MITIGATION OF AMMONIA EMISSIONS FROM CONFINED DAIRY

OPERATIONS

By

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To the Faculty of Washington State University:

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MITIGATION OF AMMONIA EMISSIONS FROM CONFINED DAIRY OPERATIONS

Abstract

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Over the past few decades, livestock agriculture in the United States (U.S.) has undergone rapid transformations. In general, concentrated animal feeding operations (CAFOs) have not only grown in size but also in tendency to concentrate in smaller geographical regions for economies of scale. Consequently, ammonia (NH_3) and other emissions from these CAFOs have prompted serious concerns because of potential adverse effects on human health and the environment. Therefore, there is a critical need to develop efficient and cost-effective technologies for mitigating NH_3 and other emissions from CAFOs.

This thesis presents technologies or techniques for mitigating NH_3 emissions from dairy operations, which include: (1) management of manure-pH, (2) anaerobic digestion and solids-liquid separation, and (3) an enhanced aerobic biological processing. The first study examined potential mitigations of NH_3 emissions from the barn and manure storage, when a pH < 6 is maintained in a closed-loop recycle of flush water as well as the effective cost reduction accruing from repetitive acid dosing. Results, from this study, revealed significant potential mitigations of

NH$_3$ emissions from dairy barns as well as in subsequent storage of effluents. Operating the flushing system in a closed-loop also indicated significant cost-benefit accruing from reduced acid dosages. The second study evaluated, in lab and field studies, the effects of anaerobic digestion (AD) and solids-liquid separation on emissions during subsequent storage and land application of effluents. Although the AD process exacerbated NH$_3$ emissions from storage of AD liquid effluents, significant reduction of NH$_3$ emissions were observed during and after land application of AD effluents. Solids/liquid separation proved to be an effective means to not only reduce solids loading to storage facilities but also in reduction in NH$_3$ losses. The third component of this research explored an enhanced biological removal of NH$_3$ from dairy wastewater. This bioprocess utilized bacterium *Alcaligenes faecalis* strain No. 4, which has an ability to carry out heterotrophic nitrification and aerobic-denitrification simultaneously in a single tank. Removal of TAN from dairy wastewater by *A. faecalis* strain No. 4 was rapid and efficient thus ensuring mitigation of NH$_3$ emissions from treated effluent. For this bioprocess to achieve high efficiency of TAN removal, however, addition of external carbon to the dairy wastewater, as well as adequate oxygen supply are necessary.

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Dedication

This dissertation is dedicated to my parents, my wife, and my son.

CHAPTER ONE

INTRODUCTION

1.1. Background

Over the past few decades, livestock agriculture in the U.S. and globally has undergone rapid transformations. In general, concentrated animal feeding operations (CAFOs) have grown in size and tendency to concentrate in smaller geographical regions to enjoy economics of scale (MacDonald and McBride, 2009; Beldman et al., 2010). However, these larger and denser spatial concentrations of animal feeding operations (AFOs) pose serious challenges to environmental quality from the huge volumes of manure produced. Areas within and in the vicinity of CAFOs may encounter challenges from emission of odor, gaseous, and particulate matter. This scenario has prompted more strict state and federal laws to regulate air quality and odor resulting from CAFOs.

The main pollutant gases from AFOs include ammonia (NH_3), hydrogen sulfide (H_2S), methane (CH_4), nitrous oxide (N_2O), and carbon dioxide (CO_2) (Ni, 2015). Ammonia and H_2S are the most recognized hazardous substances that are emitted from animal wastes (USEPA, 2008). Global ammonia (NH_3) emission is projected to more than double from 65 Tg N in 2008 to 132 Tg N in 2100 because of increased anthropogenic activities with over 50% of emissions originating from livestock and crop production systems (Sutton et al., 2013). Although total U.S. ammonia emission is about 4.8 million tons per year, animal operations produce roughly 2.5 million tons per year (approximately half of U.S. emissions) with dairy operations being responsible for about 20% of emissions from the animal industry component (Shih et al., 2006).

In CAFOs, NH_3 emissions occur from several sources including animal housing, bedding, solids-liquid separation areas, manure storage facilities, and from land application of manure (Ndegwa et al., 2008). Gaseous emissions are mostly influenced by feeding regimen, facility type and management, manure management system, and the method of land application of manure (USEPA, 2012). Possible negative impacts of NH_3 emissions include: toxicity to sensitive ecosystems, eutrophication in surface waters, and environmental acidification (Fangmeier et al., 1994; Asman et al., 1998). In addition to posing a variety of adverse health-risks to humans and animals from formation of fine airborne particulates ($PM_{2.5}$) in the atmosphere in form of ammonium nitrates and ammonium sulfates (McCubbin et al., 2002), NH_3 emission also reduces manure fertilizer-value (Sommer et al., 2006).

Manure management techniques, therefore, are necessary to mitigate odor, gaseous and particulate matter from CAFOs. This research work focused on the development of efficient and cost-effective manure management technologies for mitigating NH_3 emissions from dairy operations.

1.1.1. Potential Mitigation via Manure-pH Management

Ammonia volatilization is directly proportional to the proportion of un-ionized aqueous NH_3 in the total ammoniacal nitrogen (TAN). When temperature is held constant, pH determines the equilibrium between NH_4^+ and NH_3 in aqueous systems. The greatest increase in NH_3 release takes place between a pH of 7 and 10, and at pH values above 11 all of the TAN is in the form of free NH_3. Decreasing pH has a very important effect on NH_3/NH_4^+ equilibrium: below pH 7 NH_3 volatilization decreases and the fraction of NH_3 to TAN approaches to 0 at pH values near to 6

(Vaddella et al., 2013; Zhao et al., 2015). Lowering manure pH shifts equilibrium towards more non-volatile NH_4^+ while reducing the volatile NH_3, thus reducing potential for NH_3 volatilization.

In a review paper, Ndegwa et al. (2008) identified reduction of pH as a technically viable approach for mitigating NH_3 emissions in CAFOs. This approach is based on the principle that lowering pH reduces ammonium ion dissociation. Numerous batch studies employing acid treatments have shown significant reductions of NH_3 emissions from livestock manure (Molloy and Tunney, 1983; Stevens et al., 1989; Frost et al., 1990; Sorensen and Eriksen, 2009; Petersen et al., 2012). However, even though acids are effective in reducing pH and thereby reducing NH_3 emissions, on-farm acid treatments on CAFOs are nonexistent because it is difficult to implement these treatment systems, as acids are costly and potentially hazardous.

In large dairies with manure flush systems, manure is intermittently flushed from the concrete or slatted floor with clean or recycled water. In manure flush systems, if a closed loop recycle of flush water is practiced, repetitive dosage to adjust the pH of the recycle flush water could over time successively reduce the acid dosage. Hypothetically, therefore, this approach would not only reduce the acid cost but also enhance the feasibility of using acids to mitigate NH_3 emissions in CAFOs. The effectiveness of the aforementioned approach in NH_3 mitigation was one component of the research presented in this dissertation.

1.1.2. Potential Mitigation via Anaerobic Digestion and Solids-Liquid Separation

Anaerobic digestion (AD) process, in general, is associated with changes in manure physical-chemical properties, which may affect ammonia emission from AD effluents. The mineralization of organic nitrogen produces ammoniacal nitrogen and these results in higher TAN in the effluent manure (Sommer et al., 2006). While, AD has been effectively employed to

reduce greenhouse gases and odor emissions from manure storages (Amon et al., 2006; Novak and Fiorelli, 2010; Chadwick et al., 2011), ammoniacal-N is generally elevated. Thus, a key limitation of AD is the potential for high NH_3 volatilization from the AD effluents. Manure pretreatments and methods of manure land application, however, may impact NH_3 volatilization.

Solids-liquid separation is an efficient manure treatment method for odor control, nutrient management, and improved economics of liquid manure treatment (Zhang and Westerman, 1997). Solids-liquid separation remove significant biodegradable organic solids and associated nutrients (Zhang and Westerman, 1997); and the separated wet solids carry with them a fraction of the TAN in the water associated with the solids and through adhesion to solids (Visscher et al., 2002; Vaddella et al., 2013). Consequently, solids-liquid separation can remove one or more precursors of NH_3 and odor and substantially mitigate NH_3 emissions, and odors from the storage of liquid effluents.

The second component of this dissertation addressed two critical elements towards identification or development of strategies for managing dairy manure: (1) quantifying the role that solids separation can play in mitigating NH_3 and odor emissions from manure slurry storage, and (2) elucidating how AD of manure and mode of manure application, to land, affects NH_3 emission.

1.1.3. Potential Mitigation via Enhanced Bioprocess

Conventional nitrification-denitrification process is one of the most environmentally friendly bioprocesses for the removal of nitrogen from livestock wastewater. Biological nitrogen removal treatment consists of two stages: (i) nitrification, and (ii) denitrification. Nitrification transforms NH_3 to oxidized nitrogen compounds, and these compounds are further reduced to

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nitrogen gas via denitrification (Focht and Chang, 1975; Junter et al., 1995; Chen et al., 1998; Zhu et al., 2008; Sun et al., 2010). Unfortunately, biological removal of NH_3 in a conventional treatment system is a slow process because of low NH_3 oxidation rates designating the nitrification step as the rate-limiting process (Szogi et al., 2004; Zhu et al., 2008). This process also requires two separate reactors for nitrification and denitrification. Consequently the process requires larger and multiple reactors, which translate into higher capital investment and operation costs. Moreover, a high level of oxygen is required for nitrification as oxygen is a key parameter in the nitrification process. Furthermore, in conventional aerobic treatment of high strength ammonia wastewaters, aeration using air represents the major operating cost (Water Pollution Control Federation, 1988; Ahmed et al., 2004).

Recent studies showed that bacterium *A. faecalis* strain No. 4 has the ability to carry out heterotrophic nitrification and aerobic denitrification, thereby removing significant amounts of ammonium from wastewater containing low or high strength ammonium concentrations mainly via nitrogen (N_2) gas and microbial assimilation in a single aerobic process (Joo et al., 2005, 2006, 2007; Shoda and Ishikawa, 2014). However, no studies have used *A. faecalis* strains for treating high strength ammonium dairy wastewater. The third portion of this research, therefore, focused on assessing the utilization of this bacterium for treatment of dairy wastewater. In addition, use of pure oxygen gas instead of ambient air to further enhance this bioprocess was also investigated.

1.2. Research Objectives

The overall goal of this research was to evaluate and develop potential manure management technologies to mitigate NH_3 emissions from dairy operations. To achieve this overall goal, the following specific objectives were pursued in this research:

1. Quantify potential mitigations of NH_3 in the barn and in storage, when a pH < 6 is maintained in a closed-loop recycle of flush water.

2. Determine the effects AD and solids-liquid separations have on NH_3 emissions during manure storage and land application.

3. Develop a cost-effective bio-treatment process to mitigate emissions of NH_3 from dairy wastewater.

1.3. Dissertation Structure

This dissertation is organized into five chapters. Research background and objectives are presented in chapter 1. Each of chapters 2, 3, and 4 presents at least one potential NH_3 emissions mitigation strategy. Chapter two presents potential mitigation of NH_3 in the barn and in storage, when a pH below 6 is maintained in a closed-loop recycle of flush water as well as the cost-effectiveness of such a manure management system. Chapter three evaluates the effects AD and solids-liquid separations have on NH_3 emissions during manure storage and land application. A potential cost-effective bioprocess for mitigating emissions of NH_3 from dairy wastewater is presented in chapter four. Finally, the main conclusions of the entire research and recommendations for future studies, to advance the research presented in this thesis, are presented in chapter five.

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CHAPTER TWO

MANURE-pH MANAGEMNT FOR MITIGATING AMMONIA EMISSIONS FROM DAIRY BARNS AND LIQUID MANURE STORAGES

2.1. Abstract

Concentrated feeding operations are important sources of NH_3 emissions. Manure acidification, however, has the potential to significantly mitigate NH_3 emissions. This research investigated achievable NH_3 emission mitigation in a dairy barn when maintaining a pH < 6 in the recycle flush-water. The proof-of-the-concept was evaluated in a bench-scale study, while a pilot-scale dairy barn model was constructed to simulate actual manure flush systems. Two manure-pH management strategies were tested at the pilot-scale: (i) pH-adjustment of flush water, and (ii) sprinkler-irrigation of manure alleys with acid water between scheduled flushing events. A separate study was conducted to evaluate further NH_3 emission mitigation probable during post-collection storage of treated manure. Ammonia measurements were made using a closed-chamber method coupled with a photoacoustic IR multigas analyzer. The mean cumulative NH_3 emissions during the study period were: 47.0, 13.9 and 6.1 mg for control, pH-adjusted flush water, and irrigating manure alleys with acid water between scheduled manure-flushing events, respectively. Adjustment of the pH of recycle flush water reduced the consumption of acid by 86% within the first two cycles. Adjustment of pH in the barn further reduced NH_3 emissions during storage of barn effluents by 39% compared with storage of untreated flushed manure. The results from this research indicate significant potential mitigations of NH_3 emissions from dairy barns as well as in subsequent storage of effluents; while also demonstrating that operating the flushing system in a closed-loop results in significant cost-benefit accruing from reduced acid dosages.

2.2. Introduction

Ammonia emissions from intensive animal production systems (commonly referred to as concentrated animal feeding operations or CAFOs in the U.S.) have prompted serious concerns because of potential adverse effects on human health (McCubbin et al., 2002) and the environment in general (Fangmeier et al., 1994; Asman et al., 1998). Dairy cattle barns, in particular, are important sources of NH_3 emissions (Misselbrook and Powell, 2005; Pereira et al., 2012). The common types of dairy production system in the U.S. are tiestall barns, freestall barns, and open lots (USEPA, 2012). Emissions of NH_3, within barns, results from accumulation of urine and feces on the barn floor. The common manure handling systems used for freestall dairy barns in the U.S. are manure scrapping (or vacuuming) and flushing (USEPA, 2012). In large dairies with manure flush systems, manure is intermittently flushed from the concrete or slatted floor with clean or recycled water. Flush water is pumped from a storage tank to collect manure in the barn and convey it out to storage. The manure laden flush water is then delivered to a larger primary pond where more settling occurs before the cleaner supernatant water is allowed to flow into a secondary pond for further storage and treatment. The supernatant water from the secondary pond is usually the source for continuous closed-loop flushing purposes.

In a review paper, Ndegwa et al. (2008) identified reduction of pH as a technically viable approach for mitigating NH_3 emissions in CAFOs. This approach is based on the principle that lowering pH reduces ammonium ion dissociation, i.e., results in less proportion of the volatile ammonia species (i.e., NH_3) of ammoniacal nitrogen (AN). However, even though acids are effective in reducing pH and thereby reducing NH_3 emissions, on-farm acid treatments on CAFOs are nonexistent because it is difficult to implement these treatment systems as acids are costly and potentially hazardous. Numerous batch studies employing acid treatments have shown

significant reductions of NH_3 emissions from livestock manure (Molloy and Tunney, 1983; Stevens et al., 1989; Frost et al., 1990; Sorensen and Eriksen, 2009; Petersen et al., 2012). However, most studies did not consider implementation of this approach in the field or on full-scale commercial facilities. For example, in a manure flush system, if a closed loop recycle of flush water is practiced, repetitive dosage to adjust the pH of the recycle flush water could over time successively reduce the acid dosage; rendering the approach cost-effective. In addition to reducing the cost, this approach can also reduce the hazardousness of the acid because more dilute acids may be used to maintain the pH in a closed loop recycle system. Our hypothesis is that such an approach would not only reduce the acid or treatment cost but also enhance the feasibility of using acids to mitigate NH_3 emissions in CAFOs.

The overall goal of this research was to quantify the potential mitigations of NH_3 in the barn and in storage, when a pH < 6 is maintained in a closed-loop recycle of flush water and to determine effective cost reduction accruing from this repetitive acid dosing. To achieve this objective, and to test our hypothesis, such a system was simulated in laboratory batch experiments at the proof-of-concept stage and the system was evaluated further in a continuously operated system in a model dairy barn. An additional study to evaluate NH_3 emissions mitigation potential from storages of manure effluents from this practice against untreated manure was also conducted in lab-scale simulated storages.

2.3. Materials and Methods

2.3.1. Sample Collection and Preparation

Fresh cattle feces, fresh urine, and liquid supernatant from a secondary lagoon were collected separately in 20-L plastic tanks and stored in a walk-in cooler at 4°C prior to use in this

research. Dairy cattle manure was reconstituted by mixing thawed urine and feces samples in the ratio of 1 to 1.7 (w/w), respectively (Morse et al., 1994; Hristov et al., 2009). In a typical flush system, manure slurry is usually diluted at water to manure ratios varying from 10:1 to 2:1 resulting in total solids (TS) concentration ranging from 1 to 3% at the reception pit (Lenkaitis, 2012). In this study, the reconstituted manure was diluted with lagoon water to a TS content of 1.6% (Barker, 1996; Lenkaitis, 2012).

2.3.2. Manure Buffer Determination

The chemical buffer system in the manure slurry dictates the shape of titration curve (Stevens et al., 1989). The main buffering components in manure slurry controlling pH are NH_4^+/NH_3, CO_2/HCO_3^-/CO_3^{2-}, and CH_3COOH/CH_3COO^- (Sommer and Husted, 1995). In general, the buffering capacity of slurry is variable, and amount of acid needed to reduce the pH to certain level depends on the source of the slurry, slurry-dilution, storage condition and storage period (Stevens et al., 1989).

The variation of free NH_3-N relative to TAN with the temperature and pH is illustrated in Figure 2.1. Ammonia volatilization is directly proportional to the proportion of un-ionized aqueous NH_3 in the TAN. When temperature is held constant, pH determines the equilibrium between NH_4^+ and NH_3 in aqueous systems. It is evident that the greatest increase in NH_3 release takes place between a pH of 7 and 10, and at pH values above 11 all of the TAN is in the form of free NH_3. Decreasing pH has a very important effect on NH_3/NH_4^+ equilibrium: below pH 7 NH_3 volatilization decreases, the fraction of NH_3 to TAN approaches to 0 (all of the TAN is in the form of non volatile NH_4^+) at pH values near to 6 (Vaddella et al., 2013; Zhao et al., 2015).
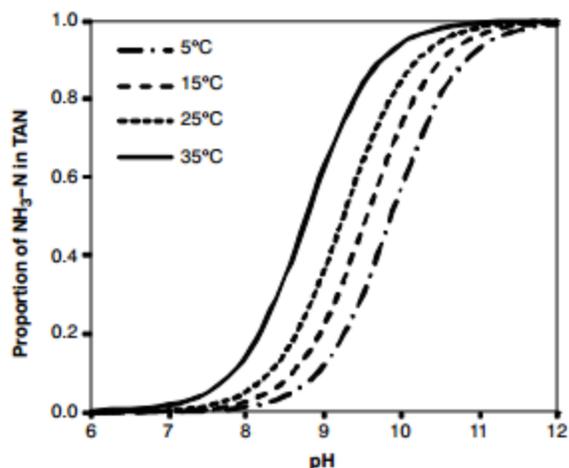
Figure 2.1 Proportion of NH_3 as a function of temperature and pH in aqueous solutions (adapted from Vaddella et al. 2013).

The buffering capacity profile of flush water, used in the current study, is presented by the titration curve in Figure 2.2. This curve was developed through titration of 450-mL reconstituted flush water samples with 1M H_2SO_4 to an end point of about pH 4.5, which was a modified version of a procedure used in previous research (Molloy and Tunney, 1983; Stevens et al., 1989). The results of titration experiments show the effect of acid dosing on pH, and thus the quantity of acid required to adjust manure pH to various specific levels. For instance, the acid dosage required to drop manure by one unit rises significantly between pH 6 and 4.5. A manure pH of 6 was thus selected as the pH-management level for manure used in this study. Lowering flush water pH from 7.6 to about 4.5 required 31 mL of acid. Total alkalinity of flush water was thus approximately the equivalent of 6.89 g $CaCO_3$ L^{-1}.

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The plot shows pH of flush water versus Volume of Acid (ml) with the fitted equation:

$$y = -1E\text{-}05x^3 + 0.0014x^2 - 0.0812x + 7.4826$$
$$R^2 = 0.9951$$
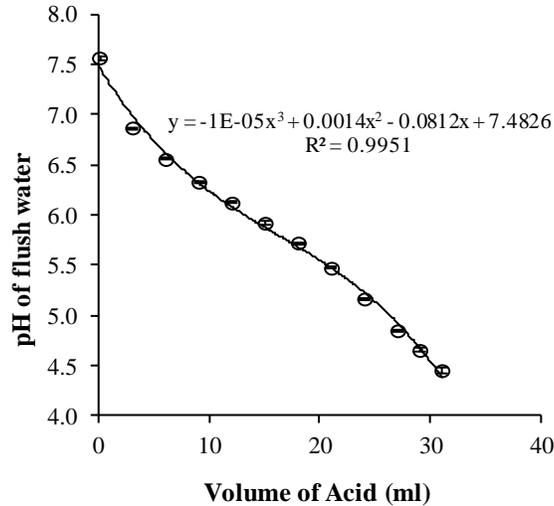
Figure 2.2 Titration curve for flush water with sulfuric acid. Vertical bars represent standard deviations from means ($n = 3$).

2.3.3. Bench Scale-Study

A schematic of the manure pH-management simulating a dairy with a flush system used in this study is shown in Figure 2.3. Preliminary experiments during proof-of-concept were carried out in 0.5-L Polypropylene graduated cylinders. A 50-g sample of reconstituted cattle slurry was mixed, in a 1-L cylinder, with 0.45 L of lagoon water to simulate manure flushing in the barn and achieve the 1.6% TS concentration. The diluted slurry, after time for exposure and reaction, was screened through a 1.4-mm sieve to simulate a typical farm practice of solids separation from the liquid (Fulhage and Pfost, 1993). The liquid stream was then left undisturbed in a sedimentation cylinder for at least 1 h to effect further solids separation via static gravity sedimentation. Most of the solids (about 60%) in flushed manure settled in about 10 min by gravity settling and detention times of 30 to 60 min are common for cattle manure in settling basins (Powers et al., 1995; Fulhage et al., 2002). The supernatant liquid fraction was next

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separated from settled sludge. Lagoon water, pretreated with acid solution (1M H_2SO_4) to a pH of 4.5 was added to this supernatant liquid fraction to make up its volume to the initial flush-water volume. Prior to volume make up, the pH of the resulting mixture, if necessary, was adjusted with sulfuric acid to 4.5. This pH-adjusted liquid was poured back into a 1-L barn cylinder, simulating the next flushing cycle. The flushing cycles were terminated when a stable pH of the flush water was achieved, i.e. when no additional acid was required to maintain pH. These tests were conducted in triplicates.
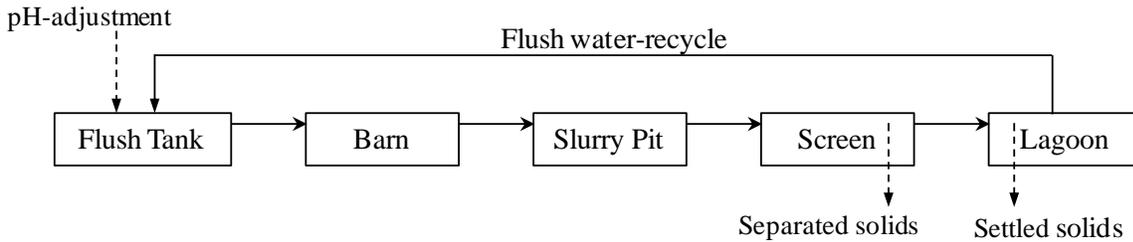
Figure 2.3 A schematic of the pH evaluation bench-scale simulation system.

2.3.4.   Pilot Scale-Study

2.3.4.1. Pilot System

The pilot-scale model of the barn system constructed to simulate a full-scale flush dairy barn system is presented in Figures 2.4 and 2.5. This model consisted of a clear Plexiglass chamber measuring 183 cm (l) × 38 cm (w) × 33 cm (h) representing the barn, two 9 cm wide manure channels representing the manure alleys, and two 5-cm diameter flush water drainage. The barn-chamber was inclined so as to provide 2% to 3% slope typical of manure alleys in dairy barns (Harner and Murphy, 1997). The pilot system was also equipped with other liquid manure

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handling accessories including: a manure tank; a flush water tank; a manure screen; a sedimentation tank; and an acid tank (Figure 2.5).
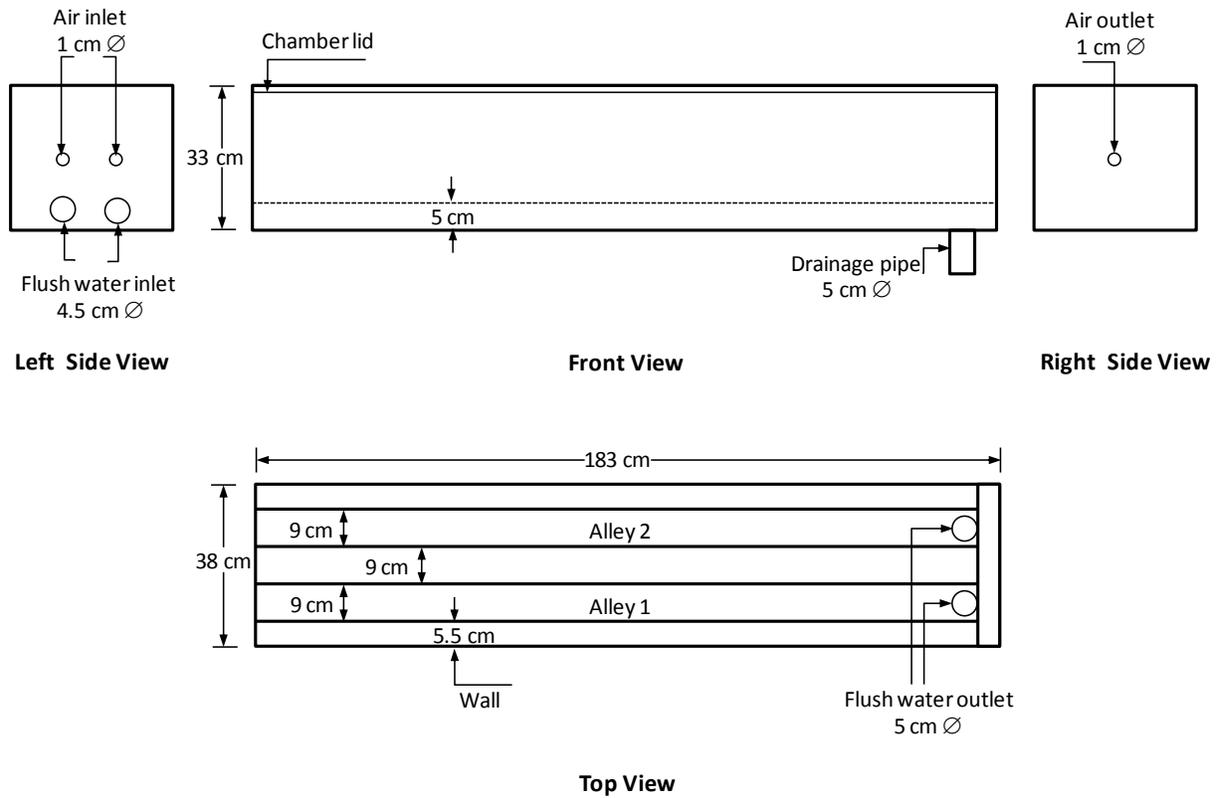
Figure 2.4 Schematic plan of the barn model at the pilot scale (not drawn to scale).
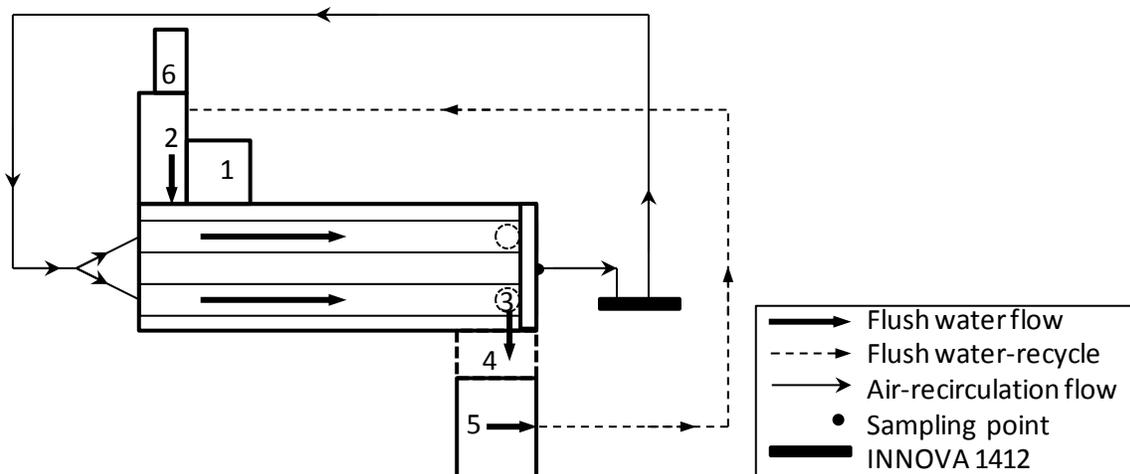
Figure 2.5 Layout of the model dairy barn system: (1) manure tank; (2) flush water tank; (3) flush-drainage outlets; (4) screen; (5) sedimentation tank; and (6) acid tank.

The following three treatments were applied to manure in the alleys: (1) flushing with untreated flush water 7.9 pH (control), (2) flushing with pH 4.5 flush water (referred to as "flushing only"), and (3) sprinkling or irrigating with pH 4.5 flush water between scheduled flushing events (referred to as "irrigation & flushing"). The pH of supplemental supernatant (referred to a stock flush water) for making up lost volumes of flush-water between flushing-cycles was initially adjusted to 4.5 with sulfuric acid.

2.3.4.2. Simulating Manure Deposition and Irrigation

The daily manure production from a Holstein cow weighing about 636 kg is approximately 48 kg (Hubbard and Lowrance, 1998). The amount of manure deposited on the barn floor is assumed to be 10% to 15% of daily manure production for an equivalent time ranging from 2.4 to 3.6 h per day (Dairy Australia, 2008). To simulate continuous manure

deposition over time as it occurs in actual barns, 54 g of fresh cattle manure (mixture of 34 g feces and 20 g urine) were uniformly hand-applied to manure alleys at three (designated as phases 1, 2, and 3) 20-min intervals during 1-h study periods. After each phase of manure deposition, the chamber lid was immediately replaced. At the end of 1 h, the manure in the alleys were flushed with 1.5 L flush water (0.75 L for each alley). The flushed effluents were screened and allowed to settle in a sedimentation tank. Supernatants from the settling process were first made up to initial flush water volume using the stock flush water. In the acid water sprinkler-irrigation system, 100 mL of stock flush water was uniformly irrigated over each manure alley two times (20-min intervals) after manure deposition.

2.3.4.3. Ammonia Emission Measurements

Ammonia emissions were measured using a closed-chamber approach similar to that used in several similar previous studies (Sommer et al., 2004; Shah et al., 2006; Ni and Heber, 2008). In a closed-chamber method, there is no air exchange between the chamber and the atmosphere and, therefore, ammonia released from manure accumulates in the chamber's headspace over time. The headspace gases are pulled into the photoacoustic IR analyzer (Model 1412, Innova AirTech Instruments, Ballerup, Denmark) via flexible Teflon tubing (0.32 cm inside diameter) and the sample is returned back to the chamber's headspace after gas concentrations measurements. Ammonia concentration in the chamber's headspace was measured continuously for approximately 20 min. Ammonia flux was calculated as the rate of concentration change in a chamber's headspace per unit surface area of the emitting surface (Parkin et al., 2003; Rochette and Eriksen-Hamel, 2008; Joo et al., 2012; Sun et al., 2014). The total cumulative NH_3 emissions

(mg) were calculated as the sum of respective emissions for each of the three 20-min sampling phases during the hourly experimental runs.

2.3.5. Manure Storage Lab Study

To evaluate NH_3 emissions from simulated post-treatment effluent storage against untreated manure storage, the storage of both effluents were simulated in plastic reactors (measuring 25.4 cm in diameter and 23.5 cm in height) in the laboratory. A set of six reactors (three replicates each for treatments and control) were used. Each reactor, with manure surface area of 386 cm^2 was loaded with 2.5 L of manure sample. Each of the reactors had 1-cm diameter headspace ventilation inlet and outlet ports. The reactor headspace air was pulled into the photoacoustic IR analyzer for NH_3 concentration measurement through one vent of the reactor and the air was returned back to the reactor's headspace through the other vent (Figure 2.6), resulting in a closed loop air circling required for measuring cumulative NH_3 concentration (Joo et al., 2012; Sun et al., 2014). Ammonia concentration measurements were continued until NH_3 saturation in the reactors headspace was observed, i.e., the analyzer continuously measured the cumulative NH_3 concentrations in the chamber headspace with time. Linear regressions of concentrations versus time data were used to measure the rate of change of concentrations, i.e., slope of the linear regressions (Parkin et al., 2003). Ammonia sampling measurements were conducted on day: 0, 1, 2, 4, 6, 8, 10, 12, 14, 17, 20, and 25.
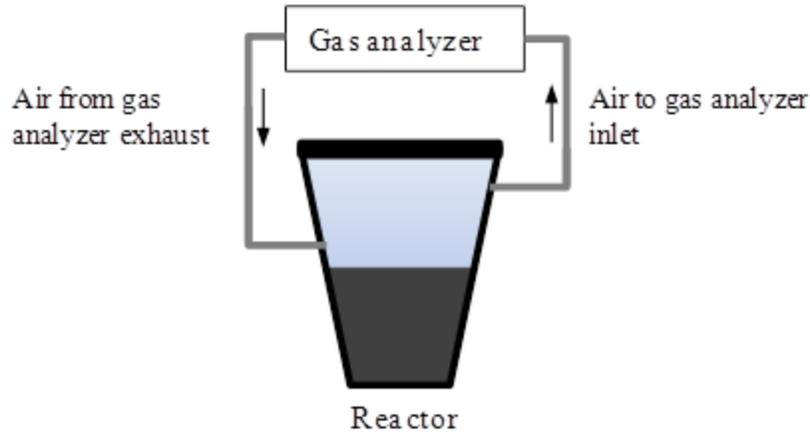
Figure 2.6 Typical reactor and ammonia gas concentration analyzer system.

2.3.6. Laboratory Analysis

The main parameters determined for the reconstituted manure and flush water included pH, total solids (TS), volatile solids (VS), and total ammoniacal nitrogen (TAN). A digital pH meter (model 290A, Orion Research Inc., MA) was used to determine pH. Total solids were determined by drying manure sample to a constant weight in a forced air oven at 105°C. To determine VS, residue sample from oven dried samples were ignited at 550°C in a muffle furnace to constant weights (APHA, 1998). Concentration of TAN was determined using the Hach Nessler colorimetric method 8038 adapted from standard methods 4500-NH_3 (APHA, 1998). The odor generation potential in the manure was determined by measuring volatile fatty acids (VFAs) at a wavelength of 495 nm using a UV-visible spectrophotometer (Hach Company, 2004).

2.4. Results and Discussion

2.4.1. Characteristics of Manure and Flushwater

The characteristics of the manure and flushwater used in this study are presented in Table 2.1. The results indicated a lower TS value of 91.5 g L^{-1} and a higher TAN value of 5.2 g L^{-1} in reconstituted manure as compared to fresh dairy cattle-manure. The dilution of scrapped feces with fresh urine during manure reconstitution possibly resulted in low TS. The relatively high TAN in the reconstituted manure is most likely caused by the mixing of fresh urine and feces (rapid formation of ammonia).

Table 2.1 Characteristics of the reconstituted manure and flush water

Parameter	Reconstituted manure	Flush water
pH	7.9 ± 0.02^{a}	7.6 ± 0.04
TS (g L^{-1})	91.5 ± 1.29	16.0 ± 0.65
VS (g L^{-1})	64.6 ± 2.23	9.1 ± 0.36
TAN (g L^{-1})	5.2 ± 0.06	1.7 ± 0.06

[a] Mean ± standard deviation ($n = 3$)

2.4.2. Effect of pH-Adjustment in the Recycle-Flush Water

Figure 2.7 represents the effects of pH-adjustment in the recycle flush water for the bench- and pilot-scale simulation studies. During the bench-scale simulation, the initial flush-water pH was 7.5 and the amount of dilute acid needed to adjust this pH to 4.5 was 30 mL. In subsequent cycles, however, the pH of the flush water was maintained at approximately 5 using only about 7 mL of the same stock of acid. The observed pH reduction pattern is in-line with basic principles of manure acidification indicating alkalinity reduction by neutralizing the carbonate fraction from manure slurry (Stevens et al., 1989). Overall, the results from the

preliminary tests demonstrated clearly the potential effectiveness of pH-adjustment of flush water in a closed-loop recycle resulting in a significant reduction (77%) in acid use over time especially after the initial acid dose.

At pilot-scale, approximately 3.9 mL of concentrated H_2SO_4 (equivalent to 107 mL of 1M H_2SO_4) for 1.5 L flush water was needed to lower initial flush-water pH from 7.9 to 4.5. The volume of acid for pH adjustment, nevertheless, decreased significantly to an average of 15 mL 1M H_2SO_4 during subsequent cycles. The acid dosage required to maintain the desired pH after the first cycle was thus approximately 86% less re-affirming the bench-scale tests.
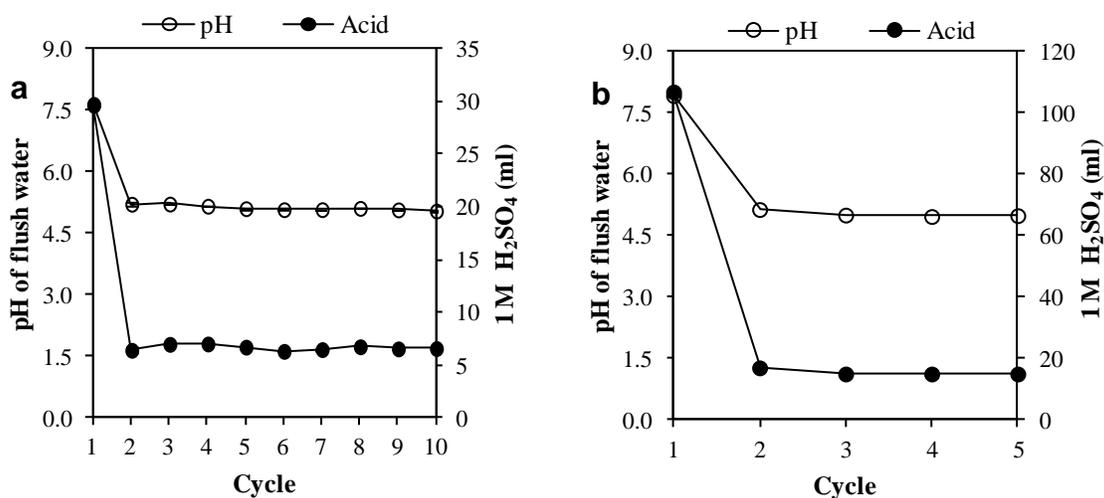
Figure 2.7 Acid consumption for adjustment and maintenance of flush water pH as a function of flush-water recycle in a closed loop: (a) bench-scale study, and (b) pilot-scale study (vertical bars in (a) represent standard errors from means, where $n = 3$).

2.4.3. Ammonia Emissions Mitigation

Figure 2.8 shows examples of typical cumulative NH_3 concentration in the pilot-scale barn for the different treatments during phases 1, 2, and 3 in one of the hourly experimental runs. The mean rate of change of NH_3 concentrations for different treatments is presented in Table 2.2. Linear regressions of the cumulative NH_3 concentrations results (R^2 ranging from 0.97 to 0.99) indicated constant NH_3 emissions during each of the three phases for both the treatments and control. The rates of NH_3 emissions for treatments and control, however, were significantly different. The rate of NH_3 emission, in phase 1, for the control was approximately 2.28 ppm min^{-1}, 0.28 ppm min^{-1} for flushing with pH-adjusted flush water, and 0.22 ppm min^{-1} for combined irrigation and flushing with pH-adjusted flush water. The respective NH_3 emissions in the next two phases (20 to 40 min and 40 to 60 min), were similar at 2.05 and 1.81 ppm min^{-1} for the control, 0.53 and 1.00 ppm min^{-1} for flushing with pH-adjusted water, and 0.26 and 0.31 ppm min^{-1} for combined irrigation and flushing with pH-adjusted water (Table 2.2). The respective NH_3 fluxes during phases 1, 2, and 3 were 0.94, 0.84 and 0.73 mg m^{-2} min^{-1} for the control, 0.12, 0.22 and 0.41 mg m^{-2} min^{-1} for flushing with pH-adjusted water, and 0.09, 0.11 and 0.13 mg m^{-2} min^{-1} for combined irrigation and flushing with pH-adjusted flush water (Figure 2.9). The total mean cumulative NH_3 emissions (mean ± standard errors, $n = 3$) during the hourly study periods were: 46.98 ± 4.3, 13.91 ± 0.59 and 6.05 ± 0.18 mg for the control, flushing-only, and combined irrigation and flushing, respectively. Cumulative NH_3 emissions from the control treatment was significantly ($P < 0.05$) greater than that from pH-adjustment treatments. Ammonia emissions for the control, in general, were 3.4 and 7.8 times greater than emissions for flushing with pH-adjusted water and for the combined irrigation and flushing with pH-adjusted water, respectively. Both manure flushing with pH-adjusted water and combined irrigation and flushing with pH-

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adjusted water showed significant reductions in NH_3 emissions (approximately 70% and 87%, respectively; Figure 2.10). Sprinkler-irrigation of pH-adjusted flush water between scheduled flushing events was thus more effective compared to flushing manure with pH-adjusted flush water only. Jensen (2002) obtained similar results (83% reduction in NH_3 concentrations in swine barn) when pH of the manure in the pits was maintained at around 5.5. In general, diverse acidification of livestock manure has shown reductions in NH_3 emissions ranging from 14 to 100% in CAFOs (Ndegwa et al., 2008). The current studies, therefore, affirm that maintaining manure pH below 6 significantly mitigates NH_3 emissions. The chemical principles underlying the success of the pH treatment related back to the alkalinity of the manure. Manures principal source of alkalinity is the bicarbonates. Acidification converts HCO_3^- to H_2CO_3 and CO_2. The proportions of bicarbonate converted to CO_2 at pH 6 and 5 are 72 and 96%, respectively (Stevens et al., 1989; Sommer and Husted, 1995). Lowering manure pH shifts equilibrium towards more non-volatile NH_4^+ while reducing the volatile NH_3 thus reducing potential for NH_3 volatilization.
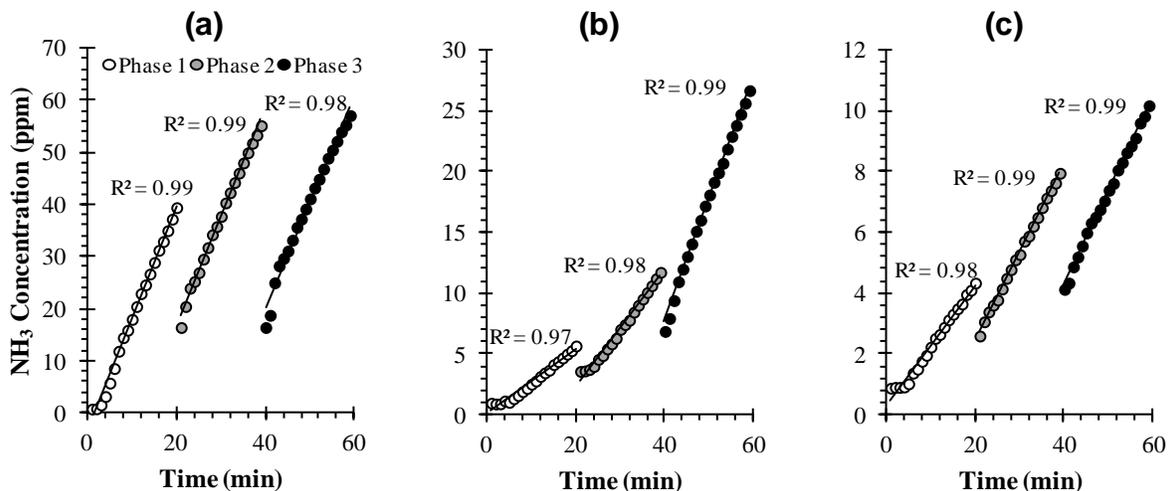
Figure 2.8 Cumulative ammonia concentration in the air in the pilot-scale barn during one full run (phases 1, 2, and 3) for: (a) control, (b) flushing-only, and (c) combined irrigation and flushing.

Table 2.2 Mean ± SE (standard error) of the rates of change ammonia concentration (ppm min^{-1}) in the chamber's headspace during the three phases

Treatments	Phase 1	Phase 2	Phase 3
Control	2.28 ± 0.31 [a*]	2.05 ± 0.17 [a]	1.81 ± 0.09 [a]
Flushing-only	0.28 ± 0.02 [b]	0.53 ± 0.02 [b]	1.00 ± 0.04 [b]
Irrigation & Flushing	0.22 ± 0.01 [b]	0.26 ± 0.02 [b]	0.31 ± 0.01 [c]

[*]Same letters (a, b, c) within the same column indicate no significant differences ($P > 0.05$); $n = 3$.
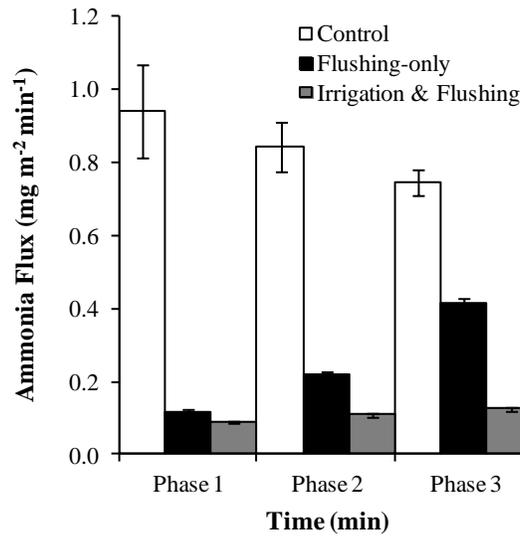
Figure 2.9 Ammonia fluxes from manure alleys in the model dairy barn during phases 1, 2, and 3 (error bars indicate standard errors (SE) from means, $n = 3$).
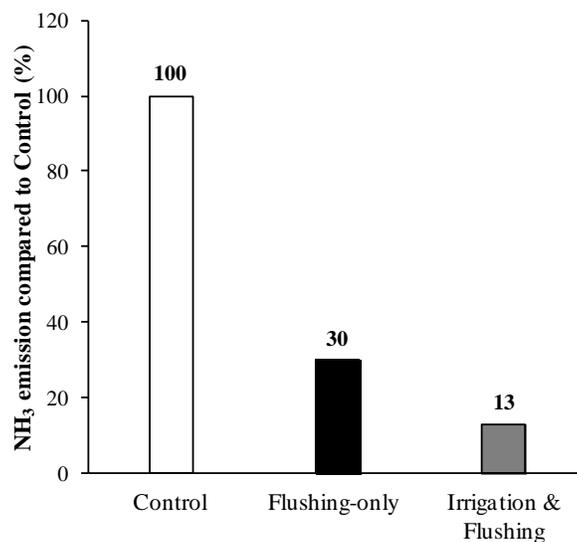
Figure 2.10 Relative ammonia emissions based on the model dairy barn for respective treatments compared to the control.

2.4.4. Post-Collection Manure Storage Study

The properties of both the treatment and control manure slurries at the start and end of the 25 d post-collection storages period are presented in Table 2.3. The pH of the flushing-only treatment effluent increased from 6.2 to 7.4 during the storage period. This increase in the manure pH during storage was probably due to consumption of VFAs by the microbial population or increased volatilization of fatty acids (Eriksen et al., 2008; Ottosen et al., 2009; Zhang et al., 2011). The VFAs concentration decreased to low levels for both control (4 to 1.3 g L^{-1}) and treated manure (4.7 to 2.3 g L^{-1}) during the course of storage. The treatment manure indicated significantly higher TS (36%) relative to the control. For comparison, Fangueiro et al. (2009) reported a similar 26% increase in dry matter content when pig slurry was acidified to pH 6 relative to untreated slurry, and attributed this increase to the addition of sulfate ions in the

acidifying H_2SO_4. The higher TS in the treatment manure relative to untreated manure could also be due to decreased NH_3 and VFAs losses from acid-treated manure.

Table 2.3 Dairy manure characteristics before and after 25 d storage

Parameters	Control		Treatment	
	Before storage	After storage	Before storage	After storage
pH	8.0 ± 0.02	8.2 ± 0.02	6.2 ± 0.03	7.4 ± 0.05
TS (g L^{-1})	18.3 ± 0.06	15.4 ± 0.08	24.9 ± 0.07	22.8 ± 0.22
VS (g L^{-1})	11.1 ± 0.02	10.9 ± 0.27	11.1 ± 0.06	11.1 ± 0.01
TAN (g L^{-1})	1.87 ± 0.20	1.66 ± 0.04	2.10 ± 0.20	2.14 ± 0.14
VFA (g L^{-1} HOAc)	4.0 ± 0.12	1.3 ± 0.01	4.7 ± 0.15	2.3 ± 0.31

All values are mean $\pm$ standard deviation ($n = 3$)

Figure 2.11 shows cumulative NH_3 emissions from post-collection storages of manure for the control and treatment during a period of 25 d. The control indicated significantly higher NH_3 emissions compared with the flushing-only treatment. Mean cumulative NH_3 lost from the storage of manure from control was 600 ± 28 mg, while the mean loss from storages of treated manure was 364 ± 15 mg. The pH adjustment reduced ammonia emission by 39% during this storage period relative to untreated manure. There were notable changes in manure-pH over the course of manure storage period from start to end. The pH of effluents for acidification treatment increased from about 6.2 to 7.4, while the pH of the control increased from 8.0 to 8.2 in 25 d. Similar pH increases were observed in previous research studies. In a three month study with cattle manure slurry acidified with sulfuric acid, Petersen et al. (2012) reported increase in manure pH from 4.5 to 6.5 compared to an increase from 7 to 7.5 for untreated manure. Sorensen and Eriksen (2009) reported increased pH (5.5 to 6.2) after few weeks of acidified cattle slurry storages using sulfuric acid. Similarly, Eriksen et al. (2008) reported pH increase from 5.5 to

above pH 7 during 11 month storage of acidified pig slurry. The increase in manure-pH observed during storages were probably due to decrease in VFAs (Zhang et al., 2011), microbial activity (Eriksen et al., 2008), decomposition of organic compounds (Sorensen and Eriksen, 2009), and degradation of dissociated organic acids (Petersen et al., 2012). Blanes-Vidal et al., (2009) attributed the increase in pH to losses of NH_3 and CO_2 from manure and decrease in acetic acids in the manure during storage.
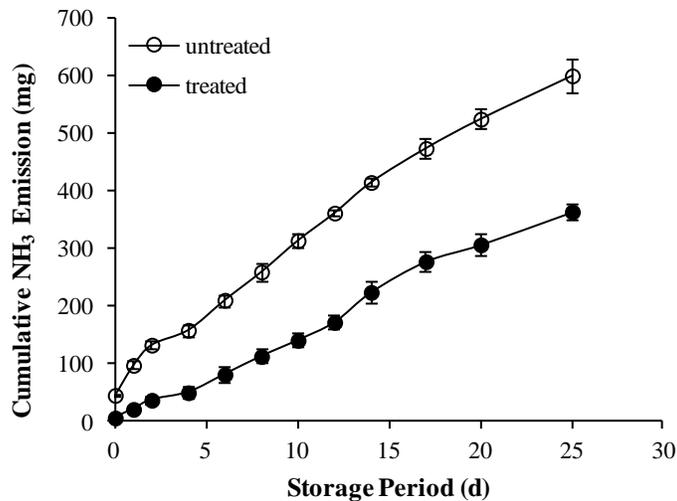
Figure 2.11 Cumulative NH_3 emissions from storages of untreated (control) and treated (flushing-only) manures (error bars indicate standard errors (SE) from means, $n = 3$).

2.5. Conclusions

The overall objective of this study was to evaluate a cost-effective strategy of mitigating NH_3 emissions from dairy barns and manure storages, in CAFOs, via manure acidification in a closed loop flush-water recycle system. The conclusions below were based on the results from this study.

1.   Adjustment of flush water pH was effective in controlling ammonia emissions within the dairy barn. The respective mean hourly cumulative NH_3 emissions were: 47.0 mg for control, 13.9 mg for pH-adjusted flush water, and 6.1 mg for sprinkling pH-adjusted flush water between flushing events.

2.   Sprinkling flush water on the manure between flushing cycles and flushing manure with pH-adjusted flush water resulted in approximately 87% and 70% reductions in NH_3 emissions, respectively; indicating that the former strategy was more effective than the latter.

3.   Operating pH-adjustment in a closed loop led to a significant reduction (86%) in acid doses immediately after the initial pH-adjustment of the raw flush water, which reflected significant cost-effectiveness of such a manure management system for mitigating NH_3 emissions.

4.   As a consequence of significantly reduced acid dose, producers can also use more dilute acids with the added benefit of lowered hazardousness of acid and the practice, in general.

5.   Post-collection storages of treated manure resulted in 39% less NH_3 loss than from storage of untreated manure during the 25 d study period. The pH-adjustment treatment resulted in less VFAs generation indicating potential odor control during post-treatment storage.

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CHAPTER THREE

EFFECTS OF ANAEROBIC DIGESTION AND SOLIDS SEPARATAION ON EMISSIONS FROM STORED AND LAND APPLIED DAIRY MANURE

3.1. Abstract

Field and laboratory studies were conducted to evaluate the effects of anaerobic digestion (AD) and solids-liquid separation on emissions during subsequent storage and land application. The lab storage tests were conducted for 21 d with manure samples obtained at the following four points in a full-scale AD system: raw manure (RM) delivery, raw manure supplemented with other substrates (AD influent), AD effluent, and AD effluent after solids-liquid separation (AD liquid effluent). Ammonia fluxes from stored AD effluent declined from 3.95 to 2.02 g m^{-2} d^{-1}. Lower NH$_3$ fluxes, however, were observed from AD liquid effluent (1.1 g m^{-2} d^{-1}) and AD influent (0.25 g m^{-2} d^{-1}). Ammonia emissions from full-scale manure storages were similar to those obtained in the lab. Results also indicated significantly lower VFA in AD effluent and AD liquid effluent compared with that from the AD influent, indicating significant reduction in odor generation potential due to AD and solids-liquid separation processes. Two manure application methods (surface application and manure injection) for both non-AD and AD manures were simulated in the lab and studied for 9 d. Surface applied non-AD manure exhibited the highest NH$_3$ flux (0.78 g m^{-2} d^{-1}), while injected AD manure led to the lowest NH$_3$ flux (0.17 g m^{-2} d^{-1}). Similar NH$_3$ emissions results were observed from the field studies. Overall, while AD of dairy manure resulted in significant increases in NH$_3$ emissions from stored effluent, the AD process significantly reduced NH$_3$ emissions following application of AD manure on land.

3.2. Introduction

Ammonia from concentrated animal feeding operations (CAFOs) is a significant source of atmospheric nitrogen pollution (Bajwa et al., 2006; Faulkner and Shaw, 2008). Possible negative impacts of NH_3 emissions include: toxicity to sensitive ecosystems, eutrophication in surface waters, and environmental acidification (Fangmeier et al., 1994; Asman et al., 1998). In addition to posing a variety of adverse health-risks to humans and animals from formation of fine airborne particulates ($PM_{2.5}$) in the atmosphere in form of ammonium nitrates and ammonium sulfates (McCubbin et al., 2002), NH_3 emission also reduces manure fertilizer-value (Sommer et al., 2006).

Anaerobic digestion (AD) offers an attractive method to treat dairy wastewater because of its ability to produce renewable energy (biogas), reduce odor emissions, harness and mitigate natural methane emissions during storage, and retain nutrients (Karim et al., 2005). During the process of AD, the organic material is broken down to mainly to methane and carbon dioxide by anaerobic bacteria in the absence of oxygen (Madsen et al., 2011). In the case of nitrogen-sourced proteins, AD degrades the macromolecules to intermediate and end products, which include volatile fatty acids (VFAs), carbon dioxide (CO_2), methane (CH_4), hydrogen gas (H_2), ammonium (NH_4^+), and reduced sulfur (Pavlostathis and Giraldogomez, 1991). During the process, organic nitrogen is partially converted to an inorganic form, increasing the total ammoniacal nitrogen (TAN = NH_3 + NH_4^+) in the effluent (Sommer et al., 2006) while also elevating total alkalinity and pH (Frear et al., 2011). Thus, while AD can effectively be employed to reduce greenhouse gases (GHG) and odor emissions from storages of effluents (Amon et al., 2006; Novak and Fiorelli, 2010; Chadwick et al., 2011), both TAN and pH are generally elevated (Sommer et al., 2006; Frear et al., 2011). During manure storage, NH_3

releases are mostly controlled by TAN concentration, manure pH and atmospheric conditions (Sommer and Husted, 1995). The loss of NH_3, CO_2, and acetic acids from manure slurry during storage results in additional pH variation at the surface of slurry over time, which may further enhance the release of gases and other odorous compounds to the atmosphere (Blanes-Vidal et al., 2009). Thus, a key negative impact of AD resides in its production of effluents with higher TAN and pH than influents, leading to the potential for high NH_3 volatilization from the effluents.

Volatile fatty acids are among the major volatile organic compounds in dairy manure and are, generally, regarded as indicators of potential odor generation from manure (Ndegwa, 2003; El-Mashad et al., 2011; Page et al., 2015). Anaerobic digestion and subsequent solids-liquid separation are effective manure treatment methods for odor control, with solids/liquid separation adding the additional benefit of improved wastewater nutrient management and improved economics of liquid manure treatment (Zhang and Westerman, 1997). Solids-liquid separation removes significant amounts of biodegradable organic solids and associated nutrients (Zhang and Westerman, 1997), which reduces both the production of VFAs and biochemical oxygen demand (BOD_5) upon subsequent storage system (Ndegwa et al., 2002). In addition, separated wet solids carry with them a fraction of the TAN both in the water associated with the solids and through adsorption to solids (Visscher et al., 2002; Vaddella et al., 2013). Consequently, solids-liquid separation can remove some solids (such as reduced carbon compounds, protein, and other nitrogen elements) responsible for the formation of NH_3 and odor, which essentially then mitigates NH_3 and odor emissions from the storage of liquid effluents. Quantifying the role that solids separation can play in mitigating emissions from manure slurry storage is thus an important element in the development of strategies for managing dairy manure.

After storage, manure is land applied as an organic fertilizer source with NH_3 emissions impacted both by upstream AD, solids/liquid separation and storage operations as well as field application methodologies. Previous work has been completed comparing NH_3 emissions following land application of both AD and non-AD cattle slurry (Amon et al. 2006) as well as the extent to which NH_3 emissions are impacted by application (King et al. 2011). Volatilization of NH_3 from land applied manure depends on several factors, such as manure characteristics, application methods, weather and field conditions (Sommer and Hutchings 2001; Huijsmans et al. 2003). Elucidating how AD of manure and mode of application, to land, affects NH_3 emission is critical to development of NH_3-emission mitigation practices and thus retention of manure fertilizer-value especially during manure applications.

The overall objective of this study was to determine the effects AD and solids-liquid separation have on emissions during manure storage and land application. Specific objectives were to: (1) determine the effects of AD and solids-liquid separation on NH_3 and odor emissions during post-AD and post-solids-liquid separation storages of flushed dairy manure, and (2) to evaluate and compare the extent of NH_3 emission following dairy manure (AD and non-AD) application using two application techniques (surface spreading and injection).

3.3. Materials and Methods

3.3.1.   Laboratory Study

3.3.1.1. Collection and Preparation of Manure and Soil Samples

Manure samples were collected from a full-scale dairy manure AD system located in Monroe, Washington State. The dairy and AD operation was comprised of a flush flume manure

handling system, producing a dilute manure stream that was subsequently thickened in a clarifier for production of a higher solids content wastewater more suitable for digestion in a mixed, plug-flow mesophilic digester (DVO, Chilton, WI). This manure stream was then co-mingled with consumer wastes (food processing byproducts, industrial greases and oils, organic fraction of muncipal solids, etc.) to enhance both biogas production and economic viability of the AD system. Samples were obtained at the following four points along the AD system: thickened raw manure (RM), thickened raw manure supplemented with other substrates (AD influent), AD effluent, and AD effluent after solids-liquid separation (AD liquid effluent). Solids/liquid separation was accomplished through the use of a rotary style manure separator DT 360 (DariTech Inc. Lynden, WA). These samples were transported to the lab in 20-L plastic buckets and frozen prior to use for this study to reduce and/or prevent biodegradation. The frozen samples were thawed at room temperature and thoroughly mixed before drawing samples for simulating both manure storages and land applications in the laboratory. Soil samples for simulating land applications were collected from the top 50 cm of the soil profile at a crop land in Pullman, WA. Soils at the site are mapped as Thatuna silt loam, and had the following physical-chemical properties in the top layer: sand 14%, silt 71% and clay 15%; organic matter 5%; water capacity 0.2 In/In, pH 6.5; CEC 17 meq/100 g (USDA-NRCS, 2014). All soils were ground and sieved in order to remove organic particles larger than 850 μm.

3.3.1.2. Instrumentation

The experimental set-up and instrumentation used in the lab study are shown in Figure 3.1. The methodology used in this study to trap NH_3 emissions from the simulated manure storage was similar to that used in previous studies (Misselbrook et al., 2005; Ndegwa et al.,

2009; Vaddella et al., 2011). Briefly, the systems consisted of sealed plastic containers or buckets (reactors) (7.57 L, 23.5 cm H, 25.4 cm dia.) to simulate manure storages under anaerobic conditions, graduated cylindrical acid traps (catalogue no. 03-007-34, Fisherbrand, Mexico), variable area flowmeters (catalogue no. 32460-42, 5% full-scale accuracy, Cole-Parmer Instrument Company, IL) to regulate reactor headspace ventilation-air flow rates, and a vacuum pump (model DAA-V715A-EB, Gast MFG Inc., MI) to pull air through the manure reactors.
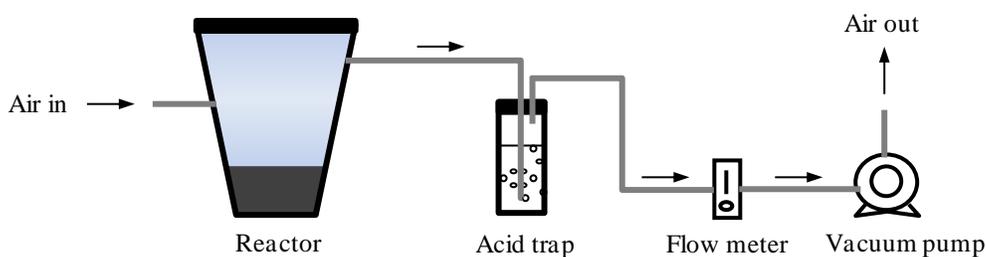
Figure 3.1 Schematic diagram of one of the simulated storages or field applications of manure

3.3.1.3. Simulated Manure Storage

Twelve of the above systems were operated to test four treatments (RM, AD influent, AD effluent, AD liquid effluent) with three replicates of each treatment. A central sealed tank evacuated with a vacuum pump was connected to 12 acid traps that were connected to the 12 reactor- systems. Each reactor, with manure surface area of 386 cm^2, was loaded with 2.5 L of test sample. Each of the reactor had 1-cm diameter headspace ventilation inlet and outlet ports. The reactors headspace ventilation air was drawn at 1 L min^{-1} (regulated by the air flowmeter) via Teflon tubing and through the acid traps to harvest emitted NH$_3$. The test lasted for approximately 21 d.

3.3.1.4. Simulated Land Application of Manure

Two manure application methods (surface application and manure injection) were simulated in triplicates for both the anaerobically digested (AD liquid effluent or AD) and non-anaerobically digested (RM or non-AD) manures, resulting in a total of 12 simultaneous simulations. A sample of 3.67 kg of prepared soil was spread in each reactor and 0.5 L of manure was applied to the soils by hand. Surface application was carried out by uniformly spreading manure onto the soil surface. Manure injection was achieved via manure injection into linear slots, approximately 2.5 cm into the soil, leaving 2.5-cm spacing between injection-slots (Fleesa and Beese, 2000). Lids were replaced immediately after manure applications.

3.3.1.5. Sampling and Analysis for Laboratory Study

Measurements of NH_3 emissions from the storage simulators were conducted for 21 d and from land application simulators for 9 d. Acid trap solutions were changed after 0.5, 1, 1.5, 2, 3, 4, 5, 7, 9, 11, 13, 16, 19 d and measurement stopped on approximately 21.5 d. The TAN concentrations in the acid traps (representing emitted NH_3) were determined using Hach Nessler colorimetric method 8038 adapted from standard method 4500-NH_3 (APHA, 1998). Ammonia emissions were evaluated based on total cumulative NH_3 (mg) emissions and NH_3 fluxes (g m^{-2} d^{-1}) (Vaddella et al., 2011). The dynamic emission rates were obtained from the regression plots of cumulative NH_3 emissions with storage time. Dynamic NH_3 emission fluxes were calculated as the quotient of the dynamic emission rates and the emitting surface areas (Parkin et al., 2003). Emissions of NH_3 from simulated land application of manure were measured following a similar protocol as that for the storages tests.

The characteristics of manure samples used in the simulation tests were determined before commencement and at the end of storage tests. Manure samples were analyzed in triplicates for total solids (TS) in a forced air oven at 105°C, and for volatile solids (VS) in a muffle furnace at 550°C in accordance with standard methods (APHA, 1998). Measurements of manure pH were made using a digital pH meter (model 290A, Orion Research Inc., MA). The potential for odor and odor generation were determined based on respective volatile fatty acids concentrations (Ndegwa, 2003; El-Mashad et al., 2011; Page et al., 2015). The calorimetric determination of VFAs in the manure samples was based on esterification of the carboxylic acids present in the sample followed by determination of esters by ferric hydroxamate reactions. The concentration of VFAs in the manure sample in mg L^{-1} of acetic acid was measured at a wavelength of 495 nm using a UV-visible spectrophotometer (Hach Company, 2004).

3.3.2. Field Study

The field studies were conducted at the location of the AD system from which manure samples for the lab-scale studies were obtained and on two dairy farms next to the AD system. Storage studies were conducted on a lagoon holding AD manure on one farm and on another lagoon holding non-AD manure at an adjacent dairy. Manure application studies were conducted on 12 field plots located at one of the dairies about 1.5 km from the AD site. The soils in this area was designated as 90% Puget silty clay loam soils with the following physical-chemical properties in the top soil layer: sand 7%, silt 62%, and clay 31%; organic matter 6%; water capacity 0.2 In/In, pH 6.7; CEC 28 meq/100 g (USDA-NRCS, 2014).

3.3.2.1. Manure Storage

Measurements of emissions from both AD and non-AD manure storages were made using a floating chamber and a photoacoustic IR analyzer (Model 1412, Innova AirTech Instruments, Ballerup, Denmark). Each of the three floating chambers covered 729 cm^2 of the emitting surface area and had volumes of 22,229 cm^3. The chamber headspace air was pulled into the photoacoustic IR analyzer for NH_3 concentration measurement through one vent of the chamber and the air was returned back into the chamber's headspace through another vent, resulting in a closed loop air circling required for measuring cumulative gas concentration (Joo et al., 2012; Sun et al., 2014). Ammonia concentration measurements were continued until NH_3 saturation in the chamber's headspace was observed. In most cases, measurements were completed within 10 min of sampling. Ammonia fluxes were calculated from linear segments of concentration versus time plot, chamber volume, and emitting surface area (Parkin et al., 2003; Rochette and Eriksen-Hamel, 2008; Joo et al., 2012a; Sun et al., 2014).

3.3.2.2. Land Application of Manure

Two manure application methods were tested: manure injection and surface application. The injector had 12 shank injectors covering a width of 4.3 m, which resulted in the shank injector spacing of 0.9 m. The research field was divided into 12 equal plots, each 8.5 m × 38.1 m (Figure 3.2). Manures (AD or non-AD) were injected into a south plot and then the injector would be lifted off the ground across the border to broadcast the same manure on the north plot. The result was triplicate tested for the two manure types and two land application methods: similar to the laboratory studies. The field experiment were conducted in mid spring (May, 2012)

for 7 d at application rates of 366 $m^3 ha^{-1}$ for AD manure and 388 $m^3 ha^{-1}$ for non-AD manure to ensure approximately the same TAN application rates.

Emissions of NH_3 from field plots were measured following a similar protocol to that of field manure storage studies. The only differences were the designs of the chambers, sampling procedure, and time of sampling. Each of the 12 chambers used in field plots covered 324 cm^2 of the plot emitting surface areas and had effective volumes of 5,726 cm^3. After manure was applied, measurements of NH_3 emissions commenced immediately. Measurements were conducted daily between 11:00 am and 12:00 pm, which was considered as the most representative time for obtaining daily average emissions (Bless et al., 1991; Joo et al., 2012b).
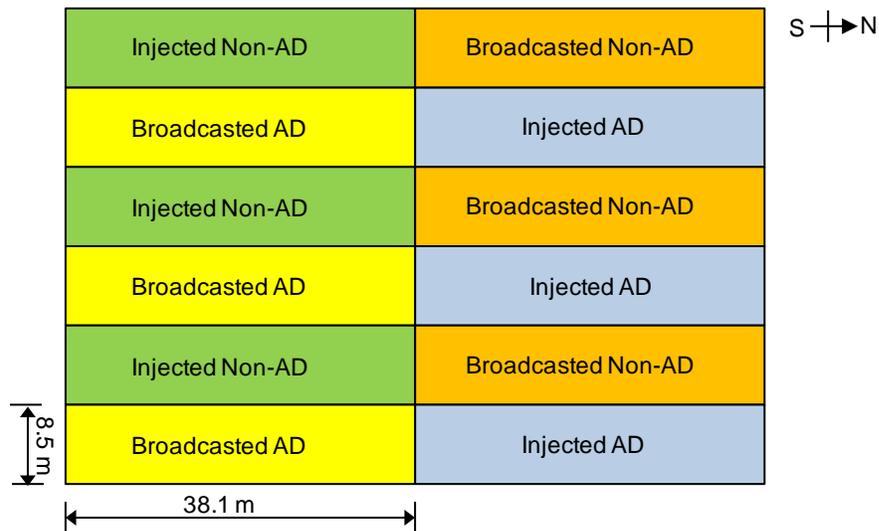
Figure 3.2 Layout of the field plots and manure application plan

3.3.3. Statistical Analysis

Treatment effects of all measures were compared across all treatments using the analysis of variance (ANOVA) procedure of SAS (SAS Institute, 2008) at the significance level of α = 0.05. Pairwise comparisons were performed using Fisher's LSD if the ANOVA indicated significant differences amongst the means (i.e., if $P < 0.05$).

3.4. Results and Discussion

3.4.1. Effects of AD and Solids-Separation on TS, VS, TAN, and VFA

The characteristics of the raw manure (RM), AD influent, AD effluent, and AD liquid effluent used in the simulation studies, and the effects of AD and solids separation on measured parameters are presented in Table 3.1. The AD influent had a 7.5% higher fraction of VS compared with raw manure samples most probably due to the co-digestion materials that were added to the raw manure. Addition of consumer wastes increased the contents of TS and VFA by 2 and 312% while reducing concentrations of TAN and pH by 23% and 1.3 units, respectively, indicating co-digestion substrates with relatively low nutrient content but lower pH (i.e. expired carbonated beverages; FOG: fats, oils, and greases).

The AD process significantly increased TAN concentration and effluent pH while decreasing TS, VS, and VFA. The increase in TAN is attributable to organic nitrogen mineralization during the digestion process (Powers et al., 1999; Sommer et al., 2006), whereas the increased pH was most likely due to higher ammonia N in the AD effluent as well as conversion of organic carbon and phosphorus to inorganic carbonate and phosphate buffer systems, leading to higher overall alkalinity and pH. Similar TAN and pH increases were observed in previous studies (Amon et al., 2006; Immovilli et al., 2008; Frear et al., 2011;

Koirala et al., 2013; Page et al., 2014). The higher VFA concentration of AD influent may be possibly due to the addition of co-digestion substrates (Frear et al., 2011; Page et al., 2015). The decrease in TS, VS and VFA concentrations in AD effluent is emblematic of anaerobic biodegradation of organic compounds during the AD process (Karim et al., 2005; Madsen et al., 2011). These results were in partial agreement with those from previous studies (Frear et al., 2011; Page et al., 2014), although the limited VS reduction (42%) and relatively high residual VFA concentration (2,468 mg L^{-1} and 55% reduction) indicate a performance below that expected of a co-digestion AD system, indicating concerns with operation or use of effective hydraulic retention times (HRT) (Frear et al., 2011).

Solids-liquid separation significantly reduced TS, VS, TAN, and VFA. The solids-liquid separation removed 64% of TS and 69% of VS from AD effluent. The concentrations of TAN and VFA were approximately 56% and 86% less in AD liquid effluent than in AD effluent (Table 3.1). The reduced TAN and VFA concentrations in AD liquid effluents relative to AD effluents were attributed to lower levels of solids in the AD liquid fraction (Zhang and Westerman, 1997) with the solids and associated water carrying a significant portion of TAN and VFA, either as part of the water or adhered to the solids.

Table 3.1 Manure characteristics and effects of AD and solids-separation on TS, VS, TAN and VFA

Treatment	Property	TS (mg kg^{-1})	VS (mg kg^{-1})	TAN (mg L^{-1})	VFA (mg L^{-1})	pH
	RM	32,700 ± 425[†]	26,600 ± 425	978 ± 58	1338 ± 342	7.03
Effect of AD	AD influent	33,300[b*] ± 450	28,600[b] ± 425	755[b] ± 15	5512[b] ± 338	5.74
	AD effluent	22,000[c] ± 104	16,600[c] ± 116	1637[c] ± 26	2468[c] ± 270	7.32
	% change	-34[‡]	-42	117	-55	28
Effect of solids-separation	AD effluent	22,000[b] ± 104	16,600[b] ± 116	1637[b] ± 26	2468[b] ± 270	7.32
	AD liquid	7917[c] ± 231	5150[c] ± 173	723[c] ± 7	352[c] ± 73	6.63

effluent				
% change	-64	-69	-56	-86	-9

TS = Total solids, *VS* = Volatile solids, *TAN* = Total ammoniacal nitrogen, *VFA* = Volatile fatty acids

RM = raw manure, *AD influent* = raw manure co-mingled with consumer wastes, *AD effluent* = anaerobically digested manure effluent, *AD liquid effluent* = anaerobically digested manure effluent after solids-liquid separation

[†] Mean ± standard deviation ($n = 3$)

[*] Mean values of each property before and after respective treatments bearing the same lower case letter (superscript) were not significantly different ($\alpha = 0.05$)

[‡] Negative signs denote reductions, while positive signs indicate increases

3.4.2. Ammonia Emissions from Manure Storages

The results of cumulative NH_3 loss and the dynamic NH_3 fluxes from simulated storages of RM, AD influent, AD effluent, and AD liquid effluent are presented in Figure 3.3. The cumulative mean NH_3 emissions during the 21-d tests were: 962 ± 47 mg from RM; 197 ± 16 mg from AD influent; 2399 ± 101 mg from AD effluent; and 870 ± 15 mg from AD liquid effluent (Figure 3.3a). Mean cumulative NH_3 emissions, within the 21 d, was significantly higher from AD effluent than from the storage of RM, AD influent, and AD liquid effluent. Emissions from RM and AD liquid effluent, however, were not significantly different. During day 1, higher NH_3 fluxes were observed from the RM (1.65 g m^{-2} d^{-1}) and AD effluent (3.95 g m^{-2} d^{-1}) storages compared with those from AD influent (0.25 g m^{-2} d^{-1}) and AD liquid effluent (1.1 g m^{-2} d^{-1}). Between days 1 and 21, the higher fluxes from RM and AD effluent decreased at constant rates to 0.70 and 2.02 g m^{-2} d^{-1}, respectively, while the fluxes from AD influent and AD liquid effluent remained approximately constant throughout the test (Figure 3.3b).

Lab simulated manure storages of AD effluent indicated significantly higher NH_3 emissions (92%) than those from AD influent (undigested manure) during the 21 d period. The increase in NH_3 emissions may be due to the inclusion of co-digestion substrates in AD influent

which could have resulted in a very low pH in the AD influent as compared with the AD effluent. Ammonia flux from the AD effluents declined from 3.95 to 2.02 $g\,m^{-2}\,d^{-1}$ compared with 0.25 $g\,m^{-2}\,d^{-1}$ from AD influent. Similar NH_3 emissions were observed from field studies. Field NH_3 emissions from storages of AD and non-AD manure ranged from 0.12 to 1.4 $g\,m^{-2}\,d^{-1}$ and 0.09 to 0.79 $g\,m^{-2}\,d^{-1}$, respectively (Figure 3.4). Immovilli et al. (2008) also showed in laboratory storage tests that the anaerobically digested cattle slurry resulted in higher (0.208 $g\,m^{-2}\,h^{-1}$) NH_3 loss compared with undigested slurry (0.121 $g\,m^{-2}\,h^{-1}$).

Post-separation storages of AD liquid effluents showed significant reductions of NH_3 emissions (64%) compared with storages of unseparated AD effluent. The reduced NH_3 loss from AD liquid effluents relative to AD effluents may be attributed to reduced TAN, after separation of solids during solids-liquid separation processes. This conclusion is consistent with observed ammonium-solids adsorption phenomenon (Visscher et al., 2002; Vaddella et al., 2013). Removal of solids and adsorbed TAN may, therefore, cause significant reductions of NH_3 emissions from stored AD liquid effluents. These results concurred with previous studies on NH_3 emission abatement via removal of biodegradable solids and the associated organic nutrients during solids-liquid separation treatment (Zhang and Westerman, 1997). Szoegi and Vannotti's (2007) study showed that a 60% TS removal resulted in a 73% reduction of NH_3 emission from swine lagoons. Based on the results reported herein and from available literature, it is evident that solids-liquid separation treatment can significantly mitigate NH_3 emissions from AD liquid effluents.
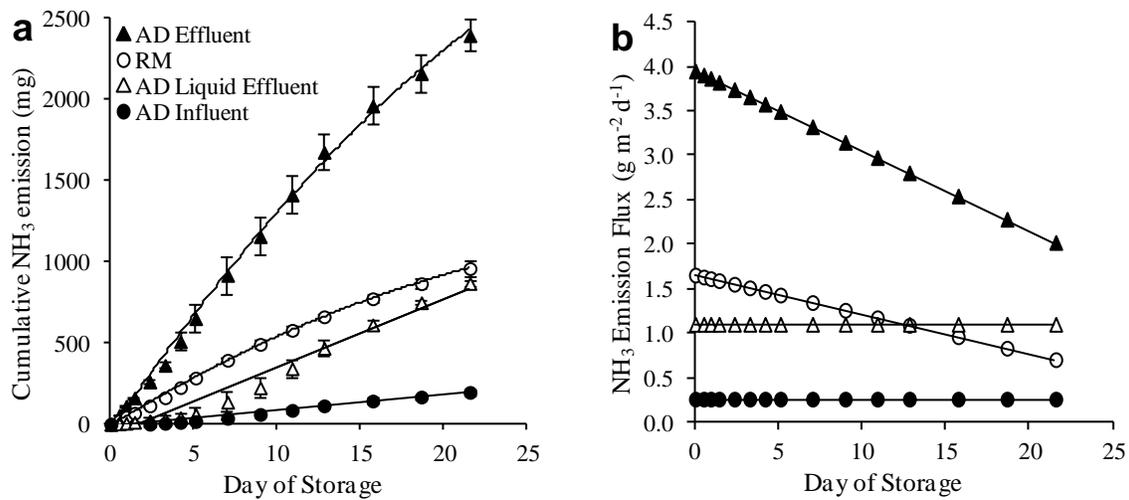
Figure 3.3 Ammonia emissions from simulated lab-scale storages: (**a**) cumulative emissions (mg), and (**b**) dynamic emissions fluxes (g m^{-2} d^{-1}). Vertical bars represent standard deviations from means ($n = 3$)
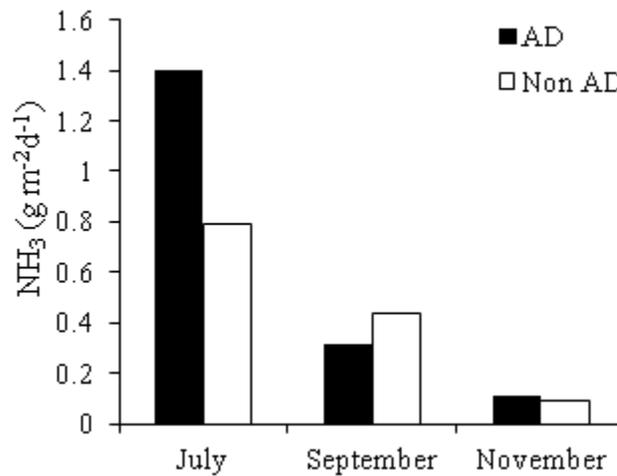
Figure 3.4 Ammonia emissions from full-scale storage of anaerobically digested (AD) and non-anaerobically digested (non-AD) manures

3.4.3. Changes in Manure Properties during Storage

Table 3.2 represents the respective characteristics of the four dairy manures (RM, AD influent, AD effluent, and AD liquid effluent) properties before and after the 21 d of storage simulation. The AD influent had the highest TS levels both at the start and end of the storage, while AD liquid effluent exhibited the lowest TS concentrations. The TS content of AD influent and AD effluent decreased by approximately 12% and 6%, respectively, during this period, while only a marginal increase in TS in AD liquid effluent was observed. The changes in VS were similar to those of TS. Evaporative moisture loss from manure surfaces can explain these observed increases (Smith et al., 2007; Page et al., 2014).

Approximately 38% and 16% reductions in TAN in the AD effluent and AD liquid effluent, respectively, were observed during the 21 d storage period. However, a 25% increase in TAN was recorded in the AD influent. This increase in the TAN content in AD influent was probably due to mineralization of organic nitrogen (Sommer et al., 2006). In a similar recent study, Page et al. (2014) also observed an increase in TAN in AD influent at the end of a three-month storage of dairy manure.

Volatile fatty acids concentrations in AD influent were the highest at the beginning and end of the storage period. These higher VFA concentrations were likely due to the higher initial biodegradable VS in the AD influent. Higher organic matter degradation usually leads to higher microbial activities resulting in more VFAs production (El-Mashad et al., 2011; Wood et al., 2012) and greater methane formation during anaerobic storage, thus in part explaining both the still high effluent VFA concentration and VFA reduction (21%) of AD influent during storage. The concentrations of VFAs were lowest in the AD liquid effluent before and at the end of storage period most likely due to its lower TS and VS contents. The results indicated that storage

of AD effluent reduced VFA by 40%, while solids-liquid separation resulted in a significantly high increase (114%) in VFA during storage. The reduction in VFA during storage of AD effluent may be attributed to the rich anaerobic culture attached to solids, which enhanced degradation of the VFA into methane during storage. Solids separation from the AD liquid effluent, on the other hand, may have significantly reduced the anaerobic community (methanogens) responsible for VFA degradation to methane, leading to the observed build-up of VFAs.

Table 3.2 Manure characteristics at the beginning and end of 21 storage simulation studies

Property		TS $(mg\ kg^{-1})$	VS $(mg\ kg^{-1})$	TAN $(mg\ L^{-1})$	VFA $(mg\ L^{-1})$
RM	Start	32,700	26,600	978	1338
	End	30,000	23,700	898	2733
	% change	-8[a]	-11	-8	104
AD influent	Start	33,300	28,567	755	5512
	End	37,300	32,511	946	4358
	% change	12	14	25	-21
AD effluent	Start	22,000	16,600	1637	2468
	End	23,300	17,200	1009	1490
	% change	6	4	-38	-40
AD liquid effluent	Start	7917	5150	723	352
	End	7856	4916	610	753
	% change	-1	-5	-16	114

TS = Total solids, *VS* = Volatile solids, *TAN* = Total ammoniacal nitrogen, *VFA* = Volatile fatty acids

RM = raw manure, *AD influent* = raw co-mingled with consumer wastes, *AD effluent* = anaerobically digested manure effluent,

AD liquid effluent = anaerobically digested manure effluent after solids-liquid separation

[a] Negative signs denote reductions, while positive signs indicate increases

3.4.4. Ammonia Emissions from Land Application of Manure

The results of cumulative NH_3 loss and the dynamic NH_3 fluxes from simulated land application of non-AD and AD manure are presented in Figure 3.5. Anaerobic digestion, in

general, regardless of manure application methods, was more effective in reducing NH_3 emissions compared with raw or undigested manure. Under surface application, AD manure emitted 56% less NH_3 than non-AD manure, while injection of AD manure resulted in 27% less NH_3 emission than injection of non-AD manure. The reduced NH_3 emission was partly attributed to the lower solids content in the AD manure compared with non-AD manure, which enhanced applied manure infiltration into the soil. Injection of non-AD manure resulted in 42% less NH_3 emissions than the surface application of non-AD manure. The difference between injected AD manure and surface-applied AD manure, however, was not significant. The results from simulation of manure injection is consistent with the work of Huijsmans et al. (2003), which showed that manure injection reduced NH_3 loss more than manure surface broadcasting or spreading.

Ammonia flux is usually the highest immediately after manure application because of the initial higher TAN concentration. Surface application of non-AD manure exhibited the highest initial NH_3 emission flux (0.78 g m^{-2} d^{-1}), while injected AD manure had the lowest initial NH_3 emission flux of 0.17 g m^{-2} d^{-1} (only 22% NH_3 flux from surface applied non-AD manure, Figure 3.5b). For similar application methods, AD manure was more effective in reducing NH_3 emissions (i.e., retained higher manure fertilizer-value) than non-AD manure.

Ammonia fluxes from treatments receiving non-AD manure dropped quickly to approximately zero within 3 d, while the fluxes from treatments receiving AD manure gradually approached zero within 5 d. These decreases were attributed to the decrease of TAN concentration in soil surface from either NH_3 emission, TAN infiltration, or TAN-nitrification. In this study, the lower solids content of AD manure may have improved AD manure infiltration into the soil so that its initial emission flux was less than that of non-AD manure and thus needed

more time to volatilize. Ammonia fluxes from applied manure were low after the first 2 d and the cumulative NH_3 emission reached 50% of its maximum within the first 1 or 2 d, explaining why most of the NH_3 emissions occurred within 5 d after manure application.

Cumulative NH_3 emissions and fluxes during 7 d after manure application in the field plots are shown in Figure 3.6. The results, in general, were similar to those obtained in laboratory studies. With respect to surface application, AD manure showed 49% less NH_3 loss compared with the non-AD manure. Injection of AD manure did not significantly reduce total NH_3 flux (Figure 3.6a) over injection of non-AD manure. The initial flux from AD manure, however, was lower than from non-AD manure (Figure 3.6b). Accordingly, application of AD manure resulted in significantly less NH_3 emission and a lower flux as opposed to non-AD manure. With respect to application methods, surface application resulted in 63% and 25% more NH_3 loss than injection of non-AD manure and AD manure, respectively. These results suggested that manure injection was a more effective method for mitigation of NH_3 emission than surface application. Ammonia fluxes were generally low after 2 d, with 50% of cumulative NH_3 emission being reached within 1 d and NH_3 emission almost complete within 5 d after manure application.

The patterns of NH_3 emission were, somewhat, different from that observed in controlled laboratory studies most probably due to differences in manure characteristics, and diurnal variation in ambient temperature, wind speed, and radiation factors within exposed field plots. In the field studies, for example, injecting AD manure reduced NH_3 emissions significantly compared with surface application of AD manure. The same test in the lab did not indicate similar significant advantage in mitigation NH_3 emissions. These discrepancies may be attributed to inadequate replication or simulation of manure injection in the lab studies to match field applications.
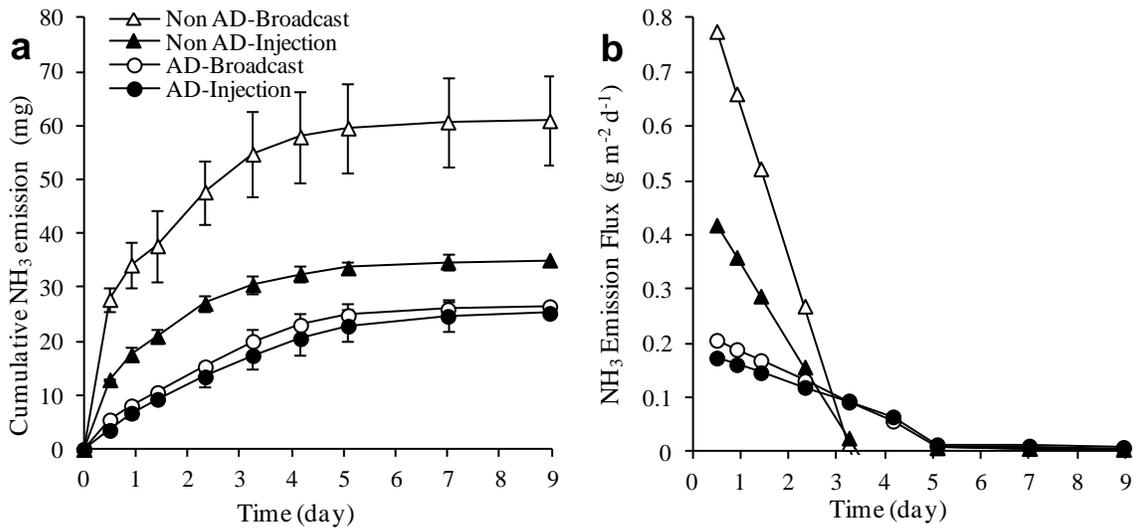
Figure 3.5 Ammonia emissions based on lab-scale land application simulation studies: (**a**) cumulative NH$_3$ emissions, and (**b**) NH$_3$ emission rates. Vertical bars in (a) represent standard deviation of means ($n = 3$)
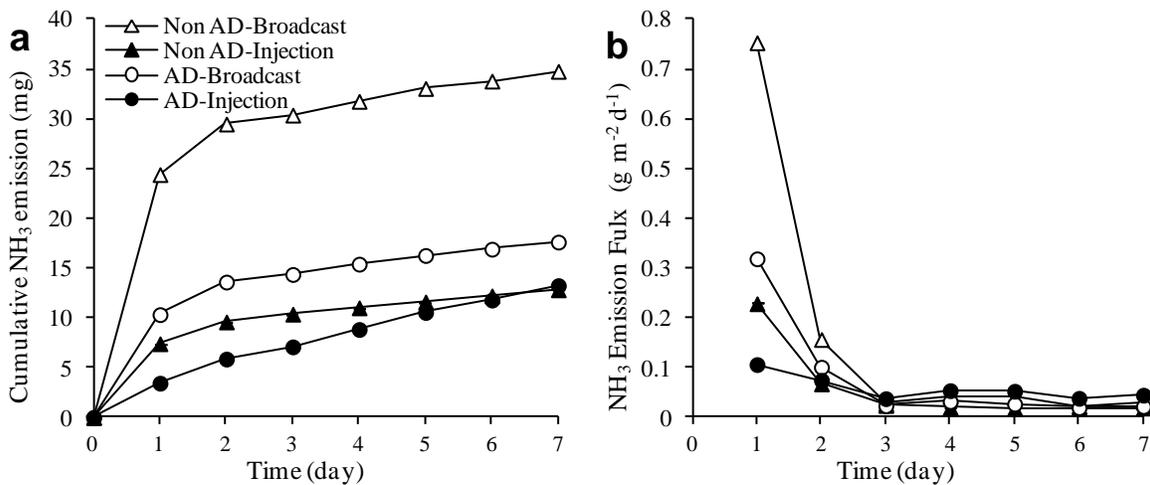
Figure 3.6 Ammonia emissions from land application of manures based on field measurements: (**a**) cumulative NH$_3$ emissions, and (**b**) NH$_3$ emission rates

3.5. Conclusions

The overall goal of this study was to investigate the effects of anaerobic digestion and solids-liquid separation on emissions from subsequent storage and land application of dairy manure. Experiments were conducted both in the laboratory and also in full-scale manure lagoons and field plots. The following conclusions were drawn from data obtained in these studies.

1. Anaerobic digestion of dairy manure increased TAN in the effluent, which exacerbated NH_3 emissions from subsequent laboratory storage of AD effluents. Over the 21 d storage period, higher NH_3 fluxes were observed from AD manure relative to undigested manure. Ammonia fluxes from the AD effluent declined from 3.95 to 2.02 g m^{-2} d^{-1} compared with 0.25 g m^{-2} d^{-1} from AD influent. Similar NH_3 emissions results were observed from the field studies. Ammonia fluxes from storages of AD and non-AD manure ranged from 0.12 to 1.4 g m^{-2} d^{-1} and 0.09 to 0.79 g m^{-2} d^{-1}, respectively.

2. Anaerobic digestion of dairy manure increased effluent pH by 1.6 units while decreasing TS, VS, and VFA by approximately 34%, 42%, and 55%, respectively.

3. Solids-liquid separation removed 64% of TS from AD effluent. The concentrations of TAN and VFA were approximately 56% and 86% less for AD liquid effluent than for AD effluent. The storages of AD liquid effluent resulted in 64% less NH_3 loss than from storage of AD effluent during the 21 d study period.

4. Regardless of manure application methods, NH_3 emissions were lower when soils received AD manure, revealing an environmental benefit of the AD process. Although there were no significant differences in NH_3 emissions between the two application methods from soil receiving AD manure in controlled lab-scale tests, manure injection was more effective in

reducing NH_3 emissions than surface application for soil receiving non-AD manure in the field study.

5.   Surface applied non-AD manure exhibited the highest NH_3 flux (0.78 g m^{-2} d^{-1}) immediately after application, while injected AD manure resulted in the lowest flux (0.17 g m^{-2} d^{-1}). Similar results were observed in the field studies: ammonia flux from surface applied non-AD manure was 0.75 g m^{-2} d^{-1}, while the loss from injected AD manure was 0.11 g m^{-2} d^{-1}.

6.   Data from the land applied manure study indicate that injection of AD manure bear the smallest risks with respect to NH_3 emissions when fertilizing with dairy manure. Manure injection is certainly environmentally friendlier than surface application because the former mitigates NH_3 emission more than the latter.

3.6. Acknowledgements

3.7. References

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CHAPTER FOUR

POTENTIAL APPLICATION OF *ALCALIGENES FAECALIS* STRAIN NO. 4 IN MITIGATING AMMONIA EMISSIONS FROM DAIRY WASTEWATER

4.1. Abstract

This research evaluated potential mitigation of NH_3 from flushed dairy manure via an enhanced aerobic bio-treatment process. The bioprocess utilizes bacterium *Alcaligenes faecalis* strain No. 4, which has abilities to carry out heterotrophic nitrification and aerobic denitrification in the same reactor. The treatment efficacy of the *A. faecalis* strain No. 4 on dairy wastewater was evaluated in aerated batch experiments. The influence of oxygen supply on TAN removal rate and extent were tested in batch systems using aeration with ordinary air and pure oxygen. These experiments were conducted at 30°C and an agitation speed of 100 rpm. Aeration with oxygen and air resulted in approximately 100% and 40% TAN removal, respectively. Intermittent (every 2 or 4 h) aeration with oxygen gas reduced oxygen consumption by 95%, while attaining nearly identical TAN removal to continuous aeration. The results revealed that adequate oxygen supply and supplementing dairy wastewater with carbon are important factors for efficient TAN removal. From the nitrogen mass balance based on the oxygen-flush system, only 4% of TAN was released as NH_3 gas, while the majority was retained in either the microbial biomass (58%) or converted to nitrogen (N_2) gas (36%). The results from this research reveal high potential for environmentally friendly bio-treatment of dairy wastewater using *A. faecalis* strain No. 4 with respect to NH_3 emissions.

4.2. Introduction

Biological treatments offer an attractive method for mitigating ammonia (NH_3) emissions from livestock wastewaters. Conventional nitrogen removal processes from wastewater traditionally consists of two stages: (i) aerobic autotrophic nitrification, and (ii) anaerobic heterotrophic denitrification. Nitrification transforms ammonium (NH_4^+) to oxidized nitrogen compounds ($NH_4^+ \rightarrow NH_2OH \rightarrow NO_2^- \rightarrow NO_3^-$); and these compounds are further reduced to nitrogen (N_2) gas via denitrification ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$) (Focht and Chang, 1975; Junter et al., 1995; Chen et al., 1998; Zhu et al., 2008; Sun et al., 2010). However, biological removal of ammonium in a conventional treatment system faces several problems including: (i) an extremely slow nitrification step, (ii) strong sensitivity to oxygen limitation, (iii) negative impacts via overloading of ammonium and organic matter, and (iv) requirement of two separate reactors for nitrification, an aerobic process, and denitrification, an anaerobic process (Joo et al., 2005; Zhu et al., 2008; Shoda & Ishikawa, 2014). The low nitrification rates in this process result in the need for long hydraulic retention times or large reactor volumes to accomplish complete NH_4^+ removal. Consequently, conventional treatment demands multiple and larger reactors and high capital and operation costs (Szogi et al., 2004; Zhu et al., 2008).

Over the past two decades, several new bio-processes for ammonium removal from municipal and domestic wastewaters have been developed, including: simultaneous nitrification and denitrification, shortcut nitrification and denitrification, anaerobic ammonium oxidation (ANAMMOX), aerobic deammonification, complete autotrophic nitrogen removal over nitrite (CANON), oxygen limited nitrification and denitrification (OLAND), advanced treatments using combination of theses process (anaerobic/oxic/anoxic process, step-feed multistage anaerobic/oxic process, and membrane bioreactors), and cell-immobilization systems. These

technologies possess promising features for NH_4^+ removal from dairy wastewaters. However, these processes also have some potential problems or limitations similar to conventional nitrogen removal processes, such as reduced nitrification rate, longer retention time, large reactor volumes, and high operational costs which limit their applications (Junter et al., 1995; Zhu et al., 2008; Sun et al., 2010).

In conventional aerobic treatment of high strength ammonia wastewaters, aeration represents the major operating cost (Water Pollution Control Federation, 1988; Ahmed et al., 2004). The use of pure oxygen gas to maintain aerobic systems has recently generated interest in wastewater treatment systems as an alternative to address the drawbacks of using air to oxygenate these systems (Brindle et al., 1998; Beutel and Horne, 1999; Casey et al., 1999; Ahmed et al., 2000; Semmens et al., 2003; Satoh et al., 2004; Palmer et al., 2009). Furthermore, use of pure oxygen to oxygenate aerobic systems has indicated potential of reducing the cost of aerating these systems (Speece, 1996). In general, systems that use pure oxygen gas rather than ambient air (21% O_2 by volume) demonstrate better oxygen transfer efficiency, are simple and compact, allow for easier gas storage and handling, and have lower operating costs. While numerous studies have evaluated aerobic treatment system using air-based aeration methods for removing ammonia from livestock wastewaters, no studies in the literature have focused the use of oxygenation using pure oxygen gas to mitigate NH_3 emissions from livestock wastewaters. Therefore, one component of this research study evaluated the use pure oxygen gas against ambient air to mitigate NH_3 emissions from dairy wastewaters.

In this study, we hypothesized that using a unique heterotrophic microorganism *Alcaligenes faecalis* strain No. 4 (*A. faecalis* strain No. 4) and aeration using pure oxygen gas would enhance the bio-treatment process to mitigate NH_3 emissions from dairy wastewaters.

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Furthermore, we anticipated that this bio-treatment process would overcome the conventional nitrification-denitrification drawbacks outlined previously in this paper. The bacterium *A. faecalis* strain No. 4 has the unique ability to consume carbon (C) and remove ammonium from wastewater mainly via nitrogen (N_2) gas and microbial assimilation in a single aerobic process (Joo et al., 2005, 2006, 2007). This is an attractive approach to biological ammonium removal from dairy wastewater because the conventional pathway of its removal via N_2 can be shortened via single aerobic process ($NH_4^+ \rightarrow NH_2OH \rightarrow N_2$). Although a few studies have been conducted with *A. faecalis* under various ammonium (low and high strength) concentrations (Joo et al., 2005, 2006, 2007; Zhao et al., 2012; Shoda & Ishikawa, 2014), to date, no studies have used *A. faecalis* strains for treating high strength ammonium dairy-cattle manure wastewater.

The goal of this study was to search for a cost-effective bioprocess for mitigation of NH_3 emissions from dairy wastewater. To achieve this goal, the following two specific objectives were formulated: (i) examine potential mitigation of NH_3 emissions from high-strength-TAN dairy-cattle wastewater using *A. faecalis* strain No. 4, and (ii) assess the enhancement of this bioprocess using pure oxygen gas, instead of air, to maintain aerobic conditions in the system.

4.3. Materials and Methods

4.3.1. Preparation of Bacterial Culture in Shaking Culture Experiments

The bacterium *A. faecalis* No. 4 was cultivated in a basal medium prepared by dissolving the following in 1 L lab grade water: 14 g K_2HPO_4, 6 g KH_2PO_4, 51 g trisodium citrate dihydrate, 6 g of $(NH_4)_2SO_4$, 0.2 g $MgSO_4 \cdot 7H_2O$ and 2 ml trace mineral solution. The trace mineral contents included (per liter): 57.1 g EDTA·2Na, 3.9 g $ZnSO_4 \cdot 7H_2O$, 7 g $CaCl_2 \cdot 2H_2O$, 5.1 g $MnCl_2 \cdot 4H_2O$, 5.0 g $FeSO_4 \cdot 7H_2O$, 1.1 g $(NH_4)_6 Mo_7O_{24} \cdot 4H_2O$, 1.6 g $CuSO_4 \cdot 5H_2O$, and 1.6 g of

CoCl$_2$·6H$_2$O (pH = 6.0). The media was autoclaved for 20 min at 120°C. A 1.2 mL of stock solution of strain No. 4 was inoculated into a 150 mL of the basal medium in a 500 mL and cultivated at 37°C at 120 strokes per minute (spm, shaker agitation rate) for 48 h prior to startup of experiments (Joo et al., 2005).

4.3.2. Dairy Wastewater Collection and Preparation

Manure samples for this study were collected from a commercial dairy farm lagoon located in central Washington, in the Pacific Northwest of the U.S.A. Samples of liquid dairy manures were transported to the lab in 20-L sealable plastic buckets and frozen prior to use for this study. The manure samples were thawed at lab temperatures prior to startup of experiments. The dairy wastewater was screened through a 1.40 mm sieve to remove debris and larger solid particles. This screeing was essential to avoid pipet-tip clogging during wastewater sampling and to ensure integrity of samples.

4.3.3. Instrumentation

The experimental set-up and instrumentation used in this study are shown in Figure 4.1 and 4.2. These systems are similar to those used in similar bio-treatments of wastewaters (Joo et al., 2005, 2006; Zhao et al., 2010; Shoda & Ishikawa, 2014). Briefly, the bioreactor system consisted of a sealed conical flask (reactor), agitation plus temperature control system (hot plate with stirrer, catalogue no. 03407-10, Cole-Parmer Instrument Company, IL) or a shaking water bath (model 50, Thermo Scientific, OH), variable area flowmeters (catalogue no. 32460-42, 5% full-scale accuracy, Cole-Parmer Instrument Company, IL) to regulate aeration in the bioreactor system, graduated cylindrical acid traps (250 mL, catalogue no. 03-007-34, Fisherbrand, Mexico),

and either an air compressor (model LA-5706, PUMA Industries Inc., TN) or oxygen tank to aerate the reactor.
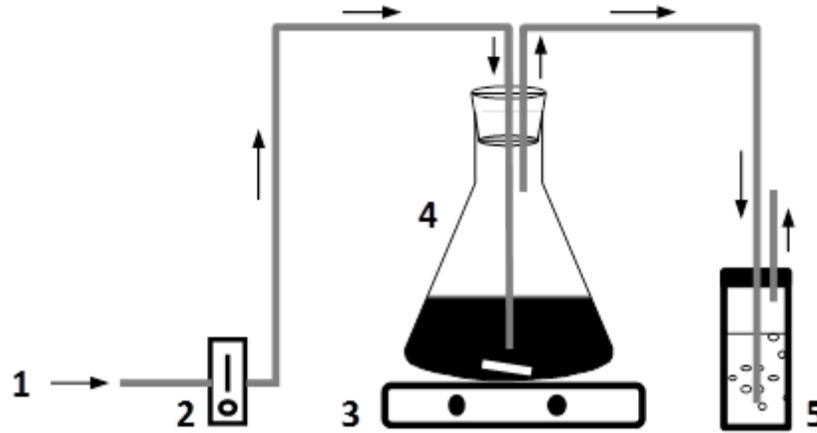
Figure 4.1 Bioreactor system for aerated batch experiments: (1) air compressor pump or oxygen-gas tank; (2) flow meter; (3) stirring hot plate; (4) reactor; and (5) acid trap. The arrows indicate the flow direction of air or oxygen.
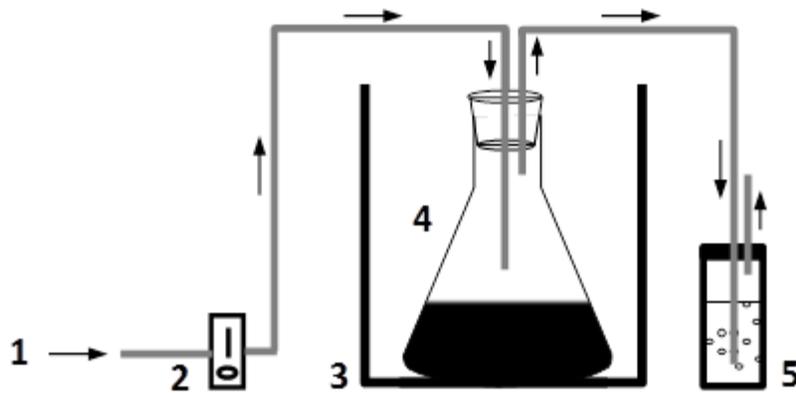

Figure 4.2 Bioreactor-headspace flushing system: (1) air compressor pump or oxygen-gas tank; (2) flow meter; (3) shaking water bath; (4) reactor; and (5) acid trap. The arrows indicate the gas flow direction.

4.3.4. Aerated Batch Culture Experiments

Two sets of aerated batch culture experiments were conducted. In the first set of phase I experiments to test the efficacy of *A. faecalis* strain No. 4 in dairy wastewaters were conducted in aerated batch studies in which air or oxygen were introduced directly into the wastewater. Batch experiments were conducted in 1 L reactors. The experimental setup is shown schematically in Figure 4.1. A sample of 50 mL of the pre-culture (10% inoculum) was introduced into each reactor containing 500 mL of dairy wastewater. The reactors were tightly sealed, stirred to ensure complete mixing of reactor contents during the process. Aeration treatments were achieved by either bubbling air or pure oxygen-gas through the reactor content. The bioreactor systems were operated for 72 h, at 30°C at air or oxygen flow rates of 0.5 L min^{-1} and agitated at 120 rpm with a magnetic stirrer. Samples of reactor contents were taken periodically for determination of viable cell number or microbial growth (at 0, 16, 32, 48, and 72 h), and for TAN and pH (every 8 h for 72 hours). The reactor headspace gas, for each reactor, was delivered via Teflon tubing to the acid trap containing 150 mL of 0.2 M H_2SO_4 solution to capture stripped NH_3 from the reactor and subsequently analyzed for TAN concentration.

In the second set, phase II, aerated batch experiments using air aeration were conducted to further examine the effects of airflow rate on TAN removal efficiency and ammonia stripping. Two sets of batch experiments were aerated using air at two different airflow rates of 0.2 and 0.5 L min^{-1}. Each batch treatment system of the dairy wastewater was inoculated with strain No. 4 and operated at 30°C and an agitation speed of 115 spm in a reciprocal shaker. All other conditions of these experiments were similar to those of the first set of experiments.

4.3.5. Reactor-Head Space Flushing Experiments

The series of experiments described in this section were formulated to evaluate enhancement of TAN removal from dairy wastewaters using *A. faecalis* strain No. 4. Reactor-headspace flushing, which is the method of aeration used in this set of experiments, refers to the process of replacing headspace gases with fresh air or oxygen gas once or at several intervals during the treatment time. The experiments were conducted in 500 mL reactors. The bioreactor systems were operated at 30°C at gas flow rates of 0.1 L min^{-1} and at 100 spm in a reciprocal shaker water bath. A schematic of the bioreactor system used in this study is shown in section 2.3 (Figure 4.2). Two sets (phases I and II) of reactor-headspace flushing experiments were conducted.

4.3.5.1. Effects of Carbon Supplementation and Oxygenation

In the phase I studies, flushing treatments included: (i) flushing with oxygen and C supplementation: 150 mL of the dairy wastewater, 15 mL (10% inoculum) of the pre-culture of strain No. 4 and 10 g trisodium citrate dehydrate were mixed in the reactor; (ii) flushing with oxygen without C-supplementation: 150 mL of the dairy wastewater and 15 mL of the pre-culture of strain No. 4 were mixed in the reactor; and (iii) flushing with air with C supplementation: 150 mL of the dairy wastewater, 15 mL of the pre-culture of strain No. 4 and 10 g trisodium citrate dehydrate 50 ml of the pre-culture of strain No. 4 were mixed in the reactor. To compare TAN removal efficiencies in these systems, samples of reactor contents were taken periodically for determination of TAN concentrations. For oxygen-flush batch treatments of strain No. 4 using dairy wastewater supplemented with C source, concentration of

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hydroxylamine (NH_2OH) and NO_3^- were also determined. Stripped ammonia from the reactor was measured following a similar procedure as that for the aerated batch studies in section 4.3.4.

4.3.5.2. Effects of Reactor Headspace-Oxygen-Flushing Durations

In phase II studies, experiments were conducted to study the impact of different flushing durations on TAN removal efficiency. The following five flushing intervals were randomly selected for evaluation: continuous and after every 2, 4, 8, and 24 h for about 5 min. For these experiments, 150 mL of the dairy wastewater, 15 mL of the pre-culture of strain No. 4 and 10 g trisodium citrate dehydrate were mixed in the reactor. To monitor TAN removal, 2 mL samples were withdrawn from the reactor periodically throughout the process time to determine respective TAN concentrations.

4.3.6. Analytical Procedures and Methods

The 2 mL samples collected periodically from respective reactors were centrifuged at 10,000 rpm and the supernatant collected for subsequent analyses. Concentrations of TAN were determined using indophenol method adapted from standard methods (Joo et al., 2007). Hydroxylamine was analyzed according to Frear and Burrell (1955) (Joo et al., 2007; Zhao et al., 2012; Yang et al., 2015). Nitrate was measured using Hach chromotropic acid method 10020 (Hach Company, 2004). A digital pH meter (model 290A, Orion Research Inc., MA) was used to determine the pH. Intracellular nitrogen content (mg-N L^{-1}) was determined by an elemental analyzer (TruSpec CHN, LECO Corporation, MI) using dry biomass. Dry cells were obtained at the end of experiment by centrifuging sample culture, followed by washing with *deionized water* and drying at 105°C for 24 h. To estimate total viable cell number (colony forming units, CFU

counts) of strain No. 4, sampled culture were diluted serially and plated on LB agar plates which were incubated for 48 h at 37°C. Total colonies appearing on the plates after this incubation periods were counted as *A. faecalis* strain No. 4 cells (Joo et al., 2005; Shoda & Ishikawa, 2014).

4.4. Results and Discussion

4.4.1. Aerated Batch Culture Experiments

The aerated batch experiments were conducted to study viability or growth of *A. faecalis* strain No. 4 and TAN removal in dairy wastewater. Aerations were achieved by introducing either ambient air or pure oxygen gas directly into the wastewater. Figure 4.3 shows the changes in *A. faecalis* strain No. 4, wastewater pH, TAN concentrations, and stripped NH_3 during 72 h of treatment period when using air and oxygen for aeration.

The initial TAN levels in the wastewater in these experiments using air and oxygen gas were similar at about 1600 mg-N L^{-1} and 1500 mg-N L^{-1}, respectively. As shown in Figure 4.3a and 4.3b, changes in TAN and stripped ammonia concentrations indicated similar trends between the two tests. The respective maximum TAN removal rates, for air and oxygen, were approximately 18.5 and 17 mg-N $L^{-1} h^{-1}$, while stripped NH_3 amounted to about 843 and 807 mg-N L^{-1} which accounted for approximately 50% of initial TAN. The wastewater pH profiles were also nearly the same for both experiments, increasing by about 0.9 and 1.3 units from the initial values for air and oxygen aerations, respectively.

The average viable cell number of *A. faecalis* strain No. 4 ranged from 1.3×10^7 to 5.6×10^8 cfu mL^{-1} for the aeration with air to 1×10^7 to 1.8×10^9 cfu mL^{-1} for aeration with pure oxygen gas, revealing growth viability of this bacterium in dairy wastewater under both aeration conditions. The viable cell number ranges of *A. faecalis* strain No. 4 compared well with

previously reported viable cell count ranges of *A. faecalis* strain No. 4 cells during treatment of other types of wastewaters. In a repeated batch experiment using *A. faecalis* strain No. 4 for treatment of the municipal wastewater, Shoda and Ishikawa (2014) reported viable cell number of strain No. 4 cells ranging between 10^8 and 10^9 cells mL^{-1}. Similarly, in a continuous experiment using strain No. 4 for treatment of the solid-free piggery wastewater, the estimated total viable cell number of *A. faecalis* strain No. 4 ranged from 1.7×10^9 to 8.8×10^9 cfu mL^{-1} (Joo et al., 2006).
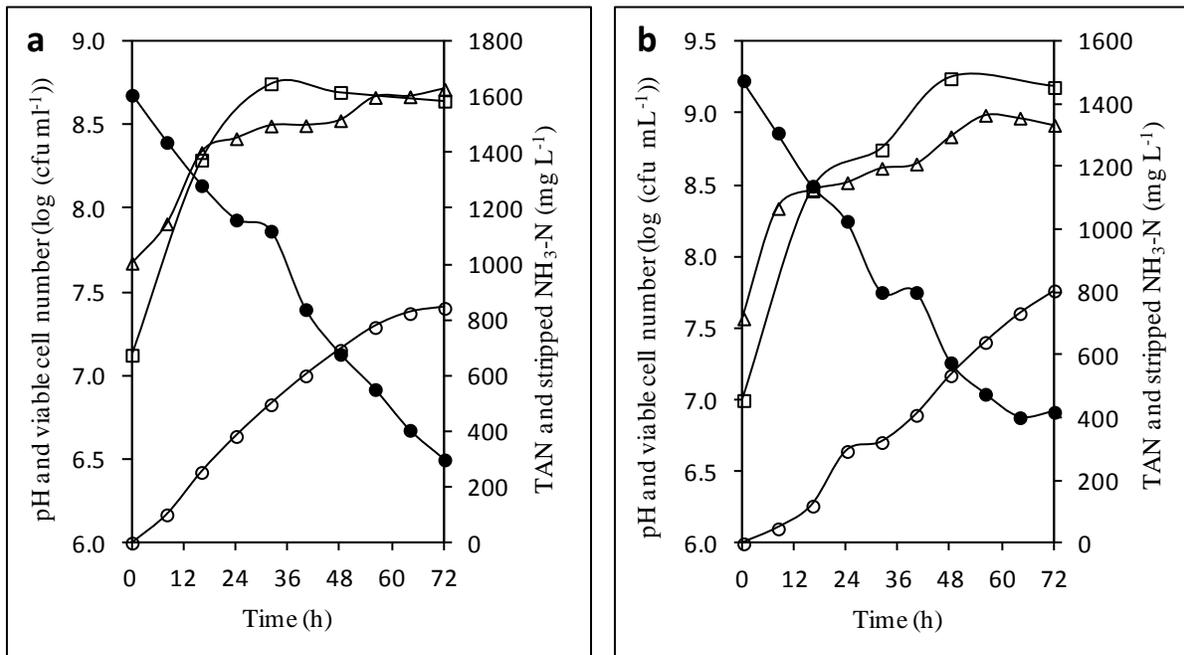
Figure 4.3 Changes in the number of strain No. 4 cells (□), pH (△), concentration of TAN (●), and stripped NH$_3$-N (○) in the aerated batch experiment of strain No.4 using dairy wastewater: (**a**) Effect of aeration using air, and (**b**) Effect of aeration using oxygen-gas.

To investigate the effects of airflow rate on TAN removal efficiency and ammonia stripping, two airflow rates (0.5 L min^{-1} and 0.2 L min^{-1}) were tested using air. The initial TAN

levels in the wastewater in these experiments were similar at about 1583 and 1578 mg L^{-1}, respectively. Figure 4.4 shows the changes in wastewater pH, TAN concentrations, and stripped NH_3 during 72 h of treatment period. When the airflow rate was held at 0.5 L min^{-1}, approximately 1216 TAN mg L^{-1} was removed, the wastewater pH increased from 7.7 to 9.1, and 785 NH_3 mg L^{-1} was stripped. On the other hand, when the airflow rate was held at 0.2 L min^{-1}, approximately 799 TAN mg L^{-1} was removed, the wastewater pH increased from 7.7 to 9.0, and only 383 NH_3 mg L^{-1} was stripped. The respective maximum TAN removal rates, for aeration at airflow rates of 0.5 and 0.2 L min^{-1}, were approximately 26.3 and 12.8 mg $L^{-1} h^{-1}$. These results showed that NH_3 stripping from at the higher airflow rate of 0.5 L min^{-1} was significantly higher (almost double) than for air aeration at 0.2 L min^{-1}. The results of higher NH_3 stripping during the aeration experiments with higher aeration rates are in line with theory and past research studies (Burton, 1992; Liao et al., 1995; Ndegwa et al., 2007).
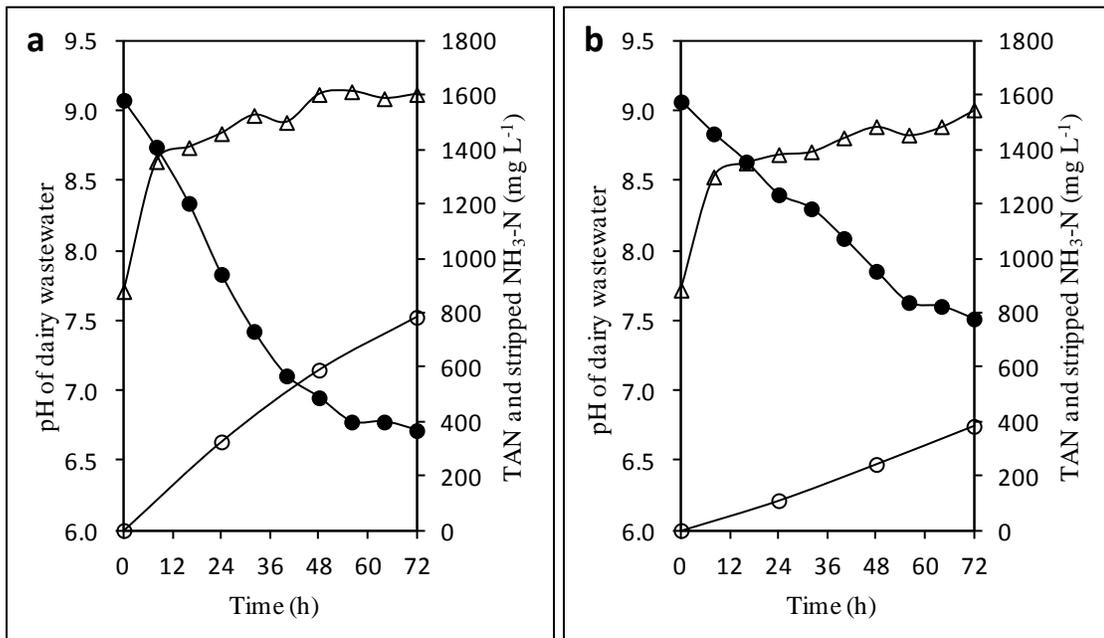
Figure 4.4 Changes in the pH ($\triangle$), concentration of TAN ($\bullet$), and stripped NH_3-N ($\circ$) at different air flow rates: (**a**) Air flow = 0.5 L min^{-1}, and (**b**) Air flow = 0.2 L min^{-1}. Each batch treatment was conducted using strain No.4 for treating dairy wastewater at 30°C and an agitation speed of 115 spm in a shaker.

4.4.2. Reactor-Headspace Flushing Experiments

4.4.2.1. Effects of Carbon Supplementation on TAN Removal

This study investigated potential enhancement of this bioprocess using an external C source to supplement C in dairy wastewater with continuous flushing of reactor-headspace with oxygen gas. Figure 4.5a shows the changes in concentrations of TAN and stripped NH_3 during 24 h batch incubation with and without C addition, respectively. The process supplemented with external C resulted in nearly 100% TAN removal from an average of 1033 mg L^{-1} to 14 mg L^{-1} within 24 h, while the wastewater pH increased only marginally from 8.5 to 8.7 (Figure 4.5b). In contrast, the process without external C supplementation virtually stalled after the first 12 h and approximately 56% TAN was removed. The total TAN removed was 63% (1064 mg L^{-1} to 399 mg L^{-1}) by 24 h. The dairy wastewater pH increased from 8.4 to 9.1 during the whole treatment time (Figure 4.5b). Results also indicated NH_3 lost via stripping was only 4% (44 mg L^{-1}) when the process was supplemented with C compared to 10% (109 mg L^{-1}) stripped when process was not supplemented with external C. These results suggest that dairy wastewater has insufficient available C during treatment with *A. faecalis* strain No. 4 and requires additional external C source to achieve 100% TAN removal. Previous studies with *A. faecalis* strain No. 4 with other types of wastewaters showed similar requirements (Joo et al., 2006; Shoda and Ishikawa, 2014). Joo et al. (2006), for example, observed about 100% ammonium removal by strain No. 4 in piggery wastewater supplemented with external C source. A more recent study by Shoda and

Ishikawa (2014), also reported complete ammonium removal by *A. faecalis* strain No. 4 in municipal wastewater supplemented with external C sources but only 20% to 30% removal rate without addition of extra C.

4.4.2.2. Effects of Rector-Headspace Flushing with Oxygen and Air on TAN Removal

The effect of the different oxygen sources (Oxygen and Air) for reactor headspace flushing on TAN removal from dairy wastewater with *A. faecalis* strain No. 4 was studied in continuous flushing batch experiments. Figure 4.5a depicts the changes in TAN concentration and stripped ammonia over a period of 24 h in batch operation experiments under continuous flush mode: (i) oxygen supply using pure oxygen gas, and (ii) oxygen supply using ambient air. The TAN removal in the batch experiment using oxygen gas was significantly higher than that in the batch experiment using air. Within the batch operation period of 24 h, the removal ratio of ammonium reached almost 100% for oxygen flushing as noted in above previous section, while it was only about 42% (TAN concentration decreased from 1,100 mg L^{-1} to 643 mg L^{-1}) for air flushing experiment. The dairy wastewater pH increased from 8.4 to 8.8 for ambient air-flush experiment (Figure 4.5b). The amount of stripped ammonia detected in the acid trap accounted for only 4% of the total TAN removal in oxygen flushing experiment and about 20% of that in air flushing experiment. The efficient TAN removal in the oxygen flushing treatments was presumably due to the process's ability to meet the oxygen demand for *A. faecalis* strain No. 4 activity (Shoda & Ishikawa, 2014).
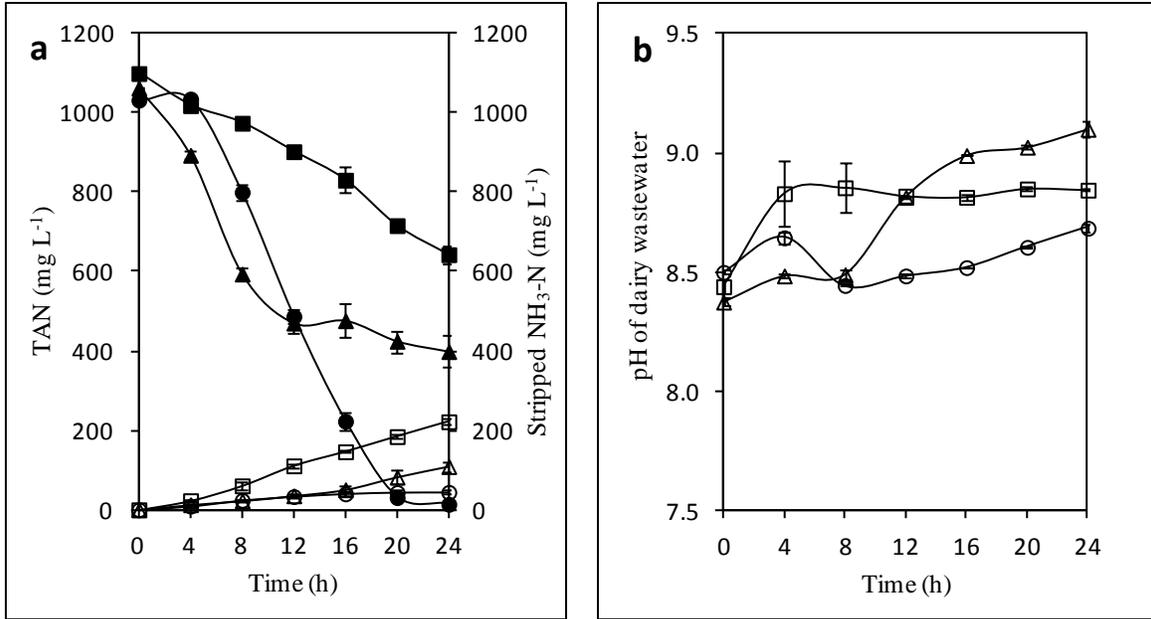
Figure 4.5 Effect of flushing reactor-headspace with air and oxygen and with and without carbon supplementation during treatment of dairy wastewater with *A. faecalis* strain No. 4: (**a**) TAN removal and ammonia stripping (circles represent oxygen and external carbon; triangles represent oxygen and without external C supplementation; squares represent air and external C supplementation). Symbols: ●/ ▲/ ■, TAN concentrations; ○/ △/ □, stripped ammonia (NH_3-N)); (**b**) dynamics of wastewater pH. Error bars indicate standard errors (SE) from means, $n = 2$.

4.4.2.3. Effects of Reactor-Headspace Frequency on TAN Removal

In this study, five flushing treatments were tested: continuous and every 2, 4, 8, and 24 h with initial average TAN concentrations ranging from 1,000-1,270 mg L^{-1}. The intermittent flushing events took approximately 5 min each to complete. The variations of the TAN removal effectiveness with flushing frequency are shown in Figure 4.6. Efficiencies of TAN removal were 99%, 97%, and 97% within 28 h under continuous flushing, flushing every 2 h, and flushing every 4 h flushing, respectively. However flushing with oxygen at intervals of 4 h and 2 h for 5 min reduced the oxygen use by more than 95% compared to continuous while still

achieving high TAN removal within the same period. Intermittent flushing of the reactor headspace at intervals of 2 or 4 h, therefore, offers a more cost-effective treatment of dairy wastewater via this bioprocess. Removal effectiveness under the 8 and 24 h flushing frequencies were only about 51% and 46%, respectively after 72 h treatments. Continuous flushing of reactor-headspace with oxygen resulted in almost 100% TAN removal within 24 h and a maximum removal rate of 59.2 mg L^{-1} h^{-1}. The maximum TAN removal rates under the 2 and 4 h flushing frequencies were 41.9 mg L^{-1} h^{-1} and 42.8 mg L^{-1} h^{-1}, respectively. Overall, the TAN removal rates under continuous flushing or flushing at intervals of 2 or 4 h were significantly higher than rates obtained in similar previous studies (Joo et al., 2005, 2006; Zhao et al., 2012; Yang et al., 2015). Treatment of high strength synthetic TAN wastewater using *A. faecalis* strain No. 4 in the aerated batch experiment showed a maximum TAN removal rate of 29 mg L^{-1} h^{-1} (Joo et al., 2005). Joo et al. (2006) measured a similar maximum TAN removal rate of about 29 mg L^{-1} h^{-1} in another similar study using *A. faecalis* strain No. 4 to treat swine wastewater supplemented with sodium citrate dehydrate as an extra C source. In another study (Zhao et al., 2012), aerated batch experiments of *A. faecalis* strain NR in basal medium with extra C and N sources yielded a maximum ammonium removal rate was reported as 26.9 mg L^{-1} h^{-1}. For another heterotrophic bacterium *Acinetobacter junii* YB, aerated batch experiments using synthetic wastewater, the maximum ammonium removal rate ranged from 8.2 to 13.7 mg L^{-1} h^{-1} (Yang et al., 2015). These results indicate that TAN removal efficiencies are dependent on oxygen concentration in the reactor-headspace and its consequent transfer into the reactor contents. Maintaining adequate oxygen supply into solution has been identified as critical for the enhanced activity of *A. faecalis* strain No. 4 and efficient ammonium removal (Shoda &

Ishikawa, 2014). This study, similarly, reveals the importance of adequate oxygen supply to the bioprocess to meet oxygen demand of this heterotrophic bacterium.
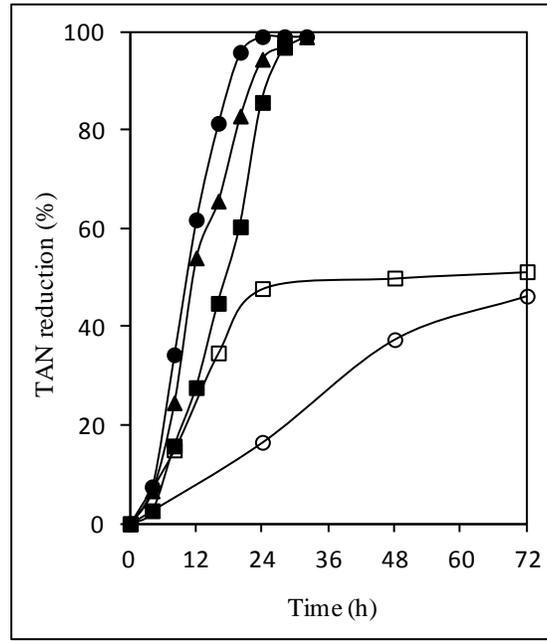
Figure 4.6 Effect of flushing frequency on TAN removal using reactor-headspace flushing with oxygen as follows: (i) continuously (●), (ii) every 2 h (■), (iii) every 4 h (▲), (iv) every 8 h (□), and (v) every 24 h (○). Flushing events every 2, 4, 8, and 24 h took only 5 minutes to complete.

4.4.3. Nitrogen Mass Balance

A balance of nitrogen was calculated for the continuous flushing with pure oxygen gas during the 24 h treatment of dairy wastewater (supplemented with external C) with *A. faecalis* strain No. 4 (Table 4.1). The nitrogen mass balance showed that TAN was nearly completely (99%) removed after 24 h. Ammoniacal nitrogen lost as NH_3-N via stripping was only 4%, while the majority was retained in microbial biomass (58%). Further nitrogen balance analysis demonstrated that the remaining nitrogen portion of approximately 36% was released via

denitrification as nitrogen (N_2) gas, while trace accumulations of NH_2OH (0.9%) and NO_3^- (3.5%) were detected during the whole process. Hydroxylamine is recognized as a critical nitrification intermediate during process of ammonium oxidation. Its low concentration, in the treated effluent, therefore, illustrates that most NH_2OH was converted to N_2 gas (Joo et al., 2005; Zhao et al., 2012). The mass balance values obtained in this study were in agreement with those from previous studies on treatment of other wastewaters (synthetic wastewater and piggery waste water) with *A. faecalis* strain No. 4 (Joo et al., 2005, 2006, 2007). For example, Joo et al. (2005) investigated ammonium removal characteristics of *A. faecalis* strain No. 4 in an aerated batch experiment using synthetic wastewaters with initial TAN ranging from 364-1,122 mg L^{-1}. After 93 h at 30°C, almost 100% removal of TAN was achieved. Subsequent nitrogen mass balance indicated that about 50% of TAN was assimilated into the cells, and 40-50% of TAN was estimated to be lost as N_2 gas. The total amount of stripped NH_3-N by aeration ranged from 2-4% of total TAN, while NH_2OH and NO_3^- contents were negligible.

Table 4.1 The mean nitrogen mass balance during treatment dairy manure supplemented with external carbon sources in batch experiments using *A. faecalis* strain No. 4 after 24 h.[a]

Initial TAN	Final TAN	Removed TAN (%)	Stripped NH_3 (%)	Assimilated TAN (%)	Denitrified TAN (%)
1033	14	1019 (99)[b]	44 (4)	600 (58)	375 (36)

[a] Experiment was conducted at 30°C at a speed of 100 spm in a shaking flask under continuous flushing of reactor-headspace with oxygen-gas. The test sample consisted of the dairy wastewater with strain No. 4, and trisodium citrate dehydrate (as external carbon source).
[b] Mean of two replicates, mg L^{-1} (percentage in parenthesis).

4.5. Conclusions

Results obtained in this research clearly exhibited the survival capacity of *A. faecalis* strain No. 4 in seeded dairy wastewater, and further demonstrated its ability to achieve efficient TAN removal, consequently mitigating ammonia emissions from dairy wastewater. Both aeration via reactor headspace flushing with oxygen and use of C addition further improved TAN removal and mitigation of ammonia emissions. Intermittent flushing of reactor-headspace with oxygen was effective for TAN removal and could yield a more cost-effective treatment than continuous flushing at the full scale. These results, in general, revealed that oxygen and C addition are important factors that, in this bioprocess, can promote fast and complete removal of TAN from dairy wastewater. Extremely low mass of TAN was lost as NH_3 gas (4%), while the majority was retained in microbial biomass (58%) or released as nitrogen gas (36%).

4.6. Acknowledgements

4.7. References

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CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

Technical and economic feasibility of several potential approaches for mitigating NH_3 emissions from dairy operations were investigated and reported in this thesis. The first approach was based on maintaining manure-pH under 6 in a closed-loop recycle of flush water. The second study evaluated the respective potentials of anaerobic digestion (AD) and solids-liquid separation on mitigation of NH_3 emissions during later storage and land application of treated manure effluents. The third study examined a bioprocess based on an enhanced nitrification-denitrification using a heterotrophic bacterium *Alcaligenes faecalis* strain No. 4 and pure oxygen. Summaries and main conclusions from these studies as well as suggestions for future studies, to advance these three research directions, are presented in the rest of this chapter.

Study 1. Manure-pH Management

Bench- and pilot-scale studies were conducted to investigate the potential mitigations of NH_3 in the barn, when a pH below 6 was maintained in the recycle-flush water. The proof-of-concept was evaluated in a bench-scale study, while a pilot-scale dairy barn model was constructed to simulate actual manure flush systems. Two manure-pH management strategies were tested at the pilot-scale: (i) pH-adjustment of flush water, and (ii) sprinkler-irrigation of manure alleys with acid water between scheduled flushing events. A separate study was conducted to evaluate further NH_3 emission mitigation probable during post-collection storage of treated manure. The mean cumulative NH_3 emissions during the study period were: 47.0, 13.9 and 6.1 mg for control, pH-adjusted flush water, and irrigating manure alleys with acid water

between scheduled manure-flushing events, respectively. Operating pH-adjustment in a closed-loop led to 86% reduction in acid doses immediately after the initial pH-adjustment of the raw flush water, which reflected significant cost-effectiveness of such a manure management system for mitigating NH_3 emissions. Adjustment of pH in the barn further reduced NH_3 emissions during storage of barn effluents by 39% compared with storage of untreated flushed manure. The results from this study, therefore: (1) indicated significant potential mitigations of NH_3 emissions from dairy barns as well as in subsequent storage of effluents, and (2) exhibited that operating a flushing system in a closed-loop results in significant cost-benefit accruing from reduced acid dosages.

Study 2. Anaerobic Digestion and Solids-Liquid Separation

Field and laboratory studies were conducted to evaluate the effects of anaerobic digestion (AD) and solids-liquid separation on emissions during subsequent storage and land application. The lab storage tests were conducted for 21 d with manure samples obtained at the following four points in a full-scale AD system: raw manure (RM) delivery, raw manure supplemented with other substrates (AD influent), AD effluent, and AD effluent after solids-liquid separation (AD liquid effluent). Over the 21 d storage period, higher NH_3 fluxes were observed from AD manure relative to undigested manure. Ammonia fluxes from the AD effluent declined from 3.95 to 2.02 g m^{-2} d^{-1} compared with 0.25 g m^{-2} d^{-1} from AD influent. Similar NH_3 emissions results were observed from the field studies. Ammonia fluxes from storages of AD and non-AD manure ranged from 0.12 to 1.4 g m^{-2} d^{-1} and 0.09 to 0.79 g m^{-2} d^{-1}, respectively. Results also indicated significantly lower VFA in AD effluent and AD liquid effluent compared with that from the AD influent, indicating significant reduction in odor generation potential due to AD and solids-liquid

separation processes. Two manure application methods (surface application and manure injection) for both non-AD and AD manures were simulated in the lab and studied for 9 d. Surface applied non-AD manure exhibited the highest NH_3 flux (0.78 g m^{-2} d^{-1}), while injected AD manure led to the lowest NH_3 flux (0.17 g m^{-2} d^{-1}). Similar NH_3 emissions results were observed from the field studies: ammonia flux from surface applied non-AD manure was 0.75 g m^{-2} d^{-1}, while the loss from injected AD manure was 0.11 g m^{-2} d^{-1}. Data from the land applied manure study indicated that injection of AD manure bear the smallest risks with respect to NH_3 emissions when fertilizing with dairy manure.

Study 3. Biological Nitrification-Denitrification

This study evaluated potential mitigation of NH_3 from flushed dairy manure via an enhanced aerobic bio-treatment process. The bioprocess utilized bacterium *Alcaligenes faecalis* strain No. 4, which has ability to carry out heterotrophic nitrification and aerobic denitrification simultaneously in the same reactor. The treatment efficacy of the *A. faecalis* strain No. 4 on dairy wastewater was evaluated in aerated batch experiments. The influence of oxygen supply on TAN removal rate and extent were tested in batch systems using aeration with ordinary air and pure oxygen gas. These experiments were conducted at 30°C and an agitation speed of 100 spm. Aeration with oxygen and air resulted in approximately 100% and 40% TAN removal, respectively. Intermittent (every 2 or 4 h) aeration with oxygen reduced oxygen consumption by 95%, while attaining nearly identical TAN removal to continuous aeration. The process supplemented with external C resulted in nearly 100% TAN removal. The results thus revealed that adequate oxygen supply, while supplementing dairy wastewater with carbon, is important for efficient TAN removal. From the nitrogen mass balance based on the oxygen-flush system,

extremely low amounts of TAN was lost as NH_3 gas (4%), while the majority was retained in microbial biomass (58%) or released as nitrogen gas (36%). Overall, these results revealed an extremely high potential for mitigation of NH_3 emissions from dairy wastewater using *A. faecalis* strain No. 4.

Recommended Future Work

1. With respect to the manure-pH management approach, evaluating the performance of operating the flushing system in a closed-loop in a full-scale dairy barn is the natural next stage. Further research studies would be also useful to investigate the effects of the proposed treatment system on the health or physical conditions of the cows' hoofs.

2. Further research is needed to elucidate the role played by other key parameters of manure (pH, temperature, ammoniacal N, etc.) and their interactions with soil properties (alkalinity, buffering capacity, cation exchange capacity, etc.), in the mechanism governing NH_3 volatilization after land application of manure.

3. The next steps towards development of the bioprocess include: (i) determinations of optimum environmental parameters (temperature, aeration level, and pH), and effects of wastewater characteristics (ammoniacal N and organic C loads, ratio of C to N, etc.) on the process, and (ii) optimization of the bioprocess in continuous flow bioreactors.