developed a “Bubble-release” model to explain the behavior characteristics of H$_2$S release. Hydrogen sulfide generated by microbial decomposition of sulfur-containing compound in the slurry exists as gas bubbles of various sizes, which contain H$_2$S and other gases (such as CO$_2$). A sudden release of bubbles, so called “burst release”, occurs when slurry is agitated. Blanes-Vidal et al., (2012c) found that H$_2$S emissions
sharply increased during slurry disturbance and then decreased in the next 2 to 20 min based on continuously H$_2$S concentration measurements. Apart from the bubble release mechanism, H$_2$S can be also affected by changes in surface pH (as H$_2$S is an acidic gas and higher pH is unfavorable of H$_2$S release). However, the effect of pH on H$_2$S release cannot be identified as measured H$_2$S emissions largely varies during and immediately after the slurry disturbance, due to the burst release (Ni et al, 2009).

4.2. Assessment and implications of considering transient-state emissions caused by slurry additions

In this study we investigated transient-state emissions of NH$_3$, CO$_2$ and H$_2$S from simulated in-barn storage conditions (slurry disturbed by frequent wastewater additions) in laboratory slurry chambers. The results regarding the addition of slurry on NH$_3$ emissions are inconclusive. The addition of slurry caused an increase in the average daily emissions of NH$_3$ during the days of addition compared to the day before the additions, but it did not show consistent results when comparing treatment vs. controls. Besides, cumulative NH$_3$ emissions were not significantly different between treatment and control except one of the experiments (B1), when cumulative emissions from disturbed slurry were higher. On the contrary, in a previous study where slurry additions were simulated as single events, emissions of NH$_3$ from frequently disturbed slurry were estimated as 43% lower than those from undisturbed slurry (Blanes-Vidal et al., 2012c). These discrepancies may be due to two reasons. First, the short-term variations in NH$_3$ emission caused by slurry disturbances. The addition itself instantly increases NH$_3$ emissions, emissions then decrease and are followed by a gradual increase (Blanes-Vidal et al., 2012c). The immediate increase in NH$_3$ emissions has been observed in previous studies (Blanes-Vidal et al., 2012c), although it could not be observed in our study, due to the lower frequency of measurements in the current study compared to Blanes-Vidal et al., (2012c). These variations in NH$_3$ emissions (i.e., increase-decrease-increase) may compensate
each other when averaged over the four treatment chambers and over a full day, causing the effect
estimate to be pulled towards its null value, i.e., not showing statistically significant differences on
averaged emissions between treatment and controls. A second reason for the higher NH$_3$ emission
from disturbed slurry in the current study could be that pH at the three layers of frequently disturbed
slurry was in the range of 7-8 (Figure 5), which has been determined as optimal swine slurry pH for
urease catalyzing urea into NH$_4^+$ and CO$_3^{2-}$ (Dai and Karring, 2014 unpublished data).

The addition of slurry caused an increase in the average daily emissions of CO$_2$ and H$_2$S during the
days (D1 and D2) of addition compared to the day before the additions (D– ). It also caused an
increase in the cumulative emissions of CO$_2$ in the treatment chambers compared to the controls
during the treatment days. This can be explained by the fact that unlike in the case of NH$_3$, slurry
disturbances always cause a peak of CO$_2$ and H$_2$S emissions, that tend to increase average
emissions. In our study, time-weighted (three measurements during 24 h) average emissions of H$_2$S
from disturbed slurry during treatment days of experiment A1, A2, B1 and B2 reached a maximum
of 1.30, 0.91, 0.82 and 1.01 mg·m$^{-2}$·min$^{-1}$, respectively (corresponding to the concentrations of 14,
10, 9 and 12 ppm, respectively). According to occupational safety and health administration (OSHA)
of United States (U.S. Dept. of Labor, 2014), prolonged exposure to 2-5 ppm H$_2$S concentrations
may cause nausea, tearing of the eyes, headaches or loss of sleep, and airway problems, while
exposure to 20 ppm can cause fatigue, loss of appetite, headache, irritability, poor memory and
dizziness.

The differences observed in disturbed and undisturbed slurry can have consequences for a good
estimation of CO$_2$ and H$_2$S emissions from wastes in future studies, but they may not be of practical
importance in the case of NH$_3$. Both CO$_2$ and H$_2$S emissions will be underestimated if they are
based on undisturbed slurry. This is of most importance in the case of H$_2$S, as emissions and
concentrations estimated from undisturbed slurry will be much lower than the actual values released
from disturbed slurry. This can lead to a significant underestimation of the occupational exposures to H\textsubscript{2}S and the potential health risks derived from these exposures. More studies involving comparison of emissions from disturbed and undisturbed slurry in full-scale buildings are needed to better evaluate the practical implications of these effects.

5. Conclusions

Emissions of ammonia and carbon dioxide remained relatively constant under steady conditions during storage. The addition of slurry caused an increase in the average daily emissions of NH\textsubscript{3}, CO\textsubscript{2} and H\textsubscript{2}S during the days of addition compared to the day before the additions. Besides, CO\textsubscript{2} and H\textsubscript{2}S emissions from treatment chambers (i.e. where slurry was added at different locations and times based on animal’s activity models) were higher than from control chambers (which was kept undisturbed during the full experimental time). However, such differences were not found when comparing NH\textsubscript{3} emissions. The differences in results between NH\textsubscript{3}, CO\textsubscript{2} and H\textsubscript{2}S, may be explained by the fact that slurry disturbances cause different short-term emission patterns in the case of NH\textsubscript{3} compared to CO\textsubscript{2} and H\textsubscript{2}S. Slurry additions cause an increase-decrease-increase pattern in NH\textsubscript{3} emissions, while in the case of CO\textsubscript{2} and H\textsubscript{2}S slurry additions cause a sharp increase in the release of these gases, which decrease to previous values shortly after the disturbance. Future estimations of CO\textsubscript{2} and H\textsubscript{2}S emissions should consider the effects that slurry disturbances have on these emissions. This is of special importance in the case of H\textsubscript{2}S, as indoor exposures and the associated health risks will be highly underestimated if the evaluation of exposures to H\textsubscript{2}S is based on emissions from slurries stored under undisturbed conditions.

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References


Figure 1. Experimental setup and schematic of the dynamic flux chambers.
Figure 2. Average ammonia emissions from slurry A and B during the day before the slurry additions started (D-), during the two days of slurry additions (D1 and D2) and during the day after the slurry addition occurred (D+). Bars indicate standard deviation. Emissions on day D+ in experiment B1 were not available.
Figure 3. Average carbon dioxide emissions from slurry A and B during the day before the slurry additions started (D-), during the two days of slurry additions (D1 and D2) and during the day after the slurry addition occurred (D+). Bars indicate standard deviation. Emissions on day D+ in experiment B1 were not available.
Figure 4. Average hydrogen sulfide emissions from slurry A and B during the day before the slurry additions started (D-), during the two days of slurry additions (D1 and D2) and during the day after the slurry addition occurred (D+). Bars indicate standard deviation.
Figure 5. pH profile from disturbed (T) and undisturbed slurry (C) after slurry mixing at the Day 0.
<table>
<thead>
<tr>
<th>Day</th>
<th>Experiment actions</th>
<th>Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>Mixing and sampling</td>
<td>Characteristics of slurry</td>
</tr>
<tr>
<td>Day 1 and 2</td>
<td>Slurry was stored without disturbance to reach a stable condition</td>
<td></td>
</tr>
<tr>
<td>Day 3 (represented as: D-)(^{[b]})</td>
<td>Start of measurements</td>
<td>Concentration of NH(_3), CO(_2), H(_2)S(^{[c]}), slurry pH(^{[d]})</td>
</tr>
<tr>
<td>Day 4 (represented as: D1) (^{[b]})</td>
<td>Treatment</td>
<td>Concentration of NH(_3), CO(_2), H(_2)S(^{[c]}), slurry pH(^{[d]})</td>
</tr>
<tr>
<td>Day 5 (represented as: D2) (^{[b]})</td>
<td>Treatment</td>
<td>Concentration of NH(_3), CO(_2), H(_2)S(^{[c]}), slurry pH(^{[d]})</td>
</tr>
<tr>
<td>Day 6 (represented as: D+)(^{[b]})</td>
<td>Subsequent measurement after treatment</td>
<td>Concentration of NH(_3), CO(_2), H(_2)S(^{[c]}), slurry pH(^{[d]})</td>
</tr>
<tr>
<td>Day 7</td>
<td>Mixing and sampling, changing slurry(^{[e]})</td>
<td>Characteristics of slurry</td>
</tr>
</tbody>
</table>

\(^{[a]}\) Four experiments in total, and the sequence of experiment was A1, B1, A2 (repeat of A1), B2 (repeat of B1).

\(^{[b]}\) Day D-: the day before disturbance; Day D1 and D2, the days of slurry disturbance; Day D+: the day after disturbance.

\(^{[c]}\) Concentration of NH\(_3\) and CO\(_2\) were measured approximately every 9 min; H\(_2\)S concentration on day 3 (D-) and 6 (D+) were measured from 11:00 to 12:00 (3 repetitions at each chamber), H\(_2\)S concentrations on day 4 (D1) and 5 (D2) were measured during each addition (one measurement) and every 8 h for all chambers.

\(^{[d]}\) Slurry pH at day 3 and 6 was measured from 12:00 to 14:00 at three depths (0.5-1 cm, 4 cm, 6 cm), slurry pH at day 4 and 5 was measured after each addition at 3 depths.

\(^{[e]}\) Change chambers already filled with another type of slurry.
Table 2. Slurry characteristics

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DM (%)</th>
<th>TAN</th>
<th>TN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>p (B, A)</td>
</tr>
<tr>
<td>A1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>8.91±0.14</td>
<td>9.52±0.15</td>
<td>0.202</td>
</tr>
<tr>
<td>Control</td>
<td>8.61±0.08</td>
<td>9.09±0.60</td>
<td>0.411</td>
</tr>
<tr>
<td>p (T, C)</td>
<td>0.124</td>
<td>0.433</td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>8.75±0.04</td>
<td>8.51±0.38</td>
<td>0.572</td>
</tr>
<tr>
<td>Control</td>
<td>8.55±0.25</td>
<td>8.85±0.53</td>
<td>0.359</td>
</tr>
<tr>
<td>p (T, C)</td>
<td>0.396</td>
<td>0.535</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>0.91±0.08</td>
<td>0.68±0.01</td>
<td>0.136</td>
</tr>
<tr>
<td>Control</td>
<td>0.80±0.06</td>
<td>0.74±0.06</td>
<td>0.139</td>
</tr>
<tr>
<td>p (T, C)</td>
<td>0.265</td>
<td>0.283</td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>0.54±0.02</td>
<td>0.66±0.04</td>
<td>0.634</td>
</tr>
<tr>
<td>Control</td>
<td>0.47±0.00</td>
<td>0.87±0.15</td>
<td>0.171</td>
</tr>
<tr>
<td>p (T, C)</td>
<td>0.069</td>
<td>0.303</td>
<td></td>
</tr>
</tbody>
</table>

*a* Average of two treatment groups (T1 and T2), and each group has eight measurements (replicate of each chamber).

*b* Average of two control chambers (replicate of each chamber).

*c* Mean ± standard deviation.

*d* n.a. : not available.

*e* p value of comparison between treatment and control, significant difference marked with * (p<0.05).

*f* p value of comparison between before and after experiment, significant difference marked with *(p<0.05).*
Table 3. Comparison (p-values from paired t-tests) between average gas emissions from slurry A and B during the day before the slurry additions started (D-), during the two days of slurry additions (D1 and D2) and during the day after the slurry addition occurred (D+)

<table>
<thead>
<tr>
<th></th>
<th>Treatment</th>
<th>Control</th>
<th>Treatment</th>
<th>Control</th>
<th>Treatment</th>
<th>Control</th>
<th>Treatment</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₃</td>
<td>p(D-, D1)</td>
<td>0.009</td>
<td>0.505 n.s.</td>
<td>0.021</td>
<td>0.208 n.s.</td>
<td>0.037</td>
<td>0.174 n.s.</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>p(D-, D2)</td>
<td>0.011</td>
<td>0.303 n.s.</td>
<td>0.024</td>
<td>0.102 n.s.</td>
<td>0.008</td>
<td>0.100 n.s.</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>p(D1, D2)</td>
<td>0.009</td>
<td>0.084 n.s.</td>
<td>0.007</td>
<td>0.002</td>
<td>0.084 n.s.</td>
<td>0.117 n.s.</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>p(D1, D+)</td>
<td>0.014</td>
<td>0.212 n.s.</td>
<td>0.009</td>
<td>0.109 n.s.</td>
<td>0.192 n.s.</td>
<td>0.053 n.s.</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>p(D1, D+)</td>
<td>0.008</td>
<td>0.067 n.s.</td>
<td>0.064 n.s.</td>
<td>0.262 n.s.</td>
<td>0.001</td>
<td>0.074 n.s.</td>
<td>0.031</td>
</tr>
<tr>
<td></td>
<td>p(D2, D+)</td>
<td>0.001</td>
<td>0.424 n.s.</td>
<td>0.237 n.s.</td>
<td>0.481 n.s.</td>
<td>0.038</td>
<td>0.054 n.s.</td>
<td>0.038</td>
</tr>
<tr>
<td>CO₂</td>
<td>p(D-, D1)</td>
<td>0.473 n.s.</td>
<td>0.398 n.s.</td>
<td>0.027</td>
<td>0.088 n.s.</td>
<td>0.055 n.s.</td>
<td>0.458 n.s.</td>
<td>0.031</td>
</tr>
<tr>
<td></td>
<td>p(D-, D2)</td>
<td>0.186 n.s.</td>
<td>0.381 n.s.</td>
<td>0.007</td>
<td>0.113 n.s.</td>
<td>0.109 n.s.</td>
<td>0.460 n.s.</td>
<td>0.075 n.s.</td>
</tr>
<tr>
<td></td>
<td>p(D-, D+)</td>
<td>0.124 n.s.</td>
<td>0.304 n.s.</td>
<td>0.009</td>
<td>0.037</td>
<td>0.109 n.s.</td>
<td>0.460 n.s.</td>
<td>0.075 n.s.</td>
</tr>
<tr>
<td></td>
<td>p(D1, D2)</td>
<td>0.447 n.s.</td>
<td>0.369 n.s.</td>
<td>0.252 n.s.</td>
<td>0.309 n.s.</td>
<td>0.396 n.s.</td>
<td>0.082 n.s.</td>
<td>0.032</td>
</tr>
<tr>
<td></td>
<td>p(D1, D+)</td>
<td>0.018</td>
<td>0.348 n.s.</td>
<td>0.164 n.s.</td>
<td>0.228 n.s.</td>
<td>0.078 n.s.</td>
<td>0.379 n.s.</td>
<td>0.078 n.s.</td>
</tr>
<tr>
<td></td>
<td>p(D2, D+)</td>
<td>0.116 n.s.</td>
<td>0.42 n.s.</td>
<td>0.015</td>
<td>0.25 n.s.</td>
<td>0.121 n.s.</td>
<td>0.587 n.s.</td>
<td></td>
</tr>
<tr>
<td>H₂S</td>
<td>p(D-, D1)</td>
<td>0.03</td>
<td>0.017</td>
<td>0.005</td>
<td>0.057 n.s.</td>
<td>0.012</td>
<td>0.01</td>
<td>0.042</td>
</tr>
<tr>
<td></td>
<td>p(D-, D2)</td>
<td>0.006</td>
<td>0.045</td>
<td>0.002</td>
<td>0.184 n.s.</td>
<td>0.025</td>
<td>0.008</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td>p(D-, D+)</td>
<td>0.014</td>
<td>0.011</td>
<td>0.009</td>
<td>0.002</td>
<td>0.038</td>
<td>0.111 n.s.</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>p(D1, D2)</td>
<td>0.064 n.s.</td>
<td>0.081 n.s.</td>
<td>0.005</td>
<td>0.078 n.s.</td>
<td>0.034</td>
<td>0.009</td>
<td>0.085 n.s.</td>
</tr>
<tr>
<td></td>
<td>p(D1, D+)</td>
<td>0.087 n.s.</td>
<td>0.005</td>
<td>0.006</td>
<td>0.016</td>
<td>0.011</td>
<td>0.37 n.s.</td>
<td>0.042</td>
</tr>
<tr>
<td></td>
<td>p(D2, D+)</td>
<td>0.403 n.s.</td>
<td>0.123 n.s.</td>
<td>0.008</td>
<td>0.019</td>
<td>0.024</td>
<td>0.014</td>
<td>0.023</td>
</tr>
</tbody>
</table>

n.s.: not significant (p>0.05)
Table 4. Comparison of cumulative gas emissions between treatment and control.

<table>
<thead>
<tr>
<th></th>
<th>NH_3</th>
<th>CO_2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1</td>
<td>A2</td>
</tr>
<tr>
<td><strong>The day before the slurry additions started (D-)</strong> (T-C)/C (%)</td>
<td>-4</td>
<td>-9</td>
</tr>
<tr>
<td>p-value</td>
<td>0.81 n.s.</td>
<td>0.65 n.s.</td>
</tr>
<tr>
<td><strong>Treatment day (D1 and D2)</strong> (T-C)/C (%)</td>
<td>19</td>
<td>-17</td>
</tr>
<tr>
<td>p-value</td>
<td>0.35 n.s.</td>
<td>0.14 n.s.</td>
</tr>
<tr>
<td><strong>The day after the slurry addition occurred (D+)</strong> (T-C)/C (%)</td>
<td>13</td>
<td>-7</td>
</tr>
<tr>
<td>p-value</td>
<td>0.03 *</td>
<td>0.54 n.s.</td>
</tr>
</tbody>
</table>

n.s. not significant (p-value>0.05 based on Student t-test).
Figure 1S. Hourly distribution of slurry additions from one animal: (□) theoretical distribution (from Eq. 1), and (■) estimated daily distribution of additions by a Poisson process (100 potential days were generated by Matlab, two of them selected randomly for this study) (①②③④: indicate the chambers of addition occurred, these numbers were randomly generated by excel).
Figure 2S. Hourly average ammonia emission pattern from one day before slurry addition, two days of slurry addition, and one day after slurry addition, where T(ave.): average of treatment; C(ave.): average of control; T(max.): maximum emissions of treatment; T(min.): minimum emissions of treatment. Arrows indicates slurry additions (number of arrows at the certain time indicates slurry addition amount: i.e., two arrows equals two additions, each additions was 431 g).
Figure 3S. Cumulative ammonia and carbon dioxide emissions from disturbed (T) and undisturbed slurry (C) at two treatment days (D1 and D2).
Figure 3S. Hourly average carbon dioxide emission pattern from one day before slurry addition, two days of slurry additions, and one day after slurry addition (experiment A1 as an example), where 
T(ave.): average of treatment; C(ave.): average of control; T(max.): maximum emissions of treatment; T(min.): minimum emissions of treatment. Arrows indicates slurry additions (number of arrows at the certain time indicates slurry addition amount: i.e., two arrows equals two additions, each additions was 431 g).
Characteristics of Pollutant Gas Releases from Swine, Dairy, Beef, and Layer Manure, and Municipal Wastewater

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Abstract

Knowledge about characteristics of gas releases from various types of biological wastes can assist developing gas pollution reduction technologies and establishing environmental regulations. Five different organic wastes, i.e., four types of animal manure (swine, beef, dairy, and layer hen) and municipal wastewater, were studied for their characteristics of ammonia (NH₃), carbon dioxide (CO₂), hydrogen sulfide (H₂S), and sulfur dioxide (SO₂) releases for 38 to 43 days in reactors under laboratory conditions. Weekly waste additions and continuous reactor headspace ventilation were supplied to simulate waste storage conditions. Results demonstrated that the physicochemical characteristics of the different types of wastes, especially the total nitrogen, total ammoniacal nitrogen, dry matter, and pH, had strong influence on the releases of the four gases. Even for the same type of waste, the variation in physicochemical characteristics affected the gas releases remarkably. Among the five waste types, layer hen manure and municipal wastewater had the highest and lowest NH₃ release potentials, respectively. Layer manure had the highest and dairy manure had the lowest CO₂ release potentials. Dairy manure and layer manure had the highest and lowest H₂S release potentials, respectively. Beef manure and layer manure had the highest and lowest SO₂ releases, respectively.

Keywords: Agriculture wastewater; air pollution; animal agriculture; wastewater storage; water pollution.
1 Introduction

Gas release is a process of gases transfer from the immediate surface of wastes (e.g., manure or wastewater) into a free air stream. Gas emission is a process of gases emanating from an enclosure (e.g., an animal building) and entering the outdoor atmosphere (Ni, 1999). Under transient conditions or when there are gas sinks in the enclosure (e.g., a biofilter), the quantity of gas release does not equal that of gas emission. Ammonia (NH$_3$), carbon dioxide (CO$_2$), hydrogen sulfide (H$_2$S), and sulfur dioxide (SO$_2$) are among the major pollutant gases released from animal manure and municipal wastewater. Excess quantities of NH$_3$ emitted from livestock and poultry farms could have negative impact on environment and ecosystems. Ammonia has also been reported to relate to livestock odor annoyance and health outcomes in residential outdoor environment (Blanes-Vidal et al., 2012b; Qamaruz-Zaman and Milke, 2012; Blanes-Vidal et al., 2014), although it is not the main component of odorants. Emission of CO$_2$ not only contributes to greenhouse effect, but also alters the manure surface pH and accelerates the NH$_3$ emission (Ni et al., 2000). Hydrogen sulfide has been identified as a prominent gaseous constituent in animal buildings and manure storages (Hooser et al., 2000). It has been considered the most dangerous gas from livestock production systems and has been responsible for animal and farm operators’ deaths in animal facilities (e.g., Beaver and Field, 2007; Oesterhelweg and Puschel, 2008). Generally, concentrations of H$_2$S ranging from 0 to 5 ppm (part per million) are detected in animal buildings. However, when manure in deep-pit is agitated by mixing during pit emptying, paramount increases in H$_2$S releases occur (Hoff et al., 2006; Blanes-Vidal et al., 2012a). Sulfur dioxide (SO$_2$) emitted to the atmosphere can form H$_2$SO$_4$ and cause acid rain. However, very little information about SO$_2$ from agriculture wastes has been reported. Knowledge about quantification and release behavior of gases from various waste sources is not only important for establishing environmental regulations, but also crucial for
the development of gas emission reduction technologies. However, direct comparison of the 
characteristics of gas releases from different pollution sources has not been found in the 
literature.

Gases are produced in organic wastes under biological decomposition, which causes 
organic matters to convert into gases. The converted substances can exist either as an ionized 
form (i.e., \( \text{NH}_4^+ \), \( \text{HCO}_3^- \)) or free gases (i.e., \( \text{NH}_3 \), \( \text{CO}_2 \)) in the wastes. Two mechanisms of gas 
releases have been reported in the literatures: 1) dissolved gases transfer from the liquid 
wastes to the air stream due to the difference in partial pressure between the liquid surface 
and the free air stream, via the so called convective mass transfer release (Ni, 1999); 2) 
aggregation and ascent of formed gas bubble break at the surface releasing to the atmosphere, 
defined as Bubble-release (Ni et al., 2009; Blanes-Vidal et al., 2010; Blanes-Vidal and 
Nadimi, 2011). Different release mechanisms are responsible for different gases. As reported 
by Ni et al. (2009), convective mass transfer governed \( \text{NH}_3 \) release due to the high solubility 
of \( \text{NH}_3 \), while bubble-release was dominant or play an important role in the releases of \( \text{CO}_2 \), 
\( \text{H}_2\text{S} \), and \( \text{SO}_2 \).

The chemical dissociation reactions that occur in the liquid wastes related to \( \text{NH}_3 \), \( \text{CO}_2 \), 
\( \text{H}_2\text{S} \), and \( \text{SO}_2 \) are expressed in Eqs. (1) – (6).

\[
\text{NH}_4^+ \rightleftharpoons \text{NH}_3 + \text{H}^+ \tag{1}
\]
\[
\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{HCO}_3^- + \text{H}^+ \tag{2}
\]
\[
\text{HCO}_3^- \rightleftharpoons \text{CO}_2^{2-} + \text{H}^+ \tag{3}
\]
\[
\text{H}_2\text{S} \rightleftharpoons \text{HS}^- + \text{H}^+ \tag{4}
\]
\[
2\text{HS}^- + 3\text{O}_2 \rightleftharpoons 2\text{HSO}_4^- \tag{5}
\]
\[
\text{HSO}_4^- + \text{H}^+ \rightleftharpoons \text{SO}_2 + \text{H}_2\text{O} \tag{6}
\]

It has been known that pH in the liquid waste is controlled by major buffer components, 
including total inorganic carbon ([TIC] = [\( \text{CO}_2 \]) + [\( \text{HCO}_3^- \]) + [\( \text{CO}_2^{2-} \]), total ammioniacal 
nitrogen ([TAN] = [\( \text{NH}_3 \]) + [\( \text{NH}_4^+ \]), and total acetic acid ([TAc] = [\( \text{HAc} \]) + [\( \text{Ac}^- \]) (Sommer
and Husted, 1995; Sommer and Sherlock, 1996). The pH and concentration of buffer components (TAN, TIC, and TAc) are different in different types of wastes, which can affect gas releases. In a stored liquid waste system, the microbial degradation and the releases of gases can change the waste characteristics and pH over time (Moller et al., 2004). For instance, oxic degradation of organic matter reduces the content of acids in solution and thereby increases pH; and anoxic processes will contribute to the formation of organic acids (e.g., VFA) and thereby reduce pH. Besides, releases of acidic gases (i.e., H$_2$S, CO$_2$) tends to increase the pH in surface liquid layer; and releases of bases (i.e., NH$_3$) tends to decrease the pH in it (Sommer and Sherlock, 1996). Conversely, change in waste pH affects the releases of NH$_3$, CO$_2$, H$_2$S and SO$_2$. Gas releases, and waste characteristics and pH present a dynamic equilibrium when the liquid waste is under steady state conditions. However, disturbances such as animal manure drops into the pit or rain falls into outdoor waste storage tanks can break the dynamic equilibrium and result in transient gas releases (Blanes-Vidal et al., 2012a).

Gaseous emissions from livestock production facilities are affected by several factors, such as animal activities, temperature, ventilation systems, farm operations (Arogo et al., 2003). Emissions of gases from different animal production systems under field condition are hardly comparable; and the effects of ambient parameters (i.e., air flow and temperature) on the emissions are difficult to determine (Saha et al., 2011). Therefore, gas releases from different wastes and their characteristics can be better investigated under controlled laboratory conditions.

Studies related to gas emissions from waste storage have mainly been focused on NH$_3$ from swine manure (Ni, 1999; Arogo et al., 2003; Gay et al., 2003; Sommer et al., 2007; Cortus et al., 2008), dairy manure (Patni and Jui, 1991; Sommer et al., 2007; Mathot et al., 2012), and layer manure (Ni et al., 2010); CO$_2$ from swine manure (Ni et al., 1999; Moller et
al., 2004; Sommer et al., 2007) and dairy manure (Mathot et al., 2012); H$_2$S from swine manure (Arogo et al., 2000; Clanton and Schmidt, 2000; Gay et al., 2003; Hoff et al., 2006; Kim et al., 2007; Moreno et al., 2010) and dairy manure (Bicudo et al., 2003; Zhao et al., 2007). There are very few studies about SO$_2$ emission from waste storage reported in the literature. Data of gas generations and releases from municipal wastewater during storage are also lacking.

Although gas releases from different wastes have been studied in the laboratories or investigated on livestock or poultry farms, so far there has been no systematic evaluation and comparison of production of different gases (NH$_3$, CO$_2$, H$_2$S, and SO$_2$) during storage under the same experimental conditions. The objective of this research is to investigate the characteristics of NH$_3$, CO$_2$, H$_2$S, and SO$_2$ releases (including quantities and patterns) from four types of animal manure (swine, beef, dairy, and layer hen) and municipal wastewater in controlled laboratory conditions.

2 Materials and Methods

2.1 Overview of the experiments

Four runs of tests were conducted to study releases of gases (NH$_3$, CO$_2$, H$_2$S, and SO$_2$) from five types of wastes, i.e., swine manure (SM), dairy manure (DM), beef manure (BM), layer hen manure (LM), and municipal wastewater (MW). Two tests were conducted for SM, DM, and MW from test runs 1 to 4 and one test was conducted for BM and LM in test run 4 (Table 1). The recorded room temperature, ventilation air temperature and air relative humidity during the tests were 20.4 ± 0.7 °C (mean ± standard deviation), 21.6 ± 0.6 °C and 21.9 ± 1.7%, respectively. The test durations were 43 days for swine manure and 38 days for the other types of wastes.

[Insert Table 1 here]

2.2 Experimental setup
The experiments were conducted in a 4.5 m x 2.7 m insulated and environmentally controlled walk-in chamber, which was maintained at approximately 20°C, in the Air Quality Laboratory at Purdue University, Indiana, USA. The chamber could house a maximum of 34 reactors for gas release experiments; but not all the reactors were used in this study.

2.2.1 Reactors

The reactors were 122 cm tall and had an inside diameter of 38 cm and made of PVC pipes. Each reactor had a fixed slip cap on the bottom and a removable slip cap that could be sealed on the top (Figure 1). The reactor was lined with 0.05-mm thick Tedlar® film on the top 64 cm of the inside walls and the “ceiling” of the reactor to create a chemically inert headspace. The height of the stainless steel air supply pipe was adjustable to allow the air inlet to always be located 15 cm above the waste surface. The air inlet included a baffle to direct the air radially in all horizontal directions. The headspace of the reactors was ventilated with fresh air from the air compressor at approximately 0.13 L s⁻¹ to simulate waste storage conditions.

Ventilation air to each reactor was supplied continuously from an air compressor except during manure additions (Figure 2). The pressure of the compressed air was reduced and stabilized by two pressure regulators connected in series. The air supply manifold (M_a, Figure 2) distributed air equally to each reactor using 0.84-mm diameter stainless steel precision orifices.

2.2.2 Measurement

Gas concentrations, ventilation rate, room temperature (T_r), and relative humidity (RH_a) and temperature (T_a) in the ventilation air were continuously measured. Signals from gas analyzers and other sensors were acquired every second using data acquisition and control.
(DAC) hardware from National Instruments Co. (Austin, TX) and a piece of custom-programmed DAC software written in LabVIEW (National Instruments). The software processed the signals every second and averaged the data every minute before saving them to a computer (Figure 2).

Controlled by the DAC system, the exhaust air from all reactors and the fresh air from the air compressor were sequentially delivered one after another to gas analyzers for gas concentration measurement. The measurement time was 10 minutes for each air source before switching to another source. Gas concentrations from each source of air were measured for at least six times a day throughout the study.

Concentrations of NH$_3$, CO$_2$, and H$_2$S/SO$_2$ in the reactor headspace air were measured with a chemiluminescence NH$_3$ analyzer (Model 17 C, Thermo Environmental Instruments (TEI), Franklin, MA), a photo-acoustic infrared CO$_2$ monitor (Model 3600, Mine Safety Appliances Co., Pittsburgh, PA) and a SO$_2$ analyzer (Model 45, TEI) combined with a H$_2$S converter (Model 340, TEI), respectively. The analyzer concentration detection limits were 1 ppb (part per billion) for NH$_3$ and 50 ppm for CO$_2$. They were 1 ppb at 60 seconds average time for H$_2$S and SO$_2$. All analyzers were calibrated using certified zero air and calibration gases prior to and after the study, and zero/span checked at least weekly during the study.

Airflow rate from each reactor was measured simultaneously with gas concentrations using a mass flow meter (0-10 L min$^{-1}$, Model 50S-10, McMillan, Georgetown, TX). Air temperature in the reactor room was monitored in four locations with type T thermocouples. A relative humidity and temperature sensor (Humitter 50 YC, Vaisala, Woburn, MA) was used inside the air supply manifold $M_a$ to monitor air relative humidity and temperature.

2.3 Waste sources and experimental schedule

Swine manure and dairy manure were collected from the deep pit of a grow/finish swine barn and a dairy manure collection pond, respectively, at two commercial farms in
Indiana, USA. Swine and dairy manure for the initial filling and weekly additions was collected in the same barn on the same day for all tests (Table 2). Layer hen manure was collected twice from two high-rise houses at a commercial farm in Ohio, USA. The municipal wastewater was collected from a municipal wastewater treatment plant in Indiana. Manure for weekly additions was stored in plastic baskets and frozen until one day before additions, except for the last addition in the layer manure reactors.

The initial reactor filling was 66 cm of manure or wastewater in each reactor. To simulate the field conditions, 5 cm of manure or wastewater were added to each reactor every week for four weeks except for SM in test 1 and test 2, which had five weekly additions.

Six samples of swine manure and three samples of other wastes were obtained on day 0. Two to six samples from each waste were collected at the end of each test. All samples’ pH value, dry matter, total kjeldahl nitrogen (TKN), and total ammoniacal nitrogen (TAN) were analyzed in the Animal Sciences Waste Management Laboratory at Purdue University.

2.4 Calculations and statistical analysis

Gas concentration data that were saved every minute were extracted by taking the last three of the ten minutes and averaged for the reason of allowing sufficient system equilibrium time. Gas release rates were approximated with gas emission rates in this study because of the relatively small volume of reactor headspace compared with air volumes in animal buildings and the fact that there were no gas sinks in the reactors. Gas release flux was the gas release per specific gas release surface area and was approximately with gas emission flux, which was calculated based on the reactor ventilation rate and the corrected gas concentrations [Eq. (7)]. Gas concentration corrections were performed according to the analyzer calibrations and Zero/Span checks.

\[
E = \frac{(C_E - C_S) \cdot Q}{S} \quad (7)
\]
where, $E$ is the gas emission flux ($\mu g \text{ s}^{-1} \text{ m}^{-2}$); $C_E$ and $C_S$ are the gas concentrations ($\mu g \text{ m}^{-3}$) of the exhaust air from the reactors and the supply air from compressor, respectively; $Q$ is the reactor airflow rate ($\text{m}^3 \text{ s}^{-1}$); and $S$ is the waste surface area of gas release ($\text{m}^2$) and equals 0.114 m$^2$ in this study.

Gas release calculation and graphing were performed in Microsoft Excel and GraphPad Prism (version 5) (La Jolla, CA, USA). Paired t-test and single factor analysis of variance (ANOVA) were used for tests of significance to compare mean values of waste characteristics and gas releases. All statistical analyses were performed using the SPSS statistical software (version 16) (SPSS, Chicago, IL, USA).

3 Results and discussion

3.1 Physicochemical characteristics of wastes

3.1.1 Characteristics of source manure and wastewater

The physicochemical characteristics of source manure (before the tests) were within values reported by Sánchez and González (2005) and Kissinger et al. (2007), while the characteristics of municipal wastewater were similar to the study reported by Xing et al. (2001) (Table 3). Statistical analysis revealed that the municipal wastewater was lower in dry matter, TKN$^{\text{wb}}$ (TKN in wet basis), TAN$^{\text{wb}}$ (TAN in wet basis), TKN$^{\text{db}}$ (TKN in dry basis) and TAN$^{\text{db}}$ (TAN in dry basis) in comparison with the beef, swine, and layer manure ($p < 0.05$), but similar to the dairy manure in dry matter, TKN$^{\text{wb}}$ and TAN$^{\text{wb}}$ ($p > 0.05$). The source layer manure had the highest pH, dry matter, and TKN in wet basis compared with all other wastes, while the source swine manure contained the highest TKN and TAN in dry basis among all the wastes ($p < 0.05$).

3.1.2 Effect of storage time

After 38 to 43 days of storage period, the lowest waste pH was found in the second test...
of municipal wastewater (MW2) with a value of pH = 5.39±0.13 and the highest pH was 8.69±0.03 in the layer manure. The dairy manure showed a significant variance between the two tests (DM1 and DM2). The pH in all waste types was lower at the end of the storage period compared with that before the storage except for test DM1 (Table 3). The pH of stored bulk liquid wastes can be mainly affected by three biochemical processes. First, hydrolysis of urea in urine by enzyme urease in feces can produce a mixture of NH$_3$, NH$_4^+$, CO$_3^{2-}$, and HCO$_3^{-}$. One mol of urea produces two mol TAN and one mol TIC. This may increase pH due to more bases substance (NH$_3$ and CO$_3^{2-}$/HCO$_3^{-}$) exist in wastewaters. Second, the balance of aerobic degradation of organic materials and anaerobic reaction processes can affect the pH. The former can reduce the concentration of acids and thereby increase the pH, and the latter can accelerate the formation of organic acids and thereby decrease the pH. Third, the release rate of gases (e.g., CO$_2$ and NH$_3$) at the wastewater surface can change the pH. Release of CO$_2$ can cause an increase in pH while release of NH$_3$ can result in a reduction in pH. The release of CO$_2$ is more easily than NH$_3$ due to its lower solubility in wastewater (Sommer et al., 2006). The pH changes in different wastes in this study could be explained with the complicated biochemical processes during the storage.

The dry matter concentrations decreased after the storage in the SM1, SM2, DM1, and MW1, but increased in other waste types towards the end of the tests. The change in dry matter concentrations could be attributed to the relative proportions of solid loss and water evaporation. A decrease in waste dry matter could be caused by the degradation of organic matter such as volatile solid (VS) and total organic compounds (TOC), while an increase could be caused by water evaporation.

The TAN concentrations in both wet basis and dry basis for all waste types at the end of the tests were higher than those before the tests. These increases could be caused by an imbalanced production of TAN in the wastes and release of NH$_3$ from the wastes. The
degradation of organic matter (e.g., urea), which is catalyzed by microorganism (e.g., urease) and the urea hydrolysis, can produce NH$_3$ (NH$_3$ in liquid phase) and NH$_4^+$ in the liquid wastes (Ni, 1999; Sommer et al., 2006). The concentrations of NH$_3$ and NH$_4^+$ in the liquid wastes could increase if the rate of NH$_3$ (NH$_3$ in gas phase) release, which could be affected by the pH, temperature, and air velocity over waste surface, was slower than the rate of NH$_3$ and NH$_4^+$ production.

3.2 Gas releases

3.2.1 Ammonia

The daily mean NH$_3$ releases from each of the reactors ranged from 53.2 to 110.6 µg s$^{-1}$ m$^{-2}$ for swine manure, 4.3 to 11.9 µg s$^{-1}$ m$^{-2}$ for dairy manure, 4 to 22.3 µg s$^{-1}$ m$^{-2}$ for beef manure, 0.1 to 636.6 µg s$^{-1}$ m$^{-2}$ for layer manure, and 0.41 to 1.4 µg s$^{-1}$ m$^{-2}$ for municipal wastewater (Table 4). The average daily mean (ADM) NH$_3$ releases (43 days for swine manure, and 38 days for other wastes) were in an ascending order from the municipal wastewater (0.9±0.5 µg s$^{-1}$ m$^{-2}$) to dairy manure (6.9±2.9 µg s$^{-1}$ m$^{-2}$), beef manure (13.95±0.31 µg s$^{-1}$ m$^{-2}$), swine manure (86.8±9.3 µg s$^{-1}$ m$^{-2}$), and layer manure (153.9±14.7 µg s$^{-1}$ m$^{-2}$).

There were no significantly differences of NH$_3$ releases among the municipal wastewater, dairy manure, and beef manure (p > 0.05). However, the NH$_3$ releases from the layer manure was statistically different compared with those from other types of wastes (p < 0.05) as shown in the daily release variations and the differences among the wastes in the box-whisker plots (Figure 3).

During the day following each weekly waste addition to the reactors, an increase of daily mean NH$_3$ releases was observed from almost all waste types (Figure 4), especially from the layer manure after the last addition on day 28. One explanation for the waste-
addition-induced high NH$_3$ releases could be that the source wastes brought in more new chemical compounds that created more favorable physicochemical conditions for NH$_3$ releases. For the layer manure, the much higher NH$_3$ releases from days 28 to 31 were related to the higher moisture concentration of the added manure on day 28 (Ni et al., 2010).

Previous studies have showed that NH$_3$ release is mainly affected by manure pH, temperature, ammoniacal nitrogen content, and ventilation rate (Chaoui et al., 2009; Rong et al., 2009; Saha et al., 2011). In the current study, NH$_3$ releases from different wastes were positively correlated with their initial TKN (correlation coefficient: $r = 0.98$, $n = 8$) and TAN (correlation coefficient: $r = 0.90$, $n = 8$).

### 3.2.2 Carbon dioxide

The highest CO$_2$ release was found from layer manure, followed by beef manure and swine manure. Dairy manure and municipal wastewater were similar in CO$_2$ releases within the same test time (i.e., MW1 and DM1, MW2 and DM2) (Figure 3). The ADM CO$_2$ releases from LM, BM, SM, DM, and MW were 9898±1179, 2788±69, 2540±101, 765±548, and 629±214 µg s$^{-1}$ m$^{-2}$, respectively (Table 4).

Releases of CO$_2$ from the two tests of dairy manure demonstrated large variations (DM1 released 76% less CO$_2$ than DM2). This difference could be due to the significant difference of initial pH between DM1 (7.96±0.43) and DM2 (5.98±0.04) shown in Table 3.

An increase in daily mean CO$_2$ releases during the first three to seven days of waste storage were observed in most tests except for MW1 and DM1 on day 1, when the CO$_2$ releases were higher than those on day 2 (Figure 4). The release patterns of CO$_2$ followed the similar trend as in the study of Fangueiro et al. (2008), who reported that an increase in CO$_2$ emissions was found immediately after land application of manure, then the emissions decreased after seven days.
The factors that influence CO₂ release have been rarely discussed in the literature individually, except for the total pig weight, manure temperature, and ventilation rate under field conditions in the CO₂ release model developed by Ni et al. (1999). The effects of pig weight and ventilation were confirmed in a recent publication by Zong et al. (2014). In this study, a positive correlation between CO₂ releases and initial dry matter content of wastes was found (correlation coefficient: r = 0.99, n = 8). The results suggest that the higher dry matter content, the higher CO₂ release could be expected. This was in agreement with the results reported by Fangueiro et al. (2008) and Mathot et al. (2012), who showed that the CO₂ emissions were generally higher in the solid fraction of separated manure than the liquid fraction and raw manure.

3.2.3 Sulfur compounds

The highest H₂S release was found in DM2, with an ADM of 4.31±0.43 µg s⁻¹ m⁻². On average, the H₂S releases from MW1, DM1, SM1, SM2, and LM were not significantly different (p > 0.05) (Figure 3).

The H₂S releases were negatively correlated with the waste pH (r = -0.72). The dairy manure in DM2 had the lowest pH among all the wastes (Table 3). On contrary, the ADM H₂S release in DM1 was only 0.05±0.00 µg s⁻¹ m⁻², demonstrating that H₂S releases could be markedly different even from the same type of manure. Layer manure, which had a relatively higher pH of 8.79±0.06, released the lowest quantities of H₂S compared with the other wastes.

An increase in H₂S release occurred one day after each manure addition in all tests except for DM1 (Figure 4). Sulfur reduction bacteria contained in fresh manure or new sulfur substrate from added manure could promote the H₂S production. This could also be the reason of the delayed release increase in this study, apart from the increase of H₂S emission found immediately after manure addition reported by Blanes-Vidal et al. (2012a).
Daily mean $\text{H}_2\text{S}$ and $\text{SO}_2$ releases showed similar skew distribution presented in Figure 3 except for the layer manure. Generally, the ADM values were higher than the median. This indicated that the peaks of $\text{H}_2\text{S}$ and $\text{SO}_2$ releases caused by manure additions could have significant effect on the total gas released from manure during storage.

When waste additions were performed weekly, the $\text{SO}_2$ releases clearly increased one day after each addition and then decreased (Figure 4). This behavior was similar to the $\text{H}_2\text{S}$ releases. However, the mechanism of $\text{SO}_2$ releases has been scarcely discussed in the literature. Little is known about the relationship between the releases of the two gases, which deserve further investigations.

4 Conclusions

This comparison study of five types of wastes under controlled laboratory environment provided a better understanding of the gas release characteristics and relevant factors affecting the releases. The following conclusions were drawn from the study.

1. Physicochemical characteristics of different types of wastes had great influence on the $\text{NH}_3$, $\text{CO}_2$, $\text{H}_2\text{S}$, and $\text{SO}_2$ releases. Even for the same type of waste (as demonstrated between DM1 and DM2, and MW1 and MW2), the variation in physicochemical characteristics could affect the gas releases remarkably. The TN, TAN, dry matter, and pH were dominant for the gas releases.

2. Concentrations of TN and TAN in the different types of wastes strongly affected the $\text{NH}_3$ releases from the wastes. Layer hen manure and municipal wastewater had the highest and lowest $\text{NH}_3$ release potentials, respectively.

3. Initial dry matter concentrations of the different types of wastes were positively correlated to the $\text{CO}_2$ releases from of wastes ($r= 0.99$). Layer manure and dairy manure had the highest and lowest $\text{CO}_2$ release potentials, respectively.

4. The pH of different types of wastes had high correlations with the $\text{H}_2\text{S}$ and $\text{SO}_2$
5. Addition of raw wastes to the stored wastes brought in new chemical compounds that could result in short-term bursts of gases releases.

6. Mitigation technologies to reduce releases of different gases from different wastes could be developed in accordance with waste characteristics.

Acknowledgement

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References


Blanes-Vidal, V., Nadimi, E.S., Sommer, S.G., 2010. A comprehensive model to estimate the simultaneous release of acidic and basic gaseous pollutants from swine slurry under different


Figure Captions

Figure 1. Schematic of the reactor design. Double-ended arrow indicates height adjustment of the air inlet pipe.

Figure 2. Diagram of the laboratory setup (not to scale). The hydrogen sulfide/sulfur dioxide and carbon dioxide analyzers had internal filters and pumps.

Figure 3. Box and whiskers plot of daily mean gas releases from five types of wastes during the entire tests, including swine manure (SM1 and SM2), dairy manure (DM1 and DM2), beef manure (BM), layer manure (LM) and municipal wastewater (MW1 and MW2). Means were shown as “+”. Line inside the box indicates the median. Bars indicate the values lower than the 5th percentile and greater than the 95th percentile as circles. Any data beyond the whiskers are shown as points. Same latters (a, b, c, d) within the same graph indicate no significant differences among the waste and test (p > 0.05).

Figure 4. Daily average emission patterns of NH3, CO2, H2S, and SO2 from swine manure (SM1 and SM2), dairy manure (DM1 and DM2), beef manure (BM), layer manure (LM) and municipal wastewater (MW1 and MW2). Arrows indicate waste additions.
Table 1. Overview of the four runs of tests.

<table>
<thead>
<tr>
<th>Waste type and test #</th>
<th>Test run #</th>
<th>Days of test</th>
<th>Number of Reactors</th>
<th>Reactor room temperature ($T_r$), °C</th>
<th>Ventilation air</th>
<th>Relative humidity ($R_{H_a}$), %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Temperature ($T_a$), °C</td>
<td></td>
</tr>
<tr>
<td>SM1</td>
<td>1</td>
<td>43</td>
<td>4</td>
<td>20.1±0.7(^1)</td>
<td>22.1±1.1</td>
<td>22.9±3.3</td>
</tr>
<tr>
<td>SM2</td>
<td>2</td>
<td>43</td>
<td>4</td>
<td>19.8±0.1</td>
<td>21.9±0.4</td>
<td>23.7±2.7</td>
</tr>
<tr>
<td>DM1</td>
<td>3</td>
<td>38</td>
<td>2</td>
<td>20.5±0.4</td>
<td>20.7±0.3</td>
<td>20.6±1.9</td>
</tr>
<tr>
<td>DM2</td>
<td>4</td>
<td>38</td>
<td>2</td>
<td>21.3±0.4</td>
<td>21.7±0.5</td>
<td>20.3±3.6</td>
</tr>
<tr>
<td>BM</td>
<td>4</td>
<td>38</td>
<td>2</td>
<td>21.3±0.5</td>
<td>21.7±0.6</td>
<td>20.3±3.7</td>
</tr>
<tr>
<td>LM</td>
<td>4</td>
<td>38</td>
<td>4</td>
<td>21.3±0.6</td>
<td>21.7±0.7</td>
<td>20.3±3.8</td>
</tr>
<tr>
<td>MW1</td>
<td>3</td>
<td>38</td>
<td>2</td>
<td>20.5±0.4</td>
<td>20.7±0.3</td>
<td>20.6±1.9</td>
</tr>
<tr>
<td>MW2</td>
<td>4</td>
<td>38</td>
<td>2</td>
<td>21.3±0.4</td>
<td>21.7±0.5</td>
<td>20.3±3.6</td>
</tr>
</tbody>
</table>

\(^1\) Mean ± standard deviation
Table 2. Test schedule

<table>
<thead>
<tr>
<th>Test day</th>
<th>Test 1 and Test 2</th>
<th>Test 3</th>
<th>Test 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Operation</td>
<td>Operation</td>
<td>Operation</td>
</tr>
<tr>
<td></td>
<td>Waste depth (cm)</td>
<td>Waste depth (cm)</td>
<td>Waste depth (cm)</td>
</tr>
<tr>
<td>1</td>
<td>Pit empty</td>
<td>Collection</td>
<td>Initial filling</td>
</tr>
<tr>
<td>2</td>
<td>Test 3</td>
<td>Collection</td>
<td>Addition</td>
</tr>
<tr>
<td>3</td>
<td>Test 4</td>
<td>Addition</td>
<td>Addition</td>
</tr>
<tr>
<td>-22</td>
<td>-21</td>
<td>Initial filling</td>
<td>66</td>
</tr>
<tr>
<td>-19</td>
<td>Pit empty</td>
<td>Collection</td>
<td>Initial filling</td>
</tr>
<tr>
<td>0</td>
<td>Collection</td>
<td>Initial filling</td>
<td>66</td>
</tr>
<tr>
<td>7</td>
<td>Initial filling</td>
<td>Initial filling</td>
<td>66</td>
</tr>
<tr>
<td>14</td>
<td>Addition</td>
<td>Addition</td>
<td>Addition</td>
</tr>
<tr>
<td>21</td>
<td>Addition</td>
<td>Addition</td>
<td>Addition</td>
</tr>
<tr>
<td>28</td>
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<tr>
<td>35</td>
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<tr>
<td>38</td>
<td>End of test</td>
<td>End of test</td>
<td>End of test</td>
</tr>
<tr>
<td>43</td>
<td>End of test</td>
<td>End of test</td>
<td>End of test</td>
</tr>
</tbody>
</table>

1. Initial and addition manure was collected in the same barn on the same day of filling or addition; 2. Initial and addition dairy manure and wastewater was collected the same day, those for addition was frozen until one day before addition; 3. Initial and addition manure and wastewater was collected the same day, those for addition was frozen until one day before addition except the last addition for layer manure reactors; 4. Depth of manure or municipal wastewater in reactors; 5. Only for animal manure, municipal wastewater was collected one day before filling.
Table 3. Characteristics of the five types of wastes (Mean ± 95% confidence interval) before and after the tests.\(^1,2\)

<table>
<thead>
<tr>
<th>Waste type and Test #</th>
<th>Samples, n</th>
<th>pH</th>
<th>Dry matter, %</th>
<th>TKN (wb(^3)), ppm</th>
<th>TAN (wb(^3)), ppm</th>
<th>TKN (db(^4)), ppm</th>
<th>TAN (db(^4)), ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before tests</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM1</td>
<td>6</td>
<td>7.00±0.01(^{cd})</td>
<td>7.82±0.15(^{b})</td>
<td>7,926±440(^{c})</td>
<td>5,538±149(^{c})</td>
<td>101,324±4,653(^{c})</td>
<td>70,869±2,231(^{c})</td>
</tr>
<tr>
<td>SM2</td>
<td>6</td>
<td>7.36±0.01(^{d})</td>
<td>8.88±0.15(^{b})</td>
<td>8,465±501(^{c})</td>
<td>6,374±266(^{d})</td>
<td>95,357±5,727(^{c})</td>
<td>71,842±3,639(^{c})</td>
</tr>
<tr>
<td>Average SM</td>
<td></td>
<td>7.18±0.10</td>
<td>8.35±0.32</td>
<td>8,195±367</td>
<td>5,956±281</td>
<td>98,340±4,057</td>
<td>71,356±2,152</td>
</tr>
<tr>
<td>DM1</td>
<td>3</td>
<td>7.96±0.43(^{a})</td>
<td>1.02±0.05(^{a})</td>
<td>639±25(^{a})</td>
<td>318±20(^{ab})</td>
<td>62,781±3,516</td>
<td>31,138±989</td>
</tr>
<tr>
<td>DM2</td>
<td>3</td>
<td>5.98±0.04(^{a})</td>
<td>1.86±0.07(^{a})</td>
<td>1,326±52(^{a})</td>
<td>377±5(^{a})</td>
<td>71,465±2,560</td>
<td>20,302±531</td>
</tr>
<tr>
<td>Average DM</td>
<td></td>
<td>6.97±0.82</td>
<td>1.44±0.34</td>
<td>983±276</td>
<td>5,956±281</td>
<td>98,340±4,057</td>
<td>71,356±2,152</td>
</tr>
<tr>
<td>BM</td>
<td>3</td>
<td>6.54±0.04(^{b})</td>
<td>8.00±0.44(^{b})</td>
<td>374±203(^{b})</td>
<td>1,047±9(^{b})</td>
<td>46,956±4,750</td>
<td>13,116±762</td>
</tr>
<tr>
<td>Average BM</td>
<td></td>
<td>6.97±0.82</td>
<td>1.44±0.34</td>
<td>983±276</td>
<td>5,956±281</td>
<td>98,340±4,057</td>
<td>71,356±2,152</td>
</tr>
<tr>
<td>LM</td>
<td>3</td>
<td>8.79±0.06(^{f})</td>
<td>33.85±2.40(^{c})</td>
<td>18,056±954(^{d})</td>
<td>5,296±682(^{c})</td>
<td>53,400±969</td>
<td>15,821±3,145</td>
</tr>
<tr>
<td>MW1</td>
<td>3</td>
<td>6.89±0.08(^{bc})</td>
<td>1.07±0.02(^{a})</td>
<td>19±10(^{a})</td>
<td>60±4(^{a})</td>
<td>18,120±1,199</td>
<td>5,563±420</td>
</tr>
<tr>
<td>MW2</td>
<td>3</td>
<td>6.61±0.22(^{bc})</td>
<td>1.20±0.10(^{a})</td>
<td>300±36(^{a})</td>
<td>88±4(^{a})</td>
<td>25,155±2,912</td>
<td>7,376±425</td>
</tr>
<tr>
<td>Average MW</td>
<td></td>
<td>6.75±0.16</td>
<td>1.14±0.07</td>
<td>247±46</td>
<td>74±12</td>
<td>21,638±3,225</td>
<td>6,469±784</td>
</tr>
<tr>
<td><strong>After tests</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM1</td>
<td>4</td>
<td>6.91±0.02(^{r})</td>
<td>6.98±0.16(^{t})</td>
<td>7,850±911(^{t})</td>
<td>6,517±228(^{u})</td>
<td>112,258±10,591</td>
<td>93,411±2,798</td>
</tr>
<tr>
<td>SM2</td>
<td>4</td>
<td>7.21±0.04(^{w})</td>
<td>8.68±0.12(^{u})</td>
<td>8,704±286(^{v})</td>
<td>7,863±96(^{w})</td>
<td>100,340±4,508</td>
<td>90,594±1,165</td>
</tr>
<tr>
<td>Average SM</td>
<td></td>
<td>7.06±0.11</td>
<td>8.3±0.60</td>
<td>8,277±562</td>
<td>7,190±482</td>
<td>106,299±7,084</td>
<td>92,002±1,803</td>
</tr>
<tr>
<td>DM1</td>
<td>2</td>
<td>8.10±0.02(^{s})</td>
<td>0.99±0.01(^{rs})</td>
<td>61±17(^{r})</td>
<td>319±5(^{rs})</td>
<td>61,660±887</td>
<td>32,171±40</td>
</tr>
<tr>
<td>DM2</td>
<td>6</td>
<td>5.85±0.02(^{a})</td>
<td>2.57±0.19(^{a})</td>
<td>1,378±93(^{r})</td>
<td>626±34(^{a})</td>
<td>53,701±2,722</td>
<td>24,484±1,854</td>
</tr>
<tr>
<td>Average DM</td>
<td></td>
<td>6.41±0.67</td>
<td>2.18±0.50</td>
<td>1,18±241</td>
<td>549±96</td>
<td>55,691±3,150</td>
<td>28,862±3,370</td>
</tr>
<tr>
<td>BM</td>
<td>3</td>
<td>6.41±0.03(^{w})</td>
<td>10.57±0.20(^{v})</td>
<td>4,047±52(^{t})</td>
<td>1,826±21(^{t})</td>
<td>38,315±642</td>
<td>17,297±437</td>
</tr>
<tr>
<td>Average BM</td>
<td></td>
<td>6.41±0.03</td>
<td>10.57±0.20</td>
<td>4,047±52</td>
<td>1,826±21</td>
<td>38,315±642</td>
<td>17,297±437</td>
</tr>
<tr>
<td>LM</td>
<td>3</td>
<td>8.69±0.03(^{y})</td>
<td>37.03±1.09(^{u})</td>
<td>19,481±633(^{u})</td>
<td>6,497±251(^{u})</td>
<td>52,709±2,592</td>
<td>17,565±754</td>
</tr>
<tr>
<td>MW1</td>
<td>2</td>
<td>6.11±0.01(^{t})</td>
<td>0.88±0.06(^{r})</td>
<td>186±7(^{r})</td>
<td>75±6(^{r})</td>
<td>21,284±725</td>
<td>8,618±1,248</td>
</tr>
<tr>
<td>MW2</td>
<td>6</td>
<td>5.93±0.13(^{r})</td>
<td>1.22±0.05(^{r})</td>
<td>38±26(^{r})</td>
<td>177±4(^{r})</td>
<td>31,539±3,065</td>
<td>14,557±723</td>
</tr>
<tr>
<td>Average MW</td>
<td></td>
<td>5.57±0.24</td>
<td>1.14±0.11</td>
<td>334±62</td>
<td>152±31</td>
<td>28,973±3,845</td>
<td>13,072±1,889</td>
</tr>
</tbody>
</table>

\(^1\) Statistical differences of each characteristic before test (within column) among the wastes were marked with different letters (a, b, c, d, e) (p<0.05); \(^2\) Statistical differences of each characteristic after test (within column) among the wastes were marked with different letters (r, s, t, u, v, w, x, y) (p<0.05); \(^3\) wet basis; \(^4\) dry basis.
<table>
<thead>
<tr>
<th>Waste type and Test run</th>
<th>NH₃ µg s⁻¹ m²</th>
<th>CO₂ µg s⁻¹ m²</th>
<th>H₂S µg s⁻¹ m²</th>
<th>SO₂ µg s⁻¹ m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>SM1 mean</td>
<td>78.4±3.1</td>
<td>2,614±72</td>
<td>0.96±0.47</td>
<td>0.01±0.00</td>
</tr>
<tr>
<td>SM2 mean</td>
<td>95.2±1.4</td>
<td>2465±63</td>
<td>1.13±0.12</td>
<td>0.05±0.01</td>
</tr>
<tr>
<td>Average SM</td>
<td>86.8±9.3</td>
<td>2,540±101</td>
<td>1.00±0.30</td>
<td>0.03±0.01</td>
</tr>
<tr>
<td>Min. SM</td>
<td>53.2</td>
<td>1941</td>
<td>0.50</td>
<td>0.00</td>
</tr>
<tr>
<td>Max. SM</td>
<td>110.6</td>
<td>3736</td>
<td>2.90</td>
<td>0.10</td>
</tr>
<tr>
<td>DM1 mean</td>
<td>9.4±0.6</td>
<td>294±15</td>
<td>0.05±0.00</td>
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<td>4.31±0.43</td>
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<td>MW2 mean</td>
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<td>Average MW</td>
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Note: numbers after “±” are standard deviations.
Figure 1. Schematic of the reactor design. Double-ended arrow indicates height adjustment of the air inlet pipe.
Figure 2. Diagram of the laboratory setup (not to scale). The hydrogen sulfide/sulfur dioxide and carbon dioxide analyzers had internal filters and pumps.
Figure 3. Box and whiskers plot of daily mean gas releases from five types of wastes during
the entire tests, including swine manure (SM1 and SM2), dairy manure (DM1 and DM2), beef
manure (BM), layer manure (LM) and municipal wastewater (MW1 and MW2). Means were
shown as “+”. Line inside the box indicates the median. Bars indicate the values lower than
the 5th percentile and greater than the 95th percentile as circles. Any data beyond the
whiskers are shown as points. Same latters (a, b, c, d) within the same graph indicate no
significant differences among the waste and test (p > 0.05).
Figure 4. Daily average emission patterns of NH$_3$, CO$_2$, H$_2$S, and SO$_2$ from swine manure (SM1 and SM2), dairy manure (DM1 and DM2), beef manure (BM), layer manure (LM) and municipal wastewater (MW1 and MW2). Arrows indicate waste additions. The last waste addition on day 35 was only applied for tests 1 and 2.
A Determination and Comparison of Urease Activity in Feces and Fresh Manure from Pig and Cattle in Relation to Ammonia Production and pH Changes

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ABSTRACT:

Ammonia emission from animal production is a major environmental problem and has impacts on the animal health and working environment inside production houses. Ammonia is formed in manure by the enzymatic degradation of urinary urea and catalyzed by urease that is present in feces. We have determined and compared the urease activity in feces and manure (a urine and feces mixture) from pigs and cattle at 25 °C by using Michaelis-Menten kinetics. To obtain accurate estimates of kinetic parameters $V_{\text{max}}$ and $K'_{m}$, we used a 5 min reaction time to determine the initial reaction velocities based on total ammoniacal nitrogen (TAN) concentrations. The resulting $V_{\text{max}}$ value (mmol urea hydrolyzed per kg wet feces per min) was $2.06\pm0.08$ mmol urea/kg/min and $0.80\pm0.04$ mmol urea/kg/min for pig feces and cattle feces, respectively. The $K'_{m}$ values were $32.59\pm5.65$ mmol urea/l and $15.43\pm2.94$ mmol urea/l for pig feces and cattle feces, respectively. Thus, our results reveal that both the $V_{\text{max}}$ and $K'_{m}$ values of the urease activity for pig feces are more than 2-fold higher than those for cattle feces. The difference in urea hydrolysis rates between animal species is even more significant in fresh manure. The initial velocities of TAN formation are $1.53$ mM/min and $0.33$ mM/min for pig and cattle manure, respectively. Furthermore, our investigation shows that the maximum urease activity for pig feces occurs at approximately pH 7, and in cattle feces it is closer to pH 8, indicating that the predominant fecal ureolytic bacteria species differ between animal species. We believe that our study contributes to a better
understanding of the urea hydrolysis process in manure and provides a basis for more accurate and
animal-specific prediction models for urea hydrolysis rates and ammonia concentration in manures
and thus can be used to predict ammonia volatilization rates from animal production.
INTRODUCTION

The emission of ammonia (NH₃) from agricultural systems is a major environmental problem. Most NH₃ emissions come from animal production, especially from manure (a mixture of urine and feces). In addition, NH₃ emission affects human and animal health [1-3]. NH₃ in manure is formed by the hydrolysis of urinary urea (CO(NH₂)₂) and is catalyzed by microbial urease that is present in feces. The enzymatic decomposition of urea into carbonic acid (H₂CO₃) and volatile NH₃ is initiated when urine and feces contact one another after being excreted. Reaction 1 represents the overall catalytic hydrolysis of urea, which enables organisms to use urea as a nitrogen source [4,5]. The enzymatic hydrolysis of urea has a half-time of 20 ms at 25 °C, and urease is among the most proficient known enzymes [6-8].

Reaction 1.

\[
\text{CO(NH}_2\text{)}_2 + 2\text{H}_2\text{O} \xrightarrow{\text{Urease}} \text{H}_2\text{CO}_3 + 2\text{NH}_3
\]

In aqueous solutions, the carbonic acid and NH₃ generated from urea hydrolysis are in equilibrium with bicarbonate (HCO₃⁻) and ammonium (NH₄⁺) ions, respectively. Consequently, urea hydrolysis is associated with a subsequent increase in pH [4]. However, in the absence of active urease, urea is a very stable molecule with a half-time of approximately 40 years at 25 °C [8,9]. The non-catalytic decomposition of urea is not hydrolysis but proceeds through an elimination reaction to form isocyanate (HNCO) and NH₃ (Reaction 2).
The NH₃ emission level from manure depends on several factors including the animal species, urinary urea concentration, fecal urease activity, pH, temperature, manure management system, and air exchange rate. Therefore, NH₃ production and emission can be reduced by altering the dietary composition, adding urease inhibitors, acidifying or cooling the manure, and modifying the house interior [2,10-15]. To develop accurate prediction models for NH₃ emission and efficient NH₃ emission-reducing strategies for both pig and cattle production systems, it is necessary to understand the enzymatic process of NH₃ formation in manure. However, accurate measurements of the urease activity in feces and manure from different animal species are still limited.

The aims of this study were to determine and compare the kinetics of urea hydrolysis as catalyzed by feces and manure from pigs and cattle and to make accurate estimates of kinetic parameters $V_{\text{max}}$ and $K'_m$. In addition, we determined the initial chemical and physical properties of feces, urine, and fresh manure and investigated the effects of pH on animal fecal urease activity. Our work shed light on the urea hydrolysis process in manure from pigs and cattle and has provided the basis for animal-specific prediction models of urea hydrolysis rates and NH₃ concentrations in manures, and thus NH₃ volatilization rates from animal production.
MATERIALS AND METHODS

Most chemicals and reagents were purchased from Sigma-Aldrich. Urea stock solutions (1 M and 4 M) were prepared by dissolving urea (Sigma 51459, puriss. p.a., ACS reagent, ≥99.5 % (T)) in ultra pure water just before use. Phosphate buffer stock solutions (400 mM) were prepared by mixing phosphate salts NaH$_2$PO$_4$·H$_2$O (Sigma S9638, ACS reagent, 98.0-102.0 %) and Na$_2$HPO$_4$·7H$_2$O (Sigma 30413, puriss. p.a., ACS reagent, ≥99 %) in certain proportions to produce pH values of 6.0, 7.0, and 8.0 according to Ruzin [16]. In addition, citric acid-Na$_2$HPO$_4$-buffered stock solution (400 mM) pH 5.0 was prepared by mixing certain amounts of citric acid (Sigma 251275, ACS reagent, ≥99.5 %) and Na$_2$HPO$_4$·7H$_2$O. A 400 mM HEPES (Sigma H3375, ≥99.5 %) buffer stock solution was titrated to pH 9.0 with 1 M NaOH. All stock solutions were prepared a few hours before each series of experiments. Concentrated (98 %) sulfuric acid (100748, Merck KGaA, Germany), Kjeltab catalyst tablets (Thompson & Capper, UK), 32 % sodium hydroxide (28225, VWR, Denmark), and boric acid (Sigma 31144) were used for the Kjeldahl analyses. A FOSS 2200 Kjeltec Auto Distillation apparatus was used for all distillations. A PHM210 pH meter with ±0.01 pH units of accuracy (Meterlab, Radiometer Analytical, Lyon, France) was used for all pH measurements. Ultra pure water from an Ultra Clear UV system (SG Water, Hamburg, Germany) was used in all experiments.

Collecting Urine and Feces Samples
Fresh urine and feces samples were collected from fattening pigs (70-100 kg) and beef cows (500-600 kg). The pigs were approximately 3-5 months of age, and they were kept in an intensive housing system with a slatted floor. The animals were given wet feed made from wheat, barley, and soya beans that was fortified with minerals and vitamins; they had free access to water. The cattle were a cross between Danish Red and Simmental races at 4-6 years of age. The cattle were kept in a loose-housing system and were primarily fed clover-grass silage supplemented with compound feed for dairy cattle. Feces and urine samples from individual animals were collected separately in clean plastic bags to ensure that there was no mixing prior to the experiments. Both the feces and urine samples were grabbed directly upon excretion from the animals to prevent any contact with the barn floor. All the samples were stored at 4 °C during transportation. Equal amounts of feces from five specimens were pooled for both pigs and cattle. In addition, equal amounts of urine from five animals were pooled and used in the experiments. Half the feces and urine pools were saved at -80 °C for later use in chemical analyses and for determining the relative urease activity at different pH values. All urease activity measurements in fresh feces and manure were conducted within two days after sample collection. The urine and feces pools were stored at 4 °C until use. However, the urease activity in thawed feces pools that had been saved at -80 °C was measured for comparison.

Ethics Statement
The urine and feces samples were collected by using a self-made “bucket on a stick” without touching the animals. The animals were never touched and were never stimulated or forced to excrete urine or feces. Because the animals experienced no “pain, suffering, anxiety or lasting harm”, approval from the Danish Inspectorate for Animal Experiments was not necessary according to the relevant Danish legislation (Bekendtgørelse af lov om dyreforsøg). The urine and feces samples used in this study were collected with permission from the animal owners.

Chemical Analyses of Feces, Urine, and Fresh Manure

Three samples of pooled feces, pooled urine, and feces:urine mixtures (at a weight:volume (w:v) ratio of 1.0:3.0 for pigs and 3.0:2.0 for cattle) were analyzed for pH, dry matter, total Kjeldahl nitrogen (TKN = Organic-N + NH₃-N + NH₄⁺-N) concentration, total ammoniacal nitrogen (TAN = NH₃-N + NH₄⁺-N) concentration, and urea nitrogen (UN = Urea-N) concentration according to Table 1. Before the pH measurements of the feces, 10 g of fresh feces were thoroughly mixed with 30 ml of ultra pure water. For the dry matter determinations, fresh feces or manure samples were evaporated to dryness in an oven at 105 °C for at least 24 h until the weights of the samples were constant. The TKN and TAN concentrations were determined by using 3 ml of urine or 2-3 g of feces or manure (samples were weighed before analysis) [17-19]. The initial urea concentration ([Urea]) in urine was calculated by subtracting the initial TAN concentration in urine [TANᵢ,urine] from the final TAN concentration [TANᵢ,urine] that was generated after the complete enzymatic
hydrolysis of urea in urine by jack bean urease (Sigma 94282, activity ~35 units/mg) and then multiplying this difference by 0.5 according to Eq. 1 because two NH₃ molecules are generated from the hydrolysis of each urea molecule. For this determination, 56 ml of pooled urine was added to 4 ml of 400 mM phosphate buffer, pH 7.0 and 20 ml of jack bean urease solution (0.1 mg/ml equaling 3.5 units/ml) for a final concentration of 0.875 units/ml in the diluted urine solution to equal 1.25 units per ml of pure urine. The reaction mixture was incubated for 8 h at 25 °C on a magnetic stirrer (mixing was performed during the first five minutes of incubation, and the reaction mixture was also stirred for 20 s at 300 rpm before each sampling). The TAN was determined after 5 min, 2 h, 4 h, 6 h, and 8 h of incubation, and at 8 h the reaction had reached completion. The final constant TAN reached upon the completion of the reaction was defined as the TANₜ,urine (Figure S1).

\[
[Urea] = 0.5 \cdot [UN] = 0.5 \cdot \left[ TAN_{f,urine} - TAN_{t,urine} \right] \tag{1}
\]

**Kinetic Measurements of Urease Activity in Feces**

The amounts and ratios of feces and urine produced by animals depend on several factors including their diet and water supply [20,21]. Some animal studies suggest that the (w:v)-ratio of feces:urine produced by fattening pigs is approximately 1:3 [20,22] and that of cattle is approximately 3:2 [21,23]. Thus, to determine the kinetics of urease activity in pig feces, mixtures (approximately 40 ml of total volume) containing 10 g of pooled feces and 30 ml of urea-phosphate buffer solution, pH
7.0 with different urea concentrations were incubated in 50 ml beakers with magnetic stirring. For the kinetic measurements of urease activity in cattle feces, mixtures (containing approximately 30 ml of total volume) containing 18 g of pooled feces and 12 ml of urea-phosphate buffer solution, pH 7.0 with different urea concentrations were incubated in beakers while stirring. The stirring rate for all these kinetic experiments was 300 rpm during the 5 min incubation. Two to three hours before the kinetic measurements, the fecal samples and all solutions were placed in a water bath at a constant temperature of 25 °C. The feces samples were subsequently prepared for the kinetic experiments; for example, to obtain a final urea concentration of 400 mM urea in a 40 ml reaction sample, 10 g of fecal sample was added to 23 ml of ultra pure water before being titrated to pH 7.0 with approximately 0.1-0.2 ml of 1 M NaOH. Afterwards, 3 ml of 400 mM phosphate buffer, pH 7.0 and 4 ml of 4.0 M stock urea solution were added. The final urea concentrations were 0.0 mM, 20 mM, 40 mM, 80 mM, 100 mM, 200 mM, 400 mM, and 600 mM for the experiments with fresh pig feces, and 0.0 mM, 10 mM, 20 mM, 40 mM, 60 mM, 80 mM, 120 mM, and 160 mM for those with fresh cattle feces. The same procedure was used for the thawed feces samples except that the final urea concentrations in the experiments were 0.0 mM, 2.0 mM, 4.0 mM, 8.0 mM, 20 mM, 40 mM, 60 mM, and 80 mM for both species. The urea hydrolysis reactions were initiated by adding the amounts of stock urea solution (1.0 or 4.0 M) corresponding to the desired final urea concentrations of the mixtures. The 1.0 M urea stock solution was used to prepare the reactions with
2.0-100 mM urea, and the 4.0 M urea stock solution was used for reactions containing 120-600 mM urea. For each substrate (urea) concentration, the amount of NH₃ nitrogen generated during the 5 min reaction time was calculated by subtracting the initial amounts of ammoniacal nitrogen in feces and urea-buffer solutions from the final amount of ammoniacal nitrogen at the end of the reaction. Thus, for the kinetic measurements of urease activity in feces, 3 ml of sample was taken from each reaction mixture after reacting for 5 min and analyzed by Kjeldahl method to determine the TAN concentration [17-19]. Experiments showed that adding 75 ml of ultra pure water and 60 ml of 32 % sodium hydroxide (NaOH) to the reactions as described in the Kjeldahl method [17-19] completely stops urease activity (there is no further increase in the TAN). Thus, no urea is hydrolyzed between the time of NaOH addition and the Kjeldahl distillation. To verify that the pH remained constant during the kinetic reaction, the pH of the mixture was measured throughout the whole reaction, from t = 0 min to t = 5 min. All experiments were performed in triplicate. The kinetics of urea hydrolysis by pig and cattle feces was characterized by determining the maximum reaction rate Vₘₐₓ and the apparent Michaelis constant Kₗₘ according to Eq. 2 and Eq. 3.

Measurements of Urease Activity in Fresh Manure

To make fresh manure, pooled feces and pooled urine samples from five specimens were mixed in (w:v)-ratios of 1.0:3.0 and 3.0:2.0 for pigs and cattle, respectively. Thus, pig manure was made by mixing 20 g of pooled pig feces with 60 ml of pooled pig urine and cattle manure was made by
mixing 60 g of pooled feces with 40 ml of pooled urine in 140 ml beakers. The fresh manure was then made homogenous by magnetic stirring at 300 rpm for 5 min before the beakers were covered with parafilm and incubated at 25 °C. TAN concentration and pH of the manure samples were measured immediately after mixing (t ~ 0), homogenization (t = 5 min), and at incubation times of 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, and up to approximately 100 h. The initial TAN of the manure (t = 0) was calculated by adding the determined TAN value of urine with that of feces. The TAN concentrations were determined by Kjeldahl method [17-19] and all experiments were performed in triplicate.

**Determining Fecal Urease Activity at Different pH Values**

The fecal urease activity was determined under buffered conditions at pH values of 5.0, 6.0, 7.0, 8.0, and 9.0. Citric acid/Na$_2$HPO$_4$ buffer at a 40 mM final concentration was used in the mixture for pH 5.0, 40 mM phosphate buffers were used for pH 6.0, 7.0, and 8.0, and 40 mM HEPES was used as a buffer for pH 9.0. The temperatures of all samples and solutions were equilibrated in a water bath at 25 °C before mixing. To directly compare the urease activity in feces from pigs and cattle, the same weights for feces and a 1.0:3.0 (w:v) ratio of feces:liquid were used for both species. According to the kinetic data, the rate of urea hydrolysis is close to a $V_{max}$ at 0.2 M urea for both pig and cattle feces and, therefore, this urea concentration was used to determine the urease activity at different pH values. Thus, 10 g of pooled pig or cattle feces was mixed with 23 ml of ultra pure water in a 50
ml beaker and the pH was adjusted to the indicated pH value by adding sulfuric acid (1 M) or sodium hydroxide (1 M). Subsequently, 3 ml of 400 mM buffer stock solution (citric acid/Na₂HPO₄ buffer, phosphate buffer, or HEPES buffer) was added to keep the adjusted pH constant. The reaction was initiated by adding 4 ml of 2 M urea stock solution to a final concentration of 0.2 M and a total volume of 40 ml. The reactions were performed at 25 °C while stirring at 300 rpm. After a reaction time of 5 min, the TAN concentration was determined [17-19]. The amount of ammoniacal nitrogen generated during the reaction was determined by subtracting the initial amounts of ammoniacal nitrogen present in feces and urea-buffer solutions. All the experiments were performed in triplicate.

**Enzyme Kinetics and Statistical Analyses**

Enzymatic reactions such as the hydrolysis of urea as catalyzed by urease can be described by Michaelis-Menten kinetics according to Eq. 2, where $V$ is the rate of the enzymatic reaction, $[S]$ is the substrate concentration, $V_{\text{max}}$ is the maximum rate of the enzymatic reaction, and $K'_m$ is the apparent Michaelis constant [24]. The data in Figure 1, Figure 2A, Figure 2B, Figure S3, Figure S4A, and Figure S4B were analyzed by using the Michaelis-Menten model.

$$V = \frac{V_{\text{max}} \cdot [S]}{(K'_m + [S])}$$

(2)
By rearranging the Michaelis-Menten equation (Eq. 2) into the Lineweaver-Burk equation (Eq. 3), a linear regression of enzymatic reaction data \( \left( \frac{1}{[S]} , \frac{1}{V} \right) \) can be used to determine the \( V_{\text{max}} \) and \( K'_m \) values for the fecal urease activity in a Lineweaver-Burk plot [25]. The data in Figure 2C, Figure 2D, Figure S4C, and Figure S4D were analyzed according to the Lineweaver-Burk equation.

\[
\frac{1}{V} = \frac{K'_m}{V_{\text{max}} [S]} + \frac{1}{V_{\text{max}}} \quad (3)
\]

A Student's t-test was used to determine if the nitrogen content, dry matter, and pH values for feces, urine, and manure samples are significantly different between pigs and cattle (Table 1), and to compare the urease kinetic values for \( V_{\text{max}} \) and \( K'_m \) between pig and cattle feces at a significance level of \( \alpha = 0.05 \) (Table 2 and Table S1). A regression analysis by phase exponential association was used to determine the maximum TAN formation level as shown in Figure 3A, Figure 3B, and Figure S1. The pH change over time was determined by the one phase association and one phase decay regression in Figures 3A and 3B. All statistical analyses were performed with GraphPad Prism.
RESULTS

Comparing the Chemical and Physical Properties of Feces, Urine, and Fresh Manure from Pigs and Cattle

The initial properties including the TKN, TAN, and UN concentrations, dry matter, and pH of feces, urine, and fresh manure from pigs and cattle were determined (Table 1). All the TKN values were higher for the pig samples than for the corresponding cattle samples. Thus, the highest TKN concentration was found in pig feces with a value of 578.8±1.2 mmol/kg and that of cattle feces was only 337.8±33.0 mmol/kg (p<0.05). The TKN values for pig and cattle urine were 350.2±2.1 mM and 261.3±0.9 mM, respectively. In addition, the TAN measurements for pig feces (39.6±4.6 mmol/kg) and urine (23.6±1.0 mM) were significantly higher than the values for cattle feces (21.2±0.4 mmol/kg) and urine (15.9±1.0 mM), respectively. In addition, the urea concentrations in the urine samples were evaluated by finding the UN values. The urea concentration of pig urine (99.2±2.5 mM) was significantly higher than it was in cattle urine (76.4±0.5 mM). The dry matter of pig feces (15.32±0.09 %) was approximately 4 % higher than it was for cattle feces (11.44±0.22 %), and the pH values of both pig feces (pH 6.89±0.01) and urine (pH 7.69±0.03) were lower than the corresponding values for cattle (pH 7.02±0.02 and 8.55±0.02, respectively) (p<0.05). With the exception of the TAN concentration in pig manure, all the values measured in fresh manure samples (combined feces and urine samples) were consistent with the expected values based on those
determined for the separate feces and urine samples and their ratios in the combined feces and urine samples. The relatively high TAN concentration in pig manure (87.2±1.6 mM; Table 1) is most likely caused by the significantly faster formation of NH$_3$ in manure from pigs than from cattle when feces and urine are mixed (Figure 3). Therefore, the initial TAN concentrations used to determine the TAN formed in the manure reactions (Figures 1, 3, 4, and Supplemental Figures S2 and S3) were calculated by adding the proportions of TAN originating from pure feces and urine (or urea stock solution) (Table 1).

Urease Activity in Feces from Pigs and Cattle

The kinetics of urea hydrolysis as catalyzed by fresh feces from pigs and cattle were investigated by first determining the rates of TAN formation in reaction mixtures containing feces and different urea concentrations (Figure 1). To obtain accurate enzymatic reaction velocities for the fecal samples, the rates of NH$_3$ formation at different urea concentrations should be determined during the initial phase of the reactions and at a time when the levels of TAN formation are sufficient to achieve significant and reliable TAN measurements by Kjeldahl method. Therefore, to identify the optimal reaction time for the initial rate measurements, the levels of TAN formed at different reaction times (5 min, 11 min, and 20 min) were determined in mixtures of pig feces and 100 mM urea and the relation between the calculated rate of TAN formation and corresponding reaction time was investigated (Figure S2). The results clearly show that the calculated rate of TAN formation
decreases significantly when the reaction time increases. Thus, the reaction rate calculated from the
TAN formed at 5 min (0.45 mM/min) was significantly higher than the rates calculated at 11 min
(0.31 mM/min) and 20 min (0.22 mM/min). Therefore, the initial rates of TAN formation were
calculated from the TAN formed during the first 5 min of the reaction (Figures 1A and 1B). The
maximum rates determined for TAN formation in reactions with pig feces and cattle feces using
regression analyses were 1.03±0.04 mM/min (R²=0.84) and 0.99±0.05 mM/min (R²=0.82),
respectively (Figures 1A and 1B). In addition, a comparison of the rates of TAN formation at
different urea concentrations for the two feces samples reveals that the maximum rate of TAN
formation is reached at a lower concentration for the cattle feces than for the pig feces (Figures 1A
and 1B). This finding indicates that pig feces require higher concentrations of urea to reach the
maximum reaction rate of TAN formation for the 5 min incubation. For comparison, the specific
rates of TAN formation, that is, the reaction rates per wet weight of fresh feces, were calculated for
all the urea concentrations (Figures 1C and 1D). The results show that pig feces are a much better
catalyst for TAN formation than cattle feces (Figures 1C and 1D). Thus, the maximum specific
rates of TAN formation for pig feces and cattle feces according to regression analyses were
4.11±0.17 mmol/kg/min (R²=0.84) and 1.61±0.07 mmol/kg/min (R²=0.82), respectively (Figures
1C and 1D). Based on the assumption that the hydrolysis of each urea molecule generates two
molecules of NH₃, the specific rates of TAN formation (mmol/kg/min) were converted into specific
reaction velocities of hydrolyzed urea ($V_0$; mmol urea/kg/min) and presented in Michaelis-Menten curves (Figures 2A and 2B) and Lineweaver-Burk plots (Figures 2C and 2D). From the Michaelis-Menten curves, the specific $V_{\text{max}}$ and $K_m$ values of the urease activity in fresh feces from pigs and cattle were determined. The $V_{\text{max}}$ was $2.06 \pm 0.08$ mmol urea/kg/min and $0.80 \pm 0.04$ mmol urea/kg/min for pig feces and cattle feces, respectively (Table 2). The $K_m$ was $32.59 \pm 5.65$ mmol urea/l and $15.43 \pm 2.94$ mmol urea/l for pig feces and cattle feces, respectively (Table 2). For comparison, the $V_{\text{max}}$ and $K_m$ values were also determined from the Lineweaver-Burk plots (Figures 2C and 2D). Both the $V_{\text{max}}$ ($1.94$ mmol urea/kg/min for pig feces and $0.75$ mmol urea/kg/min for cattle feces) and $K_m$ ($26.58$ mmol urea/l for pig feces and $12.31$ mmol urea/l for cattle feces) from the Lineweaver-Burk plots were consistent with those determined from the Michaelis-Menten curves. The urease activities in thawed pig and cattle feces pools that had been saved at $-80 \, ^\circ\text{C}$ were also evaluated by Michaelis-Menten kinetics (Figures S3 and S4), and their corresponding $V_{\text{max}}$ and $K_m$ values were calculated from the Michaelis-Menten curves (Table S1). The $V_{\text{max}}$ was $1.63 \pm 0.12$ mmol urea/kg/min and $0.51 \pm 0.01$ mmol urea/kg/min for the thawed pig feces and cattle feces, respectively. The $K_m$ was $12.84 \pm 3.03$ mmol urea/l and $2.58 \pm 0.34$ mmol urea/l for the thawed pig feces and cattle feces, respectively (Table S1). The $V_{\text{max}}$ and $K_m$ values determined from Lineweaver-Burk plots (Figures S4C and S4D) were $1.43$ mmol urea/kg/min and
9.86 mmol urea/l for the thawed pig feces, respectively, and those for thawed cattle feces were 0.53 mmol urea/kg/min and 3.08 mmol urea/l, respectively.

**Urease Activity in Fresh Manure from Pigs and Cattle**

To investigate and compare the urease activity in fresh manure from pigs and cattle, fresh feces and urine were mixed in (w:v)-ratios of 1.0:3.0 and 3.0:2.0 for pigs and cattle, respectively (Figure 3). The concentration of formed TAN and the pH increased rapidly in both types of manure. However, the rate of TAN formation in pig manure is significantly faster than it is in cattle manure. Thus, the initial velocities of TAN formation based on measurements taken at 5 min after mixing are 1.53 mM/min and 0.33 mM/min for pig and cattle manure, respectively. After approximately 30 hours, the formed TAN concentration for pig manure reaches a plateau of ~0.2 M (0.20±0.003 M; K=0.16, \( R^2=0.980 \)) and that of cattle manure reaches a plateau of ~0.14 M (0.14±0.001 M; K=0.12, \( R^2=0.998 \)) (Figure 3) as determined by regression analyses through one-phase exponential association. For both manures, the pH change was fitted with a one phase association (Figure 3; \( R^2=0.99 \) for both pig and cattle, n=30). The pH in cattle manure reaches a maximum of 8.91 after 6-8 hours, and a maximum of pH 8.70 for pig manure is obtained after reacting for 8-10 hours. This finding indicates that the pH of cattle manure changes by a total of 1.04 pH units from the initial pH of 7.87 (Table 1). For the pig manure, the pH changes by a total of 1.65 pH units from the initial pH of 7.05 (Table 1). After reaching the plateau, the pH values for both manure preparations decrease...
through one phase decay (Figure 3; R²=0.64 for pigs (n=12), and R²=0.87 for cattle (n=18)). The pH of pig manure decreases, with 0.41 units for the 12-96 hour time period, and the pH of cattle manure decreases 0.76 units in the 8-92 hour time period (Figure 3).

The pH effect on Urease Activity in Feces from Pigs and Cattle

For a direct comparison of the urease activity in pig and cattle feces at different pH values, all reactions in this experiment contained the same amount of feces. Therefore, the rate of urea hydrolysis was lower for cattle feces than for pig feces (Figure 4). The initial rates of TAN formation were within ranges of 0.78-1.06 mM/min and 0.63-0.75 mM/min for pig feces and cattle feces, respectively. For both species, the fecal urease activity varied significantly with the pH but the cattle feces is less affected by changes in pH (Figure 4). By comparison, the relative rates of TAN formation were calculated with reference to that catalyzed by pig feces at pH 7.0 (100 %, Figure 4A). The relative reaction rates of TAN formation for the pig feces were 80 %, 98 %, 81 %, and 73 % at pH values of 5.0, 6.0, 8.0, and 9.0, respectively (Figure 4B). The relative rates of TAN formation for cattle feces compared with that for pig feces at pH 7.0 were 59 %, 66 %, 70 %, 69 %, and 61 % at pH values of 5.0, 6.0, 7.0, 8.0, and 9.0, respectively (Figure 4B). Thus, the results suggest that the optimal pH for urea hydrolysis as catalyzed by fecal urease is approximately pH 7 for pig feces and between pH 7 and 8 for cattle feces.
To understand the process of NH₃ formation in animal manure, we have determined the chemical and physical properties of feces, urine, and fresh manure and characterized the urease activity in fresh feces and manure from pigs and cattle.

**Pig Samples Contain Higher Levels of Nitrogen Compounds**

The measured concentrations of TKN and TAN, and the pH values for feces, urine, and manure from pigs (Table 1) were consistent with previous results [20,22,26]. With regards to the urinary urea concentration and dry matter of feces and urine from pigs, our results were lower than those reported by Canh et al. [20]. The observed concentrations of TKN and TAN in urine and manure from cattle (Table 1) were consistent with nitrogen excretion values reported in some other studies [27-29]. In addition, the pH of the fresh manure is consistent with the values reported by those studies [28,29]. However, the amount of urea in urine and the dry matter in manure from cattle in the present study are lower than those observed by Bristow et al. [27] and Burgos et al. [29]. The differences in dry matter levels compared with other studies are likely caused by variations in water consumption between animal facilities. Furthermore, several factors including the dietary protein content, feed composition, and volume of urine produced are known to affect the composition of nitrogen compounds and their concentrations in urine and feces and lead to large variations in TKN, TAN, and urea concentrations. The fact that all TKN, TAN, and urea measurements are higher for
the pig samples than for the cattle samples (Table 1) most likely reflects that the pigs are given feedstuffs with higher protein contents, which affects the nitrogen composition of urine and feces [30]. In particular, the TKN and TAN values in pig feces are 71% and 87% higher than the values for cattle feces, respectively. The higher TAN concentrations in pig feces and urine could be caused by a more ready conversion of organic nitrogen into ammoniacal nitrogen in the pig samples than in the cattle excreta. In addition, the dry matter of the pig manure is significantly lower than it is for cattle manure, which has also been reported in other studies [29,30]. Our results also show that the pH values of feces, urine, and fresh manure from pigs are all lower than the values for cattle (Table 1).

**Pig Feces have a Higher Specific Urease Activity than Cattle Feces**

By using Michaelis-Menten kinetic analyses, we have determined the specific urease activity of fresh feces from pigs and cattle at 25 °C. We first determined and compared the activities in feces-urea mixtures with feces:liquid ratios equaling those in authentic manure from pigs and cattle (Figure 1A and 1B). The maximum rates of TAN formation in the reaction mixtures are approximately 1 mM/min for both mixtures, and the urea concentration at half-maximum reaction rates of TAN formation are very different for the reactions. Thus, to further elucidate the results and make a thorough kinetic comparison of the pig and cattle fecal urease activities, the kinetic data were converted into specific reaction velocities of hydrolyzed urea (mmol urea hydrolyzed per kg
wet feces per min, Figure 2). The kinetic analyses showed that the maximum specific urease activity and the $K'_m$ value are more than 2-fold higher for pig feces than for cattle feces. In kinetic analyses employing pure enzyme preparations, the Michaelis constant is an inverse measure of the affinity between the substrate and enzyme. Thus, the smaller the $K_m$ value, the higher the affinity [24,25]. However, with a complex biological material such as feces, the Michaelis constant of the urease activity is actually a measure of the “overall affinity” between urea and the microbial community in feces and depends on factors such as diffusion, membrane-spanning urea transporter characteristics, the urease enzyme, and other components of the urease system [4,31-33]. Most microbial ureases are intracellular and, therefore, the urea must first reach the cells in feces and then be transported across the cytoplasmic membrane before it is degraded by urease. Thus, the fact that the $K'_m$ value for pig feces (32.59±5.65 mM) is approximately two times higher than it is for cattle feces (15.43±2.94 mM) suggests that the “overall affinity” of urea is lower for pig feces than for cattle feces. This finding signifies that a lower urea concentration is required to saturate the urea hydrolysis capacity of cattle feces than that of pig feces. The differences between the fecal urease kinetic parameters of pigs and cattle may indicate that their feces are dominated by different ureolytic bacteria species.

Muck R.E. [19] previously determined the $V_{max}$ (1.17±0.19 mg urea-N/g wet feces/h) and $K_m$ (0.48±0.04 mg urea-N/g mixture) for bovine feces at 24 °C. When converted into molar
concentrations, these values roughly equal $V_{\text{max}}$ and $K_m$ values of 0.7±0.1 mmol urea/kg/min and 17.1±1.4 mmol urea/l, respectively. Thus, the kinetic parameters for cattle in our study are slightly different from those determined by Muck R.E. In contrast to the findings of Muck R.E., who used a 1 h incubation time in the urease kinetic experiments, we used a much shorter reaction time (5 min), which should give more correct initial reaction velocity measurements according to enzyme kinetic theory, and thus better $V_{\text{max}}$ and $K_m$ determinations. In addition, other researchers have previously used a value of 2 mM (2 µmol/g) for the Michaelis constant in studies of both pig and dairy-cow houses [15,34,35].

**Faster NH$_3$ Production in Pig Manure than in Cattle Manure**

The difference in the enzymatic reaction velocity of urea hydrolysis between pig and cattle feces was even more significant in authentic fresh manure when the ammoniacal nitrogen production was recorded (Figure 3). Thus, the initial velocity of TAN formation was more than 4-fold higher in fresh pig manure (1.53 mM/min) than in cattle manure (0.33 mM/min) despite the higher feces-to-urine ratio in cattle manure. That observation may be explained by factors affecting the urease activity including the different chemical composition, pH, dry matter (Table 1), and texture of pig and cattle manure and the higher concentration of urea in pig manure. According to the measured concentrations of urea in urine (Table 1) and the ratios of feces and urine in the manures, the initial urea concentrations in manure from pigs and cattle are approximately 75 mM and 30 mM,
respectively. The lower rate of pH change in pig manure than in cattle manure after reaching the maximum pH (Figure 3) suggests that less NH$_3$ vaporizes from the pig manure or/and that pig manure has a stronger buffer capacity than cattle manure close to the maximum pH.

The Effects of the pH on the Fecal Urease Activity Suggest there are Different Bacterial Communities in Feces from Pigs and Cattle

Our measurements of urea hydrolysis activity at different pH values show that the maximum urease activity for pig feces is observed at approximately pH 7, and that of cattle feces is closer to pH 8 (Figure 4). It is noteworthy that fresh pig manure has an initial pH of 7.05 and that of cattle manure is 7.87 (Table 1), which suggests that the bacterial communities in the feces from the two animal species have urease enzymes that are most efficient at the initial pH of the manure. Thus, these results indicate that the predominant ureolytic bacterial species responsible for the urea hydrolysis activity in feces are different between pigs and cattle and are adapted to species-specific conditions in the animal manures.

Implications for NH$_3$ Production and Volatilization from Manure

Our results show that TAN production is both significantly faster and higher in pig manure than in cattle manure, which is important in relation to the volatilization of NH$_3$ from the two manure types. The rate of NH$_3$ volatilization from manure is related to different factors including, for example, the urease enzyme activity, the equilibrium between NH$_3$ and ammonium, the pH, the temperature, and
the air velocity at the manure surface. Consequently, reducing the urea hydrolysis activity in manure by adding urease inhibitors, for example, will lead to a reduction in the NH₃ production and volatilization levels as reported by Varel V.H. and colleagues [12,36]. In Denmark, acidifying manure to pH < 6 is an approved and established technology to reduce the volatilization of NH₃ from animal production [14]. Our observations show that the acidification of both pig and cattle manure to pH 5-6 slightly reduces the urease activity (at a reduction of up to 10-20 %) compared with the maximum activity observed at the optimal pH values (Figure 4). A previous study showed that the microbial activity as expressed by oxygen consumption, methanogenesis, and sulfate reduction in a slurry acidified to pH 5.5 was greatly reduced relative to that of untreated slurry [37]. Together, these observations show that some metabolic processes including NH₃ formation from urea hydrolysis are almost unaffected and others are dramatically reduced or absent in acidified manure relative to normal manure.

The kinetic parameters of urease activity in feces and manure have been incorporated into the calculations and process modeling of NH₃ concentration and volatilization from manure stores and animal houses in many studies [15,19,34,35,38]. We believe that the kinetic measurement and characterization of fecal urease activity for both pigs and cattle as presented in the current study will be useful in future studies to make more accurate and animal-specific prediction
models for urea hydrolysis rates and NH$_3$ concentrations in pig and cattle manures and thus, for

NH$_3$ volatilization rates from animal production.

ACKNOWLEDGMENTS

The authors thank Dalum Landbrugsskole, pig producer Stougaard, and Infarm A/S, Denmark for providing urine and feces samples from cattle and pigs. Furthermore, we thank Infarm A/S for constructive and inspiring discussions concerning the management and acidification of manure.
REFERENCES


Figure 1. The rates of formed TAN as catalyzed by fresh pig and cattle feces. The rate of TAN formed (R. of formed TAN; panels A and B) and specific rate of TAN formed (S.R. of formed TAN; panels C and D) as catalyzed by pig feces (panels A and C) and cattle feces (panels B and D) in reaction mixtures containing fresh feces and different concentrations of urea.

Figure 2. The Michaelis-Menten kinetics of the urease activity in fresh pig and cattle feces. Michaelis-Menten curves (panels A and B) and Lineweaver-Burk plots (panels C and D) for the specific reaction velocities of hydrolyzed urea ($V_0$) as catalyzed by pig feces (panels A and C) and cattle feces (panels B and D). The curves are generated from Figure 1 data. The maximum specific $V_{max}$ and $K'_m$ values of the urease activity in fresh feces from pigs and cattle were determined from the graphic presentations. The goodness of fit values ($R^2$) were 0.84 (panel A) and 0.91 (panel C) for the pig feces and 0.82 (panel B) and 0.81 (panel D) for the cattle feces.

Figure 3. Urease activity in fresh manure from pigs and cattle. The formed TAN and changes in pH over time in fresh pig manure (panel A) and fresh cattle manure (panel B). During the first hours after mixing urine and feces, the concentration of formed TAN (open squares) and pH (filled triangles) increase rapidly in both pig and cattle manures. However, the rate of TAN formation in pig manure is significantly faster than it is in cattle manure and the TAN concentration reaches a
higher plateau in pig manure than in cattle manure. In both manures, the pH decrease continuously after reaching a maximum.

Figure 4. The effect of the pH on fecal urease activity. Urease activity at different pH values are presented as the rate of TAN formation (R. of formed TAN; panels A) and the relative R. of formed TAN compared with that of pig feces at pH 7 (panel B). The optimal pH for urea hydrolysis catalyzed by fecal urease is approximately pH 7 for pig feces and between pH 7 and 8 for cattle feces.
Table 1. The chemical and physical properties of feces, urine, and manure samples (Mean±SD; n=3). The p-value obtained in each test of significance between the values for pigs and cattle is indicated below each pair of measurements. Thus, at a significance level of 0.05 all the measured properties are significantly different between pigs and cattle except the dry matter of urine (P>0.05).

<table>
<thead>
<tr>
<th>Animal species</th>
<th>TKN (mmol/kg)</th>
<th>TAN (mmol/kg)</th>
<th>[UN] (mmol/l)</th>
<th>[Urea] (mmol/l)</th>
<th>Dry matter (%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig Feces</td>
<td>578.8±1.2</td>
<td>n.a</td>
<td>39.6±4.6</td>
<td>n.a</td>
<td>15.32±0.09</td>
<td>6.89±0.01 ^2</td>
</tr>
<tr>
<td>Cattle Feces</td>
<td>337.8±33.0</td>
<td>n.a</td>
<td>21.2±0.4</td>
<td>n.a</td>
<td>11.44±0.22</td>
<td>7.02±0.02 ^1</td>
</tr>
<tr>
<td>Pig Urine</td>
<td>n.a</td>
<td>350.2±2.1</td>
<td>23.6±1.0</td>
<td>198.4±5.0</td>
<td>1.86±0.84</td>
<td>7.69±0.03</td>
</tr>
<tr>
<td>Cattle Urine</td>
<td>n.a</td>
<td>261.3±0.9</td>
<td>15.9±1.0</td>
<td>152.7±1.1</td>
<td>3.03±0.01</td>
<td>8.55±0.02</td>
</tr>
<tr>
<td>Pig Manure</td>
<td>n.a</td>
<td>369.4±7.7</td>
<td>87.2±1.6</td>
<td>n.a</td>
<td>3.71±0.09</td>
<td>7.05±0.01</td>
</tr>
<tr>
<td>Cattle Manure</td>
<td>n.a</td>
<td>317.4±4.8</td>
<td>20.5±0.2</td>
<td>n.a</td>
<td>7.81±0.07</td>
<td>7.87±0.01</td>
</tr>
</tbody>
</table>

1 n.a.: not available.
2 pH was measured in a mixture of 1:3 (wt:v) feces and water.
3 pH was measured in a mixture of 3:2 (wt:v) feces and water.
4 Pig manure was prepared by mixing feces and urine in a (wt:v)-ratio of 1:3, and cattle manure was prepared by mixing feces and urine in a 3:2 (wt:v)-ratio. TAN and pH were measured immediately after mixing the fresh feces and urine.
Table 2. Kinetic parameters of the urease activity in fresh feces. The $V_{\text{max}}$ and $K'_m$ values of fecal urease activity from pigs and cattle were determined by Michaelis-Menten kinetic analysis (Mean±S.E.).

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Temperature (°C)</th>
<th>$V_{\text{max}}$ (mmol urea/kg/min)</th>
<th>$K'_m$ (mM)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig</td>
<td>25</td>
<td>2.06±0.08</td>
<td>32.59±5.65</td>
<td>0.84</td>
</tr>
<tr>
<td>Cattle</td>
<td>25</td>
<td>0.80±0.04</td>
<td>15.43±2.94</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P &lt; 0.001</td>
<td>P &gt; 0.05</td>
<td></td>
</tr>
</tbody>
</table>
Figure S1. Determining the urea nitrogen concentration \([\text{UN}]\) in urine. Jack bean urease was added to the urine samples for urea hydrolysis. The TAN concentration was measured at different time points and the corresponding level of formed TAN was calculated by subtracting the initial TAN \((\text{TAN}_{i,\text{urine}})\) concentration from the measured TAN \((\text{TAN}_{m,\text{urine}})\) concentration. The final constant TAN reached at the completion of the reaction was defined as \(\text{TAN}_{f,\text{urine}}\). The final concentration of formed TAN \((\text{TAN}_{f,\text{urine}} - \text{TAN}_{i,\text{urine}})\) reached at the completion of the reaction equals \([\text{UN}]\) and was used to calculate the initial urea concentration in urine.

Figure S2. The relation between the reaction time and the rate of formed TAN. Formed TAN (filled triangles) and the corresponding rate of formed TAN \((\text{R. of formed TAN}; \text{open squares})\) after different reaction times. The levels of formed TAN after 5 min, 11 min, and 20 min of reaction time were measured in mixtures containing pig feces and 100 mM urea. The highest \(\text{R. of formed TAN}\) is observed at a reaction time of 5 min.

Figure S3. Rates of formed TAN as catalyzed by thawed pig and cattle feces. The rate of TAN formation \((\text{R. of formed TAN}; \text{panels A and B})\) and the specific rate of TAN formation \((\text{S.R. of})\)
formed TAN; panels C and D) as catalyzed by thawed pig feces (panels A and C) and thawed cattle
feces (panels B and D).

Figure S4. The Michaelis–Menten kinetics of urease activity in thawed pig and cattle feces.
Michaelis-Menten curves (panels A and B) and Lineweaver-Burk plots (panels C and D) for the
specific reaction velocities of hydrolyzed urea ($V_0$) as catalyzed by thawed pig feces (panels A and
C) and thawed cattle feces (panels B and D). The curves are generated from Figure S3 data. The
goodness of fit values ($R^2$) were 0.89 (panel A) and 0.86 (panel C) for the pig feces and 0.90 (panel
B) and 0.93 (panel D) for cattle feces.
Table S1. Kinetic parameters of the urease activity in thawed feces. $V_{\text{max}}$ and $K_m$ values of the urease activity of thawed feces from pig and cattle were determined by Michaelis-Menten kinetic analysis (Mean±S.E.).
FIGURE 1

A  Pig

B  Cattle

C  Pig

D  Cattle

R. of formed TAN (mmol l⁻¹ min⁻¹)

[S$_\text{Urea}$], M

R. of formed TAN (mmol l⁻¹ min⁻¹)

[S$_\text{Urea}$], M

S.R. of formed TAN (mmol kg⁻¹ min⁻¹)

[S$_\text{Urea}$], M

S.R. of formed TAN (mmol kg⁻¹ min⁻¹)

[S$_\text{Urea}$], M
FIGURE 2

A  Pig  B  Cattle

$V_0$ (mmol urea kg$^{-1}$ min$^{-1}$)

$V_0$ (mmol urea kg$^{-1}$ min$^{-1}$)

\[
\frac{1}{V_0} = 0.0137 \frac{1}{[\text{Urea}]} + 0.516
\]

\[
\frac{1}{V_0} = 0.0164 \frac{1}{[\text{Urea}]} + 1.332
\]
FIGURE 4

A

R. of formed TAN (mmol l\(^{-1}\) min\(^{-1}\))

\[\begin{array}{cccccc}
4 & 5 & 6 & 7 & 8 & 9 & 10 \\
0.6 & 0.8 & 1.0 & 1.2 & \\
Cattle & Pig
\end{array}\]

B

% of R. of formed TAN at pH 7 for pig feces

\[\begin{array}{cccccc}
4 & 5 & 6 & 7 & 8 & 9 & 10 \\
0 & 20 & 40 & 60 & 80 & 100 \\
Cattle & Pig
\end{array}\]
FIGURE S2

- Formed TAN
- R. of formed TAN

**Time (min)**

**Formed TAN (mmol·l⁻¹)**

- 0
- 0.5
- 1
- 1.5
- 2
- 2.5
- 3
- 3.5
- 4
- 4.5
- 5

**R. of formed TAN (mmol·l⁻¹·min⁻¹)**

- 0
- 0.2
- 0.25
- 0.3
- 0.35
- 0.4
- 0.45
- 0.5

- 5; 0.45
- 11; 0.31
- 20; 0.22
- 5; 2.3
- 11; 3.4
- 20; 4.5
FIGURE S3

A  Pig  
B  Cattle

C  Pig  
D  Cattle

[Urea], M
R. of formed TAN
(mmol l⁻¹ min⁻¹)

[S. R. of formed TAN]
(mmol kg⁻¹ min⁻¹)

[Urea], M
Figure S4.

(A) Pig

(B) Cattle

(C) Pig

(D) Cattle
Emissions of ammonia, carbon dioxide, and hydrogen sulfide from swine wastewater during and after acidification treatment: Effect of pH, mixing and aeration

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ABSTRACT

This study aimed at evaluating the effect of swine slurry acidification and acidification-aeration treatments on ammonia (NH₃), carbon dioxide (CO₂) and hydrogen sulfide (H₂S) emissions during slurry treatment and subsequent undisturbed storage. The study was conducted in an experimental setup consisting of nine dynamic flux chambers. Three pH levels (pH = 6.0, pH = 5.8 and pH = 5.5), combined with short-term aeration and venting (with an inert gas) treatments were studied. Acidification reduced average NH₃ emissions from swine slurry stored after acidification treatment compared to emissions during storage of non-acidified slurry. The reduction were 50%, 62% and 77% when pH was reduce to 6.0, 5.8 and 5.5, respectively. However, it had no significant effect on average CO₂ and H₂S emissions during storage of slurry after acidification. Aeration of the slurry for 30 min had no effect on average CO₂ and H₂S emissions both during the process and from stored slurry after venting treatments. During acidification treatment, the NH₃, CO₂ and H₂S release pattern observed was related to the liquid turbulence caused by the gas bubbles rather than to biological oxidation processes in this study.

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1. Introduction

Air pollutants, such as ammonia (NH₃), hydrogen sulfide (H₂S), methane (CH₄), and nitrous oxide (N₂O), emitted from animal wastewater (slurry) can affect human and animal health and the natural environment (Hartung and Phillips, 1994; Erisman et al., 2008). Deposition of NH₃ gas and particulate ammonia can cause eutrophication of surface water and may acidify ecosystems. Ammonia release from animal waste has received much attention because the co-release of NH₃ and CO₂ determines the formation of a pH profile at the slurry surface, and so the emission of other acidic (such as H₂S and CH₃COOH) and basic compounds (Blanes-Vidal and Nadimi, 2011).

Hydrogen sulfide has been reported as a main toxic substance associated with swine operations. Acute exposure to such gases emanating from animal manure can cause severe health impairment to farm operators (Donham et al., 1982). The concentration of H₂S above stored swine slurry is usually below 1 ppm (Ni et al., 2010). However, when slurry is agitated (e.g. during emptying of slurry pits), a short-term peak of very high H₂S concentrations occur (Blanes-Vidal et al., 2009a).

Carbon dioxide is a greenhouse gas, although the contribution of CO₂ released from swine wastes to the overall greenhouse effect is very limited. Quantification of CO₂ production from swine slurry is important because the co-release of NH₃ and CO₂ determines the formation of a pH profile at the slurry surface, and so the emission of other acidic (such as H₂S and CH₃COOH) and basic compounds (Blanes-Vidal and Nadimi, 2011).

Reduction of the NH₃ emissions in Europe has become increasingly significant for more than one decade. International regulations include the Gothenburg Protocol on Long-range Transboundary Air Pollution (UNECE, 1999) (stating the necessity of reducing NH₃ emissions by 12% in 2010 relative to 1990) and the EU directives and strategies (EC, 1997, 1999), e.g. the National Emissions Ceilings Directive (NECD). In the Thematic Strategy on Air Pollution (CEC, 2005), the European Commission expressed the environmental objectives for 2020, aimed at reducing 27% of agricultural NH₃ emissions in the EU25 compared to 2000, approximately 23% of the reduction have to be met by introduction of specific abatement measures in agriculture.
In order to reduce NH3 emissions, different technologies have been developed (Ndegwaa et al., 2008). Among them, one of the effective mitigation strategies is slurry acidification, which has been approved as Best Available Technology (BAT) in Denmark (Kai et al., 2008). Previous studies have reported that about 70–85% of the NH3 release from swine slurry can be reduced by decreasing the slurry pH to 5.5 through the addition of sulfuric acid (H2SO4) (Stevens et al., 1989; Frost et al., 1990; Kai et al., 2008). Acidification also improves the mineral N fertilizer equivalence (MFE) of the slurry by 25% (Sørensen and Eriksen, 2009).

Although effect of acidification on NH3 emissions has been known for many years, its implications on the emission of other compounds, such as CO2 and H2S, has not been fully documented in the literature. The release of dissolved gases from slurry is a function of the concentration of gas present in the slurry surface in a non-ionized form, which will be affected by the slurry surface pH. Therefore, a reduction of slurry pH favors the emission of weak acid forming gases such as CO2 and H2S. Furthermore, acidification by addition of H2SO4 may increase the concentration of inorganic sulfur in the slurry, which could potentially result in an increase of H2S emission as a result of the additional sulfate provided as substrate to sulfate-reducing bacteria. However, acidification causes low pH may also inhibit the bacterial activities in slurry and limit the sulfate reduction (Eriksen et al., 2008).

Aeration has been applied to stored slurry to create aerobic environment to reduce odor potential via biological degradation of volatile fat acid (VFA) (Zhang and Zhu, 2005). However, the decrease of VFA in slurry results in an increase of slurry pH and shifts the NH4+ ⇌ NH3 + H+ equilibrium, which may contribute to increased NH3 volatilization losses (Paul and Beauchamp, 1989; Zhang and Zhu, 2005). By contrast, Clark et al. (2005) proposed a low-level of bubbling air, being intent to promote the gradual release of gases (i.e., H2S) generated in anaerobic condition at innocuous rates throughout the storage rather than to oxidize the volatile compounds in the slurry.

A new slurry acidification technology, which combines acidification with aeration, has been recently introduced in Denmark and is currently in use in more than eighty Danish fattening pig farms (Eriksen et al., 2008; Kai et al., 2008; Sørensen and Eriksen, 2009). The use of this acidification technology is expected to increase in the coming years, as it has been estimated that in 2020 between 16 and 28% of the Danish commercial fattening pig farms will use a commercially available slurry acidification technology (Aaes et al., 2008).

In this acidification-aeration technology, slurry from the in-barn storage pit is transported to a process tank, where it is acidified to a pH of 5.5 by controlled addition of concentrated sulfuric acid (H2SO4), the acidified slurry is then pumped back to the barn pit, resulting in a reduction of slurry pH to 6.0 shortly after excretion (Sørensen and Eriksen, 2009). During acidification treatment, low-level aeration is also applied, as observation and practical experience has shown that it helps to decrease the formation of foam during the addition of acid (Kai et al., 2008; Sørensen and Eriksen, 2009).

The effect of this combined acidification/aeration treatment needs to be evaluated from a wider perspective, including emissions during treatment and storage, and considering not only the targeted gas (NH3), but also other important gases (CO2 and H2S) whose emission can be affected by the treatment.

The objectives of the study were: (1) to investigate the effect of different pH levels on the emission of NH3, CO2 and H2S from swine slurry during and after acidification treatment; and (2) to compare the contribution of aeration to the efficacy of the acidification process (reduction of pH and NH3 emission) and its effect on the emission of other compounds (CO2 and H2S).

2. Materials and methods

2.1. Experimental setup and procedure

The study was conducted in a laboratory using nine dynamic flux chambers, each with a volume of 30 L and a height of 51 cm (Fig. 1S). An air inlet and an exhaust air outlet were installed in the top cap. At the center of the top cap, five other ports were installed for headspace NH3, CO2 and H2S concentration measurement, pH measurement, slurry stirring, addition of fresh slurry and acid, and slurry aeration. The inlet and outlet air ducts and the sampling points were located 3 cm below the top cap. All the ports were closed by plugs to make the chamber interface airtight during the storage. A water tap was installed in the middle of the side of each chamber for slurry sampling. Slurry was collected from under-floor deep pits of a fattening pig barn after 6–7 days accumulation from last empty. Four days before starting the first treatment, each flux chamber was filled with 20 L of homogenized swine slurry to a depth of 35 cm. During the experimental period the chambers were ventilated at a constant airflow rate of 1.9 L/min, which was maintained by a critical orifice inserted in the outflow tube, between a filter and the air pump.

The experimental design included four pH levels: non-acidified slurry (pH5.8), and slurry acidified to pH = 6.0 (A6.0), pH = 5.8 (A5.8), and pH = 5.5 (A5.5). Acidification has performed by addition of concentrated sulfuric acid while mixing with a portable paddle mixer at 500 rpm in the center of the slurry chamber. Besides, the effect of two types of venting gases: air (Ga) (for the aeration treatment) and nitrogen gas (N2, Gn) (an inert gas that creates slurry agitation with no oxidation effects) with comparison to no venting treatment (G0) were studied (Table 1). The pH level 5.5 was selected according to previous literature (Eriksen et al., 2008; Kai et al., 2008; Sørensen and Eriksen, 2009), while levels pH = 6.0 and pH = 5.8 were selected to assess the gas emission reduction achieved with a less severe acidification treatment (which involves less economical expenses related to acid addition). Aeration of 30 min at a ratio of 0.04 L s⁻¹ was applied following the velocity used in practice and our pre-test in the fresh swine manure (DM content of 5.4%) to have homogeneous bubbles coming out to the surface. The total duration of the experiment was 155 days. Slurry treatments were applied three times (days 5–8: T0, days 26–27: T1 and days 56–57: T2) during the experiment. The first treatment (T0) was performed to create acidified slurry, while, the subsequent treatments (T1 and T2) correspond to two repetitions of the acidification-aeration treatment performed on the mixture of acidified slurry and non-acidified slurry added to the flux chamber (which corresponds to the slurry added to the pit by animal excretion in commercial farms). After each acidification treatment, slurries in all flux chambers (acidified and non-acidified slurries) were stored under undisturbed conditions. The experimental procedure for each slurry chamber is summarized in Table 1.

2.2. Slurry pH and characteristics

Surface pH measurements at three different depths (0.5–1 cm, 4 cm and 6 cm from the slurry surface) were measured by a pH meter (model PHM210, Meterlab Radiometer Analytical, Lyon, France, accuracy ±0.01 pH units), at the same location at the beginning of the experiment, before each treatment, during each acidification, and 30 min after acidification.

Two slurry samples per chamber were collected immediately after filling and analyzed for dry matter (DM), total nitrogen (TN), total ammonium nitrogen (TAN), total inorganic carbon (TIC), and total sulfide (TS). Slurry subsamples were evaporated to dryness.
with a constant weight in an oven at 105 °C for 24 h, and the remaining mass was recorded as DM. TN was analyzed by Kjeldahl method, and TAN was determined using ammonium cuvette test (range from 2.5 to 60 mg L⁻¹ NH₄⁺, Lange, Germany) with 100× dilution. TIC was determined using titration with hydrochloric acid (HCl, 1 mol L⁻¹) as described by Sommer (1997). TS were determined by titration (T50 Tittrator, Mettler Toledo, Columbus, USA) with 10× dilution.

2.3. Gas concentrations and emissions

Headspace NH₃ and CO₂ concentrations were continuously measured during the 155 days experimental period using an infrared 1412 photoacoustic multi-gas analyser and a multiplexer 1309 (Innova AirTech Instruments A/S, Denmark), compensated for gas and water interferences. The detection limits for NH₃ and CO₂ were 0.2 ppm and 1.5 ppm respectively (1 atm, 20 °C). The sampling rate was 45 s, so a gas emission data from each chamber was obtained every approximately 10 min. For each of the treatment process, the NH₃ and CO₂ concentration observations were one determined by titration (T50 Tittrator, Mettler Toledo, Columbus, USA) with 10× dilution.

Concentrations of H₂S were measured during acidification to pH 6.0, 5.8 and 5.5 respectively. The slurry acidification treatment time was 26 ± 9 min, 39 ± 5 min and 52 ± 19 min for slurry acidified to pH 6.0, pH 5.8 and pH 5.5, respectively. The slurry acidification was followed for the three acidification treatments, i.e. T0 at days 5–8, T1 at days 26–27 and T2 at days 56–57.

Approximately 10 s, 10 s and 10 s respectively. Table 1 shows the concentrations of H₂S exceeded the analyser’s detection limit, and therefore precision gas detector tubes (Kitagawa, Japan; range from 0.75 to 300 ppm and 0.005%–0.16%) were used.

Gas emissions from the slurry during the experiments (E, mg m⁻² s⁻¹) were calculated as follows (Eq. (1)):

\[ E = \frac{(C_a - C_i) \cdot Q}{S} \]  

where \( C_a \) and \( C_i \) are the gas concentrations (mg m⁻³) at the outlet and inlet, respectively, \( Q \) is the airflow rate (m³ s⁻¹), and \( S \) is the emitting surface area (m²).

2.4. Statistical analysis

The differences on the initial slurry conditions (pH and compositions) from different flux chambers and the effects of pH level and aeration on NH₃, CO₂ and H₂S emissions during and after slurry treatments were studied by analysis of variance (ANOVA) at a significance level of 0.05 and Tukey’s honestly significant difference test were used for subsequent multiple comparisons. The statistical analyses were performed in Matlab version 7.110 (R2010b, Mathworks Inc. Natick, MA, USA).

3. Results and discussion

3.1. Slurry characteristics

Measured slurry pH, DM, TN, TAN, TS, TIC at day 0 were on average of 6.31 ± 0.08 pH units, 53.49 ± 2.99 g kg⁻¹, 4.94 ± 0.04 g kg⁻¹, 3.15 ± 0.06 g kg⁻¹, 1.86 ± 0.04 g kg⁻¹, 4.79 ± 0.14 g kg⁻¹ (mean ± standard deviations, \( n = 9 \)), respectively. In Table 2 the measurements for each individual flux chamber are shown. Slurry composition and pH values were in agreement with data reported in previous studies (Sánchez and Gonzalez, 2005; Fanguero et al., 2009; Sørensen and Eriksen, 2009). No statistical differences regarding pH and slurry characteristics at day 0 were found among the different flux chambers, indicating that any eventual differences in the gas measurements cannot be attributed to slurry composition differences.
Table 2
Characteristics of the slurry at day 0.*

<table>
<thead>
<tr>
<th>Flux chambers^b</th>
<th>pHnG0</th>
<th>pHnGn</th>
<th>pHnGa</th>
<th>A6.0Gn</th>
<th>A6.0Ga</th>
<th>A5.8Gn</th>
<th>A5.8Ga</th>
<th>A5.5Gn</th>
<th>A5.5Ga</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (g kg⁻¹)</td>
<td>48.81</td>
<td>51.63</td>
<td>50.22</td>
<td>53.44</td>
<td>55.68</td>
<td>58.82</td>
<td>54.14</td>
<td>54.70</td>
<td>53.96</td>
</tr>
<tr>
<td>TN (g kg⁻¹)</td>
<td>4.88</td>
<td>4.97</td>
<td>4.92</td>
<td>4.91</td>
<td>4.91</td>
<td>4.96</td>
<td>5.01</td>
<td>5.00</td>
<td>4.94</td>
</tr>
<tr>
<td>TAN (g kg⁻¹)</td>
<td>3.10</td>
<td>3.23</td>
<td>3.06</td>
<td>3.21</td>
<td>3.14</td>
<td>3.19</td>
<td>3.09</td>
<td>3.21</td>
<td>3.14</td>
</tr>
<tr>
<td>TS (g kg⁻¹)</td>
<td>1.86</td>
<td>1.83</td>
<td>1.86</td>
<td>1.94</td>
<td>1.91</td>
<td>1.83</td>
<td>1.83</td>
<td>1.83</td>
<td>1.86</td>
</tr>
<tr>
<td>TIC (g kg⁻¹)</td>
<td>4.74</td>
<td>4.63</td>
<td>4.96</td>
<td>5.02</td>
<td>4.85</td>
<td>4.72</td>
<td>4.83</td>
<td>4.78</td>
<td>4.59</td>
</tr>
</tbody>
</table>

* Each value was on an average of two measurements.
^ pHn: no acidi
cation treatment and venting treatment (corresponding to steps 3, 5 and 6 respectively

3.2. Gas emissions during slurry addition, mixing, acidi
cation treatment and venting treatment

The emissions of NH₃ and CO₂ during slurry addition, slurry mixing, addition of acid while mixing, and venting treatment (with air or N₂ bubbles) are shown in Fig. 1. Differences of NH₃ and CO₂ emissions among the flux chambers (during each treatment step)

were not significant (P > 0.05). This indicates that the measurements from the different chambers can be considered as replicates and that descriptive statistics can be performed on these. Average NH₃ and CO₂ emissions during slurry addition were 3.09 ± 0.96 mg m⁻² min⁻¹ and 146 ± 69 mg m⁻² min⁻¹, respectively (averaged from pHn; as data in acidified slurry were not obtained because slurry mixing was performed shortly after slurry addition). Average NH₃ and CO₂ emissions during slurry mixing were 1.01 ± 0.28 mg m⁻² min⁻¹ and 621 ± 194 mg m⁻² min⁻¹, respectively. Average NH₃ emissions during acidification treatment were 0.56 ± 0.17 (for A6.0), 0.63 ± 0.08 (for A5.8), 0.51 ± 0.06 (for A5.5) mg m⁻² min⁻¹, respectively (averaged for all chambers containing slurry acidified at the same pH level: 6.0, 5.8 or 5.5). These NH₃ emissions during acidification, from slurries acidified at different pH levels were not significantly different (P > 0.05). Average CO₂ emissions during acidification were 1029 ± 211 (A6.0), 1348 ± 390 (A5.8), 1328 ± 122 (A5.5) mg m⁻² min⁻¹, respectively. Although the results showed higher CO₂ emissions at pH = 5.5 and 5.8 than at pH = 6.0 during acidification, but no statistical differences were found (P > 0.05). This can be explained by the fact that the lower the pH, the longer the time required for acidification and the higher the disturbance of the slurry. Higher levels of disturbance result in more CO₂ release. Ammonia emissions averaged from treatments with venting air (Ga) and vent using N₂ (Gn) were 0.52 ± 0.12 and 0.60 ± 0.25 mg m⁻² min⁻¹, respectively, while average CO₂ emissions were 1034 ± 410 and 1202 ± 433 mg m⁻² min⁻¹, respectively. Although the averaged NH₃ and CO₂ emissions of treatments with aeration appeared to be lower than those of treatments with venting N₂, no significant difference was found between them (P > 0.05). During the entire treatment, average CO₂ emissions from slurry acidified to pH = 5.8 (A5.8) and 5.5 (A5.5) were significantly higher than from non-acidified slurry (Table 3). Averaged NH₃ emissions of the treatment with only slurry addition (pHnG0) were significantly higher than all other treatments including slurry mixing, acidification, and venting treatment (Table 3). Slurry disturbance caused by addition, mixing or other agitation (e.g. venting) resulted in a breakage of surface buffer equilibrium and a reduction of surface pH (Blanes-Vidal et al., 2012). The higher emission of NH₃ from pHnG0 treatment was because of less pH reduction caused by agitation (only slurry addition) during the treatment process, and higher pH level favors higher NH₃ emissions.

Slurry disturbance caused by the treatment had an immediate decrease of NH₃ emission (57–83%) and a sharp increase of CO₂ emission (279–443%) (Fig. 2), in agreement with Ni et al. (2009) and Blanes-Vidal et al. (2012). In the latter study, it showed a decrease of NH₃ emissions by 61–91% and an increase of CO₂ emissions by 40–1515% after slurry disturbance (slurry addition or mixing).

Hydrogen sulfide emissions during slurry treatment were more than ten thousand fold higher (by 34,674–233,770%) than the emissions before treatment. Large variations in H₂S emissions...
during acidification were observed in the study. In fact, H₂S emissions from the non-acidiﬁed slurry during slurry addition were within the range of 0.22 and 2.29 mg m⁻² min⁻¹, while, H₂S levels ranged between 4.49 and 404.07 mg m⁻² min⁻¹ were measured during slurry mixing, and between 53.88 and 718.35 mg m⁻² min⁻¹ during air or N₂ bubbling. Average H₂S emissions during treatment among the different chambers were not signiﬁcantly different (P > 0.05), except for pHnG0 and A5.8Ga (Table 3). The gas emissions patterns observed during slurry disturbances caused by slurry addition, acid addition, mixing and venting are in agreement with previous studies (Ni et al., 2000, 2009; Blanes-Vidal and Nadimi, 2011). The different gas emission patterns observed for NH₃, CO₂ and H₂S are related to the different gas transport mechanisms governing the release of each gas, which are mainly determined by volatility of each gas and its tendency to form gas bubbles. The immediate decrease in NH₃ emissions after disturbance and subsequent increase during transient state conditions after the disturbances could be explained by the formation of a pH profile under the co-release of buffer components such as CO₂ (Ni et al., 2009; Blanes-Vidal et al., 2009b, 2010; Blanes-Vidal and Nadimi, 2011). The acidification treatment caused an increase of CO₂ and H₂S emissions during the treatment process. A closed chamber with an exhaust gases ﬁlter could be used in order to minimize the emission of gases such as H₂S and CO₂ to the atmosphere during treatment.

3.3. Gas emissions during undisturbed storage after treatment

3.3.1. NH₃ emissions

Ammonia emissions from acidiﬁed and non-acidiﬁed slurry during undisturbed storage after treatment are shown in Fig. 3. Ammonia emissions from acidiﬁed slurry were signiﬁcantly lower than emissions from non-acidiﬁed slurry during all stages of storage (ﬁrst day, seven days and fourteen days after the treatment).

### Table 3

Average ammonia, carbon dioxide and hydrogen sulﬁde emissions during the treatments (mg m⁻² min⁻¹).\(^{ab}\)

<table>
<thead>
<tr>
<th>Gases</th>
<th>Flux chambers(^{c})</th>
<th>pHnG0</th>
<th>pHnGa</th>
<th>pHnGn</th>
<th>A6.0Gn</th>
<th>A6.0Ga</th>
<th>A5.8Gn</th>
<th>A5.8Ga</th>
<th>A5.5Gn</th>
<th>A5.5Ga</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₃</td>
<td>4.52 ± 1.95 a</td>
<td>1.66 ± 0.52 b</td>
<td>0.9 ± 0.08 b</td>
<td>0.80 ± 0.30 b</td>
<td>0.75 ± 0.11 b</td>
<td>0.67 ± 0.11b</td>
<td>0.75 ± 0.10 b</td>
<td>0.55 ± 0.04 b</td>
<td>0.49 ± 0.06 b</td>
<td></td>
</tr>
<tr>
<td>CO₂</td>
<td>143 ± 37 a</td>
<td>348 ± 31 ab</td>
<td>483 ± 73 ab</td>
<td>861 ± 231bc</td>
<td>1009 ± 74 c</td>
<td>1111 ± 223c</td>
<td>1181 ± 152 c</td>
<td>1091 ± 29 c</td>
<td>1091 ± 33 c</td>
<td></td>
</tr>
<tr>
<td>H₂S(^{d})</td>
<td>2.67 ± 0.54 a</td>
<td>45 ± 3 ab</td>
<td>67 ± 26 ab</td>
<td>195 ± 72 ab</td>
<td>156 ± 43 ab</td>
<td>240 ± 23 ab</td>
<td>268 ± 132 ab</td>
<td>68 ± 14 ab</td>
<td>108 ± 45 ab</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) Mean ± standard deviation.  
\(^{b}\) Same letters (a, b, c) within rows indicate no signiﬁcant differences (P > 0.05) between different pH levels and gas bubbling type.  
\(^{c}\) pHn: no acidiﬁcation; A6.0: acidiﬁcation to pH = 6.0; A5.8: acidiﬁcation to pH = 5.8; A5.5: acidiﬁcation to pH = 5.5; G0: no mixing and venting; Gn: mixing and venting N₂; Ga: mixing and venting air.  
\(^{d}\) Emissions were averaged from slurry addition, mixing and venting.

![Fig. 2. Ammonia and carbon dioxide emissions patterns from one day before treatment until eleven days after treatment. Treatment day corresponds to time = 0 to time = 24 h. Hourly averaged values are presented. Solid lines indicate average values from T1 and T2, dotted lines on both sides of solid line indicate mean ± standard deviation and mean + standard deviation. Legends for treatment are as in Fig. 1.](image-url)
 REGARDING TO COMPARISON AMONG ACIDIFIED SLURRIES (A6.0, A5.8 AND A5.5), NH₃ EMISSIONS WERE NOT SIGNIFICANTLY DIFFERENT DURING THE FIRST DAY AFTER ACIDIFICATION TREATMENT, BUT NH₃ EMISSIONS FROM SLURRY ACIDIFIED TO pH = 5.5 (A5.5) WERE SIGNIFICANTLY LOWER THAN NH₃ EMISSIONS FROM SLURRY ACIDIFIED TO pH = 6.0 (A6.0) DURING THE SEVEN DAYS STORAGE \( (P < 0.05) \) (Fig. 3). After fourteen days, there were no significant differences among the acidified slurry treatments (Fig. 3). The effectiveness of slurry acidification in reducing NH₃ emission is summarized in Table 4. Some preliminary recommendations can be made based on the results of our study. Both, pH = 5.8 and pH = 5.5 are recommended target pH’s for NH₃ emission reduction, as they provide similar NH₃ emission mitigation. When short-term effects are of interest (i.e. storage time less than seven days), a pH = 5.8 is recommended over a pH = 6.0. More studies, including full-scale studies are needed to confirm the validity of these recommendations. For slurry acidification before land application (Kai et al., 2008) and long-time storage (i.e. 14 days or even more), slurries acidified to pH = 6.0 can be applied by the reason of reducing H₂SO₄ consumption and energy conservation during acidification process.

After acidification, NH₃ emissions remained constant and low for a period of time, and then increased, as shown in Fig. 2. The length of this period of low and stable NH₃ emissions was related to the pH. Acidification treatments involving lower targeted pH resulted in longer periods of low and stable NH₃ emissions. When the slurry was acidified to pH = 6.0, NH₃ emissions were low and stable for 8.5–9 h, when they reached a 20% increase compared to emissions measured right after the treatment. However, when the slurry was acidified to pH = 5.5, the time span of low and stable NH₃ emissions after acidification ranged between 55 and 58 h. These results suggest that the time between treatments could be of two days (instead of one day, as it is common practice in Denmark). More research (including full-scale experiments) is needed to confirm the appropriateness of this recommendation. The increase in NH₃ emissions can be explained by the formation of pH profile at the slurry surface (Ni et al., 2009; Blanes-Vidal et al., 2008; Blanes-Vidal and Nadiem, 2011). In our study a pH profile was also observed in the settled slurry, being the pH at the surface (0.5–1 cm from the surface) higher than the pH at deeper layers (Fig. 4).

### Table 4

| Reduction in NH₃ emissions after acidification to pH = 6.0 (A6.0), pH = 5.8 (A5.8) and pH = 5.5 (A5.5) compared to non-acidified (pHn) slurry.\(^a\) |
|-----------------|-------------|-------------|-------------|
|                 | pHn         | A6.0        | A5.8        | A5.5        |
| One day after treatment | 4.23 ± 0.49 | 0.97 ± 0.81 | 0.80 ± 0.14 | 0.35 ± 0.07 |
| Seven days after treatment | 5.42 ± 0.10 | 3.54 ± 0.80 | 2.16 ± 0.92 | 1.42 ± 0.24 |
| Fourteen days after treatment | 5.66 ± 0.39 | 3.57 ± 0.51 | 3.06 ± 0.83 | 2.04 ± 0.03 |
| Reduction compared to non-acidified slurry (%)\(^b\) | 77 t a | 81 t ab | 92 t b |
| A6.0 | 35 u a | 60 u ab | 74 u b |
| A5.5 | 37 u a | 46 u ab | 64 u b |

\(^a\) Same letters (a, b) within rows indicate no significant differences \( (P > 0.05) \) between different pH levels. Same letters (t, u) within columns indicate no significant differences \( (P > 0.05) \) between different acidification times.

\(^b\) Averaged from the same pH level and indicated as Mean ± standard deviation.

\(^c\) The reducing efficiency was calculated by: \( R(\%) = (E_{\text{pHn}} - E_{\text{Ac}}) / E_{\text{pHn}} \times 100\% \). The Ac in this formula indicate A6.0, A5.8, A5.5.
Previous studies have reported an increase of NH$_3$ emissions during aeration (Zhang and Zhu, 2005; Amon et al., 2006). However, the results of our study suggest that the increase in NH$_3$ emission is mainly caused by the agitation created during aeration rather than the effect of the aerobic conditions in the slurry, as in our study aeration of slurry for 30 min did not have a significant effect on NH$_3$ emissions during storage compared to N$_2$ gas bubbling, which causes bubble formation, but does not contribute to oxidation processes.

### 3.3.2. CO$_2$ emissions

Fig. 3 shows the CO$_2$ emissions from stored slurry after each acidification treatment. Carbon dioxide emissions during storage were not affected by the acidification and the aeration treatments. Carbon dioxide emissions during storage were rather stable if no agitation occurred (Fig. 2). The average emission during entire acidification treatment was approximately 2–10 times higher than that of subsequent storage under undisturbed conditions (Fig. 2). The results approved the inferential conclusion of Fanguero et al. (2010), who assessed the gas emissions from sandy soil applied with acidified pig slurry and proposed that most of the dissolved CO$_2$ was lost during the acidification process since a significantly decrease of CO$_2$ emission was found during the incubation.

During the undisturbed storage after acidification treatment, emission patterns of CO$_2$ were different from those of NH$_3$, which remained low and stable for a period and increased over time. In contrast, CO$_2$ emissions showed an exponential decay during the first 2–4 h after treatment and then reached a stable release in all treatments (Fig. 2). Similar CO$_2$ emission pattern after disturbance was also observed in other studies (Blanes-Vidal et al., 2012; Ni et al., 2009). This pattern of CO$_2$ release can be explained by the CO$_2$ bubble release mechanism, low convective mass transfer and low solubility. With regard to slurry aeration, little attention has been paid to the effect of aerobic treatment on CO$_2$ emissions individually. A previous study (Loyon et al., 2007) reported that aerobic treatment combined with slurry separation of piggery waste can significantly reduce CO$_2$ emissions compared to conventional slurry storage without any treatment. The reason for the lack of research studies in CO$_2$ emissions from slurry is that they are considered negligible compared to CO$_2$ emissions from animals exhalation (Blanes and Pedersen, 2005). However, CO$_2$ emission from slurry plays an important role on the pH buffer system and its emissions significantly affect NH$_3$ emissions during storage (Ni et al., 2009).

### 3.3.3. H$_2$S emissions

Hydrogen sulfide emissions during undisturbed storage after treatment are shown in Fig. 3. Average H$_2$S emissions during the first day after treatment from non-acidified slurry and slurry acidified to pH = 6.0 (A6.0) were significantly higher than slurry acidified to pH = 5.5 (A5.5) and 5.8 (A5.8) ($P < 0.05$). However, no statistically differences were found during seven days and fourteen days of storage among treatments (Fig. 3). Generally, low slurry pH is in favor of H$_2$S release. However, in our study most of the release of H$_2$S occurs during slurry agitation (acidification treatment process) due to the low volatility of H$_2$S and its bubble formation, ascension and breakage at the surface (Ni et al., 2009; Blanes-Vidal et al., 2012). Lower target pH's resulted in longer acidification treatment times (i.e. the time required to acidify the slurry until target pH increased). As slurry mixing was performed simultaneously, a higher off-gas of H$_2$S contained in the bulk slurry occurred when the slurry was acidified to lower pH's, which may have led to lower H$_2$S emissions during subsequent storage under undisturbed conditions. An alternative mechanism to explain the reduction of H$_2$S during the first day of storage after acidification in treatments A5.5 and A5.8 could be sulfide-reducing bacteria inhibited by accumulated sulfide (Eriksen et al., 2008; Ottosen et al., 2009). Previous research also documented large variations of H$_2$S emission from stored swine slurry when it is disturbed by slurry addition and mixing (Ni et al., 2010). Finally, the results showed that aeration did not have a significant effect on H$_2$S emissions during storage.

### 4. Conclusions

Average NH$_3$ emissions from swine slurry stored after the acidification-aeration treatment, were significantly lower than average NH$_3$ emissions from non-acidified slurry stored under the same conditions. The reductions in NH$_3$ emissions during storage (compared to non-acidified slurry) were of 50%, 62% and 77%, when slurry pH was decreased to 6.0, 5.8 and 5.5, respectively. The acidification treatment had no significant effect on the average CO$_2$ and H$_2$S emissions occurring during storage of slurry after acidification (compared to emissions from non-acidified slurry stored under the same conditions). However, high peaks of CO$_2$ and H$_2$S emissions occurred during the slurry acidification process. Aeration of the slurry for 30 min had no effect on average NH$_3$, CO$_2$ and H$_2$S emissions during the storage of slurries after the treatment. However, an increase of NH$_3$, CO$_2$ and H$_2$S was observed from both venting air and N$_2$ during the treatment. Therefore, the venting air (aeration) caused sudden release of the gases was related to an increase in the liquid turbulence caused by the air bubbles rather than by biological oxidation processes.

### Acknowledgments

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### Appendix A. Supplementary material

Supplementary material related to this article can be found at http://dx.doi.org/10.1016/j.jenvman.2012.11.019.
References


Authorship Agreement

This authorship agreement concerns the published research work conducted by PhD Student Xiaorong Dai, hereinafter named as first author, Victoria Blanes-Vidal, hereinafter named co-author, and advisor, Guoqiang Zhang, hereinafter named as co-author.

The research work includes the following article:

Air pollutants emissions from animal wastewater: Assessment of the dynamic processes caused by surface disturbances

In accordance with the criteria put forward at the Vancouver Convention for authorship (www.icmje.org), the undersigned first author hereby confirms that (s)he as first author is responsible for the initial drafting of the articles, a substantial contribution of the intellectual content, the acquisition and interpretation of experimental evidence, and the finalization of the versions to be published.

Similarly, the undersigned co-author confirms that (s) he as co-author has contributed quantitatively and qualitatively to the intellectual content, the interpretation of experimental evidence, and the final approval of the versions to be published.

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The research work includes the following article:

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