Post-hydrolysis Ammonia Stripping as a New Approach to Enhance Methane Potential of High Nitrogen Feedstock

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Abstract

Anaerobic digestion (AD) is a sustainable waste management technology that primarily generates two products: biogas and digestate. The technology relies on the microorganisms' activity, which depends on several operational factors, such as pH, temperature, solid contents, and ammonia levels.

Ammonia is an inorganic form of nitrogen resulting from the biodegradation of organic nitrogen. It is considered one of the major concerns for AD operations due to its inhibitory effects on some microorganisms, particularly methanogens. A common feedstock characterized by high nitrogen content is poultry manure (PM). PM is often avoided in anaerobic digesters due to the anticipated inhibition resulting from its high ammonia levels. However, since poultry manure is one of the most widely available organic wastes, researchers have extensively investigated ways to include PM as a primary feedstock for AD.

One possible way to treat high ammonia levels in digestate is ammonia stripping, the physio-chemical separation of ammonia from a solution by introducing a stripping (carrier) gas. There are a few approaches to performing ammonia stripping in AD applications; the most commonly discussed in the literature are pre-hydrolysis and side-stream ammonia stripping. Pre-hydrolysis ammonia stripping is performed on raw feedstock after increasing pH and temperature and is usually not restricted in selecting the gas carrier. On the other hand, side-stream ammonia stripping is when a portion of the digester's working volume is filtered, and the filtrate is sent to a unit where pH and temperature are increased. The carrier gas in these systems is often limited to anaerobic gases such as biogas or steam. The third and most novel approach is post-hydrolysis ammonia stripping, conducted at an intermediate stage between hydrolysis and methanogenesis in a two-stage AD process. This configuration would address the shortcomings of the other two systems. However, there is minimal information on the feasibility and potential of this approach in the literature.

This study aims to comprehensively investigate the post-hydrolysis ammonia stripping approach through the following four phases: Phase I) Proof of Concept; Phase II) Optimization; Phase III) Assessment of Alternative Carrier Gases; and Phase IV: Comparison of Different Ammonia Stripping Configurations.

Phase I provided the proof of concept under the batch mode and compared the performance of post-hydrolysis ammonia stripping with two-stage AD and co-digestion to improve poultry manure's methane potential as the primary substrate. It was observed that ammonia stripping

successfully improved methane potential by up to 150%, whereas improvements due to two-stage AD and co-digestion were limited to 41 and 9%, respectively.

Phase II provided more insight into optimizing the ammonia stripping process. Different stripping conditions were tested (pH 7.8 (unadjusted), 9 and 10, temperature 25 (unadjusted), 40 and 55 ^{o}C, and flow rate 300 L/L/hour). The results showed that higher pH and temperature lead to higher removal efficiency. However, it was concluded that optimal conditions ultimately depend on the initial and target ammonia levels. Moreover, Analysis of Variance showed that pH and temperature were significant factors affecting the ammonia removal efficiency. In addition, it was observed that higher stripping temperatures (55 ^{o}C) enhanced the digestibility of PM and increased its methane potential more than stripping at 40 ^{o}C. It was concluded that the optimum stripping conditions were pH 9.5, temperature 40 or 55 ^{o}C, and flowrate of 100 L/L/hour to collectively increase ammonia removal while reducing the associated costs and material handling.

In Phase III, renewable natural gas (RNG) was evaluated as a stripping medium in batch testing as a potential replacement for biogas and air. Ammonia stripping with RNG yielded promising results comparable to the application of air in terms of ammonia removal and enhancing biogas production from PM (60 and 69% ammonia removal for RNG and air, respectively). In addition, a metagenomic shotgun analysis showed that most biogas production was conducted through hydrogenotrophic methanogens instead of acetoclastic methanogens, which are more susceptible to high ammonia levels.

Phase IV assessed the semi-continuous flow two-stage operation of mesophilic AD reactors coupled with different ammonia stripping configurations. Post-hydrolysis ammonia stripping successfully achieved a stable operation of PM mono-digestion at ammonia levels of 1700 and 2400 mg NH_3-N/L in the cases of stripping with air and RNG, respectively. In addition, post-hydrolysis ammonia stripping in semi-continuous flow mode may have promoted acetoclastic methanogens growth since volatile fatty acid concentrations were reduced in the digesters. Phase IV also proved that the performance of post-hydrolysis ammonia stripping is superior over pre-hydrolysis and side-stream ammonia stripping. In the semi-continuous flow reactors, post-hydrolysis ammonia stripping with air achieved on average 831 L biogas/ kg VS at an organic loading rate (OLR) of 2.6 g VS/L/day, whereas side-stream ammonia stripping resulted in average of 700 L biogas/ kg VS at OLR of 1.8 g VS/L/day and higher ammonia stripping requirements. Having said that, the base scenario (no ammonia stripping) was inhibited, indicating that both ammonia stripping configurations were considered successful in alleviating inhibitory effects of ammonia from poultry manure.

Phase IV results also proved that air stripping repeatedly outperformed RNG as stripping mediums by having higher ammonia removal efficiencies resulting in higher methane production. However, stripping with RNG is believed to have more practical advantages than air due to avoiding the risk of oxygen infiltration into the reactor. Moreover, renewable natural gas has proven to be an efficient stripping medium that is available on-site.

The final stage of Phase IV tested pre-hydrolysis ammonia stripping using air in batch mode and compared it with post-hydrolysis ammonia stripping. Pre-hydrolysis ammonia stripping provided little to no improvement to the methane potential of PM in batch mode and therefore was excluded from the semi-continuous flow experiment.

The four phases of this study demonstrated the flexibility and the superiority of post-hydrolysis ammonia stripping over the other pre-hydrolysis and side-stream ammonia stripping. In addition, post-hydrolysis ammonia stripping was proven efficient and feasible for ammonia removal and enabling the mono- or co-digestion of poultry manure. The study also showed that using RNG instead of biogas can significantly reduce the operational costs of the treatment.

Dedication

To my beloved wife and life partner, Dina

&

To my dear parents, Kamil and Diba

&

To my dearly loved siblings, Nivine, Nisrine, Khalid, Shereen, Ahmad

&

To my mentor and cherished friend, Mohamed Abdallah

Acknowledgment

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My dear Mom and Dad, Diba and Kamil, being away from you for the past four years was the hardest thing I have ever done, but your encouragement and support have motivated me to keep going. Thank you for everything! My siblings, Nivine, Nisrine, Khalid, Shereen, and Ahmad, your words of support have always driven me to do my best. This work is dedicated to you and my sweet nephews and nieces, who have always been my beacon of light.

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List of Abbreviations

AD	Anaerobic Digestion
ALK	Total Alkalinity
ANOVA	Analysis of Variance
ARE	Ammonia Removal Efficiency
BMP	Biochemical Methane Potential
C/N	Carbon-to-Nitrogen Ratio
CFC	Chicken Farmers of Canada
COD	Chemical Oxygen Demand
CSTR	Continuously Stirred Tank Reactor
DM	Dairy Manure
DO	Dissolved Oxygen
FAN	Free Ammonia Nitrogen
GC	Gas Chromatography
GHG	Greenhouse Gas
HRT	Hydraulic Retention Time
I	Inoculum
ISR	Inoculum-to-Substrate Ratio
M	Cumulative Specific Methane Production
MS	Mixed Substrates (Coffeehouse and Cheese Factory Waste)
MS100	Mixed Substrates Make Up 100% of the Substrate (Diluted)
OLR	Organic Loading Rate
P	Specific Amount of Produced Methane
PHAS	Post-Hydrolysis Ammonia Stripping
PHAS-1	Post-Hydrolysis Ammonia Stripping Using Air
PHAS-2	Post-Hydrolysis Ammonia Stripping Using Renewable Natural Gas
PM	Poultry Manure
PM100	Poultry Manure Make Up 100% of the Substrate (Diluted)
PM25:MS75	Poultry Manure Make Up 25%, Mixed Substrates Make Up 75% of the Substrate (Diluted)
PM50:MS50	Poultry Manure Make Up 50%, Mixed Substrates Make Up 50% of the Substrate (Diluted)
PM75:MS25	Poultry Manure Make Up 75%, Mixed Substrates Make Up 25% of the Substrate (Diluted)
R_m	Maximum Specific Methane Production Rate
RNG	Renewable Natural Gas
rRMSE	Relative Root Mean Square Error
sCOD	Soluble Chemical Oxygen Demand
SEED	Standard Energy Efficiency Data
SSAS	Side-Stream Ammonia Stripping
SSAS-1	Side-Stream Ammonia Stripping at 20% Recycling Ratio Using Renewable Natural Gas

SSAS-2	Side-Stream Ammonia Stripping at 10% Recycling Ratio Using Renewable Natural Gas
SSAS-3	Side-Stream Ammonia Stripping at 20% Recycling Ratio Using Air
STP	Standard Temperature and Pressure
t	Incubation Time
T	Stripping Temperature
TAN	Total Ammonia Nitrogen
TKN	Total Kjeldahl Nitrogen
TS	Total Solids
VFA	Volatile Fatty Acid
VS	Volatile Solids
WS	Wheat Straw
α-diversity	Shannon Diversity

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CHAPTER 1: INTRODUCTION

This section presents an introduction to the topic and lays out the objectives of the study. It will also cover the scope of work, contribution, and thesis organization. To avoid repetition, references in this chapter will be included in Chapter 10: References.

1.1 Problem Statement

Poultry headcount is rising globally due to the increase in population and the demand for poultry products. In fact, compared with other livestock, poultry headcount has the fastest growth rate (Michalk et al., 2019; Tauseef et al., 2013). As a result, an increasing amount of poultry manure (PM) is produced. Farm owners typically rely on their livestock manure for agricultural purposes, mainly as fertilizers. However, in many aspects, direct land application and fertilization of soil using raw manure are not ideal. Firstly, using raw manure in land applications can increase the eutrophication potential during runoffs. It can also increase soil acidity and contribute to greenhouse gas (GHG) emissions. Secondly, raw manure is associated with discomforting odors and includes many pathogens and parasites that contaminate the soil and may damage the plants. Thirdly, using raw manure as fertilizer prevents its use as a natural resource for production of renewable energy in systems such as anaerobic digestion (AD) (Battini et al., 2014; Kamalinasab et al., 2016; Williams, 2013).

AD is a sustainable technology aimed at treating organic wastes through the activity of specific microorganisms, and its byproducts include methane-rich biogas and nutrient-rich digestate. It addresses several issues with conventional manure management systems and reduces the impact of farms on the environment (Fernandez-Lopez et al., 2015). In Canada, the disposal of agricultural wastes such as manure is regulated by the Nutrient Management Act, which controls the quantities and qualities of fertilizers and other organic or inorganic materials applied on agricultural lands. Most farmers in Canada are still within their rights to land apply the manure on the farm without prior treatment since it does not violate the Act. The Act, however, undergoes amendments periodically to keep agricultural practices in line with the environmental needs. Switching PM management from land applying to AD will help poultry farmers meet the Act standards for the long term as applying digestate was proven to be more beneficial than raw manure(Adghim et al., 2020; Alfa et al., 2014).

Although PM is widely available, it is typically avoided in AD applications due to its high nitrogen content compared with other types of manure, such as cow or swine. When the organic nitrogen in urea and proteins converts to ammonia, high concentrations of ammonia inhibits

the activities of the microorganisms responsible for the AD process (Nie et al., 2015a; Wang et al., 2014). According to the Canadian Biogas Association, there are currently 45 agricultural AD reactors in Canada, none of which utilizes PM at any percentage due to the anticipated complications and possible inhibition of microorganisms that PM may induce (Canadian Biogas Association, 2022). Therefore, recent research has been focused on the pretreatment of PM by targeting its ammonia levels (Abouelenien et al., 2016; Fuchs et al., 2018). Having said that, once treated, PM can have a higher methane potential (350-500 L CH_4/kg VS) than cow or swine manure (250-350 L CH_4/kg VS) (Nie et al., 2015b).

Ammonia levels affect AD processes differently, depending on the stage and the tolerance of the microorganisms to high ammonia levels. During hydrolysis and acidogenesis, microorganisms are more resilient to high ammonia levels, and typically, these two processes are not disturbed (Wijesinghe et al., 2018). Moreover, these are the stages where most organic nitrogen is biologically converted to ammonia. On the other hand, methanogens that are responsible for methane production are susceptible to high total ammonia nitrogen (TAN) levels (Bousek et al., 2016).

Ammonia stripping is a physio-chemical treatment and is one of the most economical and practical methods to alleviate the inhibitory effects of ammonia in AD applications (Cho et al., 2014; Zhang, 2016). Its advantages lie in the simplicity of the design, application, and concept. Mainly, three factors control the efficiency of ammonia stripping: pH value, temperature, and carrier gas characteristics, i.e., type and flow rate (Rodriguez-Verde et al., 2018; Yin et al., 2019). Furthermore, ammonia stripping can be implemented at different stages of an AD plant, where its implementation may have certain advantages and disadvantages. Therefore, this research focuses on finding the optimum configuration and conditions of ammonia stripping for AD applications.

Currently, two approaches for ammonia stripping in AD applications are widely discussed in the literature: 1) Stripping of raw feedstock (pre-hydrolysis) and 2) side-stream stripping of digestate. Pre-hydrolysis ammonia stripping targets raw feedstock and occurs after the pH and/or temperature are increased to certain levels, and then a gas carrier is introduced. In this approach, any gas can be used as a carrier, as maintaining anaerobic conditions is not critical at this point. On the other hand, side-stream stripping treats a portion of the digestate in a side column after increasing its pH and/or temperature and returns the treated digestate to the digester to lower the ammonia levels. In this approach, solid/liquid separation prior to treatment is crucial to maintaining the activity of microorganisms. Moreover, air or oxygen is often avoided in this method. Studies mostly suggest using biogas as a carrier gas as it is readily

available in the plant and will keep the process completely anaerobic (Fernandez-Gonzalez et al., 2019; Serna-Maza et al., 2014).

Some shortcomings can be noticed in these two approaches. For example, pre-hydrolysis ammonia stripping only removes a portion of the present ammonia and part of the ammonia formed during the decomposition of proteins throughout the stripping, if any. However, more ammonia is expected to form during digestion when proteins are biodegraded, causing instability of microorganisms' activities. On the other hand, the restrictions of the carrier gas selection in side-stream stripping to anaerobic gases limit the efficiency of ammonia removal and increase the stripping requirements (Bousek et al., 2016). The applications of pre-hydrolysis and side-stream ammonia stripping may be advantageous in biogas plants operating one-stage AD (no separate hydrolyzer) where the feedstock is characterized by total Kjeldahl levels that are close to the inhibitory threshold, which is 2500-3000 mg NH_3-N/L (Chen et al., 2008; M. Walker et al., 2011). However, the shortcomings above indicate that the digesters may still operate under sub-optimal conditions when incorporating these technologies.

Two-stage AD reactors are becoming more common due to their advantages over one-stage AD (Park et al., 2010). Separating the hydrolysis from the methanogenesis can increase methane production and give biogas plant operators more control in avoiding sudden failures. The rise in two-stage AD reactor numbers has directed recent research on ammonia stripping towards another novel approach that could address the shortcomings in the existing approaches by taking advantage of the increase in ammonia levels in the hydrolyzers. Performing ammonia stripping after hydrolysis, hence the name post-hydrolysis ammonia stripping, can limit the rise in ammonia during methanogenesis as the concentration of organic nitrogen is low after hydrolysis. Moreover, as an intermediate step between the hydrolyzer and the digester, no liquid/solid separation is required for this approach as the methanogenic archaea presence is already minimal at this stage.

As post-hydrolysis ammonia stripping is a newer approach that was not discussed thoroughly in the literature, this study provides a comprehensive understanding of the process's efficiency, optimization, and stability. It also provides a comparison between the three ammonia stripping approaches to evaluate their advantages and disadvantages as well as be able to provide recommendations.

1.2 Research Statement and Objectives

The main goal of this research is to investigate post-hydrolysis ammonia stripping as a novel approach to perform ammonia stripping that overcomes the shortcomings of conventional

approaches. This study's central objective is to alleviate ammonia's inhibitory effects on the AD of PM using post-hydrolysis ammonia stripping and to provide the poultry farming industry with a viable solution to treat their PM. The following points summarize the specific objectives of this study:

- Provide proof of concept of the new approach and ensure its applicability to the material used in this study, poultry manure.
- Optimize the ammonia stripping treatment by testing at different operating conditions.
- Investigate the possibility of mono-digestion or co-digestion of PM at high proportions in the feedstock recipe after post-hydrolysis ammonia stripping treatment.
- Test renewable natural gas as a stripping medium.
- Investigate the impact of post-hydrolysis ammonia stripping on microbial diversity in batch mode.
- Examine the stability of the proposed treatment under semi-continuous flow operations.
- Compare the performance of the post-hydrolysis ammonia stripping with pre-hydrolysis and side-stream ammonia stripping.

1.3 Contribution

This study primarily aims to increase poultry manure's methane potential through an emerging approach to address ammonia inhibition when digesting poultry manure. It covers some noticeable gaps in the literature and provides a comprehensive understanding of post-hydrolysis ammonia stripping, which would incentivize poultry farms to use poultry manure sustainably and profitably. The knowledge gaps are presented in more detail in section 2.7 after discussing the relevant literature.

Despite the global efforts to combat climate change, many challenges must be overcome to significantly reduce the immediate impacts of anthropological activities on the environment. However, countries like Canada have already set ambitious targets to reduce GHG emissions by 40-45% by 2030 (compared to 2005) and reach net-zero emissions by 2050 (Environment and Climate Change Canada, 2021). This work contributes to these targets by advancing the technical knowledge in the field of bioenergy production and helps biogas plants to expand their services to poultry farms through incorporating an economical and efficient option for pre-treatment that can ultimately increase their biogas production.

1.4 Scope of Work

The scope of this research focuses on improving the methane potential of poultry manure using post-hydrolysis ammonia stripping efficiently and optimally. Figure 1-1 introduces the range of work and phases covered in this study. The project starts by collecting and reviewing literature related to the topic and understanding the critical knowledge gaps that must be addressed. The conducted experiments in this research aim to evaluate the ammonia removal efficiency and the enhancement of methane production under different conditions. The conditions of the first experiment were set to be the most and least conservative to understand the method's limitations. The following stages focus on optimizing the ammonia stripping conditions to reduce the cost of the treatment and testing alternatives for carrier gas. After that, the experiment is rerun on a semi-continuous flow mode and at a larger scale to ensure that the proposed treatment can result in a stable digester operation. Moreover, the proposed approach is compared with the approaches discussed in the literature, i.e., Pre-hydrolysis and side-stream ammonia stripping.

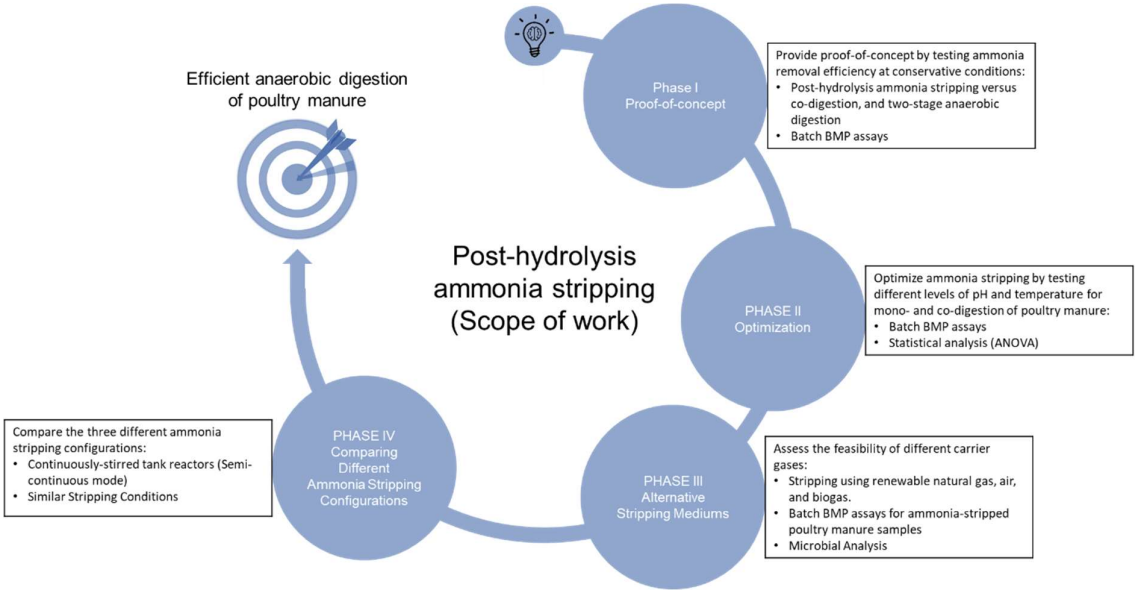


Figure 1-1: Scope of work and description of the phases of the study.

1.5 Thesis Organization

This dissertation is presented in paper format. First, a general introduction to the topic and the problem statement are provided, followed by a general literature review and a methodology chapter. Next, the results of the experimental testing, statistical analysis, and modelling conducted are provided in the technical papers. Then, the conclusions of all technical papers are integrated and discussed, followed by the final dissertation conclusion and references. The organization of the dissertation is as follows:

- Chapter 1: Introduction

This chapter introduces the topic's importance and impacts on the industry and its contribution to the industry and field advancements.

- Chapter 2: Literature Review

This chapter covers the fundamentals of ammonia inhibition, ammonia stripping, and its applications in anaerobic digestion. In addition, this chapter will summarize previous work and research results, and the knowledge gap will be highlighted.

- Chapter 3: Research Methodology

This chapter discusses the experimental plan as well as the general materials and methods used in the study.

- Chapter 4: Enhancing mono- and co-digestion of poultry manure by a novel post-hydrolysis ammonia stripping approach in a two-stage anaerobic digestion process.

This chapter presents technical paper #1, based on Phase I of the scope of work, and provides the proof-of-concept of the proposed approach.

- Chapter 5: Post-hydrolysis ammonia stripping as a new approach to enhancing the two-stage anaerobic digestion of poultry manure: optimization and statistical modelling.

This chapter presents technical paper #2, based on Phase II of the scope of work, and discusses the optimization and statistical analysis of the operating parameters of the ammonia stripping.

- Chapter 6: The applications of renewable natural gas in ammonia stripping and its impacts on microbial diversity to enhance biogas production.

This chapter presents technical paper #3, based on Phase III of the scope of work, and discusses the effect of different carrier gases on ammonia stripping and biogas production.

- Chapter 7: Comparative assessment of different ammonia stripping configurations to enhance biogas production from poultry manure.

This chapter presents technical paper #4, based on Phases IV of the scope of work. It discusses the stability of post-hydrolysis ammonia stripping under long-term operations and compares its performance to pre-hydrolysis and side-stream ammonia stripping.

- Chapter 8: Integration and general discussion of results

This chapter integrates the findings and conclusions obtained from all phases and summarizes the results under higher-rank classifications.

- Chapter 9: Conclusions and Recommendations

This chapter summarizes the conclusions that were derived/found from this research. Recommendations for future work are then presented, mentioning topics that have the potential to be developed and improved.

- Chapter 10: References

This chapter presents the full citation of the references used in the script. It was decided to gather all references in one chapter rather than after each chapter to avoid repetition.

CHAPTER 2: LITERATURE REVIEW

This section presents insights into previous studies that discussed the basics of AD, the inhibitory effects of ammonia in anaerobic digestion, ammonia stripping, and other methods for alleviating ammonia inhibition. To avoid repetition, references in this chapter will be included in Chapter 10: References.

2.1 Anaerobic Digestion

2.1.1 Overview

Anaerobic digestion (AD) is a sustainable Waste-to-Energy approach that promotes waste stabilization through the activity of certain microorganisms (Labatut et al., 2011). AD converts organic wastes into valuable and profitable products, i.e., biogas and digestate. Biogas generated from digesters can be further purified and injected into natural gas grids or used in a combined heat and power engine with minimal purification requirements. On the other hand, the nutrient-rich digestate is a stabilized fertilizer that can be used on agricultural land instead of fresh manure that may contain many parasites and pathogens (Passos et al., 2016). Figure 2-1 shows an overview of the AD inputs and outputs.

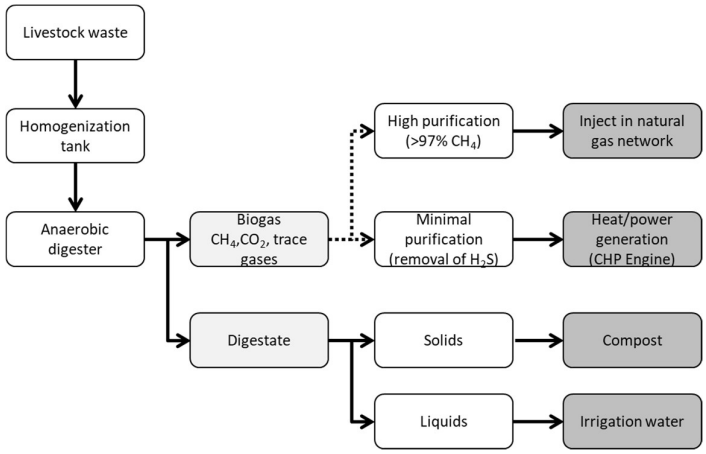


Figure 2-1: End uses of anaerobic digestion products (modified from (Cantrell et al., 2008).

Anaerobic digestion comprises four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Figure 2-2). Hydrolysis usually acts as the rate-limiting process because hydrolysis is responsible for breaking down complex organic materials, i.e., carbohydrates, proteins, and fats, into sugars, fatty acids, and amino acids. Acidogenesis usually happens faster, and it converts the outputs of hydrolysis to carbonic acids, short-chained fatty acids, and alcohols. Acetogenesis converts the broken-down acids and alcohols into acetic acids, hydrogen, carbon dioxide, and ammonia. Finally, in the last stage of AD, methanogenic

bacteria convert acetic acid as well as hydrogen and carbon dioxide into methane and carbon dioxide (Agyeman and Tao, 2014; Yin et al., 2019).

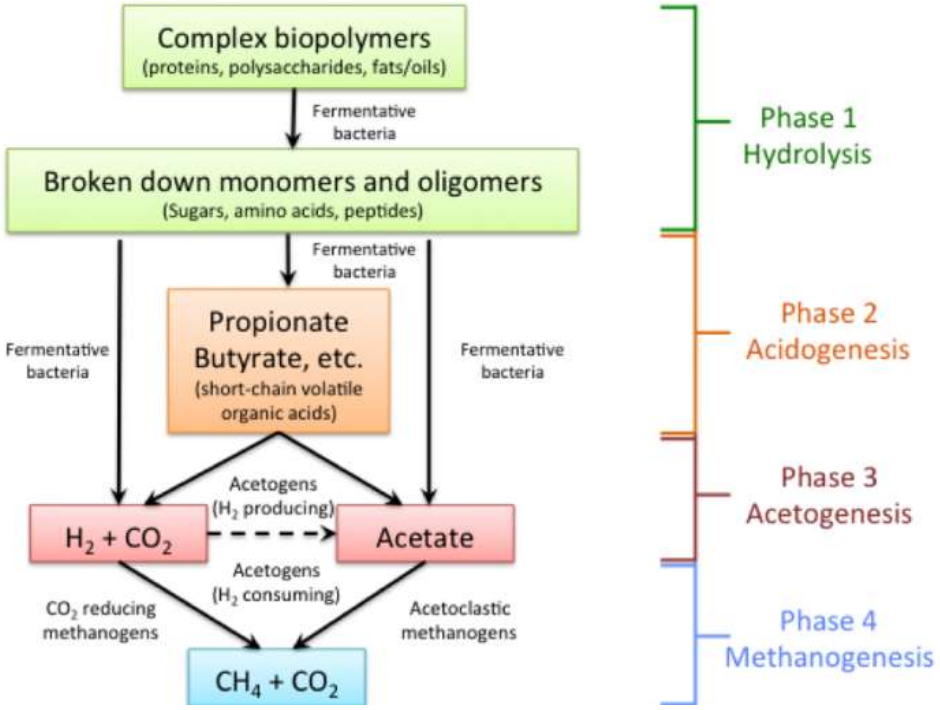


Figure 2-2: Stages of the anaerobic digestion process (extracted from BEEMS Module B7)

2.1.2 Control Parameters

The AD process mainly depends on the activity of different microorganisms; this is why optimal conditions must be attained for these microorganisms to convert organic matter to biogas. Initially, hydrolysis and acidogenesis processes prefer an acidic environment to promote hydrolytic enzymes and acidogenic bacteria cultures to grow and decompose the complex organic matter. In doing so, these two processes result in the formation of acids that lower the mixture's pH (Yin et al., 2019).

Some factors that affect the AD processes in general, and hydrolysis/acidogenesis in particular, are particle size and the solid contents of the feedstock. Hydrolysis can be improved by reducing the particle size and limiting the solid content to enhance contact between the microorganisms and the organic compounds (Agyeman and Tao, 2014).

As for methanogenic archaea, they are more susceptible to many factors that could lead to the failure of the methanogenesis stage. One of the most critical parameters that control the activities of methanogens is the pH; unlike the fermentative bacteria responsible for hydrolysis and acidogenesis, methanogens are most active when the pH is between 7 and 8.5. In addition, methanogenic archaea cannot tolerate high levels of ammonia. These are the challenges biogas

plants regularly face during their operation, and research is still ongoing to overcome these obstacles (Tsigkou et al., 2020; Zarkadas et al., 2015).

The temperature at which the reactors are operated is also among the most critical operating conditions. Most reactors are one of two types: mesophilic (operates at 35-40 ºC) or thermophilic (operates at 55-60 ºC). The main difference between these two reactor types is the species of microorganisms in charge of generating biogas. Furthermore, the types of challenges faced in each type of reactor are different as the chemical properties of the digestate are impacted by the temperature, such as the impact of temperature on the forms of ammonia present in the solution (Huang et al., 2016).

2.1.3 Inhibitory Effects of Ammonia

Ammonia, when at high levels, can inhibit the microorganisms' activities in the digester. Ammonia can be found in ionic form (NH_4^+) and free ammonia nitrogen (FAN, NH_3); FAN is also known as the volatile form of ammonia. The percentage of each form in a solution depends on the pH and the temperature, as shown in Eq 1; ammonia volatility is promoted by increasing pH and temperature (Rajagopal et al., 2013).

$$FAN\ (mg/L) = TAN \times \left(1 + \frac{10^{-pH}}{10^{-\left(0.09018\ \frac{2729.29}{T}\right)}}\right)^{-1}$$

Eq 1

where FAN is NH_3 in mg/L, TAN is the total ammonia nitrogen in mg/L, and T is the temperature in Kelvin. The equation can be represented in Figure 2-3.

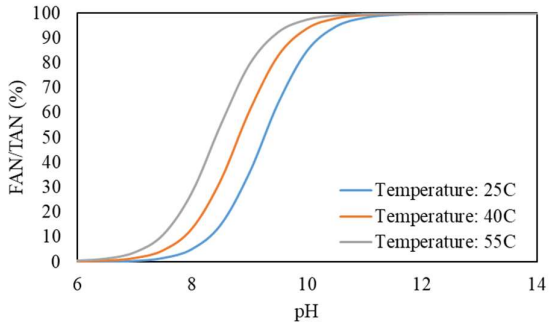


Figure 2-3:Percentage of free ammonia nitrogen under different pH and temperature conditions.

Most studies refer to FAN as the toxic form of ammonia for its ability to penetrate the cell wall of methanogenic archaea and cause proton imbalance by gaining a hydrogen atom (Figure 2-4). This process affects methanogenic activities and causes biogas production to drop or, in some cases, completely stop (Nair et al., 2014).

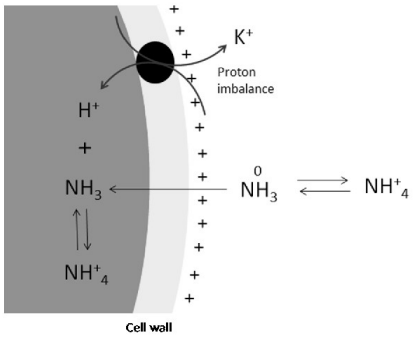


Figure 2-4: Mechanism of proton imbalance by free ammonia nitrogen (extracted from Reyes et al. (2015)).

This negative impact of FAN makes thermophilic digesters more susceptible to ammonia partial or complete inhibition as it is operated at a higher temperature than mesophilic digesters. Having said that, mesophilic reactors also face ammonia inhibition challenges, especially when handling nitrogen-rich substrates such as PM (Hadj et al., 2009).

It is difficult to determine a singular ammonia inhibitory level as a vast range is reported in the literature. This is because methanogenic archaea have numerous species, each with a certain tolerance to ammonia levels (Tian et al., 2018). In addition, microorganisms can adapt to higher levels of ammonia, but that does not mean that the inhibitory impacts of ammonia are entirely alleviated (Christou et al., 2021). Having said that, several studies agree that ammonia levels above 2500-3000 mg NH_3-N/L can be considered toxic (Chen et al., 2008; Usack & Angenent, 2015).

In addition to the ammonia that is naturally present in the urine and manure, more ammonia is formed during hydrolysis and acidogenesis of organic nitrogen in the form of protein and amino acids (Figure 2-5). This indicates that the hydrolytic enzymes and the acidogenic microorganisms are more resilient to high ammonia levels when compared with the methanogenic microorganisms (Christou et al., 2021; Park and Kim, 2016).

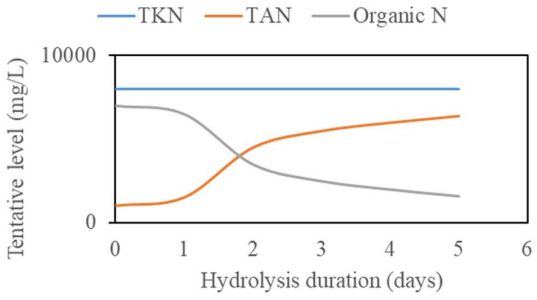


Figure 2-5: TKN, TAN, and organic nitrogen profile throughout hydrolysis.

2.2 Poultry Manure

2.2.1 Quantities

The increase in the human population and demand for meat, eggs, and other animal products has increased livestock headcount in general and poultry in specific (Figure 2-6). Poultry and chicken headcount in Canada reached 348,360,000 in 2018, which made them the most widely available livestock in Canada, followed by swine, cattle, sheep, and goats (FAOSTAT, 2018). The headcount of poultry animals has been steady in the last five years, and the resulting annual production of poultry manure is around 15,258,168 tons/year (FAOSTAT, 2021; Michalk et al., 2019; Tauseef et al., 2013). This is almost half of the 31,498,697 tons/year of manure produced by 1,391,900 dairy cows and heifers, according to Agriculture Canada (2022).

The amount of manure excreted from poultry varies depending on the type of the bird; layer chickens (for egg production) excrete around 120 kilograms of manure per 1000 chickens per day, whereas meat chickens excrete about 80 kilograms of manure per 1000 chickens per day (Williams, 2013). Despite the low excretion rate of poultry manure compared with cow manure, which is around 62 kilograms per cow per day, the high headcount of poultry makes poultry manure an abundant resource for energy recovery (Nam et al., 2016).

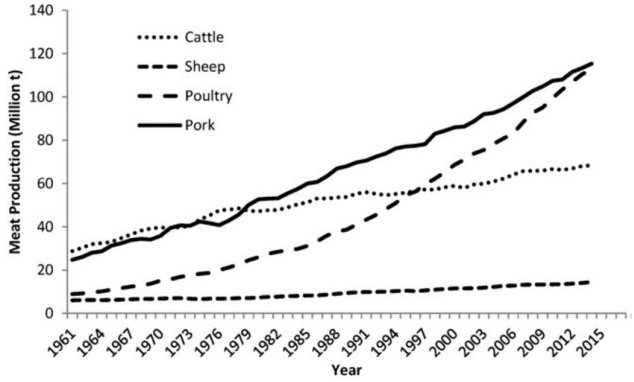


Figure 2-6: World meat production of cattle (including buffalo), sheep (including goats), poultry, and pork from 1961 to 2014 (Million t; Michalk et al.,2019).

2.2.2 Characteristics

Characteristics of the biogas plant feedstock play a significant role in controlling the stability of the digester. Therefore, it is essential to understand the feedstock's characteristics to determine the suitability and/or treatment of the feedstock before designing the digester. Manure characteristics, in general, correlate to the animal's diet and digestive system. Table 2-1 shows typical ranges of PM characteristics extracted from (Babaee et al., 2013; Dahunsi et al., 2019; Fuchs et al., 2018; Khoufi et al., 2015; Nielfa and Cano, 2015; Rodriguez-Verde et

al., 2018), however, typical values are hard to determine due to the wide range of data reported in the literature.

Table 2-1: Poultry manure characteristics from literature.

Characteristic	Range
pH	6.92-9.5
Total solids (TS, %)	25.6-92.5
Volatile solids (VS, %)	16.6-49
Chemical oxygen demand (COD, mg/L)	12550-274050
Soluble COD (sCOD, mg/L)	41400-53900
Total Kjeldahl Nitrogen (TKN, mg N/L)	2000-12000
Total Ammonia Nitrogen (TAN, mg NH_3-N/L)	800-5670
Total Alkalinity (ALK, mg $CaCO_3$/L)	1550-5320
Methane potential (untreated, L CH_4/kg VS)	159-330

As seen in Table 2-1, PM characteristics differ significantly among the studies. One of the main factors that affect the quality and the characteristics of PM is the presence of bedding materials and their type. Bedding materials are usually made of inexpensive materials such as wood shavings, hay, sawdust, and/or corn silage. These materials significantly increase poultry manure's carbon-to-nitrogen ratio (C/N). On the other hand, inorganic bedding materials, such as sand, increase the sample's ash content (Hashemi et al., 2011).

PM is noticeably drier than other types of manure typically used in biogas plants, namely cow and swine (Campos et al., 2015; Nam et al., 2016). This also affects PM's suitability in biogas plants as most plants cannot exceed 15% in TS content unless they are a high-solid anaerobic digester. The high solid contents in PM also result in practical challenges such as pumping, which contributes to PM being avoided in biogas plants.

2.3 Conventional Treatment of Poultry Manure in Canada

Poultry farms are responsible for handling the poultry manure produced on their premises. Ontario regulations set by Environment and Climate Change Canada and the Chicken Farmers of Ontario (CFC) do not entirely prohibit conventional handling methods such as spreading and land application of manure. In fact, the regulations prevent the land application and spreading of manure only over the controlled access zones. Nevertheless, the association recommends composting as a better alternative that could destroy pathogens and produce a high-quality fertilizer (Chicken Farmers of Canada, 2014). Some farmers may choose to treat poultry manure through composting to avoid contaminating the soil and reduce the amount

needed to be stored during the low-demand seasons. While controlled composting is a sustainable approach to handling manure, the only valuable output of this process is the high nutrient fertilizer, and the energy content of the manure becomes unexploitable (Fisher et al., 2019; Y. Li et al., 2018).

2.4 Tackling Ammonia Inhibition Strategies

To find efficient and economical solutions, research about overcoming ammonia inhibition challenges in biogas plants is still ongoing. There are two main streams in the literature for tackling ammonia inhibition: biological and physio-chemical approaches.

2.4.1 Biological Treatments

Biological treatments focus on understanding the resiliency of methanogens in combating ammonia inhibition. They require minimal to no modifications to the chemical properties of the digestate. However, they often need longer start-up times. In addition, as microbial characterization throughout the process is gaining more attention recently, biological treatments are subject to change significantly depending on the new findings of microbial analysis. The following sections discuss some of the most covered biological treatments in the literature.

2.4.1.1 Bioaugmentation

Fotidis et al. (2014) assessed the effects of reactor bio-augmentation on methane production. Bio-augmentation is the addition of microorganisms of a certain kind (corresponding to the need for addition) to increase the microbial population, thus increasing digestion activities. In this study, *Methanoculleus bourgensis* was selected to augment the digester as it is more tolerant to high ammonia levels. The experiment was performed on a mesophilic continuous-stir tank reactor (CSTR) digesting cattle manure and inoculated with a digested mixture of pig and cattle manure. One of the main concerns discussed was that the feasibility of augmenting was directly related to the operating conditions; added microorganisms should not be washed off from the digester without having enough retention time. This study showed that augmenting the digester alleviated the high ammonia inhibition (5000 mg NH_4/L) and increased methane production nearly five days after the addition. The potential acclimation of the microorganisms justified the increase in the methane production of the Base (no treatment) sample to higher levels of ammonia. The average increase in methane production in the augmented reactor was 31.3%.

2.4.1.2 Acclimation

Acclimation of the methanogenic microorganisms was studied by Tian et al. (2018) as an economical and feasible solution to overcome the high ammonia levels in the digester. The approach was to perform stepwise acclimation of the microorganisms in a CSTR under four phases that summed up to 133 days; ammonia levels were gradually increased from 3300 mg NH_4/ L in phase 1 to 10000 mg NH_4/ L in phase 4. The main result of this study was that the methane production in the acclimated tanks during phases 3 and 4 (ammonia levels 8000 and 10000 mg NH_4/L, respectively) was stable at more than 95% of the methane production in phase 1. However, the gas production was reduced at the earliest stages of phases 3 and 4, but it recovered before reaching half of the hydraulic retention time. The drop in methane production represented the duration needed for the acclimation of methanogenic microorganisms to adapt to the high ammonia levels. The acclimation was achieved because FAN levels were stable (800-850 mg NH_3/L) throughout the experiment by reducing the pH of the digester with the increase of ammonia levels, and the presence of trace elements such as Cobalt and Molybdenum has promoted microbial growth. Another major finding, which contradicts the findings of Fotidis et al. (2014), was that *Methanosarcina spp,* a form of acetoclastic methanogens, was more abundant (90% of the total microbial count) than hydrogenotrophic methanogens at high levels of ammonia.

The drawback of acclimation processes is that they require a significant amount of time to occur and therefore need different places on-site before operating the digester. Another disadvantage is that the biogas production due to such treatment is stable. However, it is not optimal without using acids to control the FAN/TAN level. Therefore, testing and economic feasibility must be considered before implementing such treatment.

2.4.2 Physio-chemical Treatments

Physio-chemical treatments alleviate ammonia's inhibitory effects by changing the feedstock's physical and/or chemical characteristics or the digestate. These technologies target the levels of ammonia forms (NH_4^+ and NH_3) and try to reduce them or promote NH_4^+ over NH_3. In contrast, biological treatment focuses on enhancing the resilience of the microorganisms without necessarily changing the ammonia levels. While physio-chemical methods often require more resources than biological treatments, they usually do not require long startup times and can be more efficient. The following are some of the physio-chemical technologies discussed in the literature.

2.4.2.1 pH Control

At high pH values, the FAN concentration increases and inhibits microbial activity, consequently increasing the VFAs. The pH is further lowered when VFAs are highly concentrated to reduce the FAN concentration. The interaction will probably lead to a steady-state situation with little methane generation. Akindele and Sartaj (2018a) showed that maintaining the pH at 7.0 instead of 7.5 would increase methane generation 4-times.

2.4.2.2 Temperature Control

Temperature plays a critical role in AD processes. While higher temperatures encourage the metabolic activities of microorganisms, they also promote higher concentrations of FAN, thereby increasing the toxicity in the digester. For example, Gallert and Winter (1997) studied methane production at mesophilic and thermophilic conditions with high ammonia levels, and they found that thermophilic microorganisms showed more resistance to higher ammonia levels. However, the results of this study were not in line with other studies, such as (Hejnfelt and Angelidaki, 2009), who found that mesophilic anaerobic digestion operates significantly smoother than thermophilic digesters.

2.4.2.3 Dilution

Dilution is an option to reduce ammonia concentration. However, there are many demerits of this mitigation method. Dilution tends to decrease biogas production by turning the digester from a high solid to medium or low solid content. Dilution also dramatically increases the digester size requirements, digestate volume, and digestate treatment requirements. Nielsen and Angelidaki (2008) studied the effect of dilution on anaerobic digester performance under high ammonia levels. Three different dilution agents were tested (1) water, (2) fresh manure, and (3) digested manure. In addition to the burden of additional digester size, the dilution process, at best, caused a drop of 40% in the methane potential. Thus, the authors recommended that dilution may be unfeasible compared to other efficient technologies to mitigate high ammonia concentrations.

2.4.2.4 Co-digestion

Adjusting the carbon-to-nitrogen ratio may be the most cost-effective method for mitigating high ammonia levels. Low C:N ratios reduce the efficiency of utilizing the carbon in the digester. Therefore, the adjustment is made by adding carbon-rich waste such as food waste. Adjusting C:N can regulate methane production while optimizing it will increase methane production. The best time to optimize the C:N is when the microflora is active; if the process

was subjected to inhibitory levels, the recovery would be difficult. The studies found that significantly increasing the C:N ratio prevents the microorganisms' activity because deficient ammonia levels discourage microflora growth, thereby reducing biogas production.

De Vries et al. (2012) studied the effect of co-digesting 5% of pork by-products with pig manure on the methane potential compared to the mono-digestion of pig manure. The co-digestion aimed to increase the C:N ratio to overcome the high nitrogen content. The methane potential was increased by 40% when the C:N ratio increased due to co-digestion, which proved that the suitable C:N ratio can optimize the methane potential.

In addition, co-digestion has been proven to result in positive synergetic enhancement of biogas production from a mixed substrate (Ara et al., 2015). The optimum co-digestion ratio is determined by conducting the biochemical methane potential (BMP) tests and measuring the synergistic effect of the mixed feedstock. The synergistic effect is the difference between the calculated methane yield (calculated based on each substrate's methane potential), and the co-digested feedstock's observed methane yield.

Most studies suggest that the co-digestion of PM with other substrates was inhibitory when PM formed more than 30% of the feedstock composition (VS mass basis). However, when co-digested with hog manure and dairy manure (DM) with wheat straw (WS) (Wang et al., 2012), the optimum VS mass ratios of hog:PM and DM:PM:WS were 40:60 and 39:39:22, respectively.

Anaerobic digesters often operate on complex mixtures of organic wastes. Valenti et al. (2018) studied the co-digestion of PM with a mixture of citrus pulp, olive pomace, corn silage, cattle manure, and whey at different proportions. The study suggests that the PM proportion shall not exceed 13% of the total dry weight during all trials due to its low C/N ratio of 5.5 and the possibility of inhibition due to ammonia. Rodriguez-Verde et al. (2018) applied a linear model to simulate the co-digestion of different PM and pig manure proportions until stable methane production and operating conditions were achieved. The optimum PM:pig manure ratio was 24:76, indicating once more that including PM at high concentrations may inhibit the AD process. Furthermore, the study suggested that higher PM quantities cannot be added without further treatment to remove ammonia.

2.4.2.5 Chemical Precipitation of Ammonia

Zhuang et al. (2018) assessed the performance of anaerobic digesters operated under different ammonia levels, i.e., 0, 500, and 5000 mg NH_4/L, with the addition of magnetite. The addition

of magnetite aimed to increase the microbial growth of hydrogenotrophic methanogens, which are less sensitive to higher ammonia concentrations than acetoclastic methanogens. The difference between the methanogens is that hydrogenotrophic organisms reduce the hydrogen and CO_2 produced during acidogenesis and acetogenesis to CH_4, while acetoclastic organisms generate CH_4 directly from digesting acetic acid. The results elaborated that the absence of ammonia was harmful to digestion due to the lack of buffer that agitates microbial growth. Moreover, the results showed that ammonia levels of 5000 mg NH_4/L caused the gas production to lag for approximately six days, regardless of the addition of magnetite. The addition of magnetite did not significantly impact the biogas production from the bottles with 0 or 500 mg NH_4/L. However, magnetite expedited the biogas production to yield at 32 days instead of 39 (without magnetite) when the ammonia level was 5000 mg NH_4/L. The methane yield from the bottles with magnetite was slightly less than the magnetite-free bottles; this may be attributed to the biogas production generated primarily through the reduction of hydrogen and CO_2 into biogas instead of the consumption of acetic acid.

The removal of ammonia through struvite precipitation was studied by (Wang et al., 2016). The precipitation of ammonia is possible by adding compounds of phosphorus and magnesium, which combine with the ammonia to form struvite. The study was performed on continuous flow lab-scale reactors containing NH_4 levels around 1300 $\pm$100 mg NH_4/L. The amounts of precipitating compounds added could control the ammonia removal; therefore, it could be optimized. In this study, the highest methane potential was 41.7% higher than the untreated sample, and this production corresponded to 57.6% nitrogen recovery. Despite being able to increase biogas production significantly, this method has a few drawbacks that may reduce its feasibility on a large scale. The daily produced precipitate can be around 1.5% of the total weight of the inlet feed, and this precipitate must be treated before reuse or disposal.

2.5 Ammonia Stripping

Ammonia stripping is a physio-chemical treatment that separates ammonia from the digestate by promoting ammonia volatility and introducing a carrier gas. Ammonia stripping is a well-known technology with applications in water and wastewater treatment; it is also known for its simplicity and relatively low cost (Fuchs et al., 2018). In anaerobic digestion applications, ammonia stripping has been proven to alleviate the inhibitory effects of ammonia and enhance methane production. Two approaches to conducting ammonia stripping are widely discussed in the literature: pre-hydrolysis ammonia stripping and side-stream ammonia stripping. As each approach has been proven to remove ammonia and enhance methane potential to a certain level,

certain shortcomings can be addressed to improve the ammonia stripping efficiency further. However, the mechanism of ammonia removal through stripping remains unchanged throughout these approaches, i.e., increasing ammonia removal efficiency requires increasing pH, temperature, and the carrier gas flow rate.

Bousek et al. (2016) tested the effect of carrier gas composition on ammonia removal efficiency. The test included air with different concentrations of CO_2 (from 0% to 40% CO_2), flue gas (82% nitrogen and 18% CO_2), and biogas (60% CH_4 and 40% CO_2). Air with 0% CO_2 was proven to be the most efficient carrier gas in removing ammonia; it achieved around 81% removal in four hours. On the other hand, increasing the CO_2 content in the air to 5% reduced its removal efficiency significantly to approximately 58%. The drop in ammonia removal efficiency due to increasing the CO_2 content in the carrier gas was not linear. Comparing the ammonia removal efficiency using flue gas with air that contains 20% CO_2 showed that air with 20% CO_2 had higher removal efficiency than flue gas (57 versus 45%). CO_2 drops the removal efficiency due to its high solubility in water and its ability to drastically reduce the pH of the solution, which reduces the volatility of ammonia. Biogas showed the lowest ammonia removal efficiency (16% on a model solution, 47% on sludge). The findings of this study agree with most of the literature; many studies reported biogas removal efficiency to be around 15-50% (Abouelenien et al., 2010; Bousek et al., 2016; De la Rubia et al., 2010; Serna-Maza et al., 2014). High ammonia removal using biogas (around 40-80%) was only reported at pH higher than 10, temperature above 65 ^{o}C and long stripping duration of 1-3 days (Bousek et al., 2016; Zhang et al., 2017).

2.5.1 Pre-hydrolysis

Pre-hydrolysis ammonia stripping is the stripping of raw feedstock before it enters the digester or the hydrolyzer, if applicable. This approach is common in literature because many biogas plants operate in one stage instead of two stages, i.e., all four stages of AD occur in one reactor. As this approach is concerned with the feedstock before it is added to the digester, no microorganisms are needed in the feedstock for methane production, and there are no restrictions on carrier gas selection in terms of maintaining the anaerobic conditions or introducing severe stripping conditions if needed (Bonmatı and Flotats, 2003). This is an immense advantage of this approach, as many studies suggest that air can strip more ammonia than other tested gases such as biogas, flue gas, and carbon dioxide (Bousek et al., 2016).

The literature covers a wide range of tested parameters, i.e., pH, temperature, gas flow rate, and the duration of treatment. Table 2-2 shows a summary of the reviewed studies. Pre-

hydrolysis can achieve up to 92% ammonia removal efficiency at a temperature of 65 ºC and unadjusted pH (around 7.8) (Bousek et al., 2016). Increasing the temperature and the pH can increase ammonia removal efficiency up to 98% (at a temperature of 80 ºC, pH 11.5), as observed in (Bonmatı and Flotats, 2003). Other studies also reported high removal efficiencies at milder operating conditions; (Rodriguez-Verde et al., 2018) tested ammonia stripping at a temperature of 70, 80, and 90 ºC and pH of 8, 9, and 10. However, as the flow rate was only limited to 11 L/hour, the removal efficiency was limited to 72%.

As for the impact of pre-hydrolysis ammonia stripping on methane potential, studies have reported a wide range of results due to different considerations taken by each study. For instance, Bonmatı and Flotats (2003) found that the methane yield was 75% lower than that in the Base sample due to the high pH of the substrate after the ammonia stripping treatment. On the other hand, acclimation of methanogens to high ammonia levels is essential in determining how effective the treatments are. For example, Zhang et al. (2012) found that samples treated with pre-hydrolysis ammonia stripping had around 246% more methane potential than those untreated. In contrast, Rodriguez-Verde et al. (2018) found that treated samples only have about 33% more methane potential. It is important to note that both treatments reduced ammonia levels to sub-inhibitory levels. However, treated samples by Zhang et al. (2012) had around 400 mg TAN/L, while treated samples by Rodriguez-Verde et al. (2018) had about 2000 mg TAN/L.

2.5.2 Side-stream

Side-stream ammonia stripping is another form discussed in previous studies (Table 2-2). First, a portion of the digestate from the anaerobic digester is filtered to separate the liquid from the solids. Then, the liquid is transferred to the stripping column, where the pH and/or temperature are increased to promote ammonia removal efficiency (Fernandez-Gonzalez et al., 2019). Most previous studies have tested side-stream stripping using biogas recirculation as it is readily available in biogas plants. Most importantly, it keeps the process anaerobic to preserve microbial activities (Serna-Maza et al., 2014; Zhang et al., 2017).

Zhang et al. (2017) tested side-stream stripping on a thermophilic digester running on food waste using biogas as a stripping medium. The results showed that TAN removal was successful in increasing methane potential. However, the ammonia removal efficiency was limited to 54% at a temperature of 70 ºC and a pH above 10. This removal efficiency is very low compared to that reported by Bousek et al. (2016). Nevertheless, high pH and temperature during stripping did not have an adverse effect on the digester's operations as the CO_2 in the

biogas had dropped the pH to a suitable range (Zhang et al., 2017). In a comparable study, Serna Maza et al. (2014) showed that biogas could remove up to 48% of the ammonia under pH 10 and a temperature of 70 ºC. On the other hand, biogas could only strip around 21% of the ammonia under pH 10 and a temperature of 55 ºC (Serna-Maza et al., 2014).

The low ammonia removal efficiency of biogas has led (Fernandez-Gonzalez et al., 2019) to test air side-stream stripping. Extensive analyses were conducted to evaluate the resilience of the methanogens to more challenging conditions. Ammonia stripping was conducted using air, without liquid/solid separation, at 65 ºC, unadjusted pH levels, and at an air flowrate of 60 L air/L digestate/hour. It was concluded that the aeration of the digestate and recycling it back to the digester did not directly affect biogas production. However, it restructured the abundancy of certain microorganisms by affecting ammonia levels in the digester.

Not many studies reported the impact of side-stream ammonia stripping on methane potential. The most significant improvement in methane potential was observed by Zhang et al. (2017), where side-stream stripping alleviated ammonia inhibition and improved methane yield by 193%. On the other hand, Serna-Maza et al. (2014) found that side-stream stripping had only improved methane potential by 2% due to the minimum ammonia removal and the acclimation of the methanogenic archaea in the untreated samples.

Table 2-2: Summary of previous studies about pre-hydrolysis ammonia stripping and side-stream ammonia stripping

Study	Type of substrate	Ammonia levels	Stripping method	Temperature	pH	Flow rate	Duration of stripping	Stripping mediums	Ammonia removal	Methane Improvement	Notes on Methane improvement
		mg/L	Pre- or post-hydrolysis/side stripping	ºC		L/L/hr	hours		Maximum % Removal	%	
Bonmati and Flotats (2003)	Filtered Pig slurry waste	3390	Pre-hydrolysis (Batch)	80	unadjusted, 9.5,11.5	1.2	4,5	Air	65,69, 98.8 at pH unadjusted, 9.5, 11.5	-75	Pretreated slurry led to lower methane yield due to unadjusted pH before digestion
		3680	Side Stream (continuous)	80	unadjusted, 9.5,11.5	1.2	4,5	Air	>96% at all pHs		
De La Rubia et al., (2010)	Food waste Digestate	7000-8000	Pre-hydrolysis (Batch)	35,55,70	unadjusted (7.9-8.6)	7.5,15 ,22.5	unspecified	Biogas	18.4%/day at 30 hrs for pH 8.5 and 15 l/l/hr flow	not tested	N/A
Abouelenien et al., (2010)	Chicken manure (Diluted with water to obtain a fluid mixture)	10000	Side Stream (Continuous)	55	9	60	continuous/ no retention time mentioned	Biogas	80	195 ml CH4 kg-1 VS	No Base scenario performed
		10000	Side Stream (Continuous)					Biogas with oxygen stripping	61.2	157 ml CH4 kg-1 VS	No Base scenario performed
Zhang et al., (2012)	Piggery wastewater	4950	Pre-hydrolysis (Batch)	37	9,9.5,10,11	60,120, 240,600	45	Air	92 at pH 11 and 600 l/l/hr flow	246.1	Base (no treatment) was inhibited due to ammonia levels/Highest methane corresponded to pre-treatment at pH 9.5
Serna-Maza et al., (2013)	Food waste	5100	Side stripping (Continuous)	55,70,85	unadjusted, 10	9	2 to 5	Biogas	32.4 at 85 oC and 5 hours	2.4	No significant improvement, Base (no treatment) bottle did not have any inhibition/ ammonia removal was low

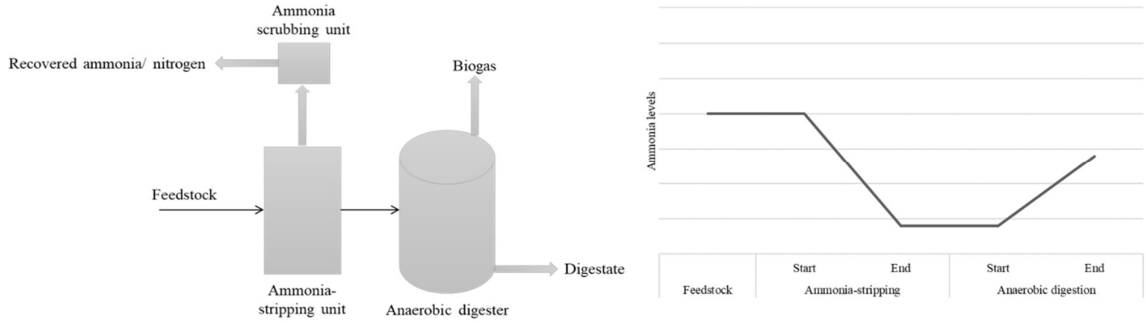
Study	Type of substrate	Ammonia levels	Stripping method	Temperature	pH	Flow rate	Duration of stripping	Stripping mediums	Ammonia removal	Methane Improvement	Notes on Methane improvement
		mg/L	Pre- or post-hydrolysis/side stripping	ºC		L/L/hr	hours		Maximum % Removal	%	
Bousek et al., (2016)	Model Solution	6000	N/A	65	unadjusted	300	4, 6, 8, 10 and 24 h	CO_2 with air at different concentrations	58	N/A	N/a
	Digestate of co-digested pig manure with maize, fodder, and sugar	6000	Side-stream stripping (Continuous)	65	unadjusted	300	4	Air	96	-3.85	Pre-treatment led to inhibition of digestion. This is because the aeration was performed on the digestate itself
		6000						Biogas	47	not tested	N/A
		6000						Flue gas	86	not tested	N/A
Zhang et al. (2017)	Food waste	5000	Side Stream (Continuous)	70	>10	9	84	Biogas	80	193	Treatment alleviated inhibition
Rodriguez-Verde et al., (2018)	Chicken manure/pig manure	7320	Pre-hydrolysis (Batch)	70,80, 90	8,9, 10	11 L/hr	2	Air	72	33.7	Treatment alleviated inhibition
Huang et al. (2019)	Swine manure (dry)	3418	Post-hydrolysis (Batch)	55	8.8, 10.2	15	24	Air	86.6	300	Treatment alleviated inhibition
Fakkaew and Polpraset (2021)	Chicken manure wastewater	1026	Pre-hydrolysis (Batch)	30, 50, 70, 80, 90	7, 9, 11	50, 400	5	Air	80	25	Improvement was limited due to increased ammonia levels after digestion

2.5.3 Post-hydrolysis

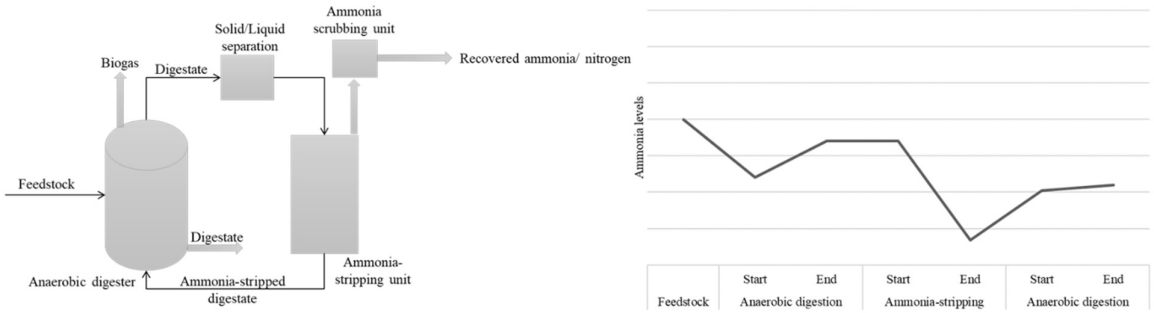
Post-hydrolysis ammonia stripping is a new approach that has recently gained much attention. Its concepts, however, are based on the accumulated knowledge and understanding of anaerobic digestion and ammonia stripping concepts. This approach was briefly discussed in Walker et al. (2011), where first kinetic modelling was used to compare different ammonia stripping configurations. However, no experimental work was conducted to verify this approach's advantages over existing approaches.

The operation of two-step reactors has proven its advantages in enhancing biogas production, which is common in North America. In addition, this separation of the hydrolysis step from the methanogenesis provides more flexibility for treatments such as post-hydrolysis ammonia stripping, where this treatment works best when the presence of the methanogenic archaea is minimal, i.e., in or after the hydrolyzer.

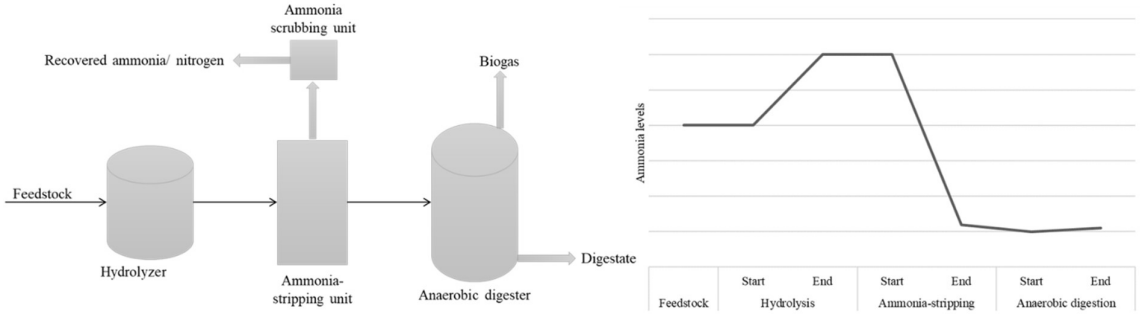
Post-hydrolysis ammonia stripping targets ammonia at its highest level in the plant and limits the further increase in ammonia downstream. Hydrolysis converts around 50-90% of the organic nitrogen to ammonia/ammonium, increasing the risk of methanogenic activity inhibition in the digester (Christou et al., 2021). Digestion converts part of the remaining 10-50% of the organic nitrogen to ammonia. As post-hydrolysis is an intermediate step between the hydrolyzer and the digester, the stripping medium can be selected based on its efficiency and practical usage rather than its suitability to maintain anaerobic conditions. Figure 2-7 tentatively demonstrates the ammonia profile in the three approaches.



(a) Pre-hydrolysis ammonia stripping



(b) Side-stream ammonia stripping



(c) Post-hydrolysis ammonia stripping (proposed approach)

Figure 2-7: Ammonia stripping configurations and tentative ammonia levels in different operational units.

There are a minimal number of studies that have experimentally tested the potential of post-hydrolysis ammonia stripping. Huang et al. (2019) studied the post-hydrolysis ammonia stripping of dry swine manure (20-36% total solids) at different treatment pH levels (8.8 and 10.2). The study proved that post-hydrolysis ammonia stripping could increase biogas potential from 25 ml/ g VS to 70-75 ml/ g VS. They concluded that both treatments led to similar methane yield because ammonia levels were at sub-inhibitory levels in both scenarios. The naturally high pH in swine manure (around 8.8) was favorable for achieving high ammonia removal efficiency of 78% after 24 hours of treatment at 55 ºC. This study proved that post-hydrolysis ammonia stripping is a feasible treatment option for high-solid swine manure (around 25% total solids). However, there is a lack of information on the feasibility of post-hydrolysis ammonia stripping for low-solids feedstock, which is more commonly used in biogas plants.

2.6 Microbial Diversity

As all functions of the biogas plant rely on microorganisms' activities, it is essential to understand how pretreatments and different types of substrates can shape the microorganisms in the digester. In addition, since ammonia causes inhibition of methanogenic activities, it

would be beneficial to identify the methanogenic and fermentative bacteria species that are more resilient to high ammonia levels.

Only a handful of previous studies had discussed the effect of ammonia stripping on the microbial communities in the digesters. Bi et al. (2020) investigated improving high-solids anaerobic digestion of poultry manure by implementing a side-stream ammonia stripping unit using biogas as a carrier gas. This study showed that ammonia stripping had primarily increased the fermentative bacteria and methanogenic archaea count compared with the Base reactor. In addition, the abundance of acetoclastic methanogens was higher than hydrogenotrophic methanogens, indicating that ammonia stripping had shaped a stronger microbial community as the former is less resilient to high ammonia levels.

Similar findings were also observed by Yin et al. (2019) when conducting hyper-thermophilic digestion of poultry manure. Bi et al. (2020) reported that *Methanosarcina* sp. was dominant at extremely high ammonia levels (above 5000 mg TAN/L). In contrast, Yin et al. (2019) reported that *Methanothermobacter* sp. was the most abundant under hyper-thermophilic conditions.

2.7 Knowledge Gap

Investigating the feasibility of post-hydrolysis ammonia stripping has been scarcely tested previously in the literature. This study will be the first to provide a comprehensive understanding of the potential and feasibility of this new approach through experimental work under batch and semi-continuous flow modes and include microbial analysis. The specific tasks that address these knowledge gaps are as outlined:

1. Implementing post-hydrolysis ammonia stripping for low-solids PM and comparing its performance to co-digestion and two-stage AD to improve methane potential.
2. Incorporating post-hydrolysis ammonia stripping and co-digestion with PM as a primary feedstock.
3. Optimizing the post-hydrolysis ammonia stripping configuration for low-solids PM using experimental data and statistical modelling.
4. Test renewable natural gas (RNG) as a stripping medium and compare its performance to biogas and air.
5. Conducting batch biochemical methane potential of PM treated with post-hydrolysis ammonia stripping using RNG.

6. Presenting microbial analysis results of post-hydrolysis ammonia stripping with air and RNG.
7. Testing the post-hydrolysis ammonia stripping under semi-continuous flow mode using lab-scale continuously stirred tank reactors (CSTRs).
8. Comparing post-hydrolysis ammonia stripping with side-stream ammonia stripping in semi-continuous flow mode.
9. Comparing post-hydrolysis ammonia stripping with pre-hydrolysis ammonia stripping in batch mode.
10. Conducting side-stream ammonia stripping with RNG instead of biogas.
11. Testing side-stream ammonia stripping at lower pH, temperature, and duration than what is discussed in the literature.

CHAPTER 3: RESEARCH METHODOLOGY

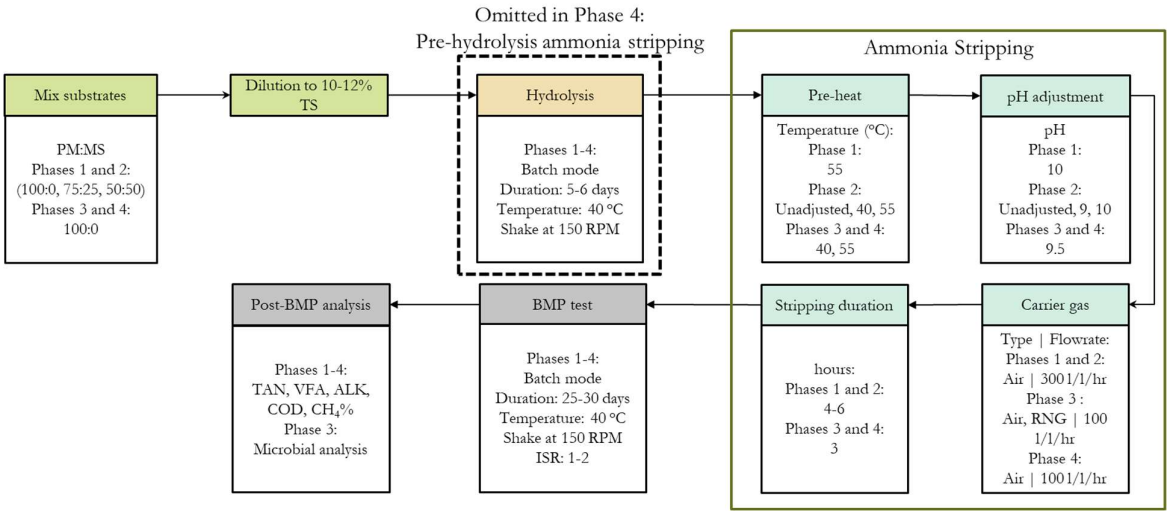
3.1 Overview

This section describes the general experimental plan and the methods used in the experiments. The detailed conditions in which the experiments were tested are presented in each technical paper. To avoid repetition, the references used for this chapter will be listed in Chapter 10: References.

3.2 Experimental Plan

The experimental plan presented in Figure 3-1 shows the test conditions of each phase of this study. As phases 1 to 3 were all in batch mode, they followed a similar approach with variations in the test setups, such as the types of substrates and the stripping conditions. Hence, the experimental plans of Phases 1-3 are summarized in Figure 3-1a, which shows the batch mode testing of the post-hydrolysis ammonia stripping (PHAS) system and different settings such as inoculum-to-substrate ratio (ISR), total solids (TS). On the other hand, the experimental plan of Phase 4, which mainly covers the semi-continuous flow mode experiment comparing PHAS with the side-stream ammonia stripping (SSAS) systems, is presented in Figure 3-1b. The figure also shows some operating parameters of the continuously stirred reactors (CSTRs) such as the organic loading rate (OLR) and hydraulic retention time (HRT). More details about each step of the experimental plan will be covered in Sections 3.3-3.6.

a)



b)

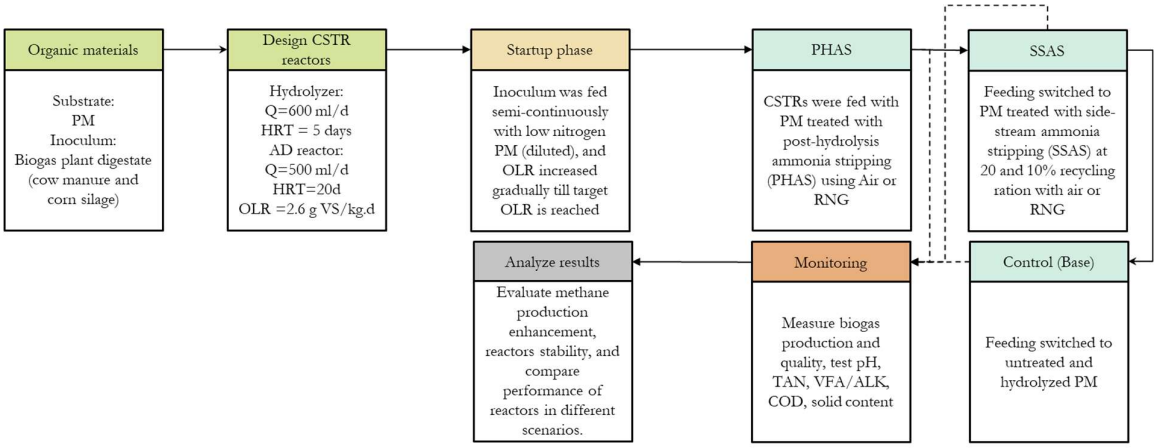


Figure 3-1: Experimental plan for a) batch mode experiments (Phases 1-4) and b) semi-continuous flow mode experiments (Phase 4).

3.3 Sample Collection

Poultry manure samples were collected from two locations throughout the study: Castor River farm (a local farm in Metcalfe, Ontario) and an egg factory in St. Isidore, Ontario. The farm had around 150 chickens, whereas the factory had 19200 chickens. Sampling was collected from layer chickens, i.e., chickens were only used for egg production. Manure from the farm was used in Phases I and II, whereas manure from the factory was used for Phases III and IV. This change is because the farm had changed its routine, and the manure quality was no longer suitable for the experiments.

Manure from the farm was already mixed with bedding materials (wood chips) and separating them was impractical (Figure 3-2). However, for Phases I and II, farm manure was suitable due to its high ammonia nitrogen despite bedding materials high in carbon content. The farm had changed its operations and introduced gravel into the bedding material, which greatly affected the quality of the poultry manure. Hence, a new location was sought for collecting poultry manure for use in Phases III and IV.

Figure 3-2: Farm poultry manure with bedding material.

Manure from the egg factory was mostly uncontaminated with any other material since the factory does not use any bedding that can be scraped out with manure (Figure 3-3). As it is more homogenous and consistent, it was selected for the remainder of the experiments.

Figure 3-3: Egg factory poultry manure.

In phases I and II, a co-substrate consisted of a mixture of cheese factory wastewater and coffee ground wastes from Ottawa. This organic mixture was collected from a biogas plant in Ottawa, which uses it as the primary feedstock. This unusual feedstock was selected as a co-substrate due to its low solid contents and high solubility and digestibility without prior treatment, unlike other common co-substrates, such as corn silage, which is a dry waste with a large particle size. The inoculum used throughout all experiments was collected from a biogas plant in Cobden, Ontario. Fresh batches were collected for each phase and used shortly after characterization. The digester operates on flushed cow manure and corn silage under mesophilic conditions. (Figure 3-4).

Figure 3-4: Inoculum used in all experiments.

3.4 Batch Biochemical Methane Potential Test

The biochemical methane potential (BMP) test is widely used to measure specific feedstock's methane potential under controlled conditions. In this study, the BMP test is divided into two steps, a hydrolysis/acidogenesis step and a methanogenesis step, each done in a separate bottle with 250-500 ml capacity (Figure 3-5). The setup of the experiment followed the guidelines set by (Holliger et al., 2016).

Figure 3-5: Hydrolysis (500 ml) and BMP (250 ml) bottles used for batch testing.

Before hydrolysis, raw poultry manure (and the co-substrate in Phases I and II) was blended after adding distilled water to reach a total solids (TS) concentration of 10-15%. The blended mixture is then sieved through a 1/4" sieve to remove large particles that cannot be digested. It is important to note here that the amount of the retained blended mixture was negligible and did not affect the characteristics of the feedstock (Figure 3-6). The sieved poultry manure is then filled in bottles leaving 30-40% of the volume as a headspace. The bottles are then purged with nitrogen gas for 1 to 1.5 minutes to ensure anaerobic conditions. The bottles are then

sealed and placed in a shaking incubator at 40 ºC and 120-150 RPM for five to six days. Characterization of the samples is conducted daily during hydrolysis to monitor the changes in TAN, VFAs, and pH.

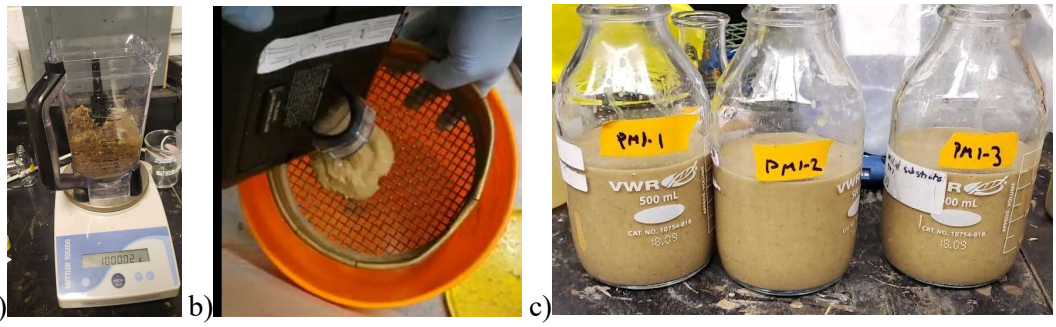


Figure 3-6: Sample preparation, a) dilution and blending, b) sifting through 1/4" sieve, c) filling substrates in hydrolysis bottles.

After the hydrolysis duration, samples are transferred into beakers/vessels for ammonia stripping (Section 3.6), or another bottle seeded with inoculum to serve as a benchmark (Base) for methane production. Although the targeted inoculum to substrate ratio (ISR) was 1-2 g VS inoculum/ g VS substrate, it was challenging to maintain a singular value due to the time that the solid contents test takes. The equation used to determine the inoculum mass needed is presented in Eq 2, and it was derived based on the ISR equation shown in Eq 3.

$$M_I = \frac{ISR \times VS_S\% \times M_{working}}{VS_I\% + ISR \times VS_S\%}$$

Eq 2

$$ISR = \frac{M_I VS_I\%}{M_S VS_S\%}$$

Eq 3

where *ISR* is inoculum to substrate VS mass ratio (g VS inoculum/g VS substrate), M_I and M_S are the masses of inoculum and substrate to be added to the BMP Bottle, respectively (g), $VS_I\%$ and $VS_S\%$ are the volatile solid percentages of the inoculum and the substrate, respectively (g), $M_{working}$ is the working volume (or mass) in the bottle, which is equivalent to 60-70% of the total volume of the bottle (g).

After filling the bottles with inoculum and substrates, nitrogen was used to purge the bottle for around 1-1.5 minutes. The bottles are then sealed and placed in a shaking incubator at 40 ºC and 150 RPM shaking speed. Biogas production was measured every day using a water displacement manometer, and a biogas sample from the headspace was collected and

characterized weekly for CH_4 and CO_2 content using Gas Chromatography (Figure 3-7). The biogas readings were correct to standard temperature and pressure (0 °C and 101 kPa) using Eq 4 from (Wijesinghe et al., 2018).

$$V_{STP} = V_{reading} \times \frac{T_s P_a}{T_i P_s}$$

Eq 4

Where V_{STP} is the volume of CH_4 (L), T_s is the standard temperature (273.15 K), P_a is atmospheric pressure (kPa), T_i is the incubation temperature (K), and P_s is standard pressure (101.325 kPa).

The experiment remained operational until the daily biogas production rate was less than 1% of the cumulative biogas production for three consecutive days (Holliger et al., 2016). The digestate of the BMP tests is characterized at the end of the experiment for ammonia, pH, VFAs, alkalinity, and COD and then stored for use in upcoming experiments.

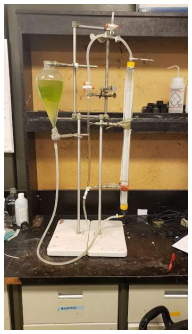
a)  b)  c)  d) 

Figure 3-7: BMP process: a) Purging with nitrogen, b) placing in a shaking incubator, c) monitoring biogas daily using a manometer, and d) biogas characterization using gas chromatography.

3.5 Continuous Stirred Tank Reactor

Conducting batch BMP tests was sufficient to understand the potential of post-hydrolysis ammonia stripping (PHAS). However, to verify the approach's feasibility under long-term semi-continuous flow operation, two continuously stirred tank reactor (CSTR) setups were designed (Figure 3-8). Each setup consisted of a hydrolyzer tank (H), an ammonia stripping unit, and a methanation tank (AD). The ammonia stripping was conducted manually every day, after which the effluent was fed to the digester.

Figure 3-8: Hydrolyzer CSTR (5 liters) and anaerobic digester CSTR (15 Liters).

The design of the reactors depends on the target organic loading rate (OLR) and the hydraulic retention time (HRT). Studies suggest that OLR can be between 1.5- 6 g VS/L/day. The design criteria of the reactors are presented in Table 3-1: CSTR Design Criteria:

Table 3-1: CSTR Design Criteria

Reactor Type	HRT (days)	Working Volume (L)
Hydrolyzer	5	3
Anaerobic Digester	20	10

The influent and the effluent for the hydrolyzer and the digester were set to 0.6 and 0.5 L/day, respectively. The hydrolyzer flow rate was higher to account for any losses during handling and treatment and to ensure that 0.5 L/day is available for the digesters. OLR of the digesters was calculated using Eq 5

:

$$OLR = \frac{Q \times VS\% \times \rho}{Working\ Volume}$$

Eq 5

where *OLR* is organic loading rate (g VS/L/day), Q is the influent and effluent flow (L/day), ρ is the density of the influent, always assumed to be 1000 kg/m^3 (kg/m^3), *VS%* is the volatile solids percentage in the influent PM, *Working Volume* is the volume the digestate occupies in the reactors which are 30-40% of the total volume (L).

With the VS of PM being around 5.52%, and a flow rate of 500 mL/day, the expected OLR is about 2.6 g VS/L/day. This is an acceptable value since it is in the range specified in the

literature. Moreover, PM can be problematic due to high ammonia levels. Therefore, an OLR in the lower range can help stabilize the reactor.

At the start of the experiment, the reactors were filled with fresh inoculum from an operating mesophilic biogas plant in Ottawa running on cow manure and corn silage. The inoculum was left under anaerobic conditions and continuous agitation for a few days without feeding to degas it and reduce its COD and VS to limit the contribution of the inoculum's organic matter to biogas production.

Feeding with diluted and treated PM started with a very low OLR (0.5 g VS/L/day) and was gradually increased to the target OLR, around 2.6 g VS/L/day. The gradual feeding was done to avoid shocks to the active microorganisms in the inoculum.

After reaching the target OLR, the scenarios in Table 3-2 started. Each scenario continued for at least three HRT or until steady-state conditions were reached before switching to the following scenario. The scenarios were planned to begin with the anticipated most conservative treatment (least impactful) and end with the least conservative one (most impactful) to maintain the activities of the microorganisms for the longest time possible.

The ammonia stripping treatment in PHAS and side-stream ammonia stripping (SSAS) will be conducted under the optimal pH, temperature, and flowrate that were observed in Phases I, II, and III; these conditions are a pH of 9.5, a temperature during stripping to be 55 ^{o}C, and a flow rate of 100 L gas/L digestate/ hour.

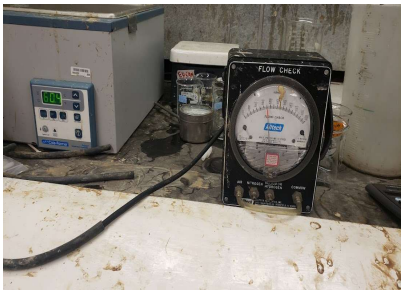
Table 3-2: CSTR experiment scenario and test conditions.

Annotation	Start (day)	End (day)	Stripping medium	Stripped Portion
Start-up	0	48	N/A	0
PHAS-1	48	126	Air	100% of hydrolysis reactor effluent (500 g/day)
PHAS-2	126	163	RNG	100% of hydrolysis reactor effluent (500 g/day)
SSAS-1	163	224	RNG	20% of reactor volume per day (2 kg per day)
SSAS-2	224	285	RNG	10% of reactor volume per day (1 kg per day)
SSAS-3	285	332	Air	10% of reactor volume per day (1 kg per day)
Base	332	400	N/A	Reactors fed with hydrolyzed PM (no treatment)

3.6 Ammonia Stripping

After hydrolysis for 5-6 days, samples were placed in beakers (or a glass cylinder for Phases III and IV) and put in a water bath where the temperature was set to 45 or 60 ^{o}C to heat the samples to 40 or 55 ^{o}C, respectively. The water bath temperature was set above the desired temperature to account for the cooling effect of the stripping medium (Figure 3-9). After reaching the desired temperature, the pH was adjusted by adding lime. Lime is added gradually

until the desired pH is reached. In Phases I and II, lime was added as a slurry, whereas in Phases III and IV, it was added directly to the PM without mixing with water first. The airflow was set to 100–300-liter air per liter digestate per hour and was measured using a flow meter (Figure 3-9). The tube that delivers the stripping medium has an aquarium stone attached to it to produce finer bubbles and increase stripping efficiency. The tube was then fixed to the bottom center of the beaker or the stripping column, and the stripping continued for the entire duration (duration varied between phases). Samples were taken every 20 or 30 minutes and tested for ammonia and pH levels. However, in Phases I and II, characterization was conducted every 2 hours. The same procedure was followed for pre-hydrolysis or side-stream ammonia stripping, although the stage where stripping was conducted was different. For instance, blended raw manure was used for pre-hydrolysis ammonia stripping, whereas side-stream ammonia stripping was performed on a portion of the digestate.

a) 

b) 
c) 

Figure 3-9: a) Checking gas flow rate using a flowmeter, b) ammonia stripping samples in beakers for Phases I and II, and c) ammonia stripping column for Phases III and IV.

It is essential to mention that whenever RNG was intended for stripping in Phases III and IV, it was performed using natural gas lines from Enbridge due to health and safety reasons in the lab. However, it was sufficient because it included high methane levels (94%), and the main impurity was ethane (5%).

Biogas stripping in Phase III was prepared in the lab by combining methane from natural gas lines and CO_2 from a high-purity CO_2 cylinder. An impermeable bag was used to collect the biogas, and it was pumped to the stripping unit using peristaltic pumps set at 100 L/L/hour.

3.7 Analytical Methods

Total solids (TS) and volatile solids (VS) were determined using standard method no. 2540 (APHA, 2005) (Figure 3-10). Volatile fatty acids (VFA) were measured using the Esterification method: Hach TNT872 (50-2500 mg CH_3COOH/L); total and soluble chemical oxygen demand (COD and sCOD) were measured using Reactor Digestion Method: Hach TNT822 (20-1500 mg COD/L); total alkalinity was measured using colorimetric method 10239: Hach TNT870 (25-400 mg $CaCO_3$/L); TAN was measured using the Phenate method and verified by Salicylate method: Hach TNT (2-47, 100-1800 mg TAN/L). The Hach tests were read using DR6000 Hach Spectrophotometer (Figure 3-11). Methane content in the biogas was measured using Gas Chromatography (GC) where helium was used as the carrier gas, the temperature of the oven, indicator, and inject port were set to 120, 120, and 130 °C, respectively, and the current was set to 100 mA.

a)  b) 

c) 

Figure 3-10: Solid contents test a) substrate and inoculum samples, b) oven for total solids analysis and c) furnace for volatile solids analysis

a) 

b) 

Figure 3-11: Chemical analysis using a) Hach TNT sets and b) Hach DR6000 Spectrophotometer

CHAPTER 4: ENHANCING MONO- AND CO-DIGESTION OF POULTRY MANURE BY A NOVEL POST-HYDROLYSIS AMMONIA STRIPPING APPROACH IN A TWO-STAGE ANAEROBIC DIGESTION PROCESS

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In the first phase of the thesis, proof of concept was needed as the post-hydrolysis ammonia stripping approach of poultry manure has not been experimentally discussed in the literature before. Therefore, this step tested post-hydrolysis ammonia stripping at conservative conditions and compared it with untreated samples. In addition to poultry manure (PM), a mixture of cheese factory wastewater and coffeehouse waste (MS) was used as co-substrates.

The objectives of Phase I include 1) testing the impact of using air as a stripping medium on biogas production, 2) investigating the ability of mono-digesting PM or co-digesting it at higher portions, and 3) monitoring ammonia levels throughout the AD stages and developing an understanding of the inhibitory levels of ammonia.

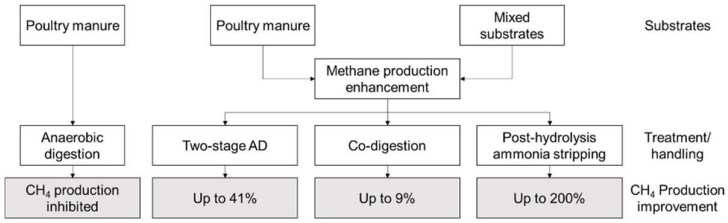
Abstract

The use of poultry manure (PM) in anaerobic digestion applications at high PM proportions is very limited due to its high nitrogen content since high ammonia levels in the feedstock can lead to unstable and sub-optimal anaerobic digestion (AD) operation. This study investigated three methods to improve methane potential from PM at high PM proportions: 1) co-digestion of PM with a mixed substrate (MS) of a cheese factory wastewater and coffee-ground wastes at PM:MS ratios of 100:0, 75:25, 50:50, 25:75, and 0:100; 2) two-stage anaerobic digestion of PM and MS at PM:MS ratios of 100:0, 75:25, and 50:50, and 3) a novel post-hydrolysis ammonia stripping at pH 10 and 55 ºC. The results indicated that co-digestion increased the methane potential in the samples due to reduced ammonia levels. A two-stage anaerobic digestion system further improved the methane potential by 21 and 41% in the cases of PM:MS at 100:0 and 75:25, while no improvement was observed in the case of PM:MS at 50:50. Post-hydrolysis ammonia stripping under the tested conditions achieved 79.2, 69, and 78.6% ammonia removal in PM:MS of 100:0, 75:25, and 50:50 and improved methane potential by 200, 150, and 64% when compared with the untreated samples.

Keywords:

Poultry manure, anaerobic digestion, mono-digestion versus co-digestion, one-stage versus two-stage AD, post-hydrolysis ammonia stripping, Gompertz modelling.

Graphical Abstract



Statement of Novelty

A considerable amount of work is present in the literature on improving the methane potential of poultry manure and other substrates characterized by high nitrogen. Nevertheless, most of these studies focus on including a low proportion of poultry manure in the feedstock. However, this study focuses on poultry manure as the main substrate to evaluate the possibility of its mono-digestion or co-digestion at higher proportions. This study also presents a new approach to performing ammonia stripping, which can address some of the shortcomings of the current ammonia stripping systems. Finally, this work also compares the results of the three different approaches to improve the methane potential.

4.1 Introduction

The continuing increase in population prompts a corresponding increase in all livestock populations, poultry being the fastest-growing livestock in the world (Michalk et al., 2019; Tauseef et al., 2013). This increase is accompanied by an increase in livestock manure production, and conventional treatment methods such as stockpiling and land application would not be viable options due to their associated negative impacts on the environment such as global warming, acidification, and eutrophication of water resources. Anaerobic digestion (AD) has been gaining attention as a sustainable alternative to conventional treatments due to its ability to stabilize organic matter and to produce clean and sustainable biogas (Adghim et al., 2019; Ara et al., 2014).

Microorganism activity is the driving force of AD and can be partially or completely inhibited by different factors including accumulation of volatile fatty acids (VFAs), ammonia nitrogen, and high sulfur content (Akindele and Sartaj, 2018b; Nielsen et al., 2013; Yan et al., 2018). Ammonia in the AD process is formed by the decomposition of organic nitrogen in meat, urea,

and manure, mainly in the form of proteins (Serna-Maza et al., 2014). Depending on temperature and pH, ammonia is present in different proportions of ionic ammonia (NH_4^+) and free ammonia nitrogen (NH_3, FAN); the latter is the main inhibitor of the microorganism activities in the AD (Bousek et al., 2016; Nair et al., 2014; Wang et al., 2012). FAN can diffuse into the methanogenic archaea cells and cause proton imbalance that inhibits the methanogenic activities (Bousek et al., 2016). Inhibitory levels of ammonia vary from a study to another depending on the types of the substrates and inoculum, level of adaptation of the inoculum, and operational conditions such as pH and temperature (Akindele and Sartaj, 2018b; Capson-Tojo et al., 2020).

Poultry manure (PM) is rich in ammonia and is often avoided in AD operations due to the expected inhibition. However, because of its large quantities and availability, recent studies have focused on pre-treatment and other strategies to include it as primary or co-feedstock in AD processes (Nie et al., 2015a). Co-digestion of PM with high carbonaceous materials has been studied as a solution to alleviate ammonia inhibition from AD, among other advantages such as the treatment of various organic wastes simultaneously (Borowski et al., 2016). Co-digestion aims to adjust the low carbon-to-nitrogen ratio (C/N) of PM into 20-30 as an optimum condition of operation by dilution with substrates of low ammonia levels (Borowski et al., 2016; Wang et al., 2012). In addition, co-digestion has been proven to result in positive synergetic enhancement of biogas production from a mixed substrate (Adghim et al., 2019; Ara et al., 2015). The optimum co-digestion ratio is determined by conducting the biochemical methane potential (BMP) tests and measuring the synergistic effect of the mixed feedstock. The synergistic effect is the difference between the calculated methane yield (calculated based on the methane potential of each substrate alone) and the observed methane yield of the co-digested feedstock.

Most studies suggest that the co-digestion of PM with other substrates was inhibitory when PM formed more than 30% of the feedstock composition (VS mass based), except when co-digested with hog manure and dairy manure (DM) with wheat straw (WS) (Wang et al., 2012) where the optimum VS mass ratios of hog:PM and DM:PM:WS were 40:60 and 39:39:22, respectively.

Although co-digestion adjusts the initial C/N ratio, ammonia often increases through the process due to further decomposition of organic nitrogen. (Wang et al., 2012) found that at lower C/N ratios of 15 and 20, total ammonia nitrogen (TAN) increased up to 66% compared

to initial TAN; whereas at higher C/N ratios, TAN remained constant throughout the BMP test and achieved higher methane potential. In another study by Wang et al., (2014), it was found that temperature can also increase the risks of ammonia inhibition and increasing C/N can reduce the inhibitory effects (Wang et al., 2014). Babaee et al. (2013) investigated the performance of the AD of poultry manure and wheat straw at different organic loading rates (OLR) and temperatures. The study concluded that AD at 35 ^{o}C resulted in a 43% increase in biogas production compared to AD at 25 ^{o}C. As for the OLR, the system was overloaded after 4 kg VS/m^3/day and inhibitory effects of ammonia became noticeable (Babaee et al., 2013).

Anaerobic digesters often run on complex mixtures of organic wastes. (Valenti et al., 2018) studied the co-digestion of PM with a mixture of citrus pulp, olive pomace (2 and 3 phases), corn silage, cattle manure, and whey, at different proportions. The study suggests that PM proportion shall not exceed 13% of the total dry weight during all trials due to its low C/N ratio of 5.5 and the possibility of inhibition due to ammonia. (Rodriguez-Verde et al., 2018) applied a linear model to simulate the co-digestion of different proportions of PM and pig manure until stable methane production and operating conditions were achieved. The optimum PM:pig manure ratio was 24:76; indicating once more that PM can inhibit the AD process at high concentrations. The study suggested that higher PM quantities cannot be added without further treatment for the removal of ammonia.

A two-stage AD (also known as two-step AD), where hydrolysis and methanogenesis stages are separated into two reactors in series, proved to be successful in increasing methane potential and methane production rate in the AD process (Lu et al., 2008; Yin et al., 2019). The hydrolytic and syntrophic bacteria convert VFAs as well as some alcohols to acetate, H_2, and CO_2 in the first reactor which are then converted to biogas by methanogenic archaea in a methanogenic reactor (Lu et al., 2008). The mesophilic hydrolysis of glucose and coffee seed skins at 35-40 ^{o}C, followed by mesophilic anaerobic digestion was tested to investigate the improvement in methane production (Malave' et al., 2015). The study proved that the two-stage AD enhanced solubility and biodegradability of the feedstock, which led to an increase in methane yield when compared with one-stage anaerobic digestion. Two-stage mesophilic AD was also investigated in more recent studies such as (Baldi et al., 2019; Tsigkou et al., 2020) who also showed that two-stage AD led to increasing methane potential and volatile solid removal when compared with one-stage AD.

While co-digestion enabled the inclusion of PM into the feedstock at low proportions, ammonia removal using air stripping was proved efficient in reducing ammonia levels and prevented inhibition of methanogens due to ammonia when PM was mono- or co-digested (Baldi et al., 2018; Nie et al., 2015a). To increase the efficiency of ammonia stripping, pH and temperature of the feedstock must be increased to expand the proportion of free ammonia nitrogen (FAN); which is the part of ammonia that can be stripped (Abouelenien et al., 2010; Nielsen et al., 2013).

Previous studies discussed two possible options in terms of the stage at which ammonia stripping could occur: pre-hydrolysis and side-stream stripping. Pre-hydrolysis ammonia stripping is when the stripping unit is located before the hydrolyzer and handles raw feedstock after pH and temperature adjustments (Bonmatı and Flotats, 2003). On the other hand, in side-stream stripping, a portion of the digestate is filtered and the liquid fraction is treated in an external column after pH and temperature adjustments, followed by recycling of the ammonia-stripped digestate back into the digester to reduce ammonia levels in the system (Pedizzi et al., 2018; Serna-Maza et al., 2014; Zhang et al., 2017). Some shortcomings can be noted in these systems based on observations from previous studies: 1) the pretreatment approach does not consider that raw samples are not hydrolyzed and therefore ammonia levels will increase significantly after being added to the digester due to the decomposition of organic nitrogen, and 2) in the side-stripping approach, stripping medium is often limited to biogas, CO_2, or nitrogen; air cannot be used as methanogens cannot survive in aerobic conditions. (Bousek et al., 2016) concluded that air is more efficient as a stripping medium when compared with CO_2 or biogas. The current study targets those shortcomings by evaluating the ammonia stripping after the hydrolysis of raw material, i.e., post-hydrolysis, in a two-stage AD before it enters the methanogenic (second) digester.

In this study, co-digestion, two-stage AD, and post-hydrolysis ammonia stripping were assessed as means to enhance methane potential from feedstock where PM is the primary substrate (PM≥50% of VS mass). A mixed substrate (MS) of a cheese factory wastewater and coffee-ground wastes was used as co-substrate as it is characterized by high carbonaceous content. Two-stage AD and ammonia stripping were applied to all tested co-digestion ratios where PM constituted 50% or more of the composition. To the best of the authors' knowledge, this is the first study to discuss the coupled effect of co-digestion and two-stage AD to enhance the BMP of poultry manure. Moreover, this study is the first to assess post-hydrolysis ammonia stripping to address the shortcomings of the current techniques (pretreatment and side-

stripping). Specifically, the main objectives of this study were to 1) evaluate the BMP of different co-digestion ratios under separated hydrolysis and methanogenesis stages (two-stage AD) compared to a single-stage AD system; 2) evaluate the impact of ammonia stripping after hydrolysis on the BMP and further ammonia formation; and 3) monitor ammonia levels and its inhibitory effects on the AD. The results of this study should help provide poultry farm operators with information about the potential of anaerobic digestion as an economic and sustainable alternative to conventional treatments or possible improvements of operating anaerobic digesters to increase methane potential.

4.2 Materials and Methods

4.2.1 Substrates and Inoculum

Poultry manure (PM) was collected from a local poultry farm in Ottawa, Canada; it was slightly mixed with bedding material and wood chips. The mixture of the cheese factory wastewater and coffee-ground wastes, hereafter notated as mixed substrate (MS), was collected from a local biogas plant site in Ontario, Canada. The inoculum (I) used, was a digestate from a biogas plant running on cow manure and corn silage. All samples were stored in buckets at 4 ºC throughout the experiment.

4.2.2 Analytical Methods

Total solids (TS) and volatile solids (VS) were determined using standard method no. 2540 (APHA, 2005). Volatile fatty acids (VFA) were measured using Hach TNT872 (50-2500 mg CH_3COOH/L); total and soluble chemical oxygen demand (COD and sCOD) were measured using Hach TNT822 (20-1500 mg COD/L); total alkalinity was measured using Hach TNT870 (25-400 mg $CaCO_3$/L). TAN was measured using the Phenate method and verified by Hach TNT (2-47 mg TAN/L). Methane content in the biogas was measured using Gas Chromatography (GC) where helium was used as the carrier gas, the temperature of the oven, indicator, and inject port were set to 120, 120, and 130 ºC, respectively, and the current was set to 100 mA.

4.2.3 Experimental Setup

This section describes the three approaches used in this study to improve methane yield of poultry manure including 1) co-digestion of untreated samples, 2) two-staged AD, and 3) post-hydrolysis ammonia stripping. A batch methane potential (BMP) test was performed on all samples from the three approaches to evaluate the respective methane yield enhancement.

To run the BMP tests, the inoculum was added to the samples at an inoculum to substrate ratio of 1 to 2 (g/g, volatile solids (VS) basis). Two Blank bottles filled with inoculum were also tested to allow the calculation of contribution from inoculum and the net cumulative biogas production from the samples. All BMP sets were tested in duplicates and placed in 250 ml serum bottles. Bottles were purged with nitrogen gas to ensure anaerobic conditions were attained. The bottles were sealed and incubated at 35 ^{o}C and agitated at a shaking speed of 100 rpm. Biogas production was measured using water displacement in a manometer every day and the methane content was determined by GC once a week. The volume of biogas was adjusted to standard temperature and pressure (STP). The mean and the standard deviation of ammonia removal and biogas production were calculated based on a sample size of 2; hence, the standard deviation was multiplied by a factor of 6.314 to compensate for the low sample size and to estimate the diversity of results at 95% confidence interval (Evans and Rosenthal, 2013). Post-BMP characterization tests were conducted to evaluate VS removal and the variation in ammonia levels.

4.2.3.1 Co-digestion of Untreated Samples

The first approach to increase the methane potential by alleviating ammonia inhibitory effects was the co-digestion of PM with MS at ratios of PM:MS of 100:0, 75:25, 50:50, 25:75, and 0:100 (VS mass-based). At this stage, no treatment or pH adjustment was performed. This stage aimed to evaluate the possible enhancement to the methane potential by reducing ammonia levels through adding high carbonaceous materials i.e., MS. Although the literature does not recommend the addition of PM at levels higher than 30-50% of the VS mass due to the anticipated ammonia inhibition, this study tested the addition of PM at 75 and 50% of the feedstock for two reasons: 1) the characteristics of PM and MS might differ from those used in previous studies, and 2) the co-digestion of untreated samples served as a Base (benchmark) to which other treatments in this study can be compared.

4.2.3.2 Two-stage Anaerobic Digestion

At this part of the experiment, the aim was to assess the possible increase in methane potential when PM constituted 50% or more of the feedstock by separating the hydrolysis stage from the methanogenesis i.e., using a two-stage AD. Thus, PM and MS were mixed at PM:MS ratios of 100:0, 75:25, and 50:50 (VS mass-based). The TS of the mixtures of PM100, PM75:MS25, PM50:MS50 was 26, 20, and 16% which can be impractical to hydrolyze due to the high TS (Zhang et al., 2017). Therefore, the TS of all samples in this stage was adjusted to 10% by

adding distilled water before the hydrolysis to ensure proper mixing and agitation of acidogenic bacteria. The diluted mixtures were hydrolyzed anaerobically at 35 ºC for six days and at a shaking speed of 100 rpm before inoculum was added to the hydrolyzed samples at VSi/VSs of 1 to 2. TAN was monitored daily, and pH was measured at the end of the hydrolysis. The duration of hydrolysis was selected based on a pretest that showed TAN and VFAs stabilizing after five to six days, which lines up with practice at biogas plants.

4.2.3.3 Ammonia Stripping

The main objective of this step was to further enhance the methane potential by the removal of ammonia from the samples through air stripping. Ammonia stripping was conducted after hydrolysis for six days on three samples: 1) hydrolyzed PM100, 2) hydrolyzed PM75:MS25, and 3) hydrolyzed PM50:MS50. The ammonia stripping was performed after hydrolysis to target ammonia at the highest levels and control the concentration of ammonia in digestate. Raw PM and MS samples were added to serum bottles at PM:MS ratios of 100:0, 75:25, and 50:50 VS mass-based and then diluted with distilled water to 10% TS. The samples were hydrolyzed for 6 days at 35 ºC and 100 rpm. After the hydrolysis, pH and temperature were adjusted to 10 and 55 ºC. pH was adjusted by adding 1M lime (CaOH) solution gradually and monitoring pH constantly. The addition of lime was monitored as it affected the dilution factor. Then, the hydrolyzed samples were air stripped at a flow rate of 300 L air/L digestate/hr via a flexible tube with an air diffuser at the end inserted to the bottom of the bottle. The bottles were placed in a water bath where the temperature was set to 60 ºC to account for the cooling effect from the airflow and to keep the temperature of the samples at 55 ºC. The temperature of the feedstock and the airflow rate were measured constantly to ensure they are accurate throughout the experiment. Ammonia stripping was continuous for six hours, ammonia and pH levels were measured every two hours. Tests were done in duplicates to ensure accurate results.

4.2.4 Kinetic Modelling for Methane Production

The experimental data were fitted to the Gompertz model (Eq 6) to evaluate the kinetic parameters describing the methane production (Kafle and Chen, 2016). The Gompertz model allows the quantification of the lag time and the biogas production rate, which are supposed to be enhanced due to implementing two-stage AD and ammonia stripping. The average values of cumulative methane production of the BMP bottles duplicates were used as the experimental (true) values, and the relative root mean square error (rRMSE) was reduced to the minimum to optimize the kinetic parameters using the SOLVER feature in excel.

$$M = P.\exp\left[-\exp\left(\frac{R_m.e}{P}(\lambda - t) + 1\right)\right]$$

Eq 6

where M is the cumulative specific methane yield (L of CH_4/kg VS), P is the specific amount of methane produced (L of CH_4/kg VS), Rm is the maximum specific methane production rate (L of CH_4/kg VS/day), λ is the lag time (days), and t is the incubation time (days).

4.3 Results and Discussion

4.3.1 Substrate Characteristics

The substrates were characterized when samples were fresh to ensure accuracy (Table 4-1). The high COD in MS makes it a preferred feedstock for AD, and an appropriate co-substrate to mix with PM to adjust the C/N ratio. VFAs in MS were 1.87-fold that in PM, which explains the low pH of 4.3 in MS samples. The high COD and VFA in MS may indicate that MS is more readily biodegradable than PM. The pH of PM was around 8.7 and was at the higher end of the range (6.9-8.5) reported in the literature (Borowski et al., 2016; Khoufi et al., 2015). Ammonia levels in PM (3843 mg TAN/L) were already in inhibitory levels as previously reported in the literature i.e., TAN > 1500-3000 mg/L (Fotidis et al., 2014). Nevertheless, inhibition is often dependent on the degree to which the inoculum is acclimatized to high ammonia levels and the substrate to inoculum ratio that could dilute the digestate to reduce the levels of ammonia. The soluble fraction of the total COD in PM and MS was 41 and 49%, respectively. The initial TAN levels of the inoculum indicated that the inoculum is acclimatized at ammonia levels around 800 mg TAN /L (TAN levels at the actual plant). However, BMP tests will determine if the process would be inhibited by the addition of PM as initial TAN levels are expected to be greater than 1000 mg TAN /L. The VS/TS ratio of PM was significantly lower than that of MS, this could be due to the presence of bedding material and wood chips residues in the PM samples that were difficult to remove, in addition to the presence of the inorganic solids in the samples.

Table 4-1: Characteristics of substrates and inoculum

Characteristics	Unit	PM	MS	I
VFA	mg CH_3COOH/L	2515 ± 106	9660 ± 170	878 ± 49
TAN	mg TAN/L	3843 ± 425	454 ± 1	813 ± 13
COD	mg COD/L	13017 ± 355	25750 ± 71	48550 ± 495
sCOD	mg COD/L	5400 ± 87	12517 ± 104	6465 ± 21
Total Alkalinity	mg $CaCO_3$/L	17360 ± 640	1001 ± 49	7740 ± 187
Total Solids	%	26.43 ± 0.62	10.84 ± 0.18	4.37 ± 0.03
Volatile Solids	%	17.43 ± 0.62	9.82 ± 0.18	3.27 ± 0.03
VS/TS	%	65.92 ± 2.51	90.58 ± 0.11	74.83 ± 0.11
pH		8.7 ± 0.2	4.3 ± 0.1	7.6 ± 0.1

4.3.2 Ammonia Levels During Hydrolysis

The hydrolysis stage aimed to break down the organic compounds by the acidogenic bacteria activity before the addition of inoculum. TAN levels started between 2500 to 3200 mg TAN/L in hydrolyzed PM100, hydrolyzed PM75:MS25, and hydrolyzed PM50:MS50, and increased to 5075, 3967, and 2805 mg TAN/L in six days, respectively. Further addition of MS i.e., hydrolyzed PM25:MS75 and hydrolyzed MS100 yielded TAN levels that are less than 1400 mg TAN/L, which is a non-inhibitory level after the addition of inoculum (Abouelenien et al., 2010; De la Rubia et al., 2010). Therefore, hydrolyzed PM25:MS75 and hydrolyzed MS100 were not included in the ammonia stripping and the BMP test.

During the hydrolysis, PM100 showed a lag of 3 days before ammonia was rapidly increased by almost 57%. This increase was preceded by a slight drop in the TAN level for two days, which could be explained by the consumption of NH_4^+ by the acidogenic bacteria to culture enough to be capable of decomposing the complex organics in the poultry manure. On the other hand, TAN started increasing from day 1 in PM75:MS25 hydrolysis and gradually reached a total 69% increase after 6 days. In terms of hydrolysis of PM50:MS50, TAN levels were stable throughout the hydrolysis and were only increased by 5%. Further increase in MS content led to very low levels of ammonia even at the end of the hydrolysis stage.

pH levels at the end of the hydrolysis of PM100, PM75:MS25, and PM50:MS50 reached 6.93, 6.47, and 6.31, respectively. The drop in pH indicated the formation of additional VFAs in the samples. The initial high pH of PM caused the pH of all hydrolyzed samples to settle on a suitable range for methanogenesis after inoculum was added without any pH adjustment. The formation of VFAs in the hydrolysis bottle was coupled with gas production (>90% CO_2)

which was due to the destruction of organic compounds; VS reductions after hydrolysis in PM100, PM75:MS25, and PM50:MS50 were 11.77±0.96, 19.68±4.66, and 12.51±1.67%.

4.3.3 BMP of Mono-digestion versus Co-digestion

The BMP results indicated that the co-digestion of PM with MS at any tested ratio yielded higher methane potential when compared with the mono-digestion of PM (PM100). The BMP of co-digesting the untreated samples was inversely proportional to the initial and final levels of ammonia in the bottles, which illustrates the inhibitory effect of ammonia on the microorganisms. The cumulative methane production of the mono- and co-digested untreated samples is presented in Figure 4-1. Methane content was measured weekly and averaged 46, 42, and 43, 41, and 43% of the biogas from PM100, PM75:MS25, PM50:MS50, PM25:MS75, and MS100, respectively.

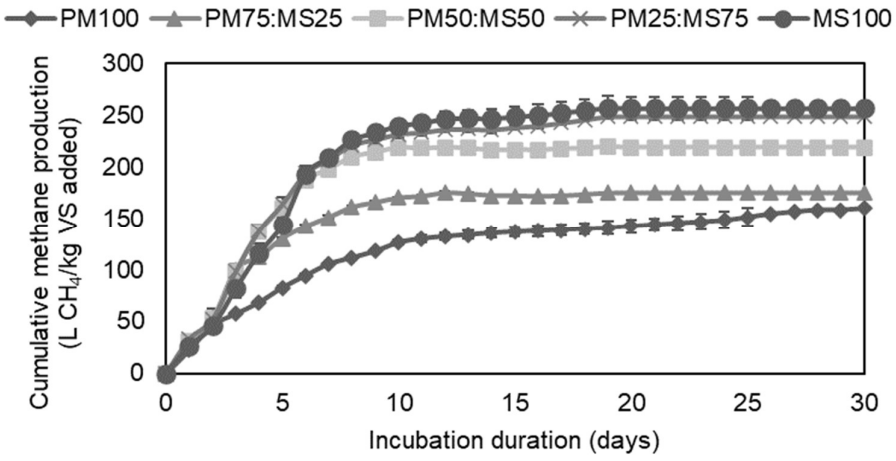


Figure 4-1: Cumulative biogas production of mono- and co-digested untreated samples.

The highest methane potential of untreated samples was achieved by MS100 (257 L CH_4/kg VS), whereas co-digesting PM with MS at PM75:MS25, PM50:MS50, and PM25:MS75 yielded 1.10-, 1.38-, and 1.57-fold the methane yield of PM100 (159 L CH_4/kg VS) and the corresponding synergistic coefficient was 0.97, 1.08, and 1.09, respectively. The improvement in methane potential due to co-digestion indicated that microorganisms' activities in PM100 and PM75:MS25 might have been inhibited due to the high ammonia levels. Moreover, co-digestion increased carbonaceous material and enhanced biodegradability by increasing the COD and VFAs. The BMP results of PM100 in this study were in line with (Wang et al., 2012) under similar ammonia levels, while methane production was inhibited in (Khoufi et al., 2015) under lower ammonia levels. The variation of the inhibition effects among different studies

could be explained by the different characteristics of PM from one study to another, in addition to the acclimation of the inoculum to high ammonia levels. In this study, no significant lag time was observed in any of the samples, and the methane production reached a plateau after two weeks. Nevertheless, methanogenic activities in untreated PM100 were partially inhibited at 3257 mg NH_3-N/L (final TAN level).

4.3.4 Two-stage versus One-stage Anaerobic Digestion

This set of tests evaluated the potential enhancement of BMP by separating the hydrolysis stage from the methanogenesis (two-stage AD). The hydrolyzed samples generally had higher BMP than the untreated samples, although initial ammonia levels were similar in the cases of untreated and hydrolyzed PM100 and PM50:MS50, whereas TAN in hydrolyzed PM75:MS25 was 15% higher than that in untreated PM75:MS25. Figure 4-2 shows the cumulative methane production from untreated and hydrolyzed samples. Separated hydrolysis (two-stage system) of PM100 and PM75:MS25 led to a 21 and 41% increase in methane potential, respectively. On the other hand, the hydrolysis of PM50:MS50 did not impact the methane yield. The increase of BMP indicates that organic compounds were more readily biodegradable due to the separate hydrolysis stage. Methane content constituted 42, 41, and 35% of the biogas from hydrolyzed PM100, PM75:MS25, and PM50:MS50, respectively.

The increase in methane yield reported in the literature such as in (Baldi et al., 2019; Tsigkou et al., 2020) were in the range of 18-26% for substrates characterized with low ammonia nitrogen like food waste and sludge. Compared with the literature, the observed enhancement of methane potential was greater than what was perceived due to the high ammonia levels in the substrates. Nevertheless, methane potential enhancement in PM75:MS25 was 58% greater than what was reported in the literature whereas methane enhancement in PM100 was in the reported range.

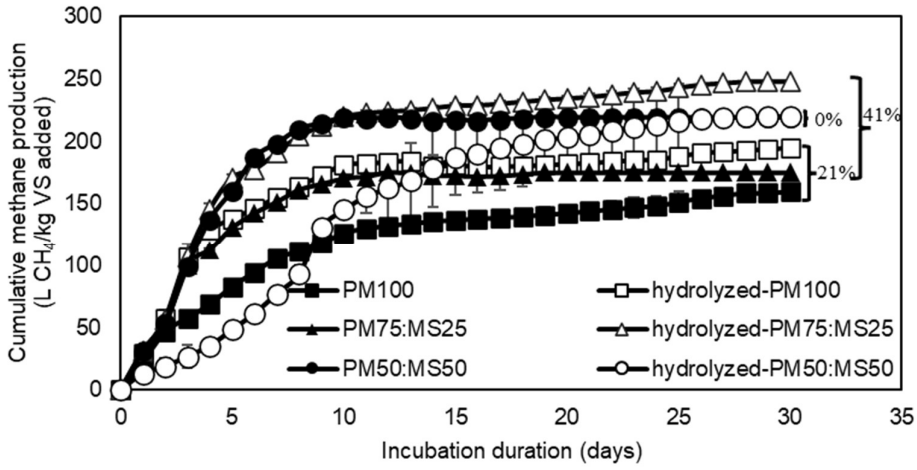


Figure 4-2: Cumulative methane potential of untreated and hydrolyzed samples

Figure 4-3 shows the results of final ammonia levels and BMP to illustrate the coupled effect of co-digestion, ammonia levels, and two-stage AD. Although ammonia levels were higher in hydrolyzed samples, they achieved higher methane potential due to the enhanced solubility and biodegradability of the organic compounds. The improvement of methane potential in hydrolyzed samples does not necessarily indicate that the process was not inhibited by elevated ammonia levels, but it indicated that other factors contributed to methane production.

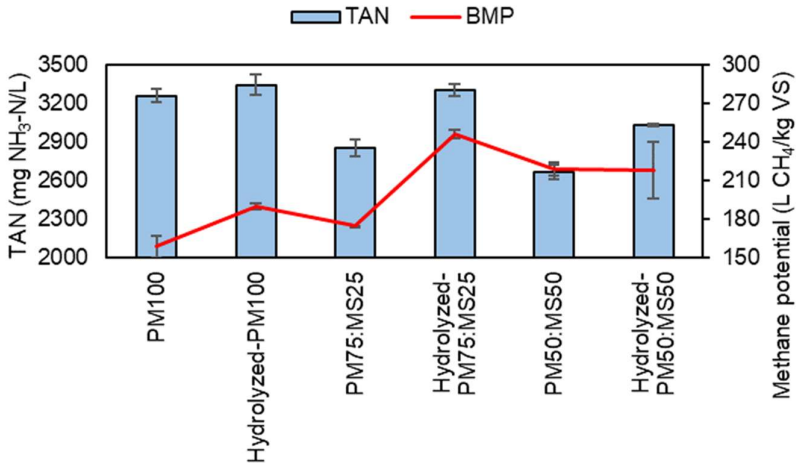


Figure 4-3: Final ammonia levels and BMP of untreated and hydrolyzed samples.

4.3.5 Ammonia Stripping Pretreatment

This phase included ammonia stripping as a pretreatment step to improve the methane potential of the substrates. PM100, PM75:MS25, and PM50:MS50 samples were hydrolyzed for six days at mesophilic conditions, after which the pH and temperature were raised to 10 and 55 °C. The stripping process of the hydrolyzed samples was performed at an airflow of 300 l air/l

digestate/hr for six hours. Ammonia levels were reduced by 79.2, 69, and 78.6% in PM100, PM75:MS25, and PM50:MS50, respectively (Figure 4-4). Ammonia removal rates were highest in the first two hours of the stripping and then reduced significantly. The removal efficiency was limited due to the drop in pH which was caused by the release of H^+ when NH_3 was formed to satisfy the NH_3/NH_4^+ equilibrium at the operating temperature and pH. Ammonia removal efficiency results were in line with (Bonmatı and Flotats, 2003; Rodriguez-Verde et al., 2018) although stripping conditions were different. The final TAN levels after stripping were between 574 and 876 mg TAN/L, which is well below inhibitory levels reported by literature (Abouelenien et al., 2016). The drop in pH throughout the ammonia stripping worked in favor of the succeeding BMP process as no further pH adjustment was required.

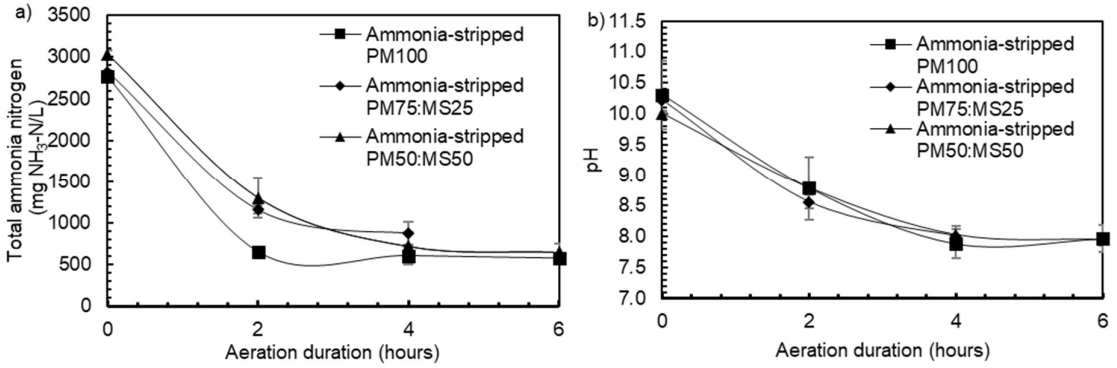


Figure 4-4: Total ammonia nitrogen (a) and pH (b) during ammonia stripping at a temperature of 55 °C and a flow rate of 300 l air/l digestate/hr.

Methane potential in ammonia-stripped PM100, PM75:MS25, PM50:MS50, was 454, 438, and 360 L CH_4/kg added VS with methane forming 48, 37, and 49% of the total biogas, respectively. These values translate to 200, 150, and 64% improvement in methane potential when compared to their respective untreated samples (Figure 4-5). However, methane yield of ammonia-stripped samples was improved by 141, 80, and 67% in PM100, PM75:MS25, and PM50:MS50, respectively when compared with hydrolyzed samples. The significant improvement in methane potential in all samples is an indication that the digestion of untreated and hydrolyzed samples was partially inhibited due to high ammonia levels. The results showed the feasibility of mono-digestion of poultry manure after ammonia stripping at pH 10 and a temperature of 55 °C.

Part of the biogas enhancement in the ammonia-stripped samples when compared with the untreated samples was due to increasing the temperature of the samples to 55 °C during the ammonia stripping. This worked as a form of thermal pretreatment, and previous studies such

as (Lu et al., 2008) proved that thermal pre-treatments of hydrolyzed samples can increase methane potential by up to 30% when compared to untreated samples.

Although ammonia removal efficiency was higher in PM50:MS50 when compared with PM75:MS25, the corresponding BMP enhancement was almost three times higher in PM75:MS25 (150% versus 64%). This indicates that all samples were below inhibitory levels after ammonia stripping. Moreover, the 64% increase in methane potential of PM50:MS50 due to ammonia stripping could denote that the methanogenic activities were partially inhibited at TAN levels of 2660 mg TAN/L.

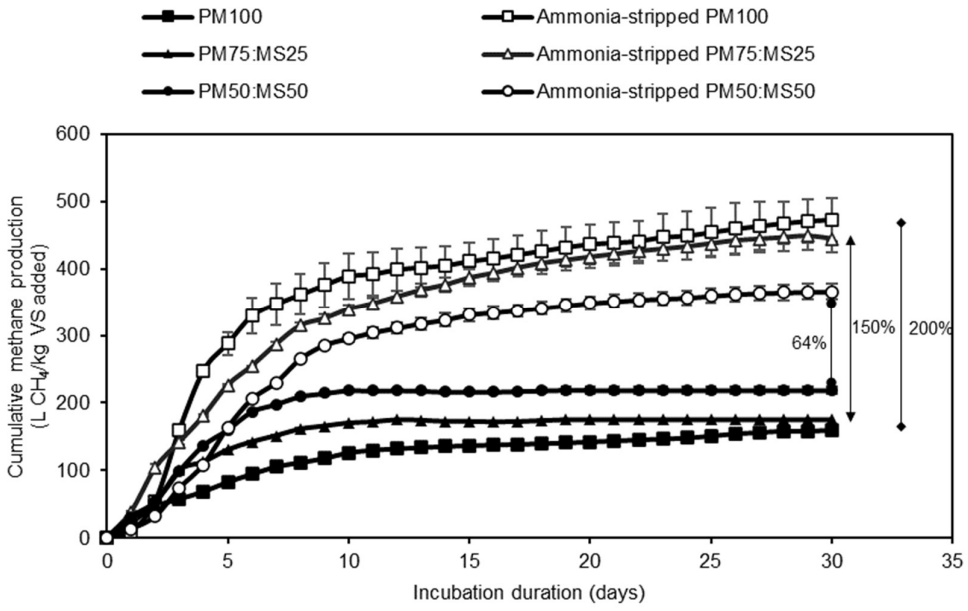


Figure 4-5: Cumulative methane potential of untreated and ammonia-stripped samples.

Poultry farms that produce larger amounts of poultry manure compared with other organic wastes can use the results of this study to transform from conventional treatments to a more sustainable and economically viable option. Outputs of this process are not limited to the biogas and digestate; a high-grade fertilizer can be produced by capturing stripped ammonia using sulfate scrubbers forming ammonium sulfate (Abouelenien et al., 2010; Fuchs et al., 2018). However, since the results presented in this study are from batch experiments, more experiments should be performed on a semi-continuous or continuous flow basis to ensure the method can provide stable operation of the anaerobic digester. Moreover, more conditions of stripping can be tested to optimize the cost of the suggested treatment.

4.3.6 Post-BMP Characterization

The digestate from all the bottles was characterized for TS, VS, total alkalinity, VFAs, and TAN. Table 4-2 shows the results of the post-BMP characterization. Two-stage AD and ammonia stripping enhanced the VS reduction in all tested scenarios. Nevertheless, VS reductions were in the range of 23-39%, which is lower than what is reported in the literature (Nie et al., 2015a). In terms of co-digestion of untreated samples, increasing the amount of MS significantly improved the VS reduction until PM50:MS50, after which further VS reduction became less noticeable. This was in line with (Borowski et al., 2016) when testing the co-digestion of sugar beet pulp residues with PM under different ratios.

Two-stage AD reduced VFAs in the digestate of co-digested samples, except in hydrolyzed-PM100 where VFAs were 66% higher than that of untreated PM100. Taking into consideration the low methane potential and the low VFAs in untreated PM100, it can be concluded that both acidogenic and methanogenic activities were inhibited due to high ammonia levels, which caused organic compounds to remain in less biodegradable forms. However, co-digestion alleviated the inhibition of acidogenic microorganisms in all co-digestion samples.

Ammonia levels were initially higher in hydrolyzed samples compared with untreated samples, and therefore the increase in TAN levels during the BMP was significantly lower in hydrolyzed samples as it started with less organic nitrogen. TAN levels increased up to 203% in untreated samples during methanogenesis compared to a maximum increase of 50% in hydrolyzed samples, which could explain why inhibition was more impactful in untreated samples although ammonia levels were lower than that of hydrolyzed samples.

Table 4-2: Characterization of the digestate from BMP. (Mean values ± standard deviation of duplicates).

Sample treatment	TS	VS (reduction)	Total Alkalinity	VFAs	TAN (increase)
	%	%	mg $CaCO_3$/L	mg CH_3COOH/L	mg TAN/L (%)
Untreated PM100	5.01±0.03	3.45±0.01 (22.99±0.1)	16953±3819	647±95	3258±51 (203±4.2)
Hydrolyzed PM100	4.25±0.08	2.78±1.16 (28.49±2.72)	12863±132	1077±45	3343±80 (-9±5)
Ammonia-stripped PM100	4.9±0.11	2.92±0.06 (26.83±0.03)	15070±42	1281±18	2596+43 (49±4)
Untreated PM75:MS25	4.72±0.09	3.24±0.09 (27.69±0.14)	30576±2174	1287±43	2854±65 (191±5)
Hydrolyzed PM75:MS25	4.43±0.06	2.98±0.05 (28.41±0.59)	12928±73	1067±57	3300±46 (31±2)
Ammonia-stripped PM75:MS25	4.36±0.06	2.63±0.03 (36.74±0.81)	10119±344	689±32	2072+52 (79±13)
Untreated PM50:MS50	4.43±0.06	3.09±0.06 (30.85±1.49)	18692±797	1237±88	2666±58 (198±7)
Hydrolyzed PM50:MS50	4.22±0.09	2.92±0.05 (35.07±0.78)	11424±50	955±37	3032±10 (50±16)
Ammonia-stripped PM50:MS50	4.51±0.06	2.74±0.05 (39.61±0.92)	10073±54	1186±0	1985+55 (114±5)
Untreated PM25:MS75	4.18±0.08	2.95±0.07 (33.75±0.45)	10057±626	1225±87	2340±25 (198 ± 10)
Untreated MS100	3.94±0.07	2.9±0.09 (33.91±1.2)	8335±91	1231±86	2052±33 (187 ± 1)

4.3.7 Kinetic Modeling for Methane Production

The experimental data of the cumulative methane potential were fitted to the Gompertz model to evaluate the kinetic parameters *P* (L of CH_4/kg VS), *Rm* (L of CH_4/kg VS/day), and λ (days) (Figure 4-6). Gompertz model successfully predicted methane potential with a maximum error of 6.4% in ammonia-stripped PM100. The results of the Gompertz model showed that ammonia stripping had significantly improved biogas production rate. However, the improvement was less noticeable when the MS proportion increased. This indicates that methanogenic activities were more inhibited in the mono-digestion of poultry manure when compared with co-digestion.

Figure 4-6 shows the predicted methane production (shown as a line) and the experimental methane production (shown as data points) and the kinetic parameters of the Gompertz model.

Correlation between the model and the predicted values was higher in untreated and hydrolyzed samples when compared with ammonia-stripped samples; Figure 4-6c shows that the deviation between the model and experimental values increased with time in ammonia-stripped PM100 and PM75:MS25. Another indication of the correlation is the R^2 (shown in Figure 4-6) which was relatively smaller in those samples compared with the untreated samples.

The model results also helped in quantifying the undistinguishable lag time in the samples; hydrolyzed-PM50:MS50 had an S-shaped curve in Figure 4-6b that indicates a lag in methane production, which can be estimated as 2.2 days from the Gompertz model. Longer lag times were generally observed in samples containing more MS which could be translated as the acclimation time of the inoculum to the new type of substrate.

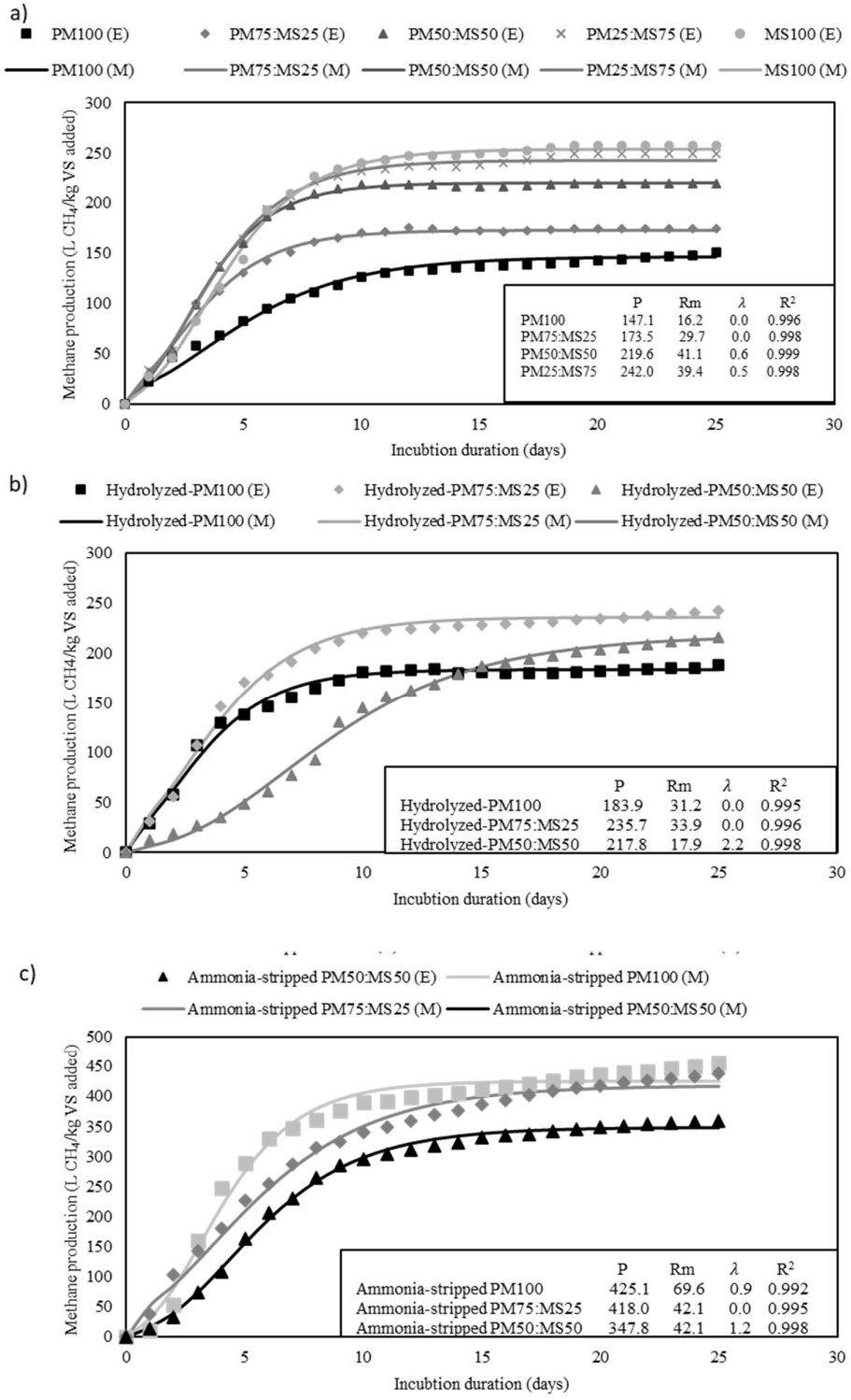


Figure 4-6: Gompertz model parameters and cumulative methane production by Gompertz model prediction (M) versus experimental results (E). a) mono- and co-digestion of untreated samples, b) hydrolyzed samples, and c) ammonia-stripped samples.

4.3.8 Conclusion

This study evaluated the possible methane potential enhancement due to separating hydrolysis from methanogenesis, co-digestion, and post-hydrolysis ammonia stripping from poultry manure. Co-digesting PM with MS at 25:75 achieved the highest methane potential among the three co-digestion ratios. Moreover, the synergistic effects of PM75:MS25, PM50:MS50, and PM25:MS75 were 0.97, 1.08, and 1.09, respectively. This indicates that co-digestion alone may not alleviate the inhibitory effects and the process will run at sub-optimal conditions. Two-stage AD enhanced the BMP of PM100 and PM75:MS25 by 22%, 42%, while no enhancement was observed in the case of PM50:MS50. Ammonia stripping after pH and temperature adjustment was able to remove 79.2±0.2, 69±4.7, and 78.6±2.9% of the ammonia in PM100, PM75:MS25, and PM50:MS50, respectively, which resulted in improvement of the methane potential of PM100, PM75:MS25, PM50:MS50 by 200, 150, and 64% when compared to their respective untreated samples. Overall, the results of the study showed that co-digestion, hydrolysis, and ammonia stripping can be combined to alleviate the inhibitory effect of ammonia and enhance the methane potential by avoiding the operation at sub-optimal conditions.

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References

The references of this Chapter are merged with the rest of the document and presented in Chapter 10: References

CHAPTER 5: POST-HYDROLYSIS AMMONIA STRIPPING AS A NEW APPROACH TO ENHANCING THE TWO-STAGE ANAEROBIC DIGESTION OF POULTRY MANURE: OPTIMIZATION AND STATISTICAL MODELING

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Since Post-hydrolysis ammonia stripping was proven successful in Phase I, the objective of phase II was to test different operating conditions to better understand the effects of pH and temperature at fixed stripping medium and flow rate. This phase also contains statistical analysis using ANOVA to determine the significance of each operational parameter i.e., pH and temperature, on the biogas production.

Abstract

Post-hydrolysis ammonia stripping was investigated as a new approach to enhance the methane potential of high ammonia substrates, such as poultry manure. The purpose of the proposed approach is to address some of the noticeable disadvantages in the existing ammonia stripping techniques, i.e., treatment of raw samples and side-stream stripping. Poultry manure (PM) and a co-substrate characterized with high COD/TAN ratio (MS) were mixed at three different ratios: PM:MS of 100:0, 75:25, and 50:50. Samples were hydrolyzed for six days to promote ammonia conversion from organic nitrogen and then stripped with air under pH values of 9 and 10 and temperatures of 40 and 55 ^{o}C. Biochemical methane potential (BMP) test results showed that post-hydrolysis ammonia stripping had alleviated ammonia inhibition and improved methane potential up to 200% when compared with untreated samples. The ammonia removal efficiency was mostly driven by pH. On the other hand, methane potential was highest in the samples treated at a higher temperature as their biodegradability was enhanced when compared with samples treated at lower temperatures. Post-BMP characterization showed that the proposed approach had also limited the increase of ammonia in the digester which ensured proper growth of methanogenic microorganisms.

Keywords:

Ammonia stripping, co-digestion, biochemical methane potential, statistical modeling.

5.1 Introduction

Anaerobic digestion (AD) of organic waste is a viable technology that reduces the carbon footprint and greenhouse gas emissions and generates revenues by producing biogas, which is

the primary source of sustainable bioenergy, and selling the digestate as a fertilizer (Ara et al., 2015; Nguyen et al., 2019). AD process performance and stability depend on several factors including feedstock characteristics (organic content, nutrients availability, nitrogen content, etc.) and operational conditions (pH, moisture content, temperature, loading rate, etc.) (Rajagopal et al., 2013; Rocamora et al., 2020). Therefore, some organic waste streams can be unsuitable feedstock for anaerobic digesters. One such case is high-nitrogen organic wastes such as poultry manure.

Poultry manure (PM) generation has been on the rise due to the continuous growth of the poultry population on a global scale (Michalk et al., 2019; Tauseef et al., 2013). However, PM is often avoided in AD processes as its high ammonia levels can lead to inhibition and instability of the AD process. The total ammonia nitrogen (TAN) in solutions is present in two forms, ionic (NH_4^+) and free ammonia nitrogen (NH_3 or FAN); where the FAN/TAN ratio is directly proportional to the pH and temperature of the solution. FAN is commonly known for being the inhibitory form of ammonia to the microbial populations in the digester and it mainly affects methanogenic activities and their growth rate (Akindele and Sartaj, 2018b; Fotidis et al., 2014; Fuchs et al., 2018).

Several studies have investigated co-digestion (Borowski et al., 2016; Wang et al., 2012) or two-stage anaerobic digestion of poultry manure (Lu et al., 2008; Yin et al., 2019) to enhance its digestibility and avoid the anticipated inhibition, however, the inhibition of the microbial microorganisms due to ammonia cannot be completely avoided without further treatment. While methods such as ultra-sonication, struvite precipitation, and acclimation of microorganisms can be used as further treatments to alleviate ammonia inhibition, these treatments are often accompanied by high operational cost and challenges and/or long startup times (Cho et al., 2014; Tian et al., 2018; Wang et al., 2016). Therefore, recent research has been focused on ammonia stripping as an economic and feasible solution to removing ammonia from poultry manure.

Ammonia stripping concept relies on increasing the volatility of the ammonia by increasing the FAN/TAN ratio through increasing pH and temperature in addition to the use of a carrier gas such as air, CO_2, or biogas (Abouelenien et al., 2010; Adghim et al., 2021; Zhang et al., 2012). The method was proven to achieve high ammonia removal efficiencies of more than 80% accompanied by improvement in methane yield that could reach up to 200%. (Adghim et al., 2021; Zhang et al., 2012, 2017). Ammonia stripping also promotes nutrient recovery from the feedstock through capturing ammonia from the carrier gas using scrubbers or traps such as

sulfuric acids, forming high-grade fertilizers that can be used for agricultural purposes (Fuchs et al., 2018).

Two concepts of ammonia stripping have been studied in the literature: 1) ammonia stripping of raw feedstock, and 2) side-stream stripping of digestate. The first approach is when the raw feedstock is stripped by a carrier gas after pH and/or temperature adjustment to promote the volatility of the ammonia, after which the ammonia-stripped feedstock is fed into the digester for biogas production (Abouelenien et al., 2010; Rodriguez-Verde et al., 2018; Zhang et al., 2012). On the other hand, side-stream ammonia stripping is when a portion of the digestate from the anaerobic digester is filtered and the filtrate is sent to an external column where pH and/or temperature are increased and the digestate is stripped, after which the ammonia-stripped digestate is returned to dilute the ammonia levels in the digester (Serna-Maza et al., 2014; Zhang et al., 2017). A wide range of ammonia removal efficiency and methane yield improvement has been reported for each approach, which makes it challenging to determine whether an approach is more superior. Moreover, each concept has certain shortcomings and research is still undergoing to resolve associated operational challenges such as clogging and effective solid/liquid separation methods (Baldi et al., 2018; Bousek et al., 2016).

In addition to the operational challenges of ammonia stripping, some conceptual disadvantages can be noticed in each approach. In the ammonia stripping of the raw feedstock approach, treatment occurs to feedstock when nitrogen is mostly in the form of organic nitrogen; 80% of the organic nitrogen is transformed into ammonia nitrogen after the hydrolysis stage (Serna-Maza et al., 2014; Wang et al., 2018). Therefore, ammonia increases significantly after the feedstock is fed to the digester and can cause inhibition due to the released ammonia. Thus, this approach is feasible when the feedstock does not contain high organic nitrogen or ammonia levels such as food waste and pig manure (Bousek et al., 2016; Zhang et al., 2012).

On the other hand, increasing the temperature and pH of digestate in the side-stream ammonia stripping approach leads to exposure of methanogens to high NH_3 levels for a certain amount of time, which can affect their activities and growth rate. Moreover, the gas in the side-stream stripping approach is mainly limited to CO_2 or biogas which were proved to be less efficient carriers than air. Nevertheless, air cannot be used in side-stream ammonia stripping as it could have a severe impact on the microbial population (Bousek et al., 2016).

Based on the understanding of the shortcomings of the existing ammonia stripping approaches, this research aims to propose and provide a proof-of-concept, and optimize a new approach called post-hydrolysis ammonia stripping. This approach will enable two-stage AD plants to

take advantage of separating hydrolysis from the methanogenesis stage by the following: 1) targeting ammonia after most organic nitrogen is biologically transformed to ammonia through hydrolysis, 2) using air as the carrier gas which ensures high removal efficiency, 3) avoiding the exposure of digestate to high FAN levels to ensure the methanogens remain unaffected, and 4) to limit further increase of ammonia in the digester.

To the best of the authors' knowledge, this would be the first study to discuss this approach and evaluate its feasibility. The proof-of-concept was established by the authors in (Adghim et al., 2021), and this work presents the optimization stage of this approach. The closest study to this concept was conducted by (Yin et al., 2019) where the effect of continuous ammonia stripping during hydrolysis as a mean to improve the hydrolysis under hyper-thermophilic temperature (70 ^{o}C) was investigated. However, the objectives and the proposed methodology of this study significantly differ from (Yin et al., 2019). The specific objectives of this study were to 1) enhance the bio-methane potential of poultry manure by alleviating ammonia inhibition using post-hydrolysis ammonia stripping, 2) test different conditions of ammonia stripping to determine optimal operating conditions, 3) assess the coupled effect of ammonia stripping and co-digestion of poultry manure with low-nitrogen co-substrate, and 4) implement response surface methodology to understand the statistical significance of the operating conditions i.e. pH, temperature, and co-digestion ratio.

5.2 Materials and Methods

5.2.1 Substrates and Inoculum

Poultry manure (PM) was collected from a local farm in Ottawa, Canada. A mixture of substrates (MS) that consists of ground coffee and cheese factory wastewater was collected from a biogas plant and used as a co-substrate to test different scenarios of co-digestion and ammonia stripping. The inoculum (I) was collected from an outlet of an anaerobic digester running on cow manure and corn silage. All samples were stored at 4 ^{o}C throughout the course of the experiment.

5.2.2 Post-hydrolysis Ammonia Stripping Experiment Setup

The ammonia stripping process started after the hydrolysis of the samples for six days at a temperature of 40 ^{o}C and a shaking speed of 100 rpm to allow the organic nitrogen conversion to ammonia nitrogen. The samples constituted mixtures of PM and MS at different volatile solids (VS) mass ratios of PM:MS 100:0, 75:25, 50:50, 25:75, 0:100. All samples were diluted

to 10% total solids (TS) with deionized water to facilitate hydrolysis and ammonia stripping. Total ammonia nitrogen (TAN) was monitored daily during the hydrolysis and the samples with low final ammonia levels were excluded from the ammonia stripping.

Five different conditions were tested to evaluate the efficiency of the ammonia stripping from the hydrolyzed samples: 1) pH and temperature remained unadjusted (Base; pH around 7.6 and temperature at 25 ^{o}C) or pH and temperature were increased to 2) 9 and 40 ^{o}C, 3) 10 and 40 ^{o}C, 4) 9 and 55 ^{o}C, and 5) 10 and 55 ^{o}C, respectively. The temperature was increased by placing the bottles in a water bath and pH was adjusted by adding lime ($Ca(OH)_2$) after the targeted temperature was met. A flexible tube was inserted to the bottom of the bottle and the other end was connected to an air supply valve. A diffuser stone was attached to the tube's end to create finer air bubbles. The air flow rate was set at 300 l air/l feedstock/ hour for four to six hours (Bousek et al., 2016). Ammonia and pH were measured every two hours to determine the ammonia removal rate and to understand the impact of stripping on pH. All hydrolysis and ammonia stripping assays were run in duplicates.

5.2.3 Batch Biochemical Methane Potential

A Batch biochemical methane potential (BMP) test was conducted to evaluate the impact of post-hydrolysis ammonia stripping on methane production. BMP test included three sets of samples: 1) untreated and non-hydrolyzed samples, 2) untreated hydrolyzed samples, and 3) ammonia-stripped samples. The first two sets imitate one-stage and two-stage AD, respectively, and the third set included all the samples treated as described in section 5.2.2. The inoculum was added to the samples at VS_i/VS_s value of 1 to 2 and the bottles were purged with nitrogen to ensure anaerobic conditions were attained. Biogas production was measured daily using water displacement and the methane content was measured every week using gas chromatography (GC). BMPs were maintained until the biogas production rate was insignificant. The results enabled different comparisons including one-stage versus two-stage anaerobic digestion, mono-digestion versus co-digestion, and untreated samples versus ammonia-stripped samples.

5.2.4 Analytical Methods

Total solids (TS) and volatile solids (VS) were determined using standard method no. 2540 (APHA, 2005). Volatile fatty acids (VFAs) were measured using Hach TNT872 (50-2500 mg CH_3COOH/L); total and soluble chemical oxygen demand (COD and sCOD) were measured using Hach TNT822 (20-1500 mg COD/L); total alkalinity was measured using Hach TNT870

(25-400 mg $CaCO_3$/L). TAN was measured using the Phenate method and verified by Hach TNT (2-47 mg TAN/L). Methane content in the biogas was measured using Gas Chromatography (GC) where helium was used as a carrier gas, the temperatures of the oven, indicator, and inject port were set to 120, 120, and 130 ºC, respectively, and the current was set to 100 mA.

5.2.5 Statistical Analysis

Analysis of Variance (ANOVA) testing of the experimental results was conducted using Design Xpert software to determine the statistical significance of pH, temperature, and MS:PM ratio on the ammonia removal efficiency by determining the P- and F-values of those parameters. The levels that were considered in the analysis are 1) pH 9 and 10, 2) temperatures 40 and 55 ºC, and 3) MS/PM of 0, 0.33, 1 which corresponds to PM100, PM75:MS25, and PM50:MS50, respectively. The testing also discloses the types of errors that occurred during the experiment and describes its randomness. Outliers are checked against the externally studentized residual, which is the deleted residual divided by its estimated standard deviation. Moreover, a polynomial regression equation of ammonia removal efficiency can be obtained from statistical modeling which will allow point estimation of ammonia removal efficiency at various pH, temperature, and MS:PM content.

5.3 Results and Discussion

5.3.1 Samples Characteristics

The samples were characterized shortly after collection to ensure characteristics did not change (Table 5-1). Poultry manure (PM) had a high pH of 8.7 and was also characterized by high ammonia levels of 3843 mg TAN/L, which makes PM a non-favorable feedstock to biogas plants due to the anticipated inhibition. The pH of PM was in the higher end of the range reported by literature (Borowski et al., 2016; Khoufi et al., 2015). Moreover, the TAN of PM was already greater than the inhibitory levels (1500-3000 mg TAN/L) reported by literature (Fotidis et al., 2014). Nevertheless, PM can be utilized when appropriate co-substrates are used or when subjected to pretreatment. Therefore, MS was used as a co-substrate due to its high COD/TAN ratio. The high volatile fatty acids (VFA) in MS indicate that it is more readily biodegradable when compared with PM. Moreover, mixing MS with PM can also affect the amount of lime needed to adjust the pH as the total alkalinity of MS is 94% less than that of PM. Unlike MS, the high solid contents value of the PM makes it impractical to hydrolyze and

aerate which gives another advantage of mixing the two substrates by reducing the amount of dilution required. The inoculum had a TAN level of 813 mg TAN/L which indicates that the microorganisms are acclimated to this level of ammonia.

Table 5-1: Characteristics of substrates and inoculum

Characteristics	Unit	PM	MS	I
VFA	mg CH_3COOH/L	2515 ± 106	9660 ± 170	878 ± 49
TAN	mg TAN/L	3843 ± 425	454 ± 1	813 ± 13
COD	mg COD/L	13017 ± 355	25750 ± 71	48550 ± 495
sCOD	mg COD/L	5400 ± 87	12517 ± 104	6465 ± 21
Total Alkalinity	mg $CaCO_3$/L	17360 ± 640	1001 ± 49	7740 ± 187
Total Solids	%	26.43 ± 0.62	10.84 ± 0.18	4.37 ± 0.03
Volatile Solids	%	17.43 ± 0.62	9.82 ± 0.18	3.27 ± 0.03
VS/TS	%	65.92 ± 2.51	90.58 ± 0.11	74.83 ± 0.11

5.3.2 Hydrolysis

Ammonia increased significantly in all samples during the six days of hydrolysis due to the conversion of organic nitrogen into ammonia nitrogen (Figure 5-1). The increase in ammonia was 42, 67, 7, and 77% in PM100, PM75:MS25, PM50:MS50, PM25:MS75, respectively. On the other hand, ammonia in MS100 reduced to 33% of its initial level. This indicates that the organic waste in MS was more readily biodegradable when compared with PM and that TAN may have been consumed by microorganisms to grow. This was also noticed in the case of PM100 where TAN levels dropped by 14% after the first two days of incubation before it increased significantly afterward.

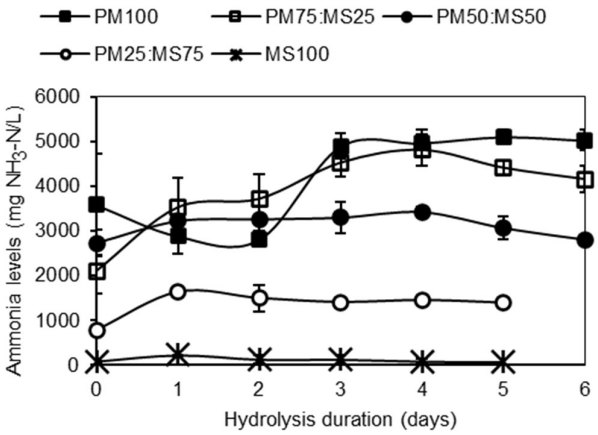

Figure 5-1: Ammonia levels during the hydrolysis stage.

The final ammonia levels were in the range of 2800 to 5100 mg TAN/L in PM100, PM75:MS25, PM50:MS50, which is expected to cause inhibition when added to the digester. On the other hand, TAN levels of PM25:MS75 and MS100 were below 1400 mg TAN/L, which is lower than the inhibitory levels reported in the literature (Abouelenien et al., 2010; De la Rubia et al., 2010). In the light of these results, PM25:MS75 and MS100 were not included in the ammonia stripping stage and the anaerobic digestion of hydrolyzed samples.

The pH of all samples dropped to a range of 6.3- 6.9 which was suitable for BMP after the addition of inoculum without the need to adjust the pH. The drop in pH indicates that the hydrolysis step was successful and indicated the formation of VFAs (data not measured), which was accompanied by some gas production (>90% CO_2). The VS reductions in PM100, PM75:MS25, and PM50:MS50 were 11.77±0.96, 19.68±4.66, and 12.51±1.67%.

5.3.3 Ammonia Removal

Ammonia stripping was performed on the hydrolyzed samples of PM100, PM75:MS25, and PM50:MS50 under different pH and temperature conditions. The increase in pH and temperature aimed to increase the FAN/TAN ratio to increase the volatility of ammonia. Figure 5-2: Final ammonia over initial ammonia (TAN/TAN_i) and pH levels during ammonia stripping of a) PM100, b) PM75:MS25, and c) PM50:MS50.. Ammonia levels started at around 4890, 3450, and 3040 mg TAN/L in PM100, PM75:MS25, and PM50:MS50, respectively. Ammonia stripping successfully reduced ammonia levels in most cases, however, the BMP test determined if these levels were inhibitory to the microorganisms' activities. All samples with an initial pH of 10 showed higher ammonia removal (average of 73%) than those set at pH 9 (average of 36%) or unadjusted (average of 14%). The ammonia removal efficiency in this study falls in line with the values reported in the literature (Bousek et al., 2016; Rodriguez-Verde et al., 2018; Zhang et al., 2012). This indicates that, in terms of ammonia stripping, the proposed approach i.e., post-hydrolysis ammonia stripping, did not encounter any different challenges when compared with the ammonia stripping of raw feedstock.

Increasing the pH was more effective than increasing temperature for removing ammonia as the removal efficiency increased on average by 74, 136, and 143% when pH was increased from 9 to 10 in PM100, PM75:MS25, and PM50:MS50, respectively; the corresponding average variations when the temperature was increased from 40 ºC to 55 ºC were 48, -10, and 53%. These observations go in line with the kinetics of increasing the volatility of ammonia.

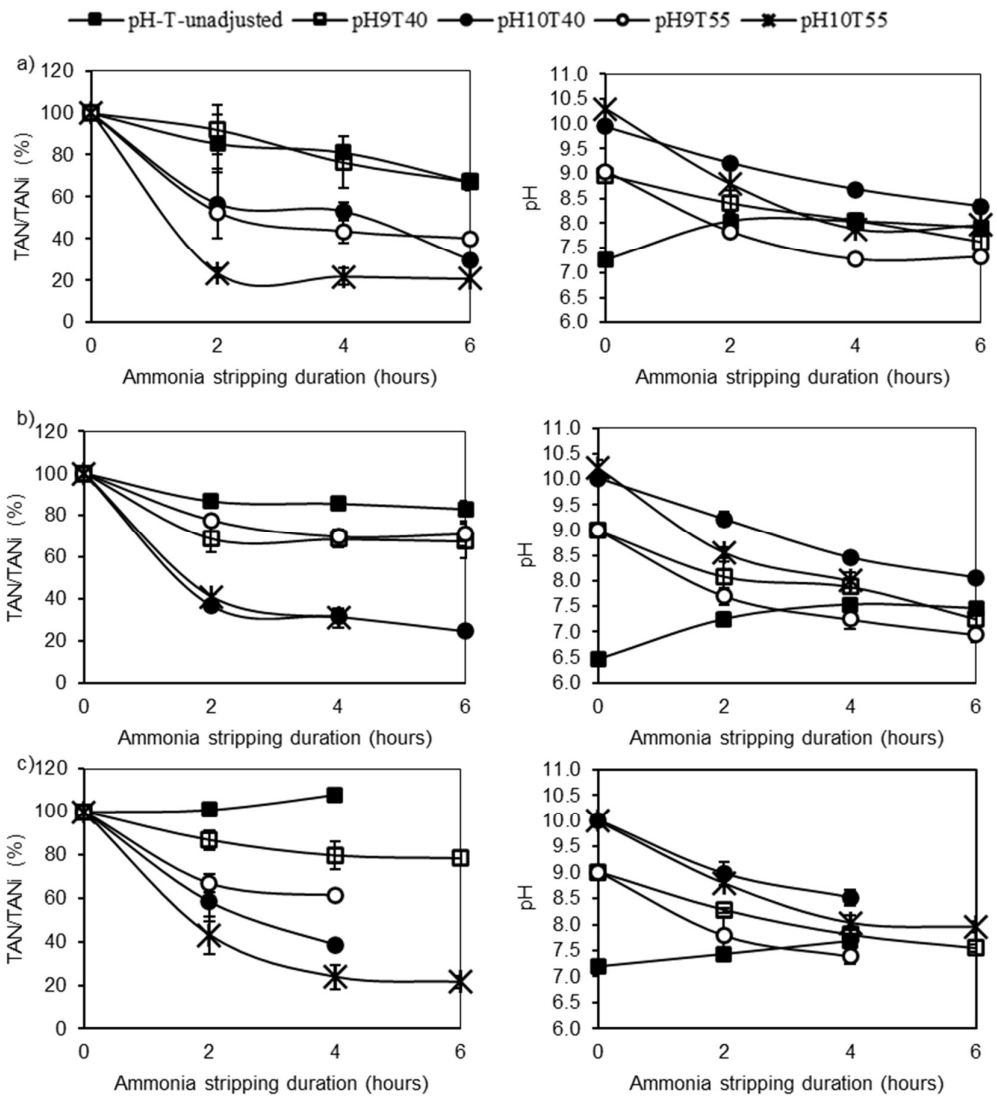

Figure 5-2: Final ammonia over initial ammonia (TAN/TAN$_i$) and pH levels during ammonia stripping of a) PM100, b) PM75:MS25, and c) PM50:MS50.

The ammonia removal efficiency was highest during the first two hours of ammonia stripping, after which it was declining due to the drop in pH, which can be explained by the release of hydrogen ions when NH_4^+ transforms to NH_3 to satisfy the equilibrium at the system's pH and temperature. Although pH drop was a limiting factor for ammonia removal, it provides an advantage when moving the feedstock to the digester as the pH will be in a suitable range for methanogens without the need for adjusting and consequently the addition of acid.

This case was not applicable when the initial conditions of pH and temperature were not adjusted. Instead, ammonia stripping led to a noticeable increase in pH in all this set. This can be explained by the removal of CO_2 which was expected to be present in significant amounts

due to the hydrolysis of the sample and the low pH of those samples. On the other hand, when pH was adjusted to 9 and 10, the amount of CO_2 was insignificant when compared with the amounts of CO_3^{-2} and HCO_3^-, which are the byproducts of the CO_2 and H_2O reactions.

The ammonia removal efficiency for the samples treated at unadjusted pH and temperature showed that the removal efficiency is highly dependent on the level of ammonia in the samples when pH is low. This was observed when comparing the removal efficiencies in the Base samples of PM100, PM75:MS25, and PM50:MS50 which were evaluated at 32, 16, and -7%, respectively. Another factor that had a role in the ammonia removal efficiency of the Base samples is the pH; as pH increased with stripping, it increased the FAN/TAN ratio allowing more ammonia to be stripped. Although PM75:MS25 had a lower initial pH (6.5) than PM50:MS50 (7.2), the stripping process eventually raised both pH values to 7.5 and 7.7, respectively.

The results of the ANOVA showed that all tested variables i.e., pH, temperature, and MS:PM were significant in determining the ammonia removal efficiency (P-value < 0.05). Moreover, a significant model was obtained with the following variables: pH, temperature, MS:PM, pH x Temperature, pH x MS:PM, $(MS:PM)^2$, and temperature x $(MS:PM)^2$.

Figure 5-3 shows the averaged impact of pH, temperature, and MS:PM on the ammonia removal efficiency. The ammonia removal efficiency increased rapidly when pH was increased from 9 to 10. On the other hand, increasing the temperature gradually increased the removal efficiency. Conversely, TAN removal and the proportion of MS in the sample did not have a linear relationship, instead, it showed that TAN removal efficiency was highest when MS:PM was closest to 0 or 1.

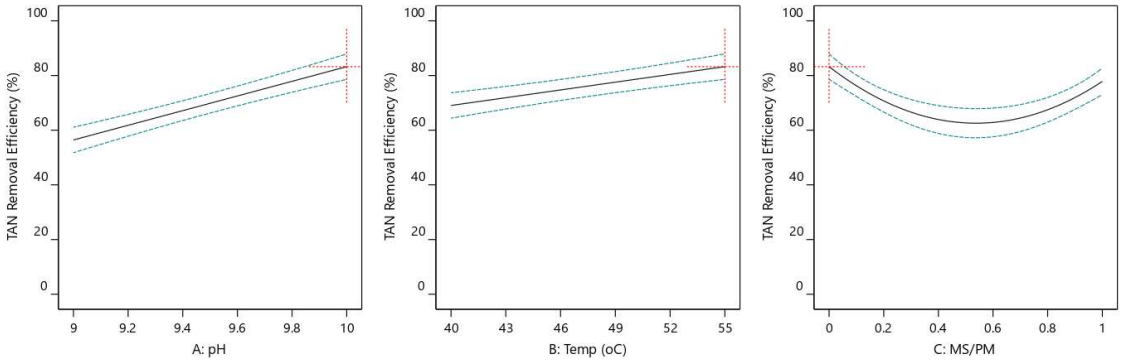

Figure 5-3: ANOVA results of averaged impact at pH 10, temperature 55 °C, and MS:PM= 0 (PM100): A) pH, B) temperature (°C), and C) MS/PM on ammonia removal efficiency.

The experimental results were checked for outliers before the polynomial model was estimated by ANOVA testing. The results of the externally studentized residuals, presented in Figure

5-4a, show that the dataset did not include any outlier (no data points had an externally studentized residual greater than 3.71 or lower than -3.71). Moreover, no pattern is visually observed in the distribution of the results, indicating that systematic errors are minimized, and the present errors are random. Finally, ANOVA model showed that the raw experimental data could be accurately described in a model presented in Eq 7 with a predicted R^2 of 0.945 (Figure 5-4b).

$$TAN\ Removal\ Efficiency\ (\%) = -435.1 + 50.3\,pH + 4.4T - 145.8\frac{MS}{PM} - 0.42\,(pH \times T) + 12.1\left(pH \times \frac{MS}{PM}\right) - 15.4\left(\frac{MS}{PM}\right)^2 + 0.73\,(T \times \left(\frac{MS}{PM}\right)^2)$$

Eq 7

where *pH* is the value set at the beginning of ammonia stripping after pH adjustment (model is obtained for *pH* values of 9 and 10), *T* is the temperature set for the ammonia stripping (model is obtained for *T* equal to 40 and 55 °C), and *MS:PM* is the ratio of MS to PM based on VS masses (model is obtained for *MS:PM* values of 0, 0.33, and 1).

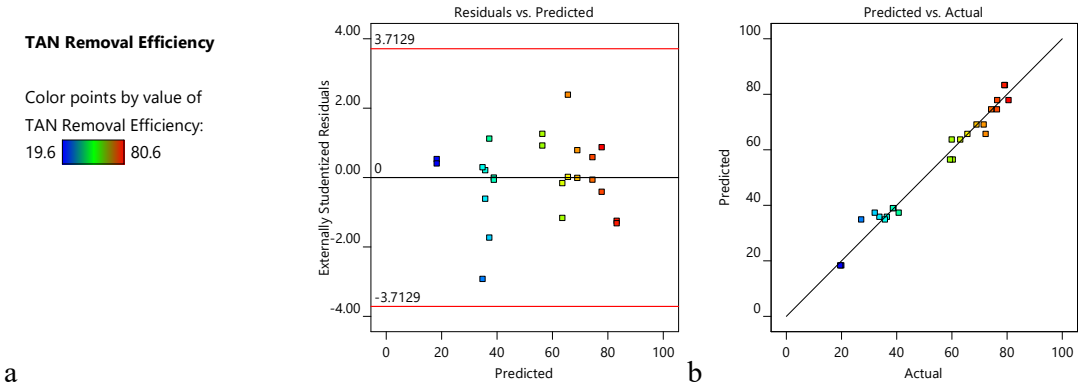

Figure 5-4: a) Externally studentized residuals graph and b) model predicted versus actual removal efficiency graph.

5.3.4 Batch Biochemical Methane Potential

5.3.4.1 Ammonia stripping impact

The impact of ammonia stripping on the methane potential was evaluated by conducting a series of biochemical methane potential (BMP) tests. Ammonia stripping alleviated the inhibitory effects of ammonia on the anaerobic digestion process and enhanced the methane potential. No evidence shows that stripping with air had any negative impacts on the methanogenesis stage. This means that air can be safely used after hydrolysis to remove

ammonia before feeding the reactor. Figure 5-5 shows the cumulative methane potential of the untreated, hydrolyzed, and ammonia-stripped samples.

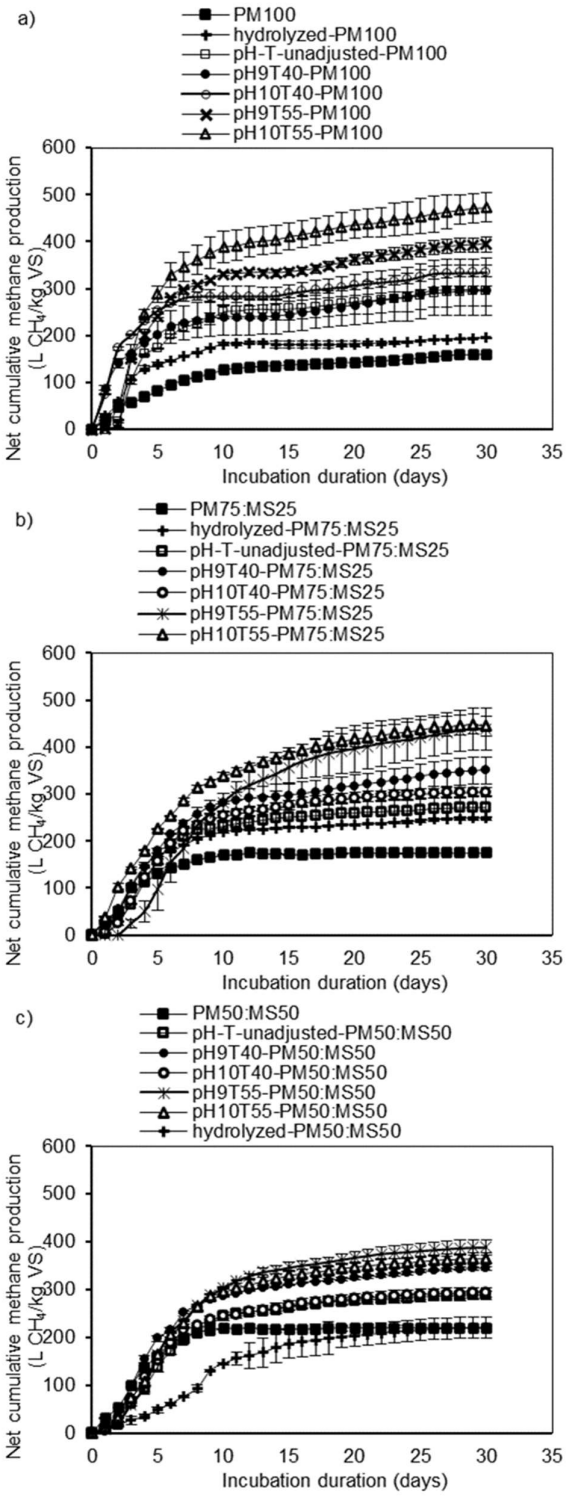

Figure 5-5: Cumulative methane potential of untreated, hydrolyzed, and ammonia-stripped sample. a) PM100, b) PM75:MS25, and c) PM50:MS50

Samples treated at a higher temperature i.e., 55 ºC yielded higher methane potential when compared to other samples even though ammonia levels were slightly higher in some cases. For instance, pH9T55-PM100 had a methane yield of 392 L CH_4/kg VS, whereas pH10T40-PM100 had a methane yield of 334 L CH_4/kg VS. The same pattern was observed in the corresponding samples of PM75:MS25 and PM50:MS50. Therefore, it can be concluded that methane yield enhancement in the treated samples was not necessarily affected by the ammonia removal efficiency or ammonia levels alone. This is because the exposure of the samples to 55 ºC for four to six hours could increase their biodegradability when compared with those treated at 40 ºC or unadjusted (room) temperature (Lu et al., 2008). The contribution of the thermal pretreatment to the enhancement of methane yield was estimated at 41% on average of all tested scenarios, while the remaining 59% was due to ammonia stripping. There was no evidence that the addition of lime had any inhibitory effects similar to what was reported by (Zhang et al., 2012) due to the addition of NaOH in their case.

Samples stripped at pH 10 and temperature of 55 ºC had up to 197 and 150% more methane potential when compared with the untreated and hydrolyzed samples, respectively. This indicates that methane production from untreated and hydrolyzed samples was partially inhibited due to high ammonia levels. Methane yield from untreated PM100, PM75:MS25, and PM50:MS50 was 153, 175, and 219 mL CH_4/g VS, which is in line with the BMP of poultry manure results reported by (Wang et al., 2012) and higher than that reported by (Khoufi et al., 2015). It is important to note that ammonia inhibition depends on the acclimation of the inoculum to high levels of ammonia which could explain the variations between the results of different studies. Samples with higher MS content tended to have lesser improvement of methane yields as ammonia levels were lower when compared with those of high PM content i.e., PM100 and PM75:MS25.

5.3.4.2 Co-digestion effect

The biochemical methane potential of the untreated and the hydrolyzed samples were directly proportional to the levels of ammonia. The increase in MS content in the samples served as a dilution to the ammonia levels and assisted in increasing the C/N ratio which led to increasing the methane yield. The synergistic effect of co-digesting untreated PM with PM:MS ratios at 75:25, 50:50, 25:75 ratios yielded 0.97-, 1.08-, and 1.09-fold the calculated methane potential. Therefore, it can be concluded that co-digestion at high PM levels was not successful without further treatments due to the high ammonia levels. Nevertheless, the results show that post-

hydrolysis ammonia stripping can alleviate ammonia inhibition and allow mono-digestion of poultry manure. In fact, the mono-digestion of ammonia-stripped poultry manure showed a higher methane yield than the corresponding co-digestion sets under similar treatments. For example, the most conservative treatment (pH10T55) resulted in methane yields of 468, 448, and 319 L CH_4/kg VS in PM100, PM75:MS25, PM50:MS50, respectively. Figure 5-6 simultaneously shows the effects of co-digestion and ammonia stripping on the methane potential.

While it can be concluded that ammonia stripping led to alleviate the ammonia inhibition from poultry manure and its mono-digestion was feasible after ammonia stripping, co-digestion of PM with MS may provide more advantages such as including various substrates in the digester and reducing the amount of chemicals and/or water needed to dilute the feedstock when the solids content is high.

Although initial and final ammonia levels were greater in the hydrolyzed PM100 and PM75:MS25 samples than untreated samples, the hydrolysis of the organic matter increased its biodegradability and led to increasing the methane potential by 41 and 21%, respectively. On the other hand, hydrolysis of PM50:MS50 did not enhance nor decrease the methane potential when compared with the untreated PM50:MS50. This emphasizes the conclusion that the effect of treatments on methane potential is less noticeable when MS content increased in the samples, which also reflects lower initial ammonia levels.

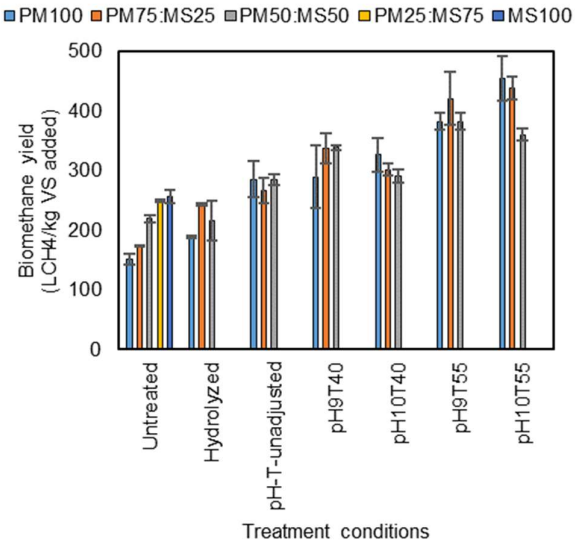

Figure 5-6: Net cumulative methane potential of untreated mixtures of poultry manure and the mixed substrates.

5.3.5 Discussion on Optimization

As the ultimate objective of the treatment is to alleviate inhibition and improve methane potential, the results prove that this is achievable with less than the most conservative treatment i.e., pH 10 and temperature 55 ºC. Optimization of the process must consider the amount of chemicals and energy used to reach a certain pH and temperature, amongst other parameters. For example, an intermediate pH level of 9.5 can be suggested for ammonia stripping. This pH level is high enough to increase the FAN/TAN ratio and would also reduce the duration of the ammonia stripping unit. Moreover, the selection of stripping conditions depends on the ammonia levels of the feedstock and the required removal efficiency to reach sub-inhibitory ammonia levels.

5.3.6 Post-BMP characterization

The digestate from all BMP bottles was characterized to better understand the effect of the different treatments on the characteristics of the digestate. The characterization included solids content, TAN, VFAs, and alkalinity.

Higher VS reduction was achieved in the samples treated at a higher temperature and pH (26-27%) in the case of PM100, compared with the samples treated at 40 ºC (23-24%). The same trend was observed in PM75:MS25 and PM50:MS50, but the difference was not as significant. This observation followed the pattern observed in BMP results. Moreover, ammonia stripping had improved the VS reduction when compared with untreated and hydrolyzed samples. Also, hydrolyzed samples had a slightly higher VS reduction than untreated samples. These improvements are justified by increasing the solubility of the samples due to higher temperatures or the separate hydrolysis of the sample.

There is a distinct effect of co-digestion on VS reduction. More MS content had led to more VS reduction due to its biodegradability, which is significantly higher than PM. For example, the untreated PM75:MS25 and PM50:MS50 showed 20, and 34% more VS reduction than PM100. Introducing the ammonia stripping had increased the VS reduction in all samples but it did not change the effect of co-digestion on VS removal.

Ammonia levels increased in all samples due to further decomposition of organic nitrogen sources in the reactors. Nevertheless, ammonia stripping effectively limited the increase in ammonia when compared with untreated or hydrolyzed samples. Average final ammonia levels in ammonia stripped PM100, PM75:MS25, and PM50:MS50 were 2390, 2330, 2260 mg TAN/L, respectively. This also indicates that the methanogenic archaea were safe at these

ammonia levels. On the other hand, methanogenic archaea were inhibited in untreated and hydrolyzed samples as final TAN levels in the PM100, PM75:MS25, and PM50:MS50 samples averaged 3290, 3050, 3013 mg TAN/L, respectively.

The volatile fatty acids to alkalinity ratio (VFA/ALK) is a common indicator of the stability of the AD process. All the samples had a VFA/ALK of less than 0.15, which indicates that there was no accumulation of VFAs in the bottles, including the untreated and hydrolyzed samples. Ammonia stripping did not have a clear effect in reducing the VFA/ALK despite the addition of lime. However, as lime was added as a slurry, the water had diluted the sample's alkalinity.

5.4 Conclusion

Post-hydrolysis ammonia stripping was investigated as a new approach to potentially alleviate the inhibition of methanogenic archaea due to high concentrations of ammonia. Different conditions of the treatment were considered including different pH, temperatures, and combinations of feedstock. The results showed that the proposed approach successfully alleviated any inhibition and limited further increase of ammonia during the digestion which secured the growth of microorganisms.

Ammonia stripping resulted in higher methane potential and VS reductions from all sample types in addition to enhancing biogas quality by increasing its methane content. Microorganisms were not affected by final TAN levels of 2300 mg TAN/L in the ammonia-stripped samples. On the other hand, untreated and hydrolyzed samples were inhibited at TAN levels of 3300 mg TAN/L. Co-digestion of poultry manure with a mixture characterized with high COD/ TAN ratio had several advantages including reducing alkalinity which reduced the amount of lime needed to adjust the pH for treatment, increasing the solubility and digestibility of the substrates, and reducing the required dilution of feedstock to facilitate its hydrolysis and ammonia stripping.

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References

The references of this Chapter are merged with the rest of the document and presented in Chapter 10: References

CHAPTER 6: THE APPLICATIONS OF RENEWABLE NATURAL GAS IN AMMONIA STRIPPING AND ITS IMPACTS ON MICROBIAL DIVERSITY TO ENHANCE BIOGAS PRODUCTION

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This technical paper compared the feasibility and applicability of carrier gases commonly used, such as biogas and air, in addition to Renewable Natural Gas, which was not discussed in the literature before (Phase III). The technical paper includes results of ammonia stripping, biogas production, as well as microbial analysis conducted at different stages of the experiment.

Abstract

The feasibility of using renewable natural gas for ammonia stripping in anaerobic digestion of poultry manure application was investigated and compared with ammonia stripping with air. Renewable natural gas and air led to comparable ammonia removal efficiencies of up to 60 and 69% under elevated pH and temperature (9.5 and 55 ºC), respectively. The consequential improvement from these treatments on biogas production was 58 and 70% for samples treated with renewable natural gas and air, respectively. Shotgun metagenomic microbial analysis revealed that the hydrogenotrophic pathway was responsible for most biogas production as the archaeal relative abundance of *Methanoculleus bourgensis* was about 95% in all digestates. Moreover, ammonia stripping did not impact the diversity of the microbial consortia but decreased the inhibitory effects on hydrogenotrophic archaea.

Keywords:

Ammonia stripping; renewable natural gas; poultry manure; anaerobic digestion; hydrolysis

6.1 Introduction

Anaerobic digestion (AD) is a well-known strategy for treating organic wastes such as animal manure and food waste to produce renewable biogas that can be used for energy and heat in an environmental-friendly approach (Ara et al., 2015; Carabeo-Pérez et al., 2021; Fernandez-Gonzalez et al., 2019). As the driving force of AD is microorganisms activities and growth, researchers in recent years have investigated some of the inhibitors to these factors (Akindele & Sartaj, 2018; Tian et al., 2018; X. Wang et al., 2018). One of the main inhibitors to methanogenic archaea activities is ammonia, specifically free ammonia nitrogen (FAN), which

is highly present in agricultural or animal waste and is a widely recognized setback when digesting poultry manure (PM) (Adghim et al. 2021; Reyes et al. 2021; Rodriguez-Verde et al. 2018).

Several methods have been proposed to alleviate the ammonia inhibition issue in the AD of PM (Fernandes et al., 2017; S. Wang et al., 2016; Yin et al., 2019). However, ammonia stripping appears to be the most economical and efficient method of treatment (Fuchs et al., 2018; H. Huang et al., 2019). One of the most recent advancements in ammonia stripping is post-hydrolysis ammonia stripping, sometimes referred to as pre-digestion ammonia stripping (Adghim et al. 2021; Huang et al. 2019; Walker et al. 2011). In this method, the effluent of the hydrolyzer in two-stage AD operations is subjected to stripping after increasing the pH and/or temperature to increase the volatility of ammonia and improve the ammonia removal efficiency (ARE). One of the distinct advantages of this approach is the flexibility of selecting a stripping medium, which could significantly affect the ARE (Bousek et al. 2016; Walker et al. 2011).

Typical stripping mediums discussed in the literature are air and biogas since they are both readily available in biogas plants (Fernandez-Gonzalez et al., 2019; Zhang et al., 2017). Bousek et al. (2016) compared air with syngas at different CO_2 percentages and found that air can achieve higher ammonia removal efficiencies (>80%), whereas the performance of syngas was inversely proportional to its CO_2 concentration. This observation is well-rooted in the literature due to the abrupt decline in pH caused by the formation of carbonic acid when the CO_2 in the carrier gas reacts with water in the digestate (Sürmeli et al., 2017). To overcome this issue, biogas stripping is conducted under harsh conditions of high pH (>10) and/or high temperature (>65 ^{o}C) and for prolonged periods (>6 hours) (K. Li et al., 2018; Serna-Maza et al., 2014; Zhang et al., 2017). The ARE reported in these studies is often capped at 40-45% due to the low gas flowrate (1-10 $L_{stripping\ gas}/L_{digestate}$/hour). However, stable operations under sub-optimal conditions were still achievable with such treatments.

Although stripping with air solves most of the issues associated with biogas stripping, biogas plant operators could be hesitant to introduce air for ammonia removal, especially at intermediate stages such as post-hydrolysis or side-stream ammonia stripping. Therefore, there is a need to find an effective alternative stripping medium that maintains anaerobic conditions and is readily available in biogas plants. Looking at the performance of biogas stripping in conjunction with the shortcomings, it is perceptible that methane is the effective component of biogas that carries the ammonia outside the system. Therefore, one alternative could be renewable natural gas (RNG), which contains at least 95% v/v methane after biogas

conditioning (S. B. Walker et al., 2018). However, there is no information in the literature about the feasibility or efficiency of using methane or RNG for ammonia stripping of AD digestate. Ammonia stripping with RNG can be a promising advancement in mitigating ammonia-inhibitory effects in AD applications. Moreover, it would enable biogas plant operators to achieve high ammonia removal efficiencies with significantly fewer chemicals (lime), energy, and time than biogas stripping, minimizing the treatment costs and maximizing the revenue.

Identifying microbial organisms in AD systems has recently gained much attention to better understand the optimal conditions and possible inhibitors of different microbial communities (St-Pierre & Wright, 2014; Yang et al., 2019). Biogas production is mainly led by acetoclastic methanogenic archaea (about 2/3rd of biogas production) and hydrogenotrophic methanogenic archaea (about 1/3rd of biogas production) in many biogas plants that run on manure or co-digestion of manure and food waste (K. Li et al., 2018). As acetoclastic archaea are significantly more vulnerable to inhibitors such as ammonia and volatile fatty acids (VFAs) than hydrogenotrophic archaea, the microbial consortia might have a different distribution in ammonia-stressed reactors (Fotidis et al., 2013; Ziganshina et al., 2017). Having said that, there is limited information available on microbial analysis of reactors that run on poultry manure at noticeably higher ammonia levels or reactors coupled with post-hydrolysis ammonia stripping systems.

This research aims to introduce RNG as a possible carrier gas for ammonia stripping applications and to evaluate its impacts on ARE and biogas production of high-nitrogen feedstock such as PM. The research also aims at covering some of the knowledge gaps in the literature regarding microbial analysis of systems coupled with ammonia stripping treatments for high nitrogen feedstock. This research intends to provide poultry farms with a deeper understanding of the renewable energy potential of PM and encourage them to consider AD as a sustainable alternative to direct land application. The specific objectives of this study are to 1) compare the ARE of air vs. RNG under similar operating conditions, 2) analyze and discuss the impact of stripping mediums on the chemical and physical properties of the stripped digestate, 3) evaluate the enhancement of biogas production due to different treatment conditions using batch methane potential tests, and 4) identify and discuss the microbial communities present in the digestate.

6.2 Methodology

6.2.1 Substrates and Inoculum

Poultry manure (PM) samples were collected from layer chickens on an egg farm in Ottawa, Canada. The manure is scraped from the floors and transported via a conveyor to an onsite pile. Since the farm does not implement any bedding systems, the collected manure had few contaminants, mainly comprised of feathers. PM samples were characterized shortly after collection and stored at 4 ºC throughout the experiment. PM had 30.0±0.3% total solids (TS) and 22.9±0.2% volatile solids (VS). The sample had high total ammonia and (TAN) total Kjeldahl nitrogen (TKN) values of 2496±74 mg/L and 12976±381 mg/L, respectively. The high organic nitrogen content, about 80.8% of TKN, indicates that ammonia fermentation could lead to excessively high ammonia levels causing inhibition of microorganism activities. Volatile fatty acids (VFA), chemical oxygen demand (COD), alkalinity, and pH of PM were 14200 ± 800 mg CH_3COOH/L, 84300 ± 920 mg COD/L, 25700 mg $CaCO_3$/L, and 8.6, respectively.

PM samples were diluted to 12% using distilled water to facilitate the hydrolysis and ammonia stripping of the samples. The dilution reduced nitrogen levels by a factor of 2.5, which was still insufficient to drop ammonia levels below previously reported inhibitory levels (above 2000-2500 mg NH_3-N/L) (Y. Chen et al., 2008; Usack & Angenent, 2015). The inoculum was collected from a mesophilic digester that operates on cow manure and corn silage near Ottawa, Canada. Its TS%, VS%, TAN, TKN, VFA, COD, ALK, and pH are 4.9±0.1% and 3.8±0.1%, 1592±5 mg NH_3-N/L, 3211±157 mg NH_3-N/L, 7300 ± 400 mg CH_3COOH/L, 48500 ± 500 mg COD/L, 10300 ±50 mg $CaCO_3$/L, and 7.7, respectively. The inoculum was characterized shortly after collection and stored at 35-40 ºC before being used.

6.2.2 Experimental Setup

6.2.2.1 Sample Preparation and Hydrolysis

The experimental setup is illustrated in the Appendices section. PM was diluted to 12% TS and blended in a food blender for only 10-15 seconds to minimize the impact of heating due to blending on hydrolysis (Holliger et al., 2016). Large contaminants like feathers were removed by sieving the blended PM through a 1/8” mesh. The sieved PM was then filled into four 500 mL bottles (hydrolysis reactors), leaving 10-15% headspace. No chemicals were added to ensure that the hydrolysis is only occurring due to biological activities. The bottles were then

purged with nitrogen to provide anaerobic conditions for the hydrolysis. The bottles were placed in a shaking incubator where the temperature and the shaking speed were set to 40 ºC and 150 rpm, respectively. Samples were collected daily for pH, TAN, TKN, and VFAs analyses. The primary purpose of this study's hydrolysis and acidogenesis stage was to promote organic nitrogen conversion to ammonia biologically. Hence, the hydrolysis setup resumed until TAN levels stabilized (around five days), after which the effluents were fully characterized.

6.2.2.2 Ammonia Stripping

Ammonia stripping was conducted in 150 mL cylinders containing 120 mL hydrolyzed PM. First, the samples were pre-heated to the stripping temperature (40 or 55 ºC) using a water bath, and then the pH was adjusted to 9.5 using lime ($Ca(OH)_2$); around 24 g lime/kg PM was needed. The pre-heating step was essential to start the stripping at the right pH level, i.e. 9.5, as pH drops when the temperature increases (Bonmatí & Flotats, 2003). Air or renewable natural gas (RNG) were used separately for stripping ammonia from the hydrolyzed PM. RNG consisted mainly of methane (94% v/v) and ethane (5% v/v). The flow rate was set to 100 L gas/L sample/ hour based on a previous study (Adghim et al. 2022), and the tube was placed at the bottom of the beaker with a diffuser at its end to supply fine bubbles. The samples were labelled as T (stripping temperature)-gas medium, i.e., T55-Air, T55-RNG, T40-Air, and T40-RNG. The stripping continued for three hours because ammonia levels had stabilized after this duration in some scenarios. Samples were taken every half an hour to measure ammonia and pH. However, the samples were fully characterized after the stripping duration was over. Each scenario was tested in triplicates.

A short side experiment was conducted to verify the advantages of RNG over biogas in terms of ammonia removal efficiency. This test aimed to understand if RNG's performance behaved more like air or biogas. Therefore, biogas stripping was tested at 55 ºC and the same stripping medium flowrate, i.e., 100 L biogas/ L sample/ hour. The pH, however, was adjusted to 10.5 instead of 9.5 to allow for more stripping duration. The biogas was prepared in the lab and consisted of 50% CO_2 and 50% CH_4 based on volume. The gas mixture was filled into 40L Tedlar bags, and the bags were connected to the stripping vessels through a peristaltic pump. The stripping duration was not pre-determined; stripping continued until pH and ammonia levels were stabilized.

6.2.2.3 Batch Biochemical Methane Potential Test

Batch biochemical methane potential (BMP) tests were set up for the raw, blended, hydrolyzed, and ammonia-stripped samples (except those treated with biogas) at a mesophilic temperature (35 °C). Samples were inoculated at an inoculum-to-substrate ratio of 1-2. The inoculum was degassed for a few days before being used in the BMP test and then used as a blank to estimate and subtract the contribution of the inoculum to biogas production and allow the reporting of net biogas production of the PM samples. Samples and inoculum were added to 250 mL BMP bottles where 30% headspace was maintained, and the samples were purged with nitrogen to ensure anaerobic conditions in the bottles. BMP bottles were then placed in a shaking incubator at 35 °C and 150 rpm. Biogas production was measured daily using water displacement, and biogas characterization was conducted weekly using gas chromatography. The BMP tests were maintained and monitored until the biogas production rate was less than 1% of the cumulative biogas production for at least three consecutive days. The digestate was fully characterized at the end of the BMP test.

6.2.3 Analytical Methods

Samples were analyzed shortly after collection at any stage to ensure accurate results. TS and VS were determined using standard method No. 2540 (APHA, 2005). VFAs were measured using the Esterification method: Hach TNT872 (50-2500 mg CH_3COOH/L); COD was measured using the Reactor Digestion Method: Hach TNT822 (20-1500 mg COD/L); total alkalinity was measured using colorimetric method 10239: Hach TNT870 (25-400 mg $CaCO_3$/L); TAN was measured by Salicylate method: Hach TNT (2-47, 100-1800 mg NH_3-N/L). Methane content in the biogas was measured using a 5'x0.125" SS 100/120 HayeSep T column fitted in a gas chromatography instrument (GOW-MAC Series 400, Bethlehem, PA). Helium was used as a carrier gas, the column, detector, and injector temperatures were set to 100, 100, and 120 °C, respectively, and the current was set to 100 mA. The temperature and pH were measured using Hach PHC20101 IntelliCAL Lab pH Probe.

6.2.4 DNA Extraction and Library Preparation

Microbial analysis was conducted by Microbiome Insights Inc, British Columbia, Canada. DNA was extracted using the Qiagen MagAttract PowerSoil DNA KF kit using a KingFisher robot. DNA quality was evaluated visually via gel electrophoresis and quantified using a Qubit 3.0 fluorometer (Thermo-Fischer, Waltham, MA, USA). Libraries were prepared using an

Illumina Nextera library preparation kit with an in-house protocol (Illumina, San Diego, CA, USA).

6.2.5 Sequencing, Data Curation, and Sequence Processing

Paired-end sequencing (150 bp x 2) was done on a NextSeq 500 in medium-output mode. Shotgun metagenomic sequence reads were processed with the Sunbeam pipeline. Initial quality evaluation was done using FastQC v0.11.5 1. Processing took part in four steps: adapter removal, read trimming, low-complexity-reads removal, and host-sequence removals. Adapter removal was done using Cutadapt v2.6 (Martin, 2015). Trimming was done with Trimmomatic v0.36 (Bolger et al., 2014) using custom parameters (Leading: 3; Trailing: 3; Sliding Window: 4:15; Minlen: 36). Low-complexity sequences were detected with Komplexity v0.3.6 (Clarke et al., 2019). High-quality reads were mapped to the human genome (Genome Reference Consortium Human Reference 37), and those mapped to it with at least 50% similarity across 60% of the read length were removed from the analysis. The remaining reads were taxonomically classified using Kraken2 with the PlusPF database from 2021-01-27 (Wood et al., 2019). For functional profiling, high-quality (filtered) reads were aligned against the Standard Energy Efficiency Data (SEED) database via translated homology search and annotated to Subsystems, or functional levels, 1-3 using Super-Focus (Silva et al., 2016).

6.3 Results and Discussion

6.3.1 Ammonia Fermentation

The incubation of poultry manure for five days increased TAN levels from around 1367±75 mg NH_3-N/L to 6895±266 mg NH_3-N/L, translating to around a 400% increase in ammonia levels. The highest rate of organic nitrogen conversion to ammonia occurred on the first day of incubation (around 192% increase in ammonia levels) and then sharply declined to 34% on the second day. The slight rise in TKN levels shown in Figure 6-1 could be due to the purging step when nitrogen was bubbled through the poultry manure to ensure anaerobic conditions and the presence of some nitrogen-fixating bacteria such as *Rhizobium* and *Azotobacter* (Fenchel et al., 2012). Since NO_3 and NO_2 levels were only increased by 173 mg N/L, it can be concluded that the fixated nitrogen was mostly converted to ammonia. However, the post-hydrolysis analysis showed that TS% reduced from 12% to 8.8%, indicating that the impact of VS destruction on TS% during the hydrolysis was higher than evaporation. This observation was also supported by the transformation rates of lipids and fats to volatile fatty acids, which increased from

6100±93 to 20810±1062 mg CH_3COOH/L. At the end of the incubation period, around 88% of organic nitrogen had been converted to ammonia, which is comparable to the 90% conversion rate observed by Sürmeli et al. (2017) for the biological hydrolysis of chicken manure. This high conversion rate of organic nitrogen during hydrolysis, followed by ammonia stripping, assists in limiting further increases in ammonia levels during methanogenesis.

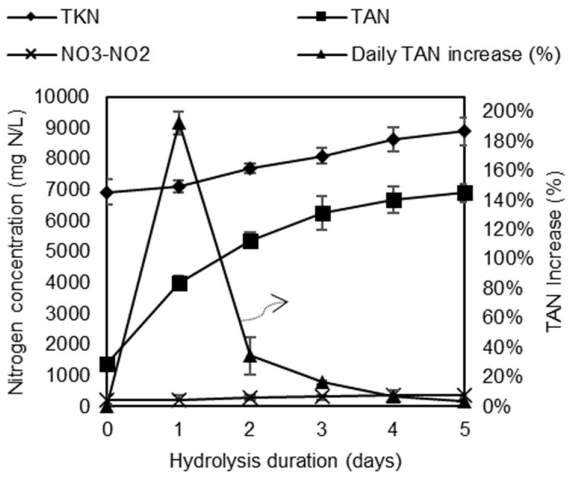

Figure 6-1: levels of different nitrogen forms and daily TAN increase during hydrolysis.

Ammonia levels were successfully reduced in all the tested scenarios except for biogas stripping (Figure 6-2a). Samples treated with air and RNG at 55 ºC displayed significantly higher AREs than those treated at 40 ºC. This is because FAN levels were higher and thus more volatile; the initial levels of FAN at pH 9.5 and temperatures of 55 and 40 ºC averaged 5212 and 4358 mg NH_3-N/L, respectively. Most of the removal occurred within the first 90 minutes for all scenarios, after which the ammonia removal rate dropped significantly due to low pH levels, which dropped the ammonia volatility. The observed ammonia removal efficiencies were in line with the previous study by the authors (Adghim et al., 2021) and other studies that observed 70-90% ammonia removal under similar treatment conditions using air (Fakkaew & Polprasert, 2021; H. Huang et al., 2019).

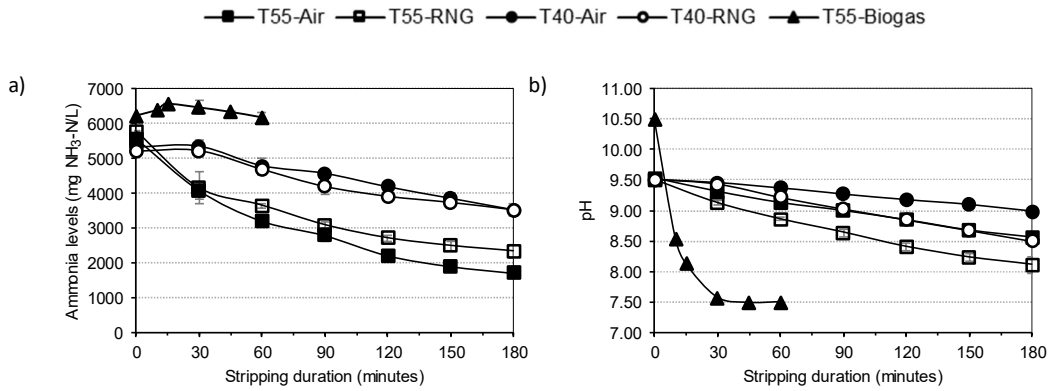

Figure 6-2: Ammonia stripping results a) ammonia levels during the treatment, and b) pH levels during the treatment.

Both air and RNG stripping were feasible in removing ammonia under the tested conditions. They showed comparable results in terms of AREs, specifically at a stripping temperature of 40 ºC, where the ammonia levels were almost identical between the sets throughout the treatment (Figure 6-2a). The ARE of air and RNG stripping at 40 ºC was 33 and 32%, respectively.

One of the perceptible differences in air and RNG performances as stripping gases is that RNG resulted in a slightly higher rate of pH drop. For example, pH dropped to an average of 9.4 and 9.2 in the first 30 minutes of stripping with air and RNG at 55 ºC, respectively. Despite this difference in pH levels, both air and RNG had achieved 28% ammonia removal, indicating that this pH level was still feasible for ammonia removal. Having said that, the effect of pH drop rate on the ARE was more evident towards the end of the stripping duration since the ARE of RNG and air at 55 ºC was 60 and 69%, respectively. The higher pH drop rate in the case of stripping with RNG could be due to the higher impurities in the used RNG (5% v/v ethane), which can react with moisture and form ethyl alcohol that can act as a weak acid (W. Chen et al., 2021).

The results of ammonia stripping using RNG are promising and noteworthy. They present several advantages when compared with using biogas as a stripping medium. RNG stripping successfully removed more than 50% of ammonia under milder conditions than those needed for stripping with biogas. The side experiment with biogas stripping at pH 10.5 and temperature 55 ºC showed that biogas was inefficient in removing ammonia under the tested conditions (Figure 6-2a). This is due to the sharp pH drop that occurred almost immediately after the stripping started, resulting from the formation of carbonic acid due to the CO_2 and H_2O reactions. The pH dropped from 10.5 to an average of 8.5 within only 10 minutes and continued

to drop until it reached 7.5 at 45 minutes (Figure 6-2b). Since the TAN and pH at the one-hour mark did not change from the 45 minutes mark, the stripping was stopped at the one-hour mark. TAN levels remained stable during stripping with variations not exceeding ±5% amongst readings. This is why ammonia removal with biogas requires pH levels higher than 10, temperatures higher than 60 ^{o}C, and prolonged durations to achieve high ammonia removals (Serna-Maza et al., 2014; Zhang et al., 2017). Considering these results, biogas stripping in the post-hydrolysis ammonia stripping was deemed infeasible and was not investigated further. The primary advantage of RNG to biogas is the low CO_2 content in the former, which avoids abrupt drops in pH and ammonia volatility (Bousek et al., 2016).

As RNG in biogas plants can be integrated with conventional natural gas pipelines, the utilization of RNG within the plant for ammonia removal purposes should not pose additional challenges than those that biogas recirculation would. Having said that, the provisions of ammonia and moisture traps must be accounted for to recover the RNG's quality before injection into the natural gas grid. Alternatively, instead of injection into the natural gas grid, the RNG used for stripping could be recirculated in a closed loop and replaced or endorsed with new RNG when needed.

6.3.2 Impact of Ammonia Stripping on Other Characteristics

Effluents of the ammonia stripping setups were fully characterized to accurately estimate what is going into the second stage of AD. Figure 6-3 shows the characteristics of the samples immediately before and after stripping for three hours. There were mainly two factors affecting the samples' characteristics during the stripping: moisture loss and ammonia stripping. TKN decreased in all models because the stripping of ammonia was significant. TKN reduction shows the impact of moisture loss and stripping together, i.e., if there is no impact from heating the sample, TKN and TAN reductions would be the same. However, TKN reduction is less due to the loss of moisture. Alkalinity had also decreased in all stripping setups. This could be explained by the limewater and CO_2 reaction, which forms a precipitate of $CaCO_3$, which also affects the ARE.

On the other hand, VFAs and COD had increased in all ammonia stripping setups except for T40-Air. Subjecting the samples to air for a prolonged period may have enabled some mesophilic aerobic activities that led to the consumption of VFAs and sCOD in T40-Air. On the other hand, stripping at 55 ^{o}C with air or RNG increased the concentrations of COD compounds by a similar value of TS increase, which was 12 and 9%, respectively. Conversely,

the impacts of evaporation on COD and VFAs were more prevalent under higher temperatures or whenever RNG was used. It is important to note that since the percentages of COD or VFA removals were not increased (<5.5%), there were no significant loss of organic compounds that would have turned into biogas during methanogenesis. The impacts of stripping on the samples' characteristics are not widely discussed in the literature. However, similar results were reported by Fakkaew and Polprasert (2021) in terms of VFA, COD, TAN, and TKN variations due to stripping. They reported a noticeably lower increase in TS compared to this study (5% versus 12%, respectively). This could be due to the high moisture content in this study compared to the other study (85% versus 70%, respectively).

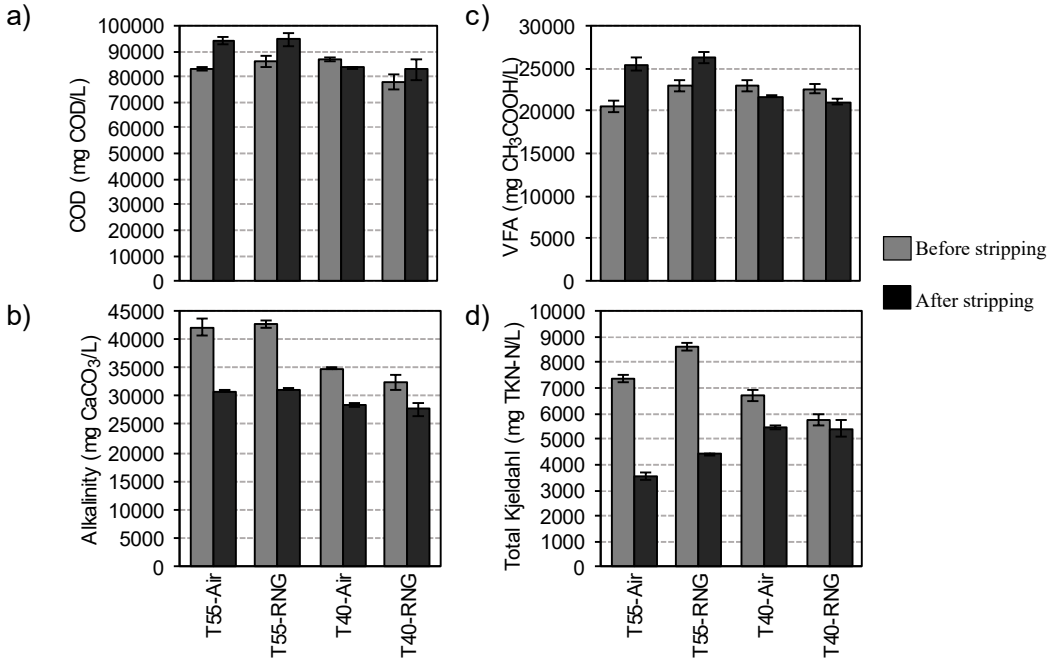

Figure 6-3: Impact of stripping on poultry manure's characteristics.

6.3.3 Methane Production

6.3.3.1 Impact of Ammonia Stripping

Biogas production was improved in all tested ammonia stripping scenarios (Figure 6-4). Hydrolyzed samples that were not subjected to any treatment encountered inhibition, limiting their methane yield to 338 ±12 L CH_4/kg VS. Post BMP characterization indicates that the low methane production was due to high ammonia levels of 3050±78 mg NH_3-N/L. On the other hand, ammonia stripping significantly improved methane yield (p-value=$2.4x10^{-8}$) by 71, 58, 30, and 24% in the cases of T55-Air, T55-RNG, T40-Air, and T40-RNG, respectively. This improvement in methane yield could be traced to the lower ammonia levels in the digestate,

which were 1867±86, 2021±37, 2352±6, and 2402±75 mg NH_3-N/L for T55-Air, T55-RNG, T40-Air, T40-RNG, respectively. The improvement of methane potential due to air stripping in this study was in line with previously reported values in the literature (60-90% improvement in biogas production) (H. Huang et al., 2019; K. Li et al., 2018).

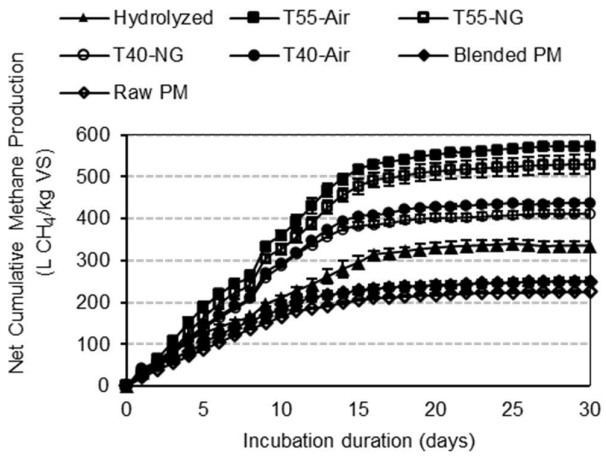

Figure 6-4: Net cumulative methane potential of ammonia-stripped, blended, and raw poultry manure.

There is no evidence that stripping with air negatively impacted methane production or the microbial population. This is because the stripping was done at an intermediate stage where methanogens' presence was already minimal. In addition, despite stripping with air, dissolved oxygen (DO) levels at the end of the stripping did not exceed 0.35 mg/L, which is well below the inhibitory levels of DO (>2 mg/L) reported by (Botheju et al., 2011). Moreover, Fakkaew and Polprasert (2021) reported that DO levels of 2.5 mg/L of stripped digestate did not impact methanogenic activities.

The impact of stripping with RNG on the methane potential is promising and yielded comparable results to stripping with air. The comparison of RNG and air in improving biogas potential was symmetrical to their effect on ARE. PM treated with RNG produced only 7% less methane than those treated with air under similar temperatures (40 or 55 ^oC). Therefore, it can be concluded that RNG is a feasible substitution for air for ammonia stripping purposes. Compared with previously reported methane yield enhancement using biogas (Nielsen et al., 2013; Serna-Maza et al., 2014), stripping with RNG can achieve higher methane production and alleviate ammonia inhibition under significantly less severe operating conditions.

It is important to note that the intended application of RNG in full-scale would be to recirculate the renewable natural gas used for ammonia stripping after passing through provisions of acidic traps to remove ammonia. This configuration would make the setup more economical than

continuous feeding of RNG and will allow for nutrient recovery in the form of ammonium-sulfate, which can be used as a fertilizer (Abouelenien et al., 2010; Yang et al., 2019).

Alongside ammonia reduction, heating the samples at 55 ^{o}C for 3-3.5 hours in the T55-Air and T55-RNG stripping setups increased the COD and VFAs levels, as indicated in Figure 6-3 and therefore enhanced the digestibility of the sample. Methane yield from T55-Air and T55-RNG was 29.7 and 28.1% more than that from T40-Air and T40-RNG samples. Comparable effects of raised temperature on methane yield were also reported by Baldi et al. (2018). Therefore, when comparing the impact of conducting the ammonia stripping at 55 ^{o}C with 40 ^{o}C on ammonia removal and methane yield, it appears crucial to consider high temperatures for ammonia stripping. Having said that, higher temperatures translate to higher energy consumption and cost. Therefore, some economic analysis may be needed to verify the economic feasibility of ammonia stripping at high temperatures.

6.3.3.2 Impact of Blending and Hydrolysis

To better understand the methane potential of poultry manure without ammonia-targeted treatment, three sets of Base samples were tested in the BMP test (Figure 6-4): 1) raw PM, 2) blended PM, and 3) hydrolyzed PM. Blending the sample and sieving it using 0.8 mm mesh slightly improved the methane potential of Raw PM. Blending often results in more significant improvements in biogas production (Agyeman & Tao, 2014; Meegoda et al., 2018). However, due to the high purity of the raw sample as well as its already small particle size, the impact of blending on methane yield in this study was not significant (maximum improvement of only 9.8%).

On the other hand, the hydrolyzed sample (representing two-stage AD) had improved the methane potential of the blended sample (representing one-stage AD) by 37.4% despite having almost the same ammonia levels (around 3000 mg NH_3-N/L). The stability and optimal biogas production in two-stage systems were also highlighted by (Nasir et al., 2012; Pan et al., 2013). Since the effluents of both setups showed similar chemical characteristics, the difference in BMP results can be justified by the substantial increase of ammonia levels by 93% during the digestion of blended PM. In contrast, ammonia levels only increased by 6% during the digestion of hydrolyzed PM. Moreover, the low pH in the separate hydrolysis/acetogenesis stage of PM increased VFAs from around 7667 to 21869 mg CH_3COOH/L by converting short-chain fatty acids and other intermediate products into acetic acid, which is readily digestible by methanogens.

Despite the evident inhibition of biogas production in all PM Base scenarios, their performance was unexpectedly close to those of less problematic types of feedstocks, such as dairy manure, which typically produces between 200-250 L CH_4/kg VS without any treatment (Huang et al., 2016; Usack and Angenent, 2015). However, it should be noted that the results in this study are based on batch BMPs, where ammonia levels at the end of digestion were determined by the inoculum-to-substrate ratio. On the other hand, accumulation of ammonia to inhibitory levels in continuous flow systems running on PM is more likely to occur since ammonia levels in the reactors will eventually be equal to the ammonia levels of the feed. Therefore, ammonia removal from PM would still be highly recommended for continuous flow applications.

6.3.4 Microbial Analysis

Thousands of microbial species belonging to Bacteria, Archaea, Fungi, or Virus kingdoms were identified using the shotgun metagenomic method. Figure 6-5 shows the microbial analysis results from the Kingdom level to the Species level. In addition, analysis before and after stripping and inoculation was conducted to understand the impacts of the treatment on microbial diversity. Both hydrolyzed and ammonia-stripped samples before inoculation showed negligible methanogenic archaea presence, indicating that conducting ammonia stripping at this intermediate stage does not compromise a significant number of methanogenic archaea.

On the other hand, stripping with air reduced the abundance of the *Firmicutes*, which is a common bacteria phylum in manure-fed bioreactors, from 45 to 34%, favoring the growth of *Actinobacteria* from 26 to 46%, which indicates that some dominant *Firmicutes* species are sensitive to oxygen and hence were impacted by the stripping treatment (St-Pierre and Wright, 2014). Both phyla are known to play a significant role in the hydrolysis and acidogenesis of organic wastes (Fernandez-Gonzalez et al., 2019; Park et al., 2016). Bacteria phyla were more diversified after methanogenesis due to the inoculation. However, *Actinobacteria* presence was negligible in all digestates due to its low tolerance to neutral to high pH levels (Ziganshina et al., 2017).

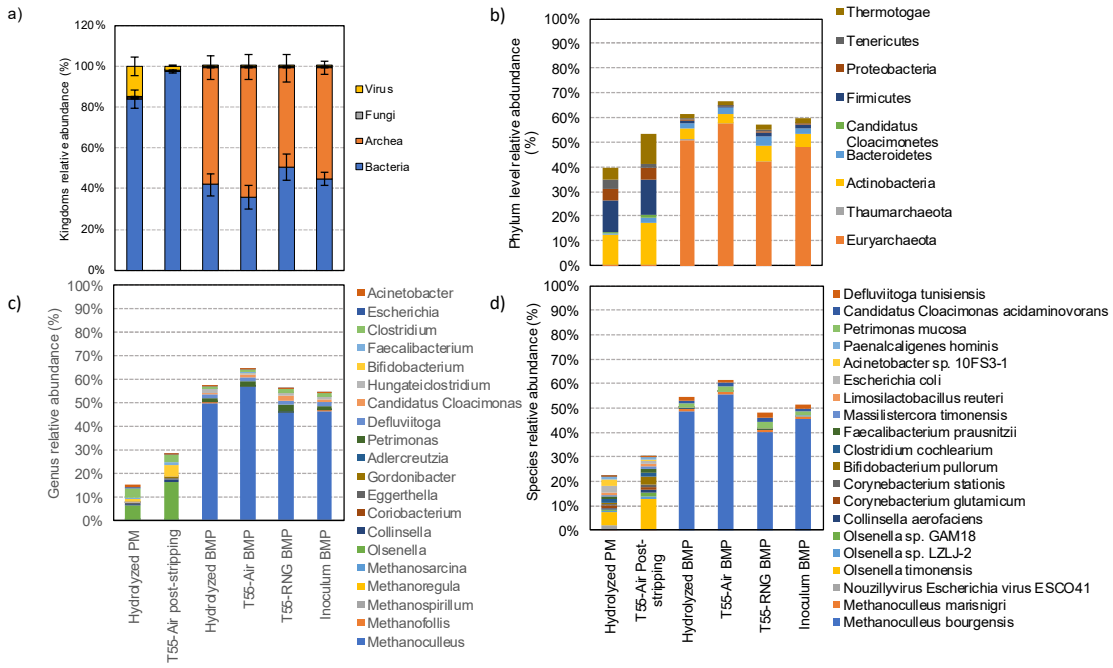

Figure 6-5: Microbial analysis a) kingdoms, b) bacterial and archaeal phyla distribution, c) top 20 bacterial and archaeal genus relative abundancies, and d) top 20 species relative abundancies

Olsenella timonensis, a newly discovered specie isolated from the human gut (Ndongo et al., 2019), was the most dominant species in the hydrolyzed and stripped samples before BMP. It constituted 23.1 and 13% of all bacteria in the hydrolyzed and ammonia-stripped PM (before inoculation), respectively. However, its presence was negligible after the BMP. There is very limited information on the functions and properties of this new bacteria species, but the results of this study suggest that it may have a high tolerance to elevated ammonia levels (at least 7000 mg NH_3-N/L).

Sulfate-reducing and sulfate-oxidizing bacteria were absent in the hydrolyzed and ammonia-stripped PM before BMP and constituted only about 2.5-3.5% of the bacteria after methanogenesis. This minor presence could be due to the higher pH during methanogenesis, which favored the methanogens growth over the growth of sulfur-reducing or sulfur-oxidizing bacteria, leading to minimized competition over carbon (Yang et al., 2019). This outcome was not surprising because the digestion of agricultural waste is not as problematic as the digestion of industrial wastes in terms of sulfur content (Barbosa Segundo et al., 2019; Hu et al., 2015).

The archaeal presence in all BMP effluents was dominated by the hydrogenotrophic methanogen, *Methanoculleus bourgensis sp.* (>95% relative abundance of archaea count), which belongs to *Methanoculleus* genus and is a common methanogen in mesophilic bioreactors (Maus et al., 2012). It was followed by *Methanoculleus marisnigri sp.,* which

accounted for 2.3-2.7% of all archaea in all BMP effluents and is classified under *Methanoculleus* genus as well. However, unlike *Methanoculleus bourgensis sp.,* this specie can utilize hydrogen and some alcohols, such as propanol and butanol, for methanogenesis (Anderson et al., 2009). This leaves only a minor presence of acetoclastic and methylotrophic methanogens, indicating that biogas production was mainly conducted through hydrogenotrophic methanogens.

Despite the similarities of the present species and abundancies among all BMP digestates, it is important to note that the sequencing in the shotgun metagenomic method had identified both active and inactive cells, which justifies why the relative abundancy results do not ideally reflect the observed enhancement in biogas production. To overcome this, the individualistic count reads of each prominent species were compared among all the analyzed samples. For example, *Methanoculleus bourgensis sp.* copies in the digestate of the hydrolyzed sample were 347718±12305 reads, only 9.2% more than the species count in the Hydrolyzed PM bottles. On the other hand, stripping with air or RNG at 55 ^{o}C increased the count of *Methanoculleus bourgensis sp.* by 34 and 17%, respectively.

Shannon diversity (α–diversity) analysis of all species was used to calculate the Evenness of species in the samples tested by the shotgun metagenomic method. The evenness graph is presented in Figure 6-6. The results showed that PM before inoculation had more Evenness amongst different species (Evenness=0.63–0.7) than the digestates at the end of the BMP (Evenness=0.4–0.52). One-way ANOVA results for samples before and after inoculation showed a significant difference in Evenness ($p=0.0015$). However, when samples before inoculation were excluded, the difference in Evenness became insignificant ($p =0.1644$). Moreover, treatment with air and RNG also led to minor variations in the microbial diversity (p from t-test = 0.1613). The inclusion of dormant cells in the count could explain the proximity of observations among the digestates because of the resulting masking effect.

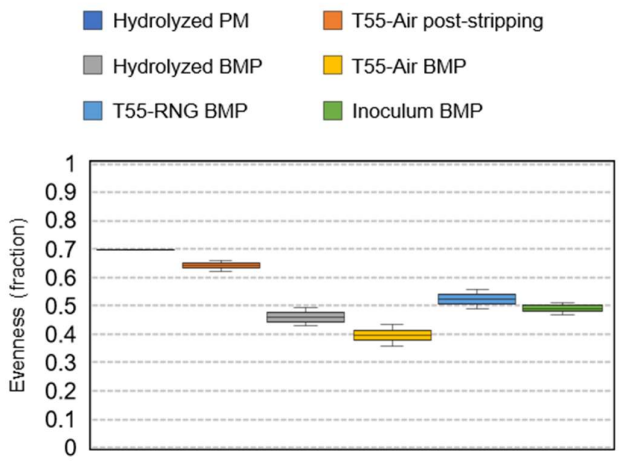

Figure 6-6: Evenness amongst species in different samples.

6.4 Conclusion

Using renewable natural gas for ammonia stripping showed promising results and effectiveness in improving the methane potential of high ammonia feedstock. Furthermore, since the ammonia removal efficiency achieved with renewable natural gas is comparable to air, renewable natural gas can be considered an efficient anaerobic stripping medium readily available in biogas plants.

Microbial analysis showed that most of the biogas was produced through the hydrogenotrophic pathway for methanogenesis, led dominantly by *Methanoculleus bourgensis sp.* in all tested scenarios.

6.5 Acknowledgment

The authors would like to thank MITACS for funding this project (MITACS 29510) and Mr. Eric Gareau for facilitating the poultry manure collection.

6.6 References

The references of this Chapter are merged with the rest of the document and presented in Chapter 10: References

CHAPTER 7: COMPARATIVE ASSESSMENT OF DIFFERENT AMMONIA STRIPPING CONFIGURATIONS TO ENHANCE BIOGAS PRODUCTION FROM POULTRY MANURE

A shorter version of this paper was accepted at Waste Management in April 2023, where the section of Pre-hydrolysis Ammonia Stripping was omitted due to word limitation.

This technical paper presents the results of Phase IV. In this phase, post-hydrolysis ammonia stripping using air and RNG was compared with side-stream ammonia stripping in semi-continuous flow mode. Additionally, post-hydrolysis ammonia stripping was compared with pre-hydrolysis ammonia stripping in batch mode. The two main objectives were to assess the stability of the post-hydrolysis ammonia stripping configuration in the long term and compare its performance with other ammonia stripping configurations.

Abstract

Poultry manure mono-digestion in semi-continuous flow mode was experimentally evaluated using two different ammonia stripping configurations aimed at reducing the inhibitory effects of ammonia 1) Post-hydrolysis (PHAS) and 2) Side-stream ammonia stripping (SSAS). Ammonia stripping operating conditions were set to pH 9.5, 55 ºC, and flowrate of 100 L gas/L/hour. Air and renewable natural gas (RNG) were tested as stripping mediums. PHAS outperformed SSAS in both air and RNG stripping. Volumetric and specific biogas production from PHAS and SSAS scenarios averaged up to 1.91 and 1.26 L/L/day and 831 and 701 L biogas/ kg VS/day under organic loading rates of 2.61 and 1.8, respectively. Pre-hydrolysis ammonia stripping was tested in batch mode and deemed unfeasible for treating poultry manure due to the release of ammonia in the digester.

Keywords:

Ammonia stripping configurations; Poultry manure digestion; anaerobic digestion; physio-chemical pre-treatment; ammonia inhibition.

7.1 Introduction

Anaerobic Digestion (AD) is an established organic waste management technology that unlocks the value of waste and converts them into valuable products, biogas and fertilizers (Baldi et al., 2018; Jain et al., 2015). As biogas production from organic waste material is gaining more ground worldwide as a means to produce sustainable bioenergy, research advancements focus on process optimization and resolving issues resulting from including

organic wastes that may cause instability in AD reactor operation. One such issue is the potential impact of ammonia inhibition during the AD of poultry manure (PM), which has resulted in PM being avoided in biogas plants or only included at small percentages of the feedstock recipe despite being an abundant waste stream (Rajagopal et al., 2013; S. Wang et al., 2016).

Ammonia inhibition is due to high levels of inorganic ammonia that result from the biodegradation of proteins and amino acids in urea, manure, and other high-protein organic matter (Park & Kim, 2016; Rajagopal et al., 2013). Although ammonia at low levels is essential to microbial growth necessary for the AD process, high levels of ammonia (>2300-2700 mg NH_3-N/L) can be toxic and lead to a reduction or complete shutdown of biogas production (Akindele & Sartaj, 2018; Y. Chen et al., 2008; Rodriguez-Verde et al., 2018; Usack & Angenent, 2015; Yenigün & Demirel, 2013).

Ammonia stripping is the most commonly used technique to mitigate ammonia inhibition in AD operation due to its relatively simple concept, applicability at large scales, and its manageable economics (Bi et al., 2020; Fuchs et al., 2018). Ammonia stripping of digestate is operated under high pH and temperature to increase ammonia volatility and improve the removal efficiency (K. Li et al., 2018; Serna-Maza et al., 2014). Recent studies have been concerned with optimizing the process by examining different aspects, specifically the stage of the stripping unit and the type of stripping gas. In two-stage AD operation, the ammonia stripping could be implemented either as Post-hydrolysis Ammonia Stripping (PHAS) or Side-stream Ammonia Stripping (SSAS). In PHAS configuration, ammonia stripping is conducted at an intermediate stage between the hydrolysis and methanogenesis phases, where ammonia fermentation is mostly completed (H. Huang et al., 2019; M. Walker et al., 2011). On the other hand, SSAS includes stripping a portion of the digestate (typically 2-5% per day) in a side column and returning the ammonia stripped digestate to the main AD reactor to reduce ammonia levels (K. Li et al., 2018; Zhang et al., 2017).

Both PHAS and SSAS systems operate at significantly different ammonia stripping tower conditions. For example, SSAS is mainly conducted through biogas recirculation, which can severely impact the buffering capacity of the material and reduce the ammonia removal efficiency. Therefore, biogas recirculation is often conducted at a very low flow rate (1-10 L biogas/ L/ hour) and relies on high volatility of ammonia at a pH level above 10, temperatures above 65 ^{o}C, and long durations of more than 24 hours (K. Li et al., 2018; Serna-Maza et al., 2014). On the other hand, PHAS systems provide more flexibility in selecting stripping gas

and can be conducted using air, which has a high removal efficiency and low impact on the buffering capacity of the material (Bousek et al., 2016). Thus, systems with air stripping can operate under pH levels of 9-10, temperatures of 40-55 ^{o}C, and durations of 1-4 hours (Fakkaew & Polprasert, 2021; H. Huang et al., 2019).

There is very limited information in the literature regarding the performance of PHAS and SSAS, and the comparison of their performance under similar testing conditions for AD of PM. Huang et al. (2019) was the only study to experimentally test the performance of PHAS of swine manure at a high solid content of 25% and observed 60-70% improvement in biogas production. However, the results were limited to batch testing. Serna-Maza et al. (2014) and Zhang et al. (2017) reported that the SSAS system successfully alleviated ammonia inhibition in the thermophilic digestion of food waste in a semi-continuous mode. Yin et al. (2019) investigated hyper-thermophilic ammonia stripping in an in-situ configuration similar to SSAS with biogas recirculation and its effect on the efficiency of different AD phases and biogas production. The study found that increasing organic loading rate (OLR) and decreasing hydraulic retention time (HRT) can increase ammonia levels to inhibitory levels (2500-3000 mg NH_3-N/L) and reduce biogas production by 75% even when ammonia stripping was still implemented. Walker et al. (2011) was the only study to compare the performance of different configurations of ammonia stripping from source-segregated food waste based on kinetic modelling. They reported that the PHAS system was more advantageous than SSAS because it resulted in higher methane yield and lower ammonia levels in the digestate. However, they stated that their conclusions were limited to modelling alone due to the lack of experimental data on the PHAS system. Considering the above, there is a lack of information on the experimental comparison between the two ammonia stripping systems.

In previous studies by the authors (Adghim et al., 2021, 2022), PHAS system was investigated for the mono- and co-digestion of PM in batch mode, and the stripping conditions were optimized at pH 9.5, temperature of 55 ^{o}C, and gas flowrate of 100 L gas/L/ hr. Furthermore, the feasibility of using renewable natural gas (RNG) as a stripping gas was investigated in PHAS, which yielded comparable performance to the application of air in terms of ammonia removal efficiency (60-70%) and biogas production enhancement (65-80%) in batch mode. However, assessing the PHAS under continuous flow long-term operation and its comparison with SSAS process is yet to be evaluated (Adghim et al., 2023).

This study aims to cover some critical knowledge gaps regarding the different ammonia stripping configurations in two-stage anaerobic digestion of poultry manure, namely PHAS

and SSAS, to provide a comprehensive comparison of the performance of the two systems, and to contribute to the ongoing optimization efforts in both systems. This study also evaluates the feasibility, performance, and stability of the PHAS and SSAS systems under semi-continuous flow mode using air vs. RNG as alternative ammonia stripping gas mediums. The performance of the different stripping configurations examined will be compared in terms of biogas production, accompanied by an in-depth discussion. The results of the present study will provide practitioners in the field, operators of poultry farms and AD plants with high nitrogen feedstock, with information about how PHAS and SSAS systems would operate continuously and the expected steady-state conditions that can be achieved.

7.2 Materials and Methods

7.2.1 Substrates and inoculum

Poultry manure (PM) samples were collected from layer chickens in an egg farm in Ottawa, Canada. The manure is scraped from the floors and transported via a conveyor to an outdoor pile. Since the farm does not implement any bedding systems, the collected manure had few contaminants, mainly comprised of feathers. Around 200 kg of PM was collected and characterized shortly after collection and before storing at -18 ºC to limit biodegradation and preserve the sample throughout the experiment. Portions of PM were thawed for one day before being used for the experiment. PM had 24.3±0.1% total solids (TS) and 16.0±0.1% volatile solids (VS). PM had high total ammonia (TAN) and total Kjeldahl nitrogen (TKN) values of 4356±123 mg/L and 9398±106 mg/L, respectively. The high organic nitrogen content, about 53.6% of TKN, indicates that ammonia fermentation could lead to extremely high ammonia levels causing inhibition of microorganism activities (W. Huang et al., 2016). Volatile fatty acids (VFA), chemical oxygen demand (COD), alkalinity, and pH of PM were 28713 ±459 mg CH_3COOH/L, 170408± 7688 mg COD/L, 39762± 320mg $CaCO_3$/L, and 8.63, respectively.

The inoculum was collected from a mesophilic digester that operates on cow manure and corn silage near Ottawa, Canada. Its TS%, VS%, TAN, TKN, VFA, COD, ALK, and pH are 4.9±0.1% and 3.8±0.1%, 1592±5 mg NH_3-N/L, 3211±157 mg NH_3-N/L, 7300 ± 400 mg CH_3COOH/L, 48500 ± 500 mg COD/L, 10300 ±50 mg $CaCO_3$/L, and 8.2 respectively. The inoculum was characterized shortly after collection and stored at 35-40 ºC before being used for the batch and the beginning of the semi-continuous flow experiments.

7.2.2 Experimental setup for semi-continuous flow two-stage AD systems

The setup for both post-hydrolysis ammonia stripping (PHAS) and side-stream ammonia stripping (SSAS) in a two-stage semi-continuous configuration consisted of three main vessels: 1) hydrolyzers, 2) ammonia-stripping units, and 3) main digesters (methanogenesis tank) in the order presented in Figure 7-1. Two identical systems were operated simultaneously under similar conditions to serve as duplicates.

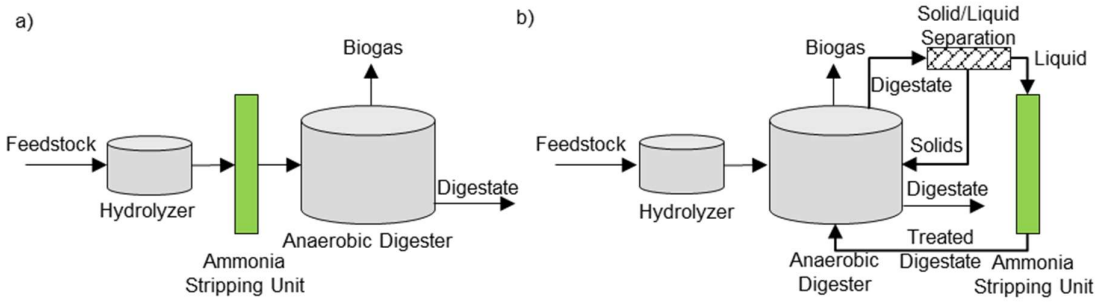

Figure 7-1:Ammonia stripping configurations a) post-hydrolysis ammonia stripping and b) side-stream ammonia stripping.

The overall duration of the experiment was 400 days, divided into different scenarios, as presented in Table 1. The experiment started with filling the main digesters with inoculum up to the working volume (10 L), and the start-up phase included feeding the reactors daily with diluted PM with reduced TAN levels (800 mg NH_3-N/L) to avoid shocking the microorganisms. Then, the feeding was increased gradually every week from 0.5 to 2.6 g VS/L/day, which was the target OLR corresponding to the selected hydraulic retention time (20 days) and working volume (10 L). Then, the reactors were fed with ammonia-stripped hydrolyzed PM in PHAS scenarios, whereas the reactors were fed with hydrolyzed PM (not ammonia-stripped) in SSAS scenarios. The stripping was conducted following the procedures listed in Section 7.2.2.4. The sequence of the scenarios was determined by anticipating the least to the most impactful scenario in terms of ammonia inhibitory effects. Therefore, the Base scenario (no treatment) was conducted at the end of the experiment. Each scenario lasted for at least three hydraulic retention time (HRT) or until the biogas production, as well as the digestate characteristics, were consistent (less than 10% variation per day) for at least one HRT (Usack et al., 2012; Usack & Angenent, 2015).

Table 7-1: Annotation and description of the different phases in semi-continuous flow reactor experiments

Annotation	Start (day)	End (day)	Stripping medium	Stripped Portion
Startup	0	48	N/A	0
PHAS-1	49	126	Air	100% of hydrolysis reactor effluent (500 g/day)
PHAS-2	127	163	RNG	100% of hydrolysis reactor effluent (500 g/day)
SSAS-1	164	224	RNG	20% of reactor volume per day (2 kg per day)
SSAS-2	225	285	RNG	10% of reactor volume per day (1 kg per day)
SSAS-3	286	332	Air	10% of reactor volume per day (1 kg per day)
Base	333	400	N/A	Reactors fed with hydrolyzed PM (no treatment)

7.2.2.1 Sample preparation

Every five days, a bucket of PM was thawed for one day, then it was used to prepare the feedstock for the following five days. PM was diluted to 10% TS and blended in a food blender for only 10-15 seconds to minimize the impact of heating due to blending on hydrolysis (Holliger et al., 2016). Large contaminants like feathers were removed by sieving the blended PM through a 1/8" mesh. The sieved PM was then stored in 5 L buckets to feed the hydrolyzers. This process is repeated throughout the experiment.

7.2.2.2 Hydrolysis reactors design

The hydrolyzers were designed based on the back-calculations for the organic loading rate (OLR), which was set to be around 2.6 g VS/L/day. The OLR was deliberately set at the lower range reported by literature (2-6 g VS/L/day) (Fernandez-Gonzalez et al., 2019; Nie et al., 2015) to have a better resolution of the impact of treatment.

Two cylindrical hydrolysis reactors were manufactured from plexiglass with a 22.9 cm diameter and 15.9 cm height. The reactors' HRT and working volume were set to 5 days and 3 liters, respectively. The hydrolysis HRT was selected based on a previous study (Adghim et al., 2023). The reactors design provisioned sampling ports to incorporate feeding and decanting, as well as a heating rod and thermometer.

The reactors were placed on shakers and set at 50 rpm, the maximum rpm to achieve stable rotation and proper mixing of the reactor's contents. The reactors were heated using a thermocouple rod inserted into the center of the reactor, and the heating was controlled by connecting both the heating rod and the thermometer to a temperature control device, which was programmed to stop heating when the temperature of the material reached 40 ^{o}C and restart

when the temperature dropped to 35 °C. The reactors were fed once a day with 600 mL of diluted PM (10% TS), and the same amount was decanted to maintain the working volume.
The primary purpose of the hydrolysis step in this experiment was to ferment organic nitrogen and convert it to ammonia through biological pathways. Therefore, no acid or alkali was added to enhance or expedite hydrolysis. Furthermore, the temperature of the reactor was set to 40 °C, which does not induce the thermal breakdown of organic compounds, but provides hydrolytic enzymes and acidogenic microorganisms a suitable environment to utilize the organic compounds and transform them into hydrolysis products (Fisher et al., 2019; Romero-Güiza et al., 2014).

7.2.2.3 Main digesters design

Two identical reactors were used as methanogenesis vessels (AD1 and AD2) throughout this experiment. The design criteria were similar to the hydrolysis reactors, except for HRT, which was set to 20 days. This HRT was determined based on a previous biochemical methane potential (BMP) test conducted in a previous study (Adghim et al., 2023). The diameter and height of the reactors were 30.5 and 27.9 cm, respectively. The reactors were heated using a similar configuration to the hydrolysis reactors. The reactors were fixated on shakers at the speed of 35 rpm, which was the maximum achievable speed to ensure proper mixing and stability of the reactor. The larger volume of the main digesters compared to the hydrolyzers required less rotational speed to achieve proper reactor stability. The proper mixing was evaluated visually and by weekly monitoring of changes in total solids at the bottom of the reactors to detect sedimentation. The biogas was collected in 40 L gas-impermeable bags, which were emptied every day to measure the volume of the produced biogas. Biogas production was adjusted to standard temperature and pressure at 0 °C and 1 atm. Methane content in the biogas was measured using gas chromatography (GC). The pH of the digestate was recorded daily and immediately after decanting to avoid changes in pH due to CO_2 desorption and drop in temperature, which could lead to an increase in pH levels and erroneous representation of the digestate's condition.

7.2.2.4 Ammonia stripping unit

Ammonia stripping treatment was conducted every day from day 49 till day 333 (the start of the Base scenario). In PHAS, the hydrolysis tanks' daily effluent was treated before entering the main digester. In SSAS, the recycling ratio, i.e., the percentage of the reactor's working volume to be treated and fed back to the digester, was initially set at 10% per day. However,

an abrupt drop in biogas production was noticed, and therefore the recycling ratio was adjusted to 20% per day for the first three HRTs of the side-stream ammonia stripping scenario to facilitate the transition to a 10% recycling ratio. The decanted digestate for treatment was passed through a 1/8" mesh to separate the solids and liquids. The liquid/solid separation step was necessary for the SSAS setup to avoid exposing methanogens to the stripping conditions, which could lead to slowing their growth. Then, the solids were returned to the digesters, and the liquids underwent ammonia stripping before being added to the reactor.

The ammonia stripping treatment for both PHAS and SSAS systems started by pre-heating the hydrolyzed PM or the decanted digestate to the stripping temperature (55 ^{o}C) using a pre-heated oven for 20 minutes, and then the pH was adjusted to 9.5 using lime ($Ca(OH)_2$) addition; the amount of lime for the PHAS system was about 24 g lime/ kg PM, whereas in SSAS the amount of lime varied between 12-30 g lime/kg PM due to changes in alkalinity and recycling ratios. The pre-heating step was essential to occur before pH adjustment as pH drops when the temperature increases (Bonmatí & Flotats, 2003). Then, the modified PM was added to two elongated glass cylinders where stripping was conducted. The temperature was controlled by circulating hot water from a water bath around the cylinder. Air (PHAS-1 and SSAS-3) and renewable natural gas (PHAS-2, SSAS-1, and SSAS-3) were used separately as stripping mediums. As an equivalent alternative to RNG, natural gas lines from the building were used for stripping, and it consisted mainly of methane (94% v/v) and ethane (5% v/v). The flow rate was set to 100 L gas/L sample/ hour for three hours based on the authors' previous work (Adghim et al., 2022). The carrier gas tube was connected to the bottom of the stripping vessel. The stripped material was then fed to the main digester through a funnel.

7.2.3 Pre-hydrolysis versus post-hydrolysis ammonia stripping

As many biogas plants implement a one-stage AD system, where all four AD phases occur in the same single tank, PHAS system becomes inapplicable, leaving only two options for ammonia stripping: 1) Pre-hydrolysis (Pre-digestion) ammonia stripping or 2) SSAS. Therefore, this study also evaluates the viability of pre-hydrolysis ammonia stripping. Furthermore, it compares it with PHAS in an independent batch experiment; procedure is different than the semi-continuous flow experiment described in previous sections. The batch experiment consisted of pre-hydrolysis and post-hydrolysis ammonia stripping columns as well as biochemical methane potential (BMP) to understand the impact of hydrolysis and whether

organic nitrogen transforms into ammonia during stripping, allowing more removal of ammonia.

The PM samples were prepared as mentioned in Section 7.2.2.1 for both pre-hydrolysis ammonia stripping and PHAS systems. In the pre-hydrolysis ammonia stripping scenario, the sample is sent directly for ammonia stripping in an elongated glass column with a gas diffuser at the bottom. In PHAS (batch mode), the sample was hydrolyzed in air-tight 500 ml bottles for five days at 40 ^{o}C and 150 rpm in a shaking incubator. After hydrolysis, the sample is taken to a similar glass column as the one used for pre-hydrolysis ammonia stripping. Before stripping, the samples were pre-heated to the required temperature (40 or 55 ^{o}C), and then the pH was adjusted to 9.5 using lime. The air flow rate was set to 100 L air/L sample/hour. Stripping continued for three hours; samples were taken every 30 minutes for pH, temperature, and ammonia measurements. After stripping, the samples were characterized to determine COD, ALK, TKN, TAN, and solid contents. Next, the samples were added to 250 ml bottles and inoculated with the same inoculum mentioned in Section 7.2.1 at an inoculum-to-substrate ratio of 1-2 till a working volume of 175 ml was attained. The BMP test continued until biogas production was less than 1% for at least three consecutive days (Holliger et al., 2016). The digestate was fully characterized after BMP, and the methane content of the biogas was measured once a week using gas chromatography (GC).

7.2.4 Analytical methods

Samples were analyzed shortly after collection at any stage to ensure accurate results. TS and VS were determined using standard method No. 2540 (APHA, 2005). VFAs were measured using the Esterification method: Hach TNT872 (50-2500 mg CH_3COOH/L); COD was measured using the Reactor Digestion Method: Hach TNT822 (20-1500 mg COD/L); total alkalinity was measured using colorimetric method 10239: Hach TNT870 (25-400 mg $CaCO_3$/L); TAN was measured by Salicylate method: Hach TNT (2-47, 100-1800 mg NH_3-N/L). Biogas characterization was conducted using a 5'x0.125" SS 100/120 HayeSep T column fitted in a gas chromatography instrument (GOW-MAC Series 400, Bethlehem, PA). Helium was used as a carrier gas, the column, detector, and injector temperatures were set to 50, 185, and 50 ^{o}C, respectively, and the current was set to 50 mA.

7.3 Results and Discussion

7.3.1 Ammonia fermentation

Ammonia levels in the diluted PM fed to the hydrolyzer reactors were around 4225 ± 612 mg NH_3-N/L throughout the first 280 days of the experiment, after which a new batch of PM was collected from the same location. However, the diluted PM from the second batch had higher ammonia levels of 6129 ± 485 mg NH_3-N/L. Due to hydrolysis, ammonia from the first and second batches increased to 5125 ± 540 and 7117 ± 392 mg NH_3-N/L, respectively. Such high ammonia levels increase the risk of inhibition in the digester and may cause a complete shutdown of biogas production. Therefore, ammonia stripping becomes essential to improve the digestion of PM (Y. Chen et al., 2008; Rajagopal et al., 2013; Zhuang et al., 2018).

The increase in ammonia due to hydrolysis translates to final TAN/TKN ratios of 68 and 86%, leaving an average of 32 and 14% of TKN as organic nitrogen at the end of hydrolysis of batches 1 and 2, respectively. The ammonia fermentation from the first batch was lower than that observed in a previous study by Adghim et al. (2023) and Sürmeli et al. (2017), where about 88-90% of TKN consisted of ammonia after biological hydrolysis of PM. This could be due to short-circuiting in the reactor. To amend this, the hydrolysis reactors were switched to a batch mode for SSAS-1 till the end of the experiment. Switching hydrolysis to batch mode increased TAN/TKN levels from 68 to 94%. This indicates that biological hydrolysis at 40 ºC was sufficient for ammonia fermentation without the need for elevated temperatures or the addition of acids or alkaline that promote a faster hydrolysis rate (Hejnfelt & Angelidaki, 2009; Yin et al., 2019).

7.3.2 Post-hydrolysis ammonia stripping

As presented in Figure 7-2a, ammonia levels in the digesters started at 710±38 mg NH_3-N/L since it was only filled with digestate from the biogas plant. Before the PHAS-1 (ammonia stripping with air) scenario started, the ammonia levels increased gradually to 1068±40 mg NH_3-N/L during the start-up phase with OLR increasing from 0.5 to 2.6 g VS/L/day, which was done to avoid shocking the microorganisms. On day 49, the reactors were fed with PHAS-1 feed, and ammonia levels increased to 1742±170 mg NH_3-N/L over 35 days and stabilized at that level until the end of PHAS-1 (day 126). PHAS-1 consistently removed 71-75% of ammonia before the ammonia-stripped PM was fed to the main reactors, which aligns with reported values by Adghim et al. (2023) and Huang et al. (2019).

The PHAS-1 treatment led to a stable volumetric biogas production (VBP) of 1.91±0.3 L/L/day and specific biogas production (SBP) of 831±59 L biogas/kg VS/day, as shown in Figure 7-2b and 2c, with a stable average methane content of 66±2%. Such high biogas and methane production levels indicate that PHAS can reliably make the mono-digestion of PM possible. Moreover, the observed biogas production highlights the great potential of PM as a biogas feedstock. Compared with more common livestock manure used as biogas feedstock such as cow and swine manure, PM has higher organic content leading to higher biogas production. Biogas production from cow and swine manure is typically around 225 and 500 L biogas/ kg VS, whereas biogas production from PM was repeatedly reported at around 700-800 L biogas/kg VS after ammonia-targeted treatments (Bi et al., 2020; Qiao et al., 2011).

Figure 7-2a shows that ammonia levels in the main digesters during PHAS-1 were equivalent to the ammonia levels in their influent and remained below the widely accepted inhibitory levels (>2500 mg NH_3-N/L) reported by Chen et al. (2008). The VS mass balance around the main digesters indicated that 78% of the influent VS mass was converted to biogas. No information was reported in the literature about VS removal due to PHAS treatment to compare with this study. However, the observed VS removal in this study was in line with VS removal reported by Li et al. (2018) for treating poultry manure in an SSAS system. This VS removal was also reflected in the COD removal, as 69% of the influent's total COD was destroyed (Figure 7-3a). These indicators prove that PHAS-1 successfully achieved stable biogas production and alleviated the inhibitory effects of ammonia.

Despite the partial ammonia fermentation that occurred during PHAS-1 and PHAS-2 in the hydrolysis stage referred to in Section 7.3.1, the difference between the main digester's influent TAN and the effluent TKN levels indicate low to no additional ammonia fermentation in the main digester, which helped maintain the reactor's stability. The lack of additional ammonia fermentation in the main digester may indicate the lack of fermentative bacteria responsible for breaking down proteins and amino acids into ammonia.

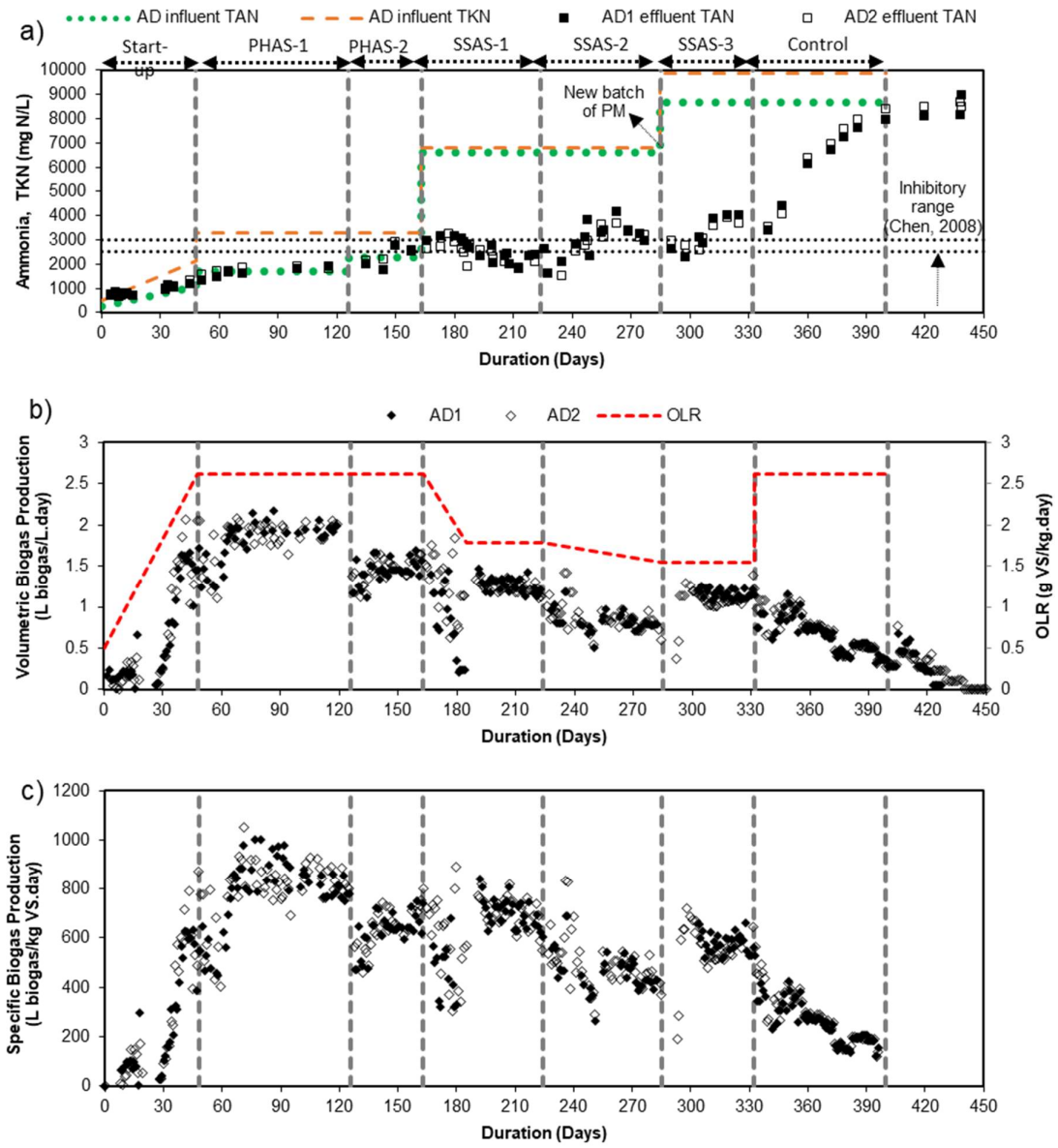

Figure 7-2: Performance indicators of AD reactors: (a) influent and effluent ammonia levels, (b) volumetric biogas production, and (c) specific biogas production.

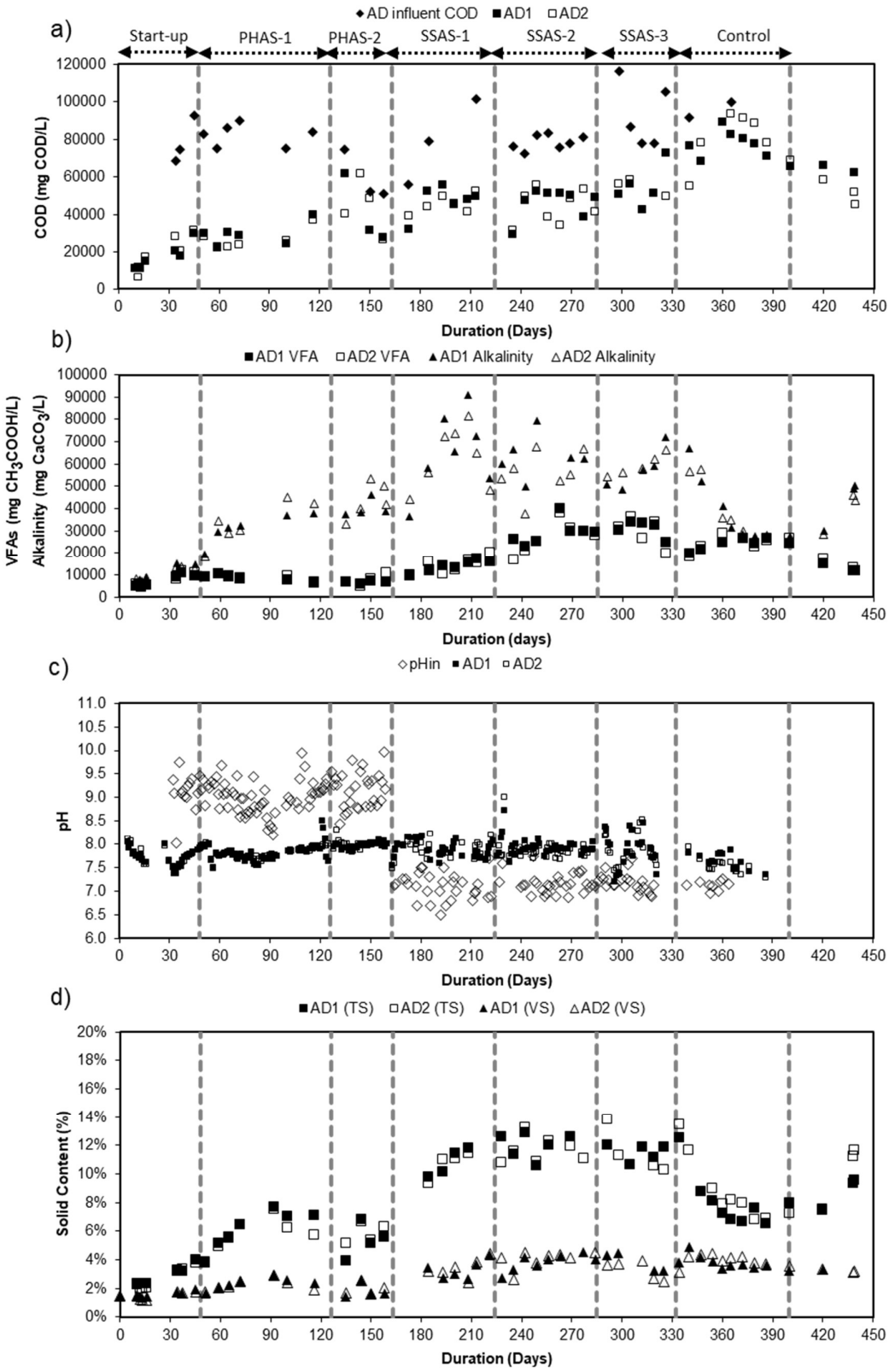

Figure 7-3: Performance conditions of anaerobic reactors a) COD, 2) VFA and alkalinity, 3) pH, and 4) solid contents.

Interestingly, the batch experiment in the study by Adghim et al. (2023) showed that VFAs were not significantly consumed during digestion, and most biogas production was conducted through the hydrogenotrophic pathway. However, in the PHAS-1 semi-continuous flow system, VFA levels dropped from 24636±2534 mg acetic acid/L in the feed to 8725±940 mg acetic acid/L in the digester (Figure 7-3b), indicating that acetic acid consumption contributed to biogas production through acetoclastic methanogens. This indicated that the continuous ammonia stripping treatment had alleviated the ammonia inhibitory effects on acetoclastic methanogens.

The pH in the AD reactors was reasonably consistent throughout the treatment phases due to the high alkalinity levels resulting from lime addition during the treatment. During the startup stage, pH dropped from 8.1 to 7.5 and then increased to 7.7 after PHAS-1 started and stabilized for the remainder of the experiment with minimal variations, as shown in Figure 7-33c.

At day 126, the ammonia stripping treatment switched to PHAS-2 (ammonia stripping with RNG) at the same tower conditions of PHAS-1, i.e., pH 9.5, 55 ^{o}C, and 100 L RNG/L/hr. The ammonia removal efficiency of RNG was 50-58%, less than that of air (71-75%). Lower ammonia removal efficiency by the application of RNG increased the digestate's ammonia levels from 1742±170 mg NH_3-N/L in PHAS-1 to 2354±401 mg NH_3-N/L, close to the inhibitory levels (Y. Chen et al., 2008). This led to a sudden drop in SBP from 831±59 to 680±48 L biogas/kg VS_{added}/day (18.2% drop). The VS and COD removal consequentially reduced from 78 and 69% in PHAS-1 to 68% and 43% in PHAS-2. However, biogas production was stable and consistent throughout the PHAS-2 scenario duration. The proportionality between ammonia levels and biogas production in this continuous experiment was also observed in a previous study by the authors where batch experiment comparing air versus RNG as stripping mediums was conducted (Adghim et al., 2023). Despite the decrease in biogas production, PHAS with RNG successfully maintained a VFA/ALK ratio below 0.3 as shown in Figure 7-3b.

The reactors needed a short time to stabilize after transitioning from PHAS-1 to PHAS-2 (4-6 days) because the feed properties were comparable in both phases, except for the higher ammonia levels in PHAS-2. This also indicates a level of acclimation of the methanogens to the PM and partly high ammonia levels. Since the digestate characteristics and the biogas production were within 10% for over 20 days, PHAS-2 was stopped at day 162 (1.85 HRT).

7.3.3 Side-stream ammonia stripping

On day 163, the ammonia stripping treatment switched to side-stream ammonia stripping (SSAS), starting with RNG and then air. Initially, it was intended to treat 10% of the reactor volume per day per day in order to be in line with conditions previously discussed in the literature (Serna-Maza et al., 2014; Yin et al., 2019; Zhang et al., 2017). However, the reactors showed signs of stress during the first 10 days of ammonia stripping treatment with a 10% per day recycling ratio, and biogas production almost plummeted due to the VFA increase (Figure 7-2b and Figure 7-3b). Therefore, the treatment ratio was increased to 20% per day at day 154, and the OLR was reduced to 1.8 g VS/L/day (Figure 7-2a) in an attempt to remedy the reactors and restore stable biogas production. The biogas production remained unstable for an additional 20 days but increased gradually, after which it stabilized at SBP of 703±60 L biogas/kg VS_{added}/day until the end of SSAS-1 (day 203). The biogas production was slightly higher than PHAS with RNG (PHAS-2) and less than PHAS with air (PHAS-1), +3.4% and -15.4%, respectively. Ammonia levels in the reactors were reduced from 2700 to 1900 mg NH_3-N/L due to the SSAS-1 treatment, which consistently achieved similar ammonia removal efficiency as PHAS-1 (60-65%). The biogas production results show the superiority of the PHAS treatment over SSAS as PHAS achieved higher biogas production at significantly lower stripping requirements; SSAS-1 treated 20% of the reactor's working volume per day, whereas PHAS treated the incoming flow from the hydrolyzer, which was equivalent to 5% of the reactor's working volume.

Switching the feeding from ammonia-stripped PM to hydrolyzed PM led to increased VFAs in the digester due to the higher concentration of VFAs in the hydrolyzed PM than in ammonia-stripped PM. However, this increase was accompanied by an increase in alkalinity due to the higher lime influx needed for treating higher amounts in SSAS-1 than in PHAS. Therefore, the VFA/ALK was maintained below 0.3, and the stability of the reactor was not compromised (Holliger et al., 2016).

The SSAS-1 treatment required almost double the amount of lime to raise the digestate's pH to 9.5 compared with PHAS because the treated amount of digestate was higher in SSAS-1. However, both systems required the same lime dosage per volume (18 g lime per kg digestate). As a result, the TS and the VS in the AD reactors steadily increased from 6.3 to 11.6% and from 2.6 to 4.25%, respectively (Figure 7-3d). A similar increase in TS% was reported by Zhang et al. (2017). The addition of inorganic lime cannot justify the increase in VS%, raising concerns about the possibility of unintended sedimentation at the bottom of the reactor during

the PHAS scenarios. To verify the significance of sedimentation in the reactors, the contents of the reactors were decanted on day 436 (the end of the experiment), and the solids at the bottom of the reactors were scraped and added to the rest of the reactor contents. If sedimentation were significant throughout the experiment, the TS measurement would be significantly higher than 9-10% (the feed's total solids). However, the TS in AD1 and AD2 were 9.5 and 11.5%, respectively, indicating no significant settlement throughout the experiment. The treatment of 20% of the reactor's volume at 55 ^{o}C for three hours every day may have contributed to increasing the total and volatile solids content.

In large-scale plants, removing 20% of the reactor's working volume per day for 2-3 hours for treatment would disrupt the operation (K. Li et al., 2018; Serna-Maza et al., 2014; Zhang et al., 2017) and lead to large treatment units. Therefore, after the performance of the AD reactors was stabilized with SSAS-1 conditions, the recycling ratio was dropped to 10% per day (SSAS-2) while maintaining OLR and other stripping tower conditions the same, i.e., pH 9.5, 55 ^{o}C, and RNG flowrate of 100 L RNG/L digestate/ hour, in order to assess the stability of the reactor at lower treatment proportions.

SSAS-2 remained operational from day 243 to day 303 (3 HRTs). The impact of reducing the treatment proportion was reflected in the biogas production and ammonia levels almost immediately after the treatment was switched to a 10% recycling ratio. The VBP and SBP dropped from 1.16 L/L/day in SSAS-1 to 0.88 L/L/day in SSAS-2 and from 703±60 L biogas/kg VS_{added} in SSAS-1 to 475±40 L biogas/ kg VS_{added} in SSAS-2, respectively. These drops were mainly due to ammonia levels increasing rapidly from 1900 to 3186±218 mg NH_3-N/L. Having said that, the SSAS-2 treatment successfully maintained stable continuous operation, despite being under sub-optimal conditions. The methane content in biogas was reduced from around 65% in SSAS-1 to 58% in SSAS-2.

These sub-optimal conditions of SSAS-2 also led to VFA accumulation at around 29722 ± 1199 mg acetic acid/L, which increased from 14086 ± 1843 mg acetic acid/L in SSAS-1. Also, due to the reduced amount of lime added for the treatment, the alkalinity dropped from 66856 to 55622 mg $CaCO_3$/L. As a result, the VFA/ALK ratio increased to 0.51, above the recommended 0.3 ratio (Nguyen et al., 2019; Reyes et al., 2015). However, there was no apparent effect of these challenges on the pH of the effluent, as it was stabilized at 7.9 throughout the experiment (Figure 7-3c). This pH level is favorable for methanogenic archaea and can reduce the competition over carbon with sulfur-reducing or sulfur-oxidizing bacteria since these types of bacteria often prefer lower pH levels, which explains why the drop in

biogas production, as well as methane content, was not drastic (Fotidis et al., 2014; Rajagopal et al., 2013).

The ammonia removal efficiency in SSAS-2 did not differ from SSAS-1 since they were conducted at similar tower conditions (both achieved 60-65% ammonia removal). However, ammonia levels increased in the main digesters due to the lower recycling ratio, which eventually led to higher ammonia levels in the ammonia stripping unit effluent in SSAS-2 than SSAS-1 (1050 mg NH_3-N/L in SSAS-2 compared to 600 mg NH_3-N/L in SSAS-1).

The last treatment (SSAS-3) represented the use of air in side-stream ammonia stripping at a 10% recycling ratio and under similar stripping tower conditions, i.e., pH 9.5, 55 ^oC, and flowrate of 100 L air/L digestate/hour. Recently, the use of air in SSAS has been of interest due to its possibility to achieve higher ammonia removal efficiencies in a shorter time compared with other gases and its lower impact on the digestate's buffer capacity (Bousek et al., 2016).

A new sample was collected from the farm for this part of the experiment. Fortunately, the collection time and conditions were similar to the first batch, which minimized the differences in the manure's characteristics, specifically the moisture content. Moreover, the farm also confirmed that no diet or operations changes occurred that could significantly affect the quality of manure. However, ammonia levels in the second batch were higher than in the first batch, increasing ammonia levels during SSAS-3 despite achieving higher ammonia removal efficiency (70 ± 3%) than SSAS-2 (Figure 7-2a). As a result, the ammonia levels of hydrolyzed PM from the second batch reached 8100 mg NH_3-N/L, compared to 6000 mg NH_3-N/L from the first batch.

Despite the slight increase of the digestate's ammonia levels during SSAS-3, VBP and SBP increased to 1.14 L biogas/L/day and 565 ±43 L biogas/kg VS_{added}/day, respectively. The increase in biogas production was accompanied by a significant drop in VFAs from 31720 to 20694 mg CH_3COOH/L. The reactors' alkalinity in SSAS-3 remained similar to SSAS-2 (58568 mg $CaCO_3$/L) because the lime dosage and treatment proportions remained the same. As a result, VFA/ALK reduced to 0.35, providing a more stable operation than observed in SSAS-2 when RNG was used. This indicates that the second batch of poultry manure may have been more readily biodegradable than the first batch, which could be evident by observing the improvement in VFA consumption.

One of the concerns when using air for ammonia stripping, especially for SSAS systems, is that it could lead to the toxification of methanogens when the treated portion is fed back to the

reactor. However, the high biogas production observed in SSAS-3 conducted in this study shows no evidence of inhibition due to using air. Moreover, dissolved oxygen (DO) was measured immediately after stripping with air and was found close to 2.5-3 mg/L and declined rapidly (within 20 minutes) to below 0.2 mg/L. A similar conclusion was also observed by (Fernandez-Gonzalez et al., 2019), where it was reported that even with no liquid/solid separation, air SSAS did not negatively impact biogas production. However, it shifted the microbial presence towards hydrogenotrophic methanogens.

It should be noted the recycling ratio in this study was higher than in previous SSAS studies discussing food waste (Serna-Maza et al., 2014; Zhang et al., 2017). Having said that, there are some key differences between the current study and the above studies that compelled the higher recycling ratio in this study. First, most SSAS studies discussed thermophilic digesters with temperatures set to 55-60 ^{o}C, whereas this study discussed mesophilic digesters. Moreover, treatment conditions, including recycling ratio and stripping tower conditions, depend on the feedstock's ammonia levels and removal efficiency. Poultry manure in this study had ammonia levels reaching 6000-8100 mg NH_3-N/L before treatment. On the other hand, the food waste ammonia levels discussed in Serna-Maza et al. (2014) and Zhang et al. (2017) were limited to 4000-6000 mg NH_3-N/L, allowing less recycling ratios to be effective.

7.3.4 Base scenario

The last phase of this study was to assess the reactors' performance when no ammonia stripping treatment was applied at any stage. On day 333, the reactors were fed with hydrolyzed PM characterized by high ammonia levels nearing 6000-8100 mg NH_3-N/L at the initial OLR (2.6 g VS/L/day). As a result, the reactors' biogas production started declining steadily and stabilized at VBP 0.524 L biogas /L digestate/day and SBP of 154±20 L biogas/kg VS/day. The SBP during the control scenario was 81, 77, 78, 68, and 73% less than PHAS-1, PHAS-2, SSAS-1, SSAS-2, and SSAS-3, respectively. Moreover, the methane content dropped to 40% of the total biogas production, whereas it was stable at 58-65% during the previous phases. In addition, the VS removal dropped from 52-78% in previous phases to 17% in the Base scenario, indicating clear signs of inhibition. Interestingly, the drop in biogas production was not abrupt, indicating some degree of acclimatization of methanogens to high ammonia levels.

The drop in biogas production due to high ammonia levels was accompanied by increased VFA and COD levels of the reactors' effluents. In addition, due to the discontinued lime addition in this phase, alkalinity dropped from around 70000 to 28000 mg $CaCO_3$/L, equivalent to the

alkalinity levels of the hydrolyzed PM fed to the reactor. As a result, VFA/ALK ratio increased to around 0.9, leading to a drop in pH to 7.46±0.08, which is still favorable for methanogenic microorganisms. However, with ammonia levels rapidly increasing to 8100±100 mg NH_3-N/L, it was clear that the process was inhibited and operated under sub-optimal conditions. TKN levels also increased significantly to 10111 mg TKN-N/L by the end of the Base scenario.

After day 400, the feeding stopped. The reactors were monitored for biogas production and other characteristics. Biogas production gradually dropped to 0.05 L biogas/L/day. Only a few properties were different from the end of the Base scenario. Due to the prolonged period after feeding, it was noticed that COD and VFA concentrations decreased to 53206 mg COD/L and 12000 mg CH_3COOH/L, respectively, due to the continuation of biogas production. The sudden increase in alkalinity and TS at the end of the experiment was due to scraping the solids precipitated at the bottom, which included some of the lime particles that were added during the treatments.

The Base scenario results showed that the mono-digestion of poultry manure is not feasible without further treatment. Treatment with PHAS and SSAS improved biogas production by 5.3- and 4.5-fold, respectively. In addition, other performance parameters, such as ammonia, VFA/ALK, and VS removal, were all improved significantly due to the stripping treatments.

In Adghim et al. (2023), hydrolyzed PM resulted in about 340 L CH_4/kg VS in the batch mode. However, ammonia levels were capped at 3300 mg NH_3-N/L because the accumulation of ammonia was not possible. On the other hand, the continuous feeding of the reactors in the current study allows for ammonia accumulation, which led to methane yield of only 61 L CH_4/kg VS/day at ammonia levels of 8100 mg NH_3-N/L.

7.3.5 Discussion of results

7.3.5.1 Discussion on post-hydrolysis versus side-stream ammonia stripping

All treatments in this study showed that mitigating ammonia inhibition and allowing the mono-digestion of poultry manure is feasible at different degrees. For instance, PHAS systems treated all incoming effluent from the hydrolyzer (500 ml/day per reactor), whereas SSAS treated higher volumes of the digestate (1000-2000 ml/day per reactor) to compete with the performance of PHAS systems. However, even at these high recycling ratios, PHAS outperformed SSAS and maintained a more stable operation of the reactors in terms of biogas production and digestate characteristics (680-830 L biogas/kg VS/day versus 475-701 L

biogas/kg VS/day). Moreover, PHAS successfully alleviated ammonia inhibitory effects at a higher OLR than SSAS (2.6 and 1.8, respectively).

Despite the advantages of PHAS, there are some situations where the use of PHAS is not applicable. One example is in biogas plants operating a one-stage AD configuration, where building a side-stream ammonia stripping may be more logical than building an additional reactor for hydrolysis intended specifically to allow PHAS, which would require additional space, operation and maintenance, and capital costs.

Despite the two systems having different approaches, some mirroring effects were observed when using air or RNG, where air treatment always led to higher biogas production. However, using air in biogas plants may be unfavorable for several reasons, such as avoiding the need to implement a solid/liquid separation prior to the treatment in the SSAS case or the risk of air infiltration to the main digester. Therefore, it was essential to find an alternative carrier gas that could achieve similar ammonia removal efficiency of air, which RNG proved successful at.

7.3.5.2 Discussion on selected parameters for side-stream ammonia stripping

This part of the discussion addresses the significant differences between the stripping conditions conducted throughout the SSAS in this study and those more common in the literature. The determination of the stripping tower conditions depends mostly on the type of carrier gas used. For example Serna-Maza et al. (2014) and Zhang et al. (2017) used biogas for ammonia removal, which can significantly reduce the pH of the solution due to its high CO_2 content if pumped at high flowrates. Therefore, in all these studies, biogas flowrate is often limited between 1-10 L biogas/ L digestate /hour, the temperatures were set to 65-75 ^{o}C, pH was increased above 10, and the duration of stripping ranged between 1-3 days per treatment batch. The high temperature and pH translate to high energy and material demands that can be burdensome to the plant.

Alternatively, using air in Fernandez-Gonzalez et al. (2019) and RNG and air in this study, higher ammonia removal efficiency was achievable at significantly lower pH and temperature requirements than biogas recirculation. Moreover, if ammonia stripping columns were operated in batch or semi-batch modes, the required hours for treatment using air or RNG compared with biogas would be 17-21 hours per week versus 168 hours per week with biogas, respectively. This means that systems with an efficient carrier gas will require significantly less energy and alkaline materials to achieve high ammonia removal efficiency, whether in PHAS or SSAS. Having said that, the carrier gas flowrate using air or RNG was almost 5-10 times

the flowrate reported for biogas stripping. This may pose a challenge in finding suitable and affordable air or RNG pumps or using multiple pumps per stripping column. There is a clear trade-off between the amount of energy and material (lime) needed to achieve high ammonia removal efficiency and the carrier gas flow rate, regardless of whether it is a PHAS or an SSAS system.

7.3.5.3 Discussion on air versus RNG as stripping mediums

Ammonia stripping with RNG is a new concept, and it shows promising results regarding alleviating ammonia inhibition and achieving stable biogas production. Compared with air, RNG has lower ammonia removal efficiency and consequentially less improvement in biogas production. However, when compared with the Base (no treatment) scenario, which was inhibited due to high ammonia levels, it is clear that RNG would still be considered a viable stripping medium. Moreover, in situations where anaerobic conditions must be maintained during stripping, RNG can be more advantageous than air due to its high purity (>98% CH_4) preventing risk of oxygen toxicity. Having said that, stripping conditions may need to be modified to achieve higher ammonia removal efficiency. Moreover, RNG can offer other advantages as an efficient anaerobic stripping alternative to the widely discussed stripping gases in literature, i.e., biogas, steam, or nitrogen. Additionally, unlike nitrogen, RNG is available in-situ and can achieve higher ammonia removal efficiency than biogas and water steam under significantly gentler conditions, i.e., lower pH, temperature, and duration of treatment (Bousek et al., 2016; K. Li et al., 2018).

7.3.6 Pre-hydrolysis ammonia stripping (Batch Experiment)

Batch experiments were conducted to identify the impact of biological hydrolysis prior to the stripping treatment, and whether organic nitrogen is converted to ammonia during the stripping. Pre-hydrolysis ammonia stripping was also discussed by (M. Walker et al., 2011) as a possible ammonia stripping configuration. Regarding ammonia removal, stripping after hydrolysis was significantly advantageous at both stripping temperatures (40 and 55 ^{o}C) as ammonia removal efficiency reached 34 and 69% in PHAS versus 19 and 27% in pre-hydrolysis ammonia stripping for treatment at 40 and 55 ^{o}C, respectively. This difference in performance between the two systems is due to the higher FAN levels in PHAS than in pre-hydrolysis ammonia stripping, despite being under a similar FAN/TAN ratio. This is because the hydrolysis step in PHAS converted around 88% of organic nitrogen into ammonia, leading to ammonia levels of 5432 ±169 mg NH_3-N/L at the beginning of the stripping treatment.

On the other hand, ammonia levels at the start of the pre-hydrolysis ammonia stripping were limited to 1165 and 1613 mg NH_3-N/L for the 40 and 55 ^oC scenarios, respectively, which led to lower concentrations of volatile ammonia. The ammonia level profile during stripping shown in Figure 7-4 indicates that in the case of pre-hydrolysis ammonia stripping at temperature 55 ^oC, there was a slight increase in ammonia levels, indicating that pre-heating the sample as well as the continuous heating during stripping transformed some organic nitrogen into ammonia (around 323 mg Organic N-N/L were converted to ammonia). However, a stripping temperature of 40 ^oC did not lead to converting organic nitrogen to ammonia. In PHAS, the samples were already hydrolyzed; therefore, no organic nitrogen changes were observed at 40 or 55 ^oC.

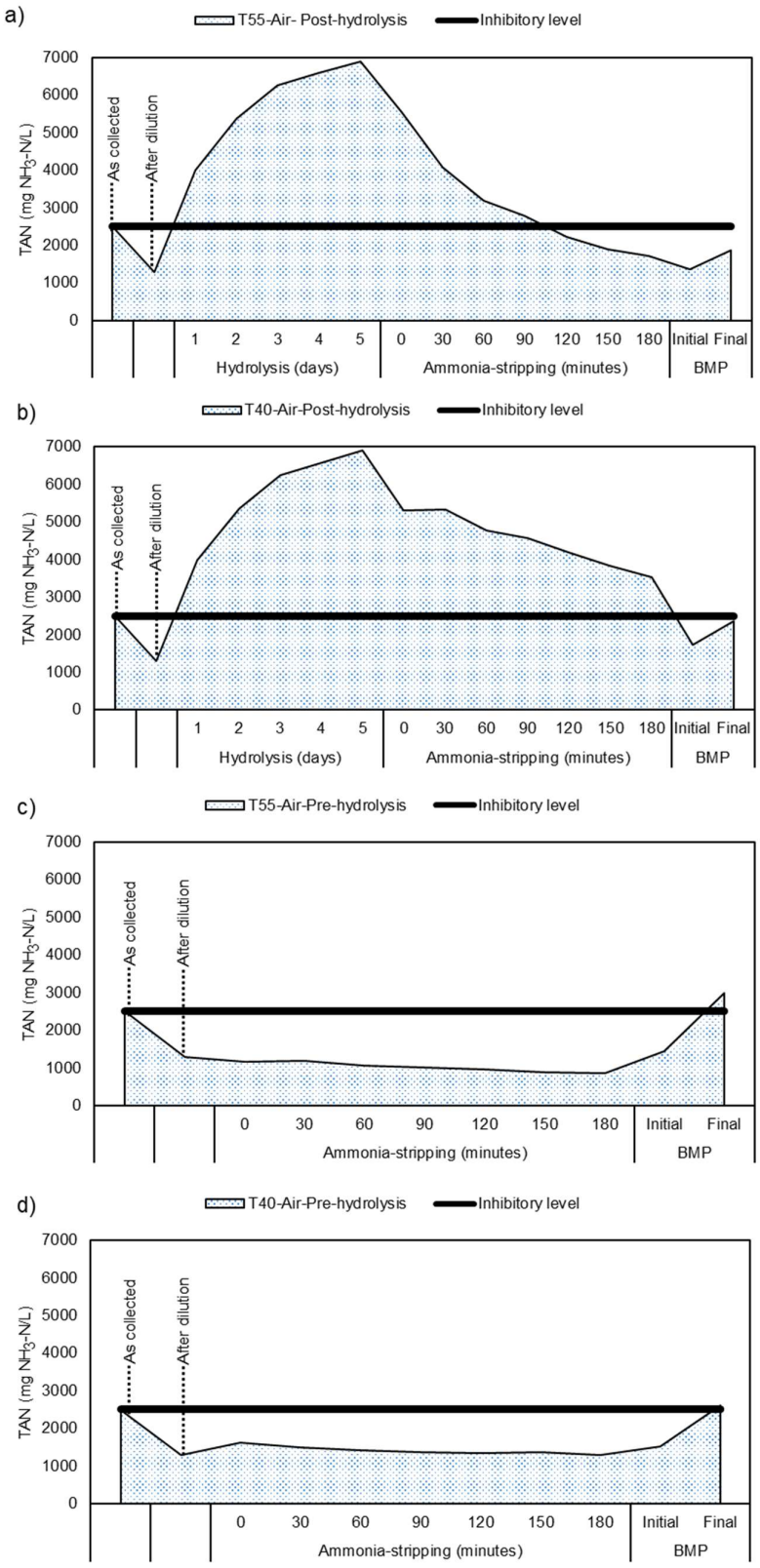

Figure 7-4: Ammonia profile during as collected, dilution and blending, ammonia stripping, and BMP test. a) and b) post-hydrolysis ammonia stripping at 55 and 40 °C; c) and d) pre-hydrolysis ammonia stripping at 55 and 40 °C.

In terms of the methane potential test (BMP), two Base scenarios were used to compare the performance of pre-hydrolysis ammonia stripping with PHAS 1) blended PM used for pre-hydrolysis ammonia stripping and 2) Hydrolyzed PM used for PHAS. Figure 7-5 shows the results of net cumulative methane potential. As expected, treatment of pre-hydrolysis ammonia stripping at 40 ºC did not significantly reduce or improve methane production compared with Blended PM (P=0.15) due to the low ammonia removal efficiency. On the other hand, an improvement of 34% in biogas potential was observed in samples treated with pre-hydrolysis ammonia stripping at 55 ºC. Interestingly, this improvement is considered inefficient compared with the other Base's methane potential (hydrolyzed PM), which achieved almost equal methane potential (333 and 331 L CH_4/kg VS_{added} in hydrolyzed and pre-hydrolysis ammonia stripping at 55 ºC, respectively). This clearly indicates that pre-hydrolysis ammonia stripping is unfavorable for high-nitrogen feedstock because of the low ammonia removal efficiency and the sudden increase in ammonia due to the degradation of organic-nitrogen during the digestion led to inhibition of the microorganism's activity. Therefore, it is recommended that biogas plant operators select either SSAS or change their operation to two-stage AD to incorporate PHAS. However, pre-hydrolysis ammonia stripping may still be feasible for low-nitrogen feedstock if the TKN is not significantly high (around 3000 mg TKN-N/L or less). In light of these results, the pre-hydrolysis ammonia stripping system was not investigated in the semi-continuous flow mode in this study.

On the other hand, methane potential from the hydrolyzed PM was enhanced by 71 and 24% due to the PHAS treatment at 55 and 40 ºC, respectively. Moreover, ammonia levels within the digester increased only by 37% in both PHAS scenarios, whereas the corresponding increments were due to pre-hydrolysis ammonia stripping. Treatment at 55 and 40 ºC were 107 and 71%, respectively.

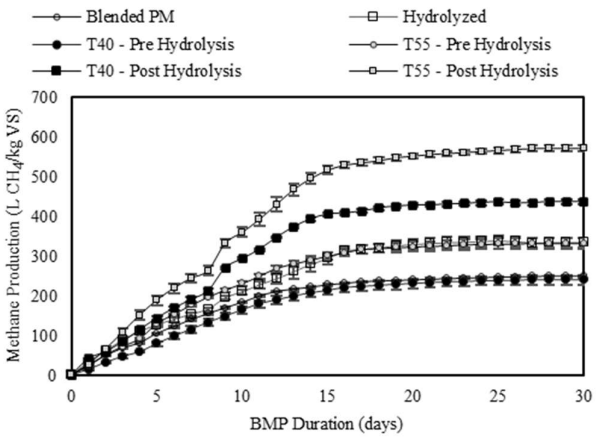

Figure 7-5: Methane production results of the batch testing of pre- versus post-hydrolysis ammonia stripping.

7.4 Conclusions

This study investigated the performance of the three possible ammonia stripping configurations intended to reduce ammonia levels in poultry manure to improve its methane potential based on experimental results. Amongst the three ammonia stripping approaches, post-hydrolysis ammonia stripping was the most advantageous and flexible configuration as it resulted in the highest biogas production and maintained sub-inhibitory levels with more efficiency than side-stream and pre-hydrolysis ammonia stripping. Side-stream ammonia stripping also proved successful in achieving stable biogas production, however, it required more treatment to compete with post-hydrolysis ammonia stripping. Batch experiment results showed that stripping before hydrolysis is unfeasible for high-nitrogen feedstock due to low ammonia removal and conversion of organic nitrogen into ammonia in the digester.

7.5 Acknowledgment

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7.6 References

The references of this Chapter are merged with the rest of the document and presented in Chapter 10: References

CHAPTER 8: INTEGRATION OF RESULTS

This chapter will combine and integrate conclusions of all the phases discussed in this dissertation and present them in terms of higher classifications, such as optimal ammonia stripping conditions, optimal ammonia stripping configuration, and biogas production.

8.1 Optimal Ammonia Stripping Conditions

8.1.1 Effect of pH

The effect of pH on ammonia removal was demonstrated in Phases I and II, and the optimal results were used later in Phases III and IV. The study covered a different range of pH to understand its impacts on the ammonia stripping kinetics when combined with other factors, i.e., temperature and flowrate. Therefore, ammonia stripping was tested at pH unadjusted (between 7-7.5), 9, 9.5, and 10. Based on the recommendations of Phase II, the pH value of 10 was advantageous over pH 9 or 7-7.5, reinstating that pH is a significant factor in increasing ammonia volatility. However, due to practical considerations such as lime requirements and handling, and to reduce equipment wear and maintenance requirements, a pH of 9.5 was selected for Phases III and IV without compromising the process efficiency by controlling other factors such as temperature, flow rate, and switching to an elongated stripping column design.

8.1.2 Effect of Temperature

The stripping temperature was thoroughly discussed in Phases I and II due to its vital role in ammonia volatility and removal. Ideally, an optimized system would require less temperature for ammonia removal to reduce the energy demands for raising the temperature throughout the stripping treatment. Therefore, this study was limited to 40 and 55 ^{o}C to represent the two temperatures that biogas plants most commonly use (mesophilic or thermophilic) in-situ for their hydrolyzers and/or digesters. The results of Phase II show that the ammonia removal at 55 ^{o}C was always more significant than that at 40 ^{o}C. Moreover, ammonia stripping at 55 ^{o}C followed by mesophilic anaerobic digestion produced more biogas than samples treated at 40 ^{o}C due to more solubilization of the material. Therefore, in the semi-continuous system experiment (Phase IV), the stripping temperature was only set at 55 ^{o}C.

8.1.3 Effect of Carrier Gas Flowrate

Carrier gas flow rate is an important factor determining ammonia removal efficiency. This study covered different air flow rates, including 300, 200, 100, and 50 L gas/ L digestate/ hour.

For proof-of-concept purposes in Phase I, 300 L/L/hour showed high ammonia removal efficiencies of 78%. However, the flow rate versus pH investigation experiment presented in the supplementary data of Phase IV showed that pH 9.5 and flowrate of 100 L/L/hour would show optimum results for ammonia stripping. Also, comparing the ammonia removal efficiency achieved with 300 L/L/hour and 100 L/L/hour at the same initial pH, it can be noted that the ammonia removal kinetics were more rapid in the former. However, the final ammonia removal efficiency was comparable, indicating that pH and temperature had more impact on the efficiency of the ammonia removal than the flow rate. Therefore, a flow rate of 100 L/L/hour was set for Phases III and IV.

8.1.4 Effect of Carrier Gas Type

One of the primary focuses of this study was the carrier gas type, i.e., air, renewable natural gas (RNG), or biogas. The literature presents air as the best-performing carrier gas, but it is often limited to pre- or post-hydrolysis ammonia stripping configurations. Moreover, no study discussed the use of RNG as a stripping medium despite its advantages as a stripping medium. Therefore, RNG was investigated for the first time as a carrier gas. The comparable performance between RNG and air led concluded that RNG is a feasible and applicable carrier gas, which can provide several advantages over biogas stripping. Biogas is widely discussed in the literature, specifically in side-stream ammonia stripping systems. However, stripping conditions are often described as severe since it is often conducted at high temperatures (>65 ^{o}C), high pH (>10), and stripping durations (1-3 days).

On the contrary, stripping with air or RNG requires significantly less energy and material (pH 9.5, temperature 55 ^{o}C) and shorter durations (2-3 hours). However, higher flow rates (50-300 L/L/hour) are required to achieve high ammonia removal efficiency. Comparatively, both systems would require similar volumes of carrier gas per treatment cycle. Moreover, comparing operating conditions using air or RNG versus biogas would highly depend on the type of stripping column used in situ, i.e., continuous flow or batch.

8.2 Ammonia Stripping Configuration

8.2.1 Post-hydrolysis ammonia stripping

Post-hydrolysis ammonia stripping in two stage AD process is the main focus of this study. Throughout Phases I to IV, post-hydrolysis ammonia stripping was tested under different conditions, which proved to be effective in reducing ammonia inhibitory effects and improving

the methane production from using poultry manure (PM) as feedstock. This shows this configuration's degree of flexibility and applicability compared to pre-hydrolysis or side-stream ammonia stripping.

Post-hydrolysis ammonia stripping resulted in stable PM digestion under batch and semi-continuous flow modes. In the latter, there was evidence that post-hydrolysis ammonia stripping improved the diversity of microorganisms during digestion because of the observed consumption of acetic acid in the digester. On the other hand, batch experiments, specifically in Phase III, showed that post-hydrolysis ammonia stripping had alleviated the ammonia inhibitory effects on hydrogenotrophic methanogens.

The application of post-hydrolysis ammonia stripping in the mono- and co-digestion of PM was a viable treatment option. Without treatment, the digestion of PM was provenly inhibited, as post-hydrolysis ammonia stripping enhanced biogas production by up to 81%. It is essential to note that the optimized conditions found in this study are applicable for the specific conditions of this study and possibly other low- to medium-solid feedstock with similar ammonia levels covered in this study (around 6000 mg NH_3-N/L). However, feedstock with higher ammonia levels may require more ammonia removal. Ideally, the ammonia of the feedstock after treatment should be less than 2000 mg NH_3-N/L because the risk of inhibition increases after this threshold.

8.2.2 Side-stream Ammonia Stripping

Side-stream ammonia stripping is a very common method of ammonia stripping. It has been discussed more thoroughly than post-hydrolysis ammonia stripping in the past decade. However, some evident shortcomings exist in the methods covered in the literature. In Phase IV, both systems were compared under semi-continuous flow mode and similar ammonia stripping conditions. The results of this phase were particularly interesting because despite treating twice the amount of digestate suggested in the literature for side-stream ammonia stripping and reducing the organic loading rate, it was still significantly outperformed by the post-hydrolysis ammonia stripping treatment. Having said that, compared to no treatment (Base), side-stream ammonia stripping successfully reduced the inhibitory effects of high ammonia levels and was operational for the long term.

Interestingly, implementing side-stream ammonia stripping with air yielded higher biogas production than when RNG was used. This was mirrored in both batch and semi-continuous flow modes of post-hydrolysis ammonia stripping discussed in Phases III and IV.

8.2.3 Pre-hydrolysis Ammonia Stripping

Pre-hydrolysis ammonia stripping was tested to highlight the role of hydrolysis in the post-hydrolysis ammonia stripping configuration and to test if organic nitrogen significantly converts to ammonia during the stripping treatment. The results showed a clear advantage of post-hydrolysis over pre-hydrolysis ammonia stripping. The ammonia removal in pre-hydrolysis ammonia stripping was limited because of the sample's low initial ammonia levels; most of the nitrogen is in the form of organic nitrogen. The total Kjeldahl nitrogen and ammonia analysis at the end of the stripping indicates that only insignificant amounts of organic nitrogen (<500 mg/L) have been converted to ammonia, which constituted less than 10% of the initial TKN. This is considered very low compared to the 88% achieved through biological hydrolysis in the post-hydrolysis ammonia stripping approach.

Pre-hydrolysis ammonia stripping may still be an effective treatment for feedstock with TKN levels closer to the inhibitory levels (2500-3000 mg TKN-N/L), where treatment is still necessary to lower ammonia levels.

CHAPTER 9: CONCLUSIONS AND RECOMMENDED FUTURE WORK

This dissertation covered a comprehensive investigation of the potential and feasibility of post-hydrolysis ammonia stripping as a novel approach to mitigate the inhibitory effects of ammonia on the anaerobic digestion (AD) process. After extensive experimental analyses, post-hydrolysis ammonia stripping was deemed as a viable treatment and its advantages over existing ammonia stripping configurations (pre-hydrolysis and side-stream ammonia stripping) were proven.

The batch experiment presented at the beginning of the study (Phase I) proved that co-digestion and two-stage AD process alone were not effective to alleviate ammonia inhibitory effects when PM constituted 50% or more of the feedstock recipe. Therefore, it was concluded that ammonia removal was necessary to enhance the biogas potential of PM. Post-hydrolysis ammonia stripping achieved around 78% ammonia removal and 150% enhancement of biogas production compared to untreated hydrolyzed poultry manure.

The statistical analysis presented in the optimization phase (Phase II) showed that pH, temperature, and co-digestion ratio were statistically significant to control the ammonia removal efficiency. It was also discussed that ammonia stripping at higher temperatures, i.e., 55 ^{o}C was more advantageous for biogas production than stripping at lower temperatures as it improved the solubilization of the digestate. This also indicated that pH can be reduced to 9.5 without compromising the ammonia removal efficiency or biogas production.

Phase III showed that renewable natural gas (RNG) applications as a stripping medium were successful in batch mode and achieved comparable ammonia removal efficiency and biogas production improvement to those obtained with air. However, biogas was briefly tested for ammonia removal at a similar flowrate of air and RNG and failed to achieve any significant ammonia removal, hence deemed as an unviable option for post-hydrolysis ammonia stripping under the set optimal conditions obtained from Phase II.

Post-hydrolysis ammonia stripping, tested in a semi-continuous flow mode (Phase IV), successfully achieved a stable operation at high biogas production of 831 and 680 L biogas/ kg VS/day at ammonia levels nearing 1700 and 2400 mg NH_3-N/L using air and RNG stripping, respectively. In addition, the semi-continuous flow experiment showed that post-hydrolysis ammonia stripping may have alleviated ammonia inhibitory effects on acetoclastic methanogens growth due to the observed 65% reduction in volatile fatty acids concentration in the digesters.

Phase IV also showed that post-hydrolysis ammonia stripping was more favorable than pre-hydrolysis ammonia stripping in batch mode and side-stream ammonia stripping in semi-continuous flow mode. Pre-hydrolysis ammonia stripping results showed that the conversion of organic nitrogen to ammonia during the stripping treatment is limited and therefore limits the ammonia removal efficiency. Moreover, most organic nitrogen eventually converts to ammonia during digestion, leading to ammonia inhibition. Therefore, pre-hydrolysis ammonia stripping can be recommended for biogas plants running a one-stage AD with slightly high nitrogen feedstock (TKN levels around 2500-3000 mg N/L).

In the semi-continuous flow mode experiment, side-stream ammonia stripping achieved lower biogas production than post-hydrolysis ammonia stripping with air. However, compared with the Base scenario (no treatment), it was observed that both post-hydrolysis and side-stream ammonia stripping treatments could achieve stable operations of biogas production. Furthermore, it was shown in both configurations that air as a stripping medium had always achieved higher ammonia removal efficiency and, consequently, biogas production. RNG can be used as an alternative to biogas recirculation or other anaerobic gases that may be either inefficient or require transportation and handling.

9.1 Recommendations for Future Work

As the main focus of this work was to cover some key knowledge gaps in the literature regarding the understanding of post-hydrolysis ammonia stripping, this study included extensive work in the lab. However, since the post-hydrolysis ammonia stripping has proven its stability under long-term operation and is advantageous to other stripping configurations, at least experimentally, the following are some recommendations for future work:

- The modelling in this study was limited to Gompertz Modelling and Statistical Modelling. Future work can include other common models, such as ADM1 and incorporate the post-hydrolysis ammonia stripping to predict the performance of the digesters.
- The stripping unit effluent gas in this study was always discarded instead of being recycled or adsorbed, as it was not a determining factor for the project's scope. However, future work can investigate nitrogen recovery from the stripping unit's effluent gas.
- Before commercialization, it is important to operate a pilot-scale post-hydrolysis ammonia stripping unit in order to identify possible complications with full-scale applications and to compare the results with those obtained from the ideal lab environment.

- The economic and environmental aspects of the post-hydrolysis ammonia stripping alone or compared with other ammonia stripping configurations were discussed lightly in this study. However, a comprehensive life cycle assessment and life cycle costing can provide much insight into the environmental and economic benefits of the suggested treatments.

9.1.1 Scaling up

Several factors affect the scaling up of AD of PM and must be taken into consideration. For example, as shown in all phases of the study, PM is considered a dry waste with at least 20% total solids. Such high solid contents concentration is not ideal for continuously fed stirred tank reactors, which are more common for biogas plants. Therefore, farmers may have to consider including a high-moisture component for up-scaling the process, which could be wash water, wastewater, another low-solid co-substrate, or a mixture of those.

Moreover, poultry farms include different types of birds such as egg layers, chicks, meat poultry, and others. As each bird type is fed with a different diet, the manure characteristics are different. Therefore, it is important to characterize the manure of each poultry type individually and together as a recipe based on the quantities produced on-site.

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CHAPTER 11: APPNEDICES

11.1 Phase I Appendices

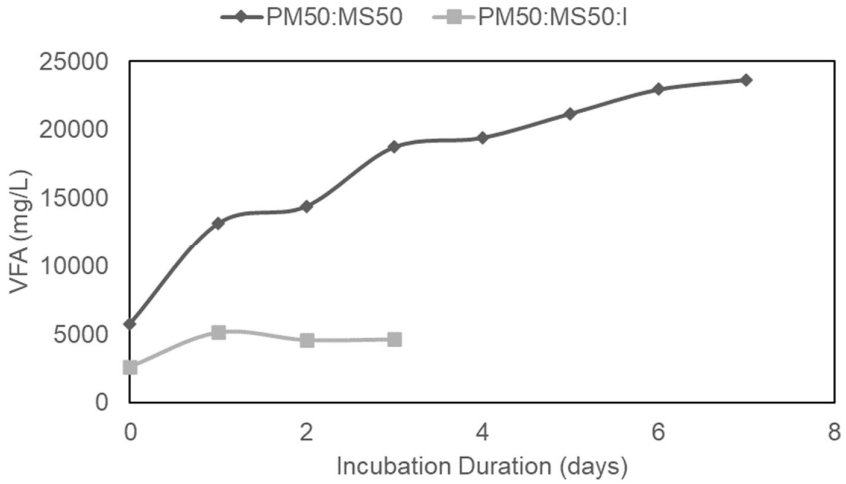

Figure Appendix 11-1: Pre-test for phase 1 to test hydrolysis time based on VFA accumulation. PM50:MS50 indicates no inoculation; PM50:MS50:I indicate inoculation at ISR of 2 g VS inoculum/g VS substrate.

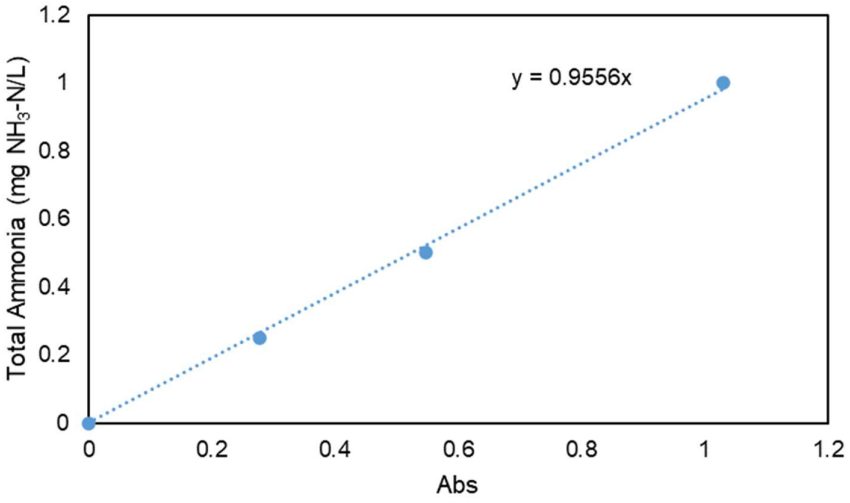

Figure Appendix 11-2: Calibration curve for phenate method in Phase 1.

Figure Appendix 11-3: Ammonia stripping bottle in water bath for Phases I and II.

Figure Appendix 11-4: Batch BMP bottles placed in the shaking incubator.

Table Appendix 11-1: Amounts of substrates and inoculum added to BMP bottles in Phase 1 with an estimation of initial ammonia.

Sample	Substrates added (g)		Inoculum added (g)	Initial TAN
	PM	MS	I	mg TAN/L
PM100-1	15.00	0.00	160.00	1072.73
PM100-2	15.00	0.00	160.00	1072.73
I1	0.00	0.00	175.00	813.00
I2	0.00	0.00	175.00	813.00
pH10T55-PM100-1	21.16	0.00	74.32	761.52
pH10T55-PM100-2	30.26	0.00	106.44	758.76
pH10T55-PM75:MS25-1	18.85	11.15	98.49	850.55
pH10T55-PM75:MS25-2	18.85	11.15	98.49	804.65
pH10T55-PM50:MS50-1	13.48	23.93	89.05	744.68
pH10T55-PM50:MS50-2	14.19	25.18	97.57	785.84
hydrolyzed-PM100-1	34.37	0.00	138.87	1606.96
hydrolyzed-PM100-2	37.23	0.00	150.57	1606.35
hydrolyzed-PM75:MS25-1	22.27	13.17	116.23	1520.85
hydrolyzed-PM75:MS25-2	23.54	13.93	122.75	1521.46
hydrolyzed-PM50:MS50-1	5.32	9.45	36.88	1438.39
hydrolyzed-PM50:MS50-2	3.48	6.18	25.44	1361.23
PM75:MS25-1	11.09	5.68	158.33	981.35
PM75:MS25-2	11.09	5.69	158.36	981.27
PM50:MS50-1	7.45	11.34	156.27	894.90
PM50:MS50-2	7.45	11.31	156.28	895.04
PM25:MS75-1	3.87	17.55	153.59	807.17
PM25:MS75-2	3.86	17.54	153.60	807.03
MS100-1	0.00	24.95	150.09	709.47
MS100-2	0.00	24.96	166.25	718.18
hydrolyzed-PM50:MS50-3	10.29	18.28	84.09	750.86

11.2 Phase II Appendices

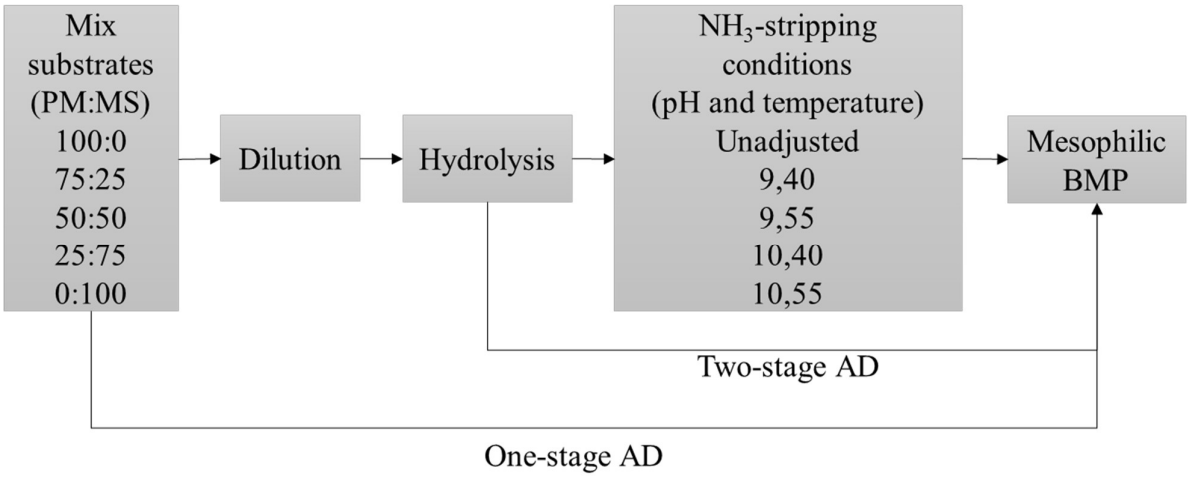

Figure Appendix 11-5: Paper #2 supplementary data (S1). Phase II experimental plan.

Table Appendix 11-2: Supplementary data for technical paper #2. Post-BMP Characterization of digestate.

Sample Type	Treatment	TS %	VS % (VS reduction %)	COD mg COD/L	sCOD mg COD/L	Total Alkalinity mg $CaCO_3$/L	VFAs mg CH_3COOH/L	TAN mg NH3-N/L (TAN increase %)
PM100	Untreated	5.01 ± 0.03	3.45 ± 0.01 (22.99 ± 0.1)	25556 ± 2476	10364 ± 262	16953 ± 3819	647 ± 95	3258 + 51 (203.7 ± 4.24)
	hydrolyzed	4.25 ± 0.08	2.78 ± 1.16 (28.49 ± 2.72)	49138 ± 4630	5849 ± 247	12863 ± 132	1077 ± 45	3343 + 80 (113.25 ± 10.79)
	pH-T-unadjusted	4.28 ± 0.05	2.81 ± 0.04 (28.4 ± 0.63)	41924 ± 3292	11704 ± 208	14321 ± 686	1260 ± 55	3257 + 90 (198.82 ± 0.42)
	pH9T40	3.93 ± 0.12	2.68 ± 0.06 (24.15 ± 1.83)	19010 ± 1905	9209 ± 111	11999 ± 2090	834 ± 55	2386 + 109 (142.11 ± 12.16)
	pH10T40	3.99 ± 0.09	2.72 ± 0.1 (23.52 ± 3.1)	18214 ± 790	8215 ± 125	10026 ± 266	531 ± 32	2288 + 53 (167.34 ± 4.89)
	pH9T55	4.9 ± 0.11	2.92 ± 0.06 (26.83 ± 0.03)	37261 ± 655	15785 ± 81	15070 ± 42	1281 ± 18	2596 + 43 (193.4 ± 5.12)
	pH10T55	5.08 ± 0.18	2.9 ± 0.06 (27.47 ± 1.75)	16977 ± 166	15354 ± 713	11191 ± 1354	941 ± 89	2303 + 59 (202.94 ± 0.01)
PM75:MS 25	Untreated	4.72 ± 0.09	3.24 ± 0.09 (27.69 ± 0.14)	46211 ± 3023	6012 ± 203	30576 ± 2174	1287 ± 43	2854 + 65 (190.81 ± 5.08)
	hydrolyzed	4.43 ± 0.06	2.98 ± 0.05 (28.41 ± 0.59)	47217 ± 3919	5995 ± 302	12928 ± 73	1067 ± 57	3300 + 46 (116.96 ± 3.05)
	pH-T-unadjusted	4.1 ± 0.04	2.78 ± 0.06 (32.99 ± 1.2)	49838 ± 2445	11477 ± 600	11877 ± 106	885 ± 78	2981 + 64 (118.38 ± 5.08)
	pH9T40	4.68 ± 0.27	2.92 ± 0.19 (30.85 ± 5.49)	51125 ± 5563	12345 ± 593	12608 ± 495	951 ± 59	2597 + 119 (120.06 ± 6.64)
	pH10T40	4.58 ± 0.07	2.74 ± 0.06 (36.01 ± 0.38)	55241 ± 3046	10112 ± 288	10834 ± 420	725 ± 78	2005 + 45 (149.77 ± 1.18)

Sample Type	Treatment	TS %	VS % (VS reduction %)	COD mg COD/L	sCOD mg COD/L	Total Alkalinity mg $CaCO_3$/L	VFAs mg CH_3COOH/L	TAN mg NH3-N/L (TAN increase %)
	pH9T55	4.75 ± 0.15	2.94 ± 0.08 (29.1 ± 2.04)	41571 ± 3811	5834 ± 122	11572 ± 319	811 ± 57	2646 + 104 (99.65 ± 18.27)
	pH10T55	4.36 ± 0.06	2.63 ± 0.03 (36.74 ± 0.81)	23094 ± 57	4872 ± 62	10119 ± 344	689 ± 32	2072 + 52 (150.53 ± 10.17)
PM50:MS 50	Untreated	4.43 ± 0.06	3.09 ± 0.06 (30.85 ± 1.49)	47306 ± 5444	5984 ± 126	18692 ± 797	1237 ± 88	2666 + 58 (197.84 ± 6.93)
	hydrolyzed	4.22 ± 0.09	2.92 ± 0.05 (35.07 ± 0.78)	39468 ± 1609	6185 ± 127	11424 ± 50	955 ± 37	3032 + 10 (116.8 ± 8.91)
	pH-T-unadjusted	4.11 ± 0.07	2.79 ± 0.05 (38.36 ± 0.15)	46822 ± 267	6206 ± 138	11696 ± 235	878 ± 44	2994 + 66 (100.38 ± 5.49)
	pH9T40	4.43 ± 0.03	2.81 ± 0.04 (37.99 ± 1.35)	41028 ± 3526	6095 ± 189	11546 ± 507	813 ± 34	2613 + 122 (112.67 ± 10.49)
	pH10T40	4.51 ± 0.1	2.73 ± 0.07 (41.11 ± 2.91)	38947 ± 3752	4539 ± 252	9680 ± 279	784 ± 44	2053 + 74 (123.49 ± 4.25)
	pH9T55	4.54 ± 0.14	2.82 ± 0.11 (38.09 ± 2.7)	46342 ± 5494	5244 ± 175	10325 ± 254	825 ± 30	2403 + 68 (111.41 ± 3.39)
	pH10T55	4.51 ± 0.06	2.74 ± 0.05 (39.61 ± 0.92)	40985 ± 3929	7241 ± 2744	10073 ± 54	1186 ± 0	1985 + 55 (159.4 ± 3.63)
PM25:MS 75	Untreated	4.18 ± 0.08	2.95 ± 0.07 (33.75 ± 0.45)	42750 ± 2893	5937 ± 464	10057 ± 626	1225 ± 87	2340 + 25 (198.03 ± 9.59)
MS100	Untreated	3.94 ± 0.07	2.9 ± 0.09 (33.91 ± 1.2)	42049 ± 2544	5446 ± 155	8335 ± 91	1231 ± 86	2052 + 33 (187.42 ± 0.93)

Table Appendix 11-3: Optimization of flowrate (using 50 L air/L digestate/hour)

Time (min)	pH	TAN DF	TAN Reading 1	TAN Reading 1	TAN (average) mg NH_3-N/L	TAN removal %
0	9.76	130.89	24	23.7	3121.7265	
20	9.65					
30	9.54	123.05	21.3	22	2664.0325	14.7%
60	9.46	91.07	30.2	29.4	2713.886	13.1%
90	9.36	79.96	26.8	27.1	2154.922	31.0%
120	9.28	81.6	26.5	25.6	2125.68	31.9%
150	9.2	80.28	24.4	24.6	1966.86	37.0%

Table Appendix 11-4: Optimization of flowrate (using 100 L air/L digestate/hour)

Time (min)	pH	TAN DF	TAN Reading 1	TAN Reading 1	TAN (average) mg NH_3-N/L	TAN removal %
0	9.67	130.09	25.4	26	3343.313	
20	9.6					
30	9.53	89.8	31.2	31	2792.78	16.5%
60	9.42	81.96	33.1	32.8	2700.582	19.2%
90	9.36	80.46	26.5	27.2	2160.351	35.4%
120	9.29	74.51	31.1	28.9	2235.3	33.1%
150	9.16	61.7	27.6	27.3	1693.665	49.3%

Table Appendix 11-5: Additional testing for flowrate 100 L air/L digestate/hour.

Duration of Stripping (hrs)	TAN (mg/L)				TAN (removal) %				pH			
	0	**0.75**	**1.5**	**2**	**0**	**0.75**	**1.5**	**2**	**0**	**0.75**	**1.5**	**2**
PM100 pHun-T40-1	6761	6156	6248	6405	0.0%	9.0%	7.6%	5.3%	6.78	6.97	6.98	6.98
PM100 pHun-T40-2	6761	5914	6050	6362	0.0%	12.5%	10.5%	5.9%	6.78	6.98	6.97	7.01
PM100 pH9.5-T40-1	5592	2911	2503	2487	0.0%	47.9%	55.2%	55.5%	9.55	9.48	9.11	9.06
PM100 pH9.5-T40-2	5636	2848	2414	2630	0.0%	49.5%	57.2%	53.3%	9.52	9.41	9.04	8.93
PM100 pH9.5-T55-1	5423	2507	1971	1784	0.0%	53.8%	63.7%	67.1%	9.55	9.12	8.88	8.78
PM100 pH9.5-T55-2	5581	2645	2081	1660	0.0%	52.6%	62.7%	70.3%	9.55	9.08	8.79	8.66
PM75:MS25 pHun-T40-1	4301	4553	4290	4168	0.0%	-5.9%	0.2%	3.1%	6.36	6.38	6.48	6.49
PM75:MS25 pHun-T40-2	4301	4633	4272	4337	0.0%	-7.7%	0.7%	-0.9%	6.36	6.34	6.41	6.45
PM75:MS25 pH9.5-T40-1	3728	1743	1474	1572	0.0%	53.2%	60.5%	57.8%	9.48	9.08	9	8.92
PM75:MS25 pH9.5-T40-2	3613	1752	1537	1669	0.0%	51.5%	57.4%	53.8%	9.48	9.08	9.02	8.91
PM75:MS25 pH9.5-T55-1	3457	1662	1231	946	0.0%	51.9%	64.4%	72.6%	9.53	9.17	9.12	8.92
PM75:MS25 pH9.5-T55-2	3525	1283	1159	902	0.0%	63.6%	67.1%	74.4%	9.5	9.05	9.06	8.8
PM50:MS50 pHun-T40-1	3297	2952	2837	3425	0.0%	10.5%	14.0%	-3.9%	5.02	5.02	5.04	5.04
PM50:MS50 pHun-T40-2	3297	2872	2837	3267	0.0%	12.9%	14.0%	0.9%	5.02	5.03	5.04	5.03
PM50:MS50 pH9.5-T40-1	2678	1161	916	851	0.0%	56.7%	65.8%	68.2%	9.5	9.36	8.87	8.9
PM50:MS50 pH9.5-T40-2	2693	1205	1035	964	0.0%	55.2%	61.6%	64.2%	9.49	9.24	8.79	8.84
PM50:MS50 pH9.5-T55-1	2623	763	502	389	0.0%	70.9%	80.9%	85.2%	9.52	8.91	8.76	8.76
PM50:MS50 pH9.5-T55-2	2622	778	488	296	0.0%	70.3%	81.4%	88.7%	9.51	8.93	8.77	8.78

11.3 Phase III Appendices

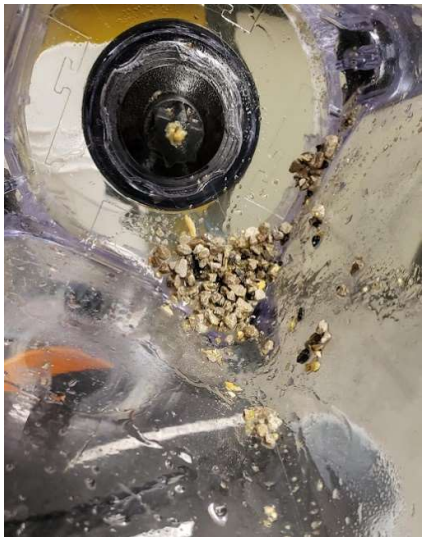

Figure Appendix 11-6: Gravel in the farm manure that reduced the manure quality.

Figure Appendix 11-7: Poultry manure outdoor pile at the egg factory to replace farm manure. Used for Phases III and IV.

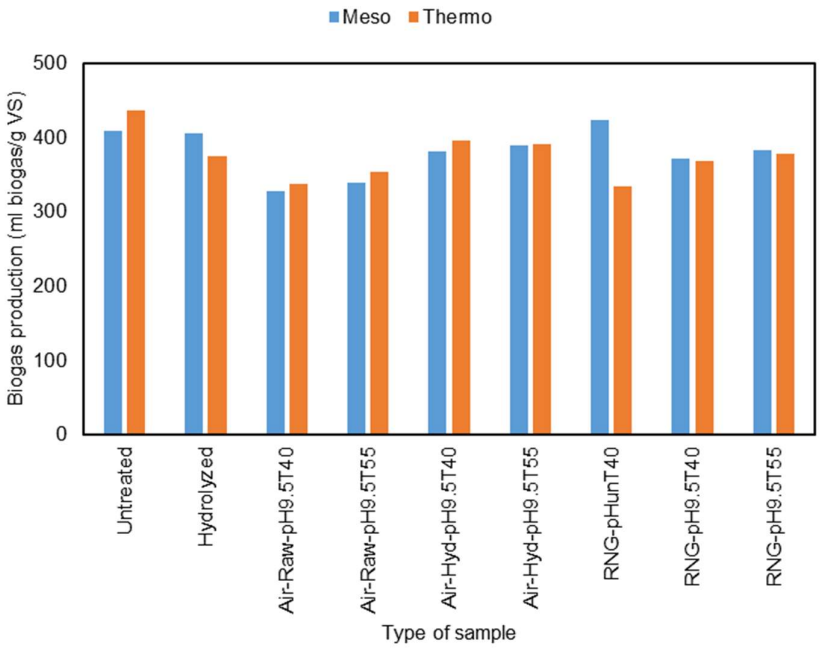

Figure Appendix 11-8: unsuccessful run for mesophilic and thermophilic BMP tests due to problems with inoculum and gas leaking. Thermophilic experiment was dropped from the experimental plan afterwards due to lack of thermophilic digesters in Canada.

Figure Appendix 11-9: Ammonia stripping setup used for Phase III.

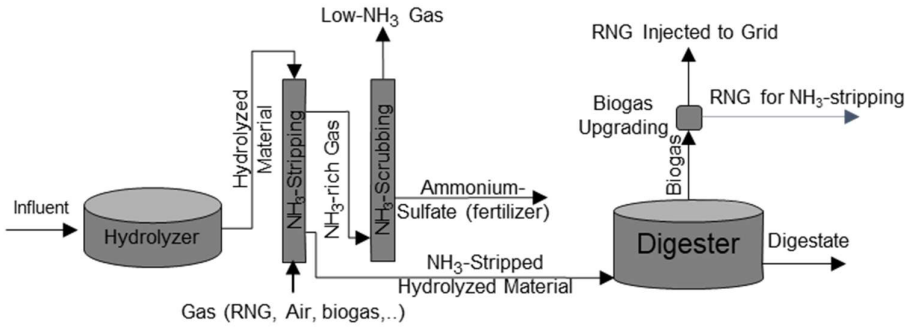

Figure Appendix 11-10: Graphical abstract of technical paper #3. Incorporating RNG in post-hydrolysis ammonia stripping.

Table Appendix 11-6: Post-BMP Characterization in Phase III.

Sample type	TS Final	VS Final	TAN	VFA	ALK	COD	TN	NO_3-NO_2	TKN	BMP
	%	%	mg NH_3-N/L	mg CH_3COOH/L	mg $CaCO_3$/L	mg COD/L	mg N/L	mg NO_3-NO_2-N/L	mg TKN-N/L	L CH_4/kg VS
T55-Air	5%	3%	1867	13468	17502	44640	3110	191	2920	564
T55-NG	5%	3%	2021	12880	17375	43804	3434	189	3235	522
T40-NG	4%	3%	2402	13250	17762	43925	3760	198	3576	408
T40-Air	4%	3%	2352	12530	17109	43780	3838	182	3722	435
Raw PM	5%	6%	2882	10441	17433	47290	4262	223	4030	224
Hydrolyzed PM	4%	3%	3047	11789	14694	42345	3812	188	3630	338
Blended PM	4%	3%	2971	11448	19713	38915	4429	199	4230	246

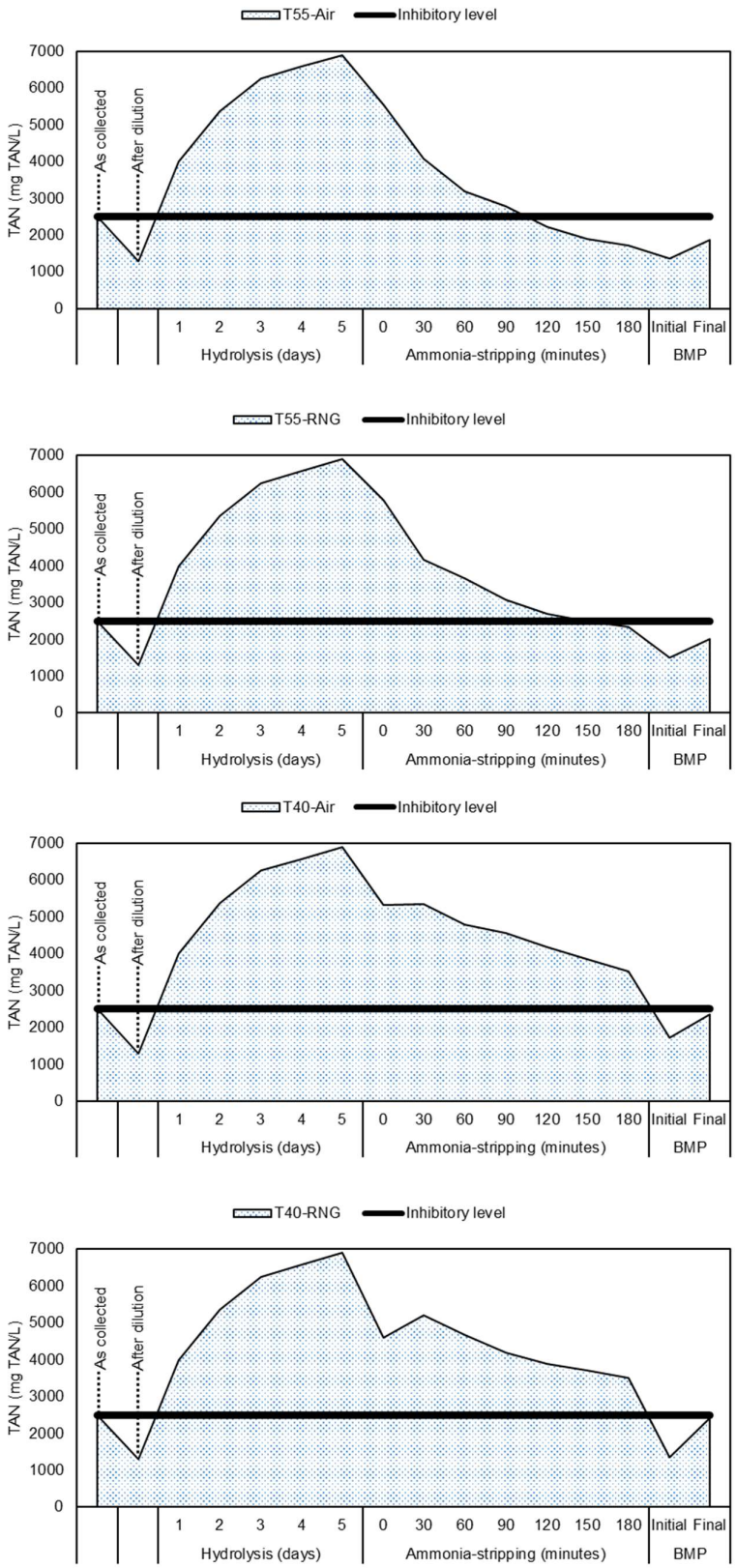

Figure Appendix 11-11: Ammonia profile using air and RNG stripping in batch mode.

11.4 Phase IV Appendices

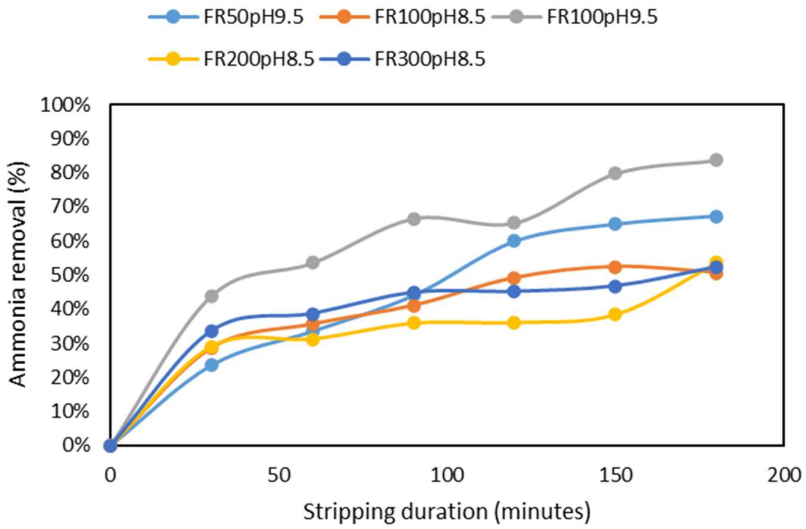

Figure Appendix 11-12 technical Paper #4 supplementary figure. Optimization step to test different flowrates with different pH.

Figure Appendix 11-13: Hydrolysis reactors in the semi-continuous experiment in Phase IV.

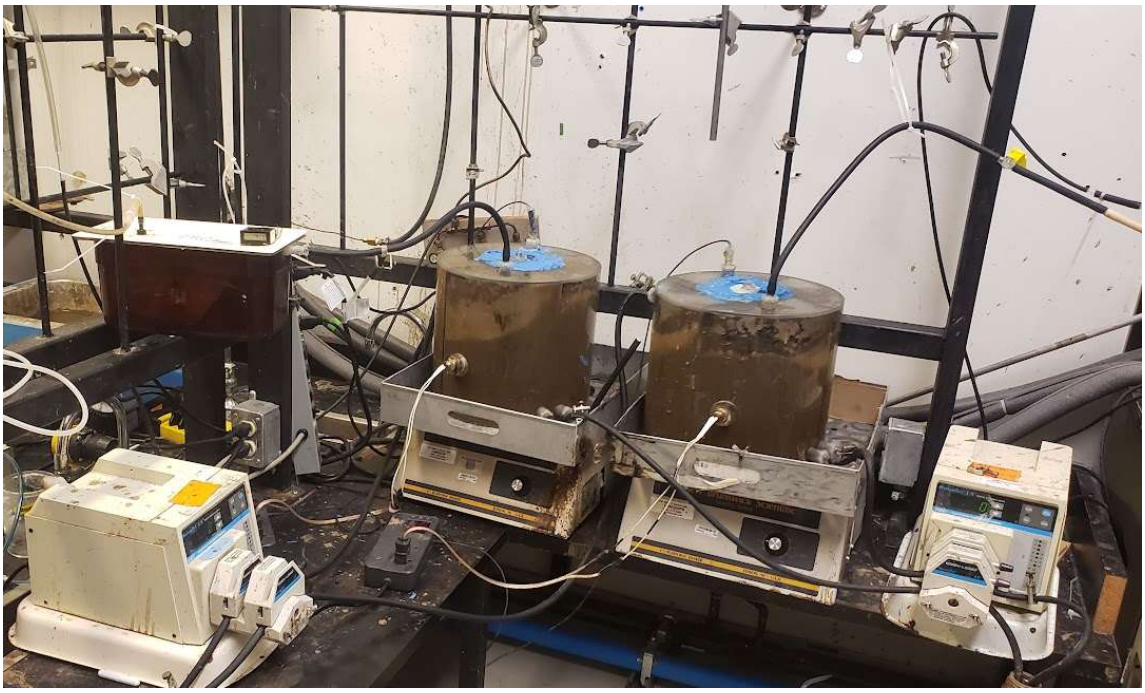

Figure Appendix 11-14: Main anaerobic digesters in the semi-continuous experiment in Phase IV.

Figure Appendix 11-15: Ammonia stripping column in the semi-continuous experiment in Phase IV (air stripping).

Figure Appendix 11-16: Ammonia stripping of 2 Liters per reactor using renewable natural gas conducted in fume hood. Mixers are only used for foam control in the headspace.

Figure Appendix 11-17: Foam control in the semi-continuous stripping column.

Figure Appendix 11-18: Programmable temperature controller for heating semi-continuous flow reactors.