PASSIVE REDUCTION OF METHANE EMISSIONS FROM A HOG WASTE LAGOON
USING A FLOATING BIOFILTER SYSTEM


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A thesis submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Master of Science in the Department of Environmental Sciences and Engineering in the Gillings School of Global Public Health.


Chapel Hill
2016

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# ABSTRACT

Katherine Lenore Broadwater: Passive Reduction of Methane Emissions from a Hog Waste
Lagoon Using a Floating Biofilter System
(Under the direction of Stephen Whalen)

Roughly 9 million hogs reside in NC in confined animal feeding operations, where waste is stored in anaerobic, open-air lagoons. Methane ($CH_4$) is an important greenhouse gas emitted from lagoons, but there are no regulatory standards. This study evaluates the efficacy of passive biofiltration as a low-cost approach to reducing $CH_4$ emissions from lagoons. Methane emission from a representative lagoon averaged 4.2 g $m^{-2}$ $d^{-1}$. Laboratory experiments showed that a community of $CH_4$ oxidizing bacteria initially colonizing a Growstone support medium and subsequently suspended over the lagoon surface was capable of oxidizing 25% of emitted $CH_4$. However, <1% of the emitted $CH_4$ from the lagoon was oxidized by the $CH_4$ oxidizing community after field deployment. Laboratory experiments indicated high $NH_3$ sensitivity of the methanotroph community initially colonizing the Growstones. Ammonia is universally emitted in open-air waste storage lagoons, suggesting that a passive biofilter is not viable for mitigating $CH_4$ emissions.

# ACKNOWLEDGEMENTS

This research project was nothing short of a team effort, an endeavor I could not have hoped to begin or finish alone. To Steve Whalen, thank you for the incredible amount of patience and guidance you showed toward me. Whether in the classroom, the lab, or on the drives to and from the field, you taught me a great deal and I will always be grateful to you for being such an excellent advisor. In conjunction with Steve, thank you to Michael Aitken and to Glenn Walters for affording me the opportunity to work on this fascinating project. To Jill Stewart, thank you for serving on my committee and for teaching me to navigate the complexities of scientific literature. D.J. Fedor, thank you for your excellent work as part of the ESE Design Center that provided us with the necessary materials for this project. I also want to thank our field site contacts for allowing us on their farm.

To UNC staff members, Jack Whaley, Robin Whitely, Rebecca Gunn, Rhoda Cerny, and many others, I am so grateful to you for your kindness and for sharing your wisdom and knowledge with me. Each of you has made my UNC experience that much better.

To my father, Steve Broadwater, you have been a never ending source of support and I could never have asked for a better father. Thank you for making home a place to which I would always want to return. To my family, especially Steven and Allison Broadwater (my older and much wiser siblings) and their respective loved ones, you are my rock and my horizon. To my friends, particularly Sofia Goodrich for buying those plane tickets and Monica Rolls for bestowing upon me the greatest honor of a best friend, I have appreciated every gesture of your love and support. I am nothing without each of you.

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% WHC          percent water holding capacity

AMO          ammonia monooxygenase enzyme

CAFO          confined animal feeding operation

FELCG          field-exposed laboratory colonized Growstones

FEUCG          field-exposed uncolonized Growstones

GC          gas chromatography

$K_s$          apparent half-saturation constant

LCG          laboratory colonized Growstones

MMO          methane monooxygenase enzyme

pMMO          particulate methane monooxygenase enzyme form

$Q_{10}$          temperature coefficient

sMMO          soluble methane monooxygenase enzyme form

$T_{opt}$          optimum temperature

UCG          uncolonized Growstones

$V_{max}$          maximum rate of CH4 oxidation

**CHAPTER 1: INTRODUCTION**

Over the past few decades, swine production in North Carolina has followed a national

trend from small, family-owned operations to large factory farms, focused on efficiency (Doorn

et al., 1997; Hribar, 2010; MacDonald & McBride, 2009; Webb, 2016a). North Carolina is

currently the second largest swine producing state, housing nearly 10 million animals (Doorn et

al., 1997; Webb, 2016a). Swine in these confined animal feeding operations (CAFOs) are held in

parlors housing hundreds of head where waste falls through a slatted floor (Hribar, 2010;

MacDonald & McBride, 2009). The preferred method of waste management in NC is to flush

waste into an anaerobic, open-air lagoon and to spray the liquid phase onto crops as fertilizer

(Hribar, 2010). This waste can adversely affect the quality of air, surface water and groundwater,

and can therefore negatively impact human health (Hribar, 2010). Nationwide, anaerobic lagoons

account for 61% of total $CH_4$ emissions from animal waste (Sharpe & Harper, 1999). Methane is

a critical greenhouse gas with a higher warming potential than $CO_2$ on a per mole basis and

allowing $CH_4$ to increase in the atmosphere unchecked enhances global warming (Le Mer &

Roger, 2001; Wuebbles & Hayhoe, 2002). Current swine waste management systems are low

cost, easy to maintain, and focused on controlling offsite transport of nitrogenous nutrients in

response to regulatory requirements. To date, no management practices are aimed directly at

mitigating $CH_4$ release from swine waste storage lagoons, although a few operations do cover

their lagoons and capture $CH_4$ for energy production (Peach, 2014). Passive biofiltration,

whereby $CH_4$ is oxidized by methanotrophic bacteria, is a potential low-cost approach that can

be easily incorporated into current management practices and has previously proven effective at

reducing $CH_4$ emission from artificial anaerobic dairy effluent in a bench scale study (Pratt et al., 2013). Here, we extend this concept from the laboratory to a field environment and from dairy to swine waste, in order to evaluate whether passive biofiltration is a viable method for mitigating $CH_4$ emission from anaerobic lagoons that commonly store swine waste on North Carolina CAFOs.

## CHAPTER 2: LITERATURE REVIEW

### 2.1 Methane in the Atmosphere

Methane is one of the most important trace gases in the atmosphere (Conrad, 1996) as it is both radiatively and chemically active (Shukla et al., 2013). Analyses of air, trapped in polar ice cores, give a record of the change the atmospheric $CH_4$ mixing ratio has undergone pre- and post-industrialization (Shukla et al., 2013). Specifically, the pre-industrial mixing ratio has increased exponentially from about 715 nL $L^{-1}$ to about 1500 nL $L^{-1}$ by the late 1970s (Conrad, 2009; Kirschke et al., 2013). Thereafter, direct measurements collected regularly from the troposphere at clean air sites show a slower, but sustained, increase of about 12 nL $L^{-1}$ in the 1980s, a decrease in the growth rate of about 4 nL $L^{-1}$ in the 1990s, followed by a stabilization of the atmospheric $CH_4$ levels at about 1773 nL $L^{-1}$ from 2000 until 2007 (Conrad, 2009; Kirschke et al., 2013). Since that time, the atmospheric mixing ratio has increased annually to a level of 1833 nL $L^{-1}$ in 2014. As of 2009, the global average $CH_4$ mixing ratio had increased by a factor of about 2.5 over pre-industrial levels. This increase is proportionately greater than the simultaneous increase in $CO_2$ (Dlugokencky at al., 2011).

Models used to estimate global GHG emissions are often poorly constrained due to uncertainties regarding emissions measurements from many important point sources. However, top-down and bottom-up models that follow IPCC AR5 guidelines for the treatment of uncertainties yield the most robust estimates by identifying and constraining the magnitudes of important terms in the contemporary atmospheric $CH_4$ budget (Kirschke et al., 2013). Between 2000 and 2009, these models estimate that natural sources emitted 218 to 347 Tg $CH_4$ $y^{-1}$,

dominated by emissions from wetlands, which show a source strength ranging from 175 to 217 Tg $y^{-1}$. Emission estimates from anthropogenic $CH_4$ sources exceeded natural sources, accounting for 48 to 61% of the total atmospheric burden of 548 to 678 Tg $y^{-1}$. Roughly 60% of anthropogenic emissions are attributed to agriculture and waste. The other 40% of anthropogenic emissions result from biomass burning and fossil fuel exploitation. Methane oxidation by hydroxyl radicals, mostly in the troposphere, represented the dominant sink of the atmospheric budget, accounting for 528 Tg $CH_4$ $y^{-1}$, or 84%, of the total sink. Other contributors to the loss of atmospheric $CH_4$ include consumption by methanotrophs in aerated soils (4%) and loss to the stratosphere (12%). The sum of all of these sinks is 632 Tg $y^{-1}$. Overall, the contemporary atmospheric $CH_4$ budget shows an increase, dominated by human activities (Kirschke et al., 2013).

Methane is an important contributor to the greenhouse effect. Though $CH_4$ has a relatively short residence time in the atmosphere relative to $CO_2$, about 10 years, its ability to absorb infrared radiation makes it 20 to 30 times more efficient on a per mole basis than $CO_2$ as a greenhouse gas (Le Mer & Roger, 2001; Wuebbles & Hayhoe, 2002). The last two centuries have seen an increase in radiative forcing directly attributed to increases of greenhouse gases such as $CH_4$. Total radiative forcing by greenhouse gases is about 2.77 W $m^{-2}$ and the direct contribution of $CH_4$ to radiative forcing is 0.5 W $m^{-2}$, about 18% of the total (Dlugokencky et al., 2011).

In addition to being radiatively important in the atmosphere, $CH_4$ is chemically active and involved in initiating complex reactions that help determine the concentrations of key components of the atmosphere (Le Mer & Roger, 2001; Wuebbles & Hayhoe, 2002). Reactions with $CH_4$ and CO are key sinks for hydroxyl (OH) radicals (Wuebbles et al., 1989). Reactions

with OH are the main removal mechanisms for atmospheric pollutants, essentially acting as a detergent in the troposphere (Prinn, 2003). Where nitrogen oxide levels are sufficiently high, reactions of $CH_4$ with OH in the troposphere leads to the formation of $O_3$ (Wuebbles & Hayhoe, 2002). Tropospheric $O_3$ negatively impacts plant productivity by affecting the uptake of $CO_2$, thereby increasing radiative forcing by $CO_2$. The increase in tropospheric $O_3$ can also exacerbate respiratory and cardiac diseases (Myhre et al., 2013). An increase in stratospheric $CH_4$ has led to a reduction in stratospheric $O_3$, which in turn, allows for an increased flux of UV to the troposphere (Wuebbles & Hayhoe, 2002).

## 2.2 Microbial Methane Production and Consumption

### 2.2.1 Methanogenesis

Methanogens all belong in the domain *Archaea* and in 26 genera more than 60 species of methanogens have been recorded (Le Mer & Roger, 2001; Whalen, 2005). All known methanogens are affiliated with the phylum *Euryarchaeota* and are obligate anaerobes (Borrel et al., 2011). Methanogens span several orders and families, but all share the same unique characteristic of using simple substrates to gain energy and produce $CH_4$ (Conrad, 2007). Further, all methanogens utilize the enzyme methyl coenzyme-M (methyl-CoM) reductase (MCR) for substrate reduction (Borrel et al., 2011). Although the phylogenetic diversity of methanogens is widespread, their metabolic pathways are highly specialized such that methanogens are categorized taxonomically by the select few substrates they are able to use to produce $CH_4$ (Borrel et al., 2011).

Based on the limited number of simple metabolizable substrates ($H_2 + CO_2$, acetate, formate, primary and secondary alcohols, and methylated compounds such as methanol, methylamines, dimethylsulphur) five trophic groups of methanogens have been categorized (Le

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Mer & Roger, 2001). These substrates are used in three main metabolic pathways for $CH_4$

production; hydrogenotrophic methanogenesis, acetoclastic methanogenesis, and methylotrophic

methanogenesis (Borrel et al., 2011).

Hydrogenotrophic methanogens utilize $H_2$ as an electron donor for the reduction of $CO_2$:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \text{ (Equation 1)}$$
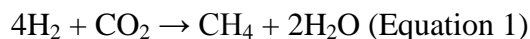
This type of catabolism is the most common and is found among most methanogenic taxa,

including the genus *Methanosarcina* (Conrad, 2007). $H_2$ is first oxidized to protons and the

electrons are then used for the reduction of $CO_2$ (Conrad, 2007).

Acetotrophic methanogens convert acetic acid by cleavage to $CH_4$ and $CO_2$:

$$CH_3COOH \rightarrow CH_4 + CO_2 \text{ (Equation 2)}$$
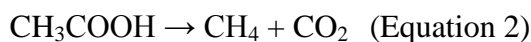
This pathway is less common than hydrogenotrophic methanogenesis; the only two genera of

methanogens able to catabolize acetate are *Methanosarcina* and *Methanoseta* (Conrad, 2007).

Specifically, the carboxyl group of the acetate is oxidized to $CO_2$ and the methyl group is

reduced to $CH_4$ (Borrel et al., 2011; Conrad, 2007; Whalen, 2005).

Methylotrophic methanogenesis is common in the genus *Methanosphaera* and some

species of the genus *Methanobacterium* (Borrel et al., 2011). *Methanobacterium spp*. use the

substrates $H_2 + CO_2$ and formate and is found in habitats such as animal feces, anaerobic

digesters, and the compost soils of rice paddies. *Methanosphaera spp*. require both $H_2$ and

methanol for use as substrates and cannot grow on methanol alone, unlike many other methanol-

using methanogens. They are typically found in the feces of humans and rabbits (Boone, 2000).

Methyl compounds, such as methanol, acetate, and dimethyl sulfide are catabolized by donating

the methyl group to corrinoid proteins, forming methyl-corrinoid ($CH_3$-corrinoid). This $CH_3$-

corrinoid complex is an intermediate pathway that transfers the methyl group to CoM, forming

methyl-CoM. This methyl-CoM complex is then reductively demethylated to yield $CH_4$ in the same process as the terminal step of $CO_2$ reduction (Boone, 2000). The process requires the oxidation of an additional methyl group to $CO_2$ to provide necessary electrons for the overall reduction to occur (Borrel et al., 2011).

Effectively, $CH_4$ production is the culmination of several types of bacteria working to degrade organic matter in aerobic and anaerobic conditions, ultimately providing substrates necessary for methanogenesis, the final step of decomposition (Segers, 1998). Hydrolytic bacteria degrade polysaccharides to monomers such as sugars like hexose. Primary fermentation bacteria convert sugars to various types of alcohols, fatty acids, acetate, $CO_2$ and to $H_2$ (Boone, 2000). Syntrophic bacteria degrade alcohols and volatile fatty acids such as lactate, butyrate and propionate to acetate, $CO_2$ and $H_2$, which can then serve as methanogenic substrates (Boone, 2000; Borrel et al., 2011; Whalen, 2005). Homoacetogens ferment hexoses directly to acetate as a sole end product, while chemolithotrophic acetogens utilize $CO_2$ and $H_2$ to produce acetate and $H_2O$. In both cases, the end product can fuel acetogenic methanogenesis (Boone, 2000; Conrad, 2007).

Substrate availability provides an environmental control on rates of methanogenesis. When $CO_2$ is the product of the oxidization of organic matter, it is generally available for use as an electron acceptor for hydrogenotrophic methanogens, although $H_2$ is often in short supply. Other electron acceptors besides $CO_2$ in the environment, such as $O_2$, $NO_3^-$, $SO_4^{-2}$, Mn (IV), or Fe (III), can also preferentially accept electrons generated from the degradation of organic matter by various microbial groups. Accordingly, bacteria using these electron acceptors out-compete methanogens for common substrates (i.e. acetate), thus inhibiting acetogenic methanogenesis (Boone, 2000).

Beyond substrate availability, other environmental factors impact rates of methanogenesis; temperature can have a significant influence. Specifically, temperatures between 30 and 40˚C are optimum for methanogenesis (Le Mer & Roger, 2001; Whalen, 2005). Low temperatures not only reduce activity of methanogens and thus reduce $CH_4$ production, but also adversely impact the activity of hydrolytic and fermentative microorganisms providing methanogenic substrates (Conrad, 2007; Le Mer & Roger, 2001). These microbes are actually more sensitive to temperature change than methanogens themselves (Le Mer & Roger, 2001). Higher temperatures increase the rate of reduction of electron acceptors, ultimately increasing the rate of $CH_4$ production. Temperature also alters the ratio of hydrogenotrophic to acetotrophic methanogenesis. Acetotrophic methanogenesis dominates at lower temperatures and hydrogenotrophic methanogenesis dominates at higher temperatures (Conrad, 1996). Lower temperatures limit the activities of $H_2$-producing syntrophs, which then limits the rate of production of methanogenic substrates, negatively impacting hydrogenotrophic methanogens, in particular (Borrel et al., 2011).

In addition to temperature and substrate availability, pH may also influence rates of methanogenesis. Although most known methanogens are neutrophilic, there is growing evidence that wetland methanogens are at least acid-tolerant. Methane production in wetlands would be limited by the generally acidic environment, but there have been studies showing isolated strains of methanogens that maintain significant activity at pH as low as 3.1. Optimal growth for most methanogens occurs at a pH of 7, but species of methanogens have been isolated that show optimal growth at pH values as low as 4.7 (Goodwin & Zeikus, 1987; Segers, 1998). Field studies of the relationship between  pH and $CH_4$ production give highly variable results, likely due to secondary factors involved in $CH_4$ production, such as the influence of pH on the activity

of microbes involved in providing methanogenic substrates (Goodwin & Zeikus, 1987; Whalen, 2005).

## 2.2.2 Methane Oxidation

Methanotrophic bacteria play a fundamental role in the global $CH_4$ cycle, oxidizing more than half of the total $CH_4$ produced and mitigating its release to the atmosphere (Reeburgh, 2003). There are two biologically mediated pathways for $CH_4$ oxidation including the well-characterized aerobic $CH_4$ oxidation and the less well documented anaerobic $CH_4$ oxidation. In the latter, sulfate, as opposed to oxygen, is the terminal electron acceptor (Borrel et al., 2011; Hinrichs & Boetius, 2002). Anaerobic $CH_4$ oxidation has only been reported in marine environments, as well as hypersaline environments and thus, its significance in freshwater and soil environments remains uncertain (Shukla et al., 2013; Whalen, 2005) and will not be discussed further. Most aerobic methanotrophs are only able to grow on $CH_4$, but a few can utilize methanol, formate, formaldehyde and methylamine (Borrel et al., 2011). Aerobic methanotrophs represent a subset of obligatory $C_1$ eubacteria, the methylotrophs (Borrel et al., 2011; Whalen, 2005).

The overall process of aerobic $CH_4$ oxidation is a series of steps from $CH_4$ to methanol to formaldehyde to formate to finally, $CO_2$:

$$CH_4 \rightarrow CH_3OH \rightarrow HCHO \rightarrow HCOOH \rightarrow CO_2 \qquad \text{(Equation 3)}$$

This oxidation pathway provides the energy and the carbon source, in the form of formaldehyde, for growth (Borrel et al., 2011; Whalen, 2005).

In all known aerobic methanotrophs, the unique enzyme $CH_4$ monooxygenase (MMO) catalyzes the first step in Equation 3, oxidizing $CH_4$ to methanol with molecular oxygen (Hanson & Hanson, 1996). Accordingly, this enzyme can be used as a functional biomarker for detecting

aerobic methanotrophs in environmental samples (Borrel et al., 2011)  MMO is able to

metabolize a large number of substrates due to a lack of specificity (Hanson & Hanson, 1996).

 The location of MMO is a defining characteristic of aerobic $CH_4$ oxidizing bacteria; a

soluble iron-containing form (sMMO) and a copper-containing membrane bound (particulate)

form (pMMO) (Borrel et al., 2011; King, 1992; Shukla et al., 2013). The former has a restricted

distribution, while the latter is ubiquitous to all methanotrophs (Borrel et al., 2011; Conrad,

2007). The particulate form, pMMO, has a narrower substrate specificity and lower $O_2$

requirement relative to sMMO and the biochemistry is better defined (Whalen, 2005).

Aerobic methanotrophs are characterized as Type I or Type II, differentiated by the

pathway used to assimilate formaldehyde (Borrel et al., 2011; Hanson & Hanson, 1996). Type I

methanotrophs utilize the ribulose monophosphate pathway, while the Type II methanotrophs

employ the serine pathway (Borrel et al., 2011). Functionally, aerobic $CH_4$ oxidizing bacteria are

described as high- or low-affinity (Segers, 1998). High-affinity methanotrophs dominate at low

(atmospheric) $CH_4$ mixing ratios where $O_2$ is high and are generally categorized as Type I

(Hanson & Hanson, 1996). Low-affinity methanotrophs are found in environments where $CH_4$ is

not growth limiting and dissolved $O_2$ concentrations may be low; these communities are

generally dominated by Type II microbes (Hanson & Hanson, 1996; Segers, 1998).  The

methanotrophs responsible for high-affinity $CH_4$ oxidation remain uncultured and are therefore

poorly characterized. The transition point between high- and low-affinity populations is

considered to be between 100 to 1000 $\mu L\ L^{-1}$ $CH_4$ in the environment (Segers, 1998).

Several environmental factors may affect aerobic methanotrophy, notably, the availability

of $O_2$ and $CH_4$, temperature, moisture content and texture of soils, pH, and $NH_4^+$ (Shukla et al.,

2013). Highest rates of $CH_4$ oxidation are generally reported at oxic/anoxic interfaces in lake and

wetland sediments, where both $O_2$ and high concentrations of $CH_4$ occur simultaneously (Shukla et al., 2013).  Similarly, high rates of $CH_4$ oxidation are found in oxygenated landfill cover soils (Whalen, 2005). The temperature response of $CH_4$ oxidation is highly variable and depends on the $CH_4$ mixing ratio (Shukla et al., 2013). At low $CH_4$ mixing ratios, diffusion limitation restricts the rate of $CH_4$ oxidation and there is minimal temperature response (Whalen & Reeburgh, 1996). At high $CH_4$ mixing ratios, enzymatic activity is the dominant influence and a pronounced temperature response of $CH_4$ oxidation is reported (Whalen & Reeburgh, 1996). High soil moisture content and fine textured soils restrict diffusion of $O_2$ and $CH_4$ to methanotrophs (Shukla et al., 2013). Extremely low soil moisture content induces water stress, increasing soil compaction and reducing the activity of methanotrophs (Shukla et al., 2013). There is no consistent response of $CH_4$ oxidizers to pH. Methane oxidation has been reported in acid peat soils with pH values between 3.6 and 4.5, indicating some level of acid tolerance (Whalen, 2005). Methane oxidation has been reported in soil and aqueous environments showing pH values from 3 to 9 and pure cultures have been grown at pH values from 5 to 9 (Shukla et al., 2013). Finally, the presence of $NH_4^+$ inhibits $CH_4$ oxidation at the cellular level due to competitive inhibition of MMO. There seems to be some correlation between the extent of inhibition and $NH_4^+$ concentration in the environment. However, $NH_4^+$ inhibition is not universally observed (Shukla et al., 2013). In some cases, the presence of $NH_4^+$ can increase $CH_4$ oxidation rates when $NH_4^+$ is in limiting concentrations and is the nitrogen source for growth (Borrel et al., 2011; Conrad, 1996).

Methane oxidation is not limited to methanotrophs. *Nitrosococcus spp.* are chemoautotrophic $NH_3$-oxidizing bacteria which obtain energy for $CO_2$ fixation by oxidizing $NH_3$ to nitrite via the Calvin-Benson cycle; this directly contrasts with methanotrophs that use

formaldehyde as the major carbon source for synthesis of cell material. The responsible enzyme, ammonium monooxygenase (AMO) is structurally similar to MMO and is also relatively nonspecific, allowing $NH_4^+$ oxidizers to oxidize $CH_4$ and incorporate the $CO_2$ into cellular material, but at rates that preclude growth (Hanson & Hanson, 1996).

**2.3 Methane in Agriculture**

Globally, industrial agriculture is a major source of both economic benefits and atmospheric pollutants. Agricultural lands occupy 37% of the earth's land surface and account for 52 and 84% of the global anthropogenic emissions of the radiatively important gases $CH_4$ and $N_2O$, respectively (Smith et al., 2008). Rice agriculture and enteric fermentation are the primary sources of agricultural $CH_4$ emissions, with the source strength of the former estimated between 31 and 112 Tg $y^{-1}$ and the latter estimated between 76 and 92 Tg $y^{-1}$ (Dlugokencky et al., 2011). Emissions from enteric fermentation have seen drastic increases on a global scale since 2010, while emission increases from other agricultural sources have remained constant (Yusuf et al., 2012). The drastic increase of $CH_4$ emission from enteric fermentation is largely due changes in methods of livestock production. Scales of economy have shifted such that US and international livestock production has transitioned from small, family-owned farms to much larger, corporate-owned enterprises. Livestock are held in large, confined animal feeding operations (CAFOs) that are equipped to hold hundreds of head. CAFO types are defined by size, type of animal, and the stage of growth accommodated by the facility (Hribar, 2010; MacDonald & McBride, 2009).

Following the national trends of consolidating livestock, North Carolina has seen the number of hogs increase, but the number of swine producing facilities decrease (MacDonald & McBride, 2009; NCDENR, 2016). From 1991 to 1997, the number of hogs expanded from 4.5 million to 10 million in NC and 8.8 million hogs were recorded in 2015, second only to Iowa

(Doorn et al., 1997; Webb, 2016a). NC has around 2,400 major swine facilities, 4,000 active hog

waste lagoons, and 650 inactive hog waste lagoons (NCDENR, 2016). According to 2012 data

supplied by the USDA, NASS, and the North Carolina Field Office, hog sales compromise about

22% of total agricultural sales in NC (Webb, 2016b).  Pork production is a significant part of the

overall state economy, accounting for over 8000 jobs with revenue topping $2.5 billion a year

(Hribar, 2010; Webb, 2016b). Consolidating livestock introduces waste management problems

(NCDENR, 2016). The most common method of waste management, practiced in areas of the

Midwest, uses slurry handling systems in open-air pits and land application for use as fertilizer

(Hagenstein, 2003). In contrast, the most common method of waste management in NC is the use

of anaerobic lagoons and sprayfields (NCDENR, 2016). Effluent is flushed from confinement

houses to lagoons and the liquid phase can be used as a fertilizer, usually applied to

bermudagrass (Hagenstein, 2003).

Although these methods of waste management are low in maintenance and cost, there are

negative consequences for human and environmental health. Odors from hog lagoons and

sprayfields cause human respiratory issues ranging from irritation to chronic lung disease. Most

research is focused on these types of human health problems, as well as on the environmental

problem of pollution of surface and ground waters by nitrogen (Hribar, 2010; NCDENR, 2016).

The contribution that regional hog production practices can have on GHG emissions, particularly

$CH_4$, is relatively unstudied. Sharpe & Harper (2002) and Sharpe et al. (2002), however, reported

emission ranging from 0.8 to 6.2 g $CH_4$ $m^{-2}$ $d^{-1}$ in NC and GA waste hog lagoons. This is

substantially higher than the average of 0.1 g $m^{-2}$ $d^{-1}$ released from wetlands (Whalen, 2005), the

largest natural source of $CH_4$ emission, suggesting that lagoons could be a significant point

source of $CH_4$ emissions in the southeastern US.

Initial regulations governing swine waste management in North Carolina in the 1990s were aimed at minimizing offsite transport of nitrogenous nutrients from lagoon/spray field systems by requiring waste applications to receiving fields at the agronomic rate for the host crop. Subsequently, the 2007 Swine Waste Environmental Performance Standards Act was passed by the NC legislature to include standards for odor control, $NH_3$ emissions, and pathogens and soil and water contamination for new waste management systems. Although the act eliminated future permitting for traditional lagoon/sprayfield technologies, existing operations employing this methodology were grandfathered. There is currently no regulatory incentive for mitigating $CH_4$ emission from lagoons, although a few NC CAFOs employ covers to capture biogas for energy production. Short of legislative action, technologies to reduce $CH_4$ emissions from lagoons must be cheap and simple to integrate into existing lagoon/sprayfield waste management systems to be widely adopted, as the majority of NC CAFOs will continue to employ this waste management technique for the foreseeable future (Noel, 2002; North Carolina General Assembly & Morgan, 1997; North Carolina General Assembly & Albertson, 2007; Peach, 2014).

**2.4 Study Objectives**

The overall objective of this study was to evaluate whether a passive biofilter is an effective technology for mitigating $CH_4$ emissions from anaerobic swine waste storage lagoons in North Carolina. To this end we:

1. Field-tested a passive biofilter consisting of a porous medium colonized in the laboratory by a community of $CH_4$ oxidizing bacteria to determine the effectiveness of the design at mitigating $CH_4$ release across the lagoon surface to the atmosphere;

2. Evaluated the physiological properties (threshold and capacity for $CH_4$ consumption) of the $CH_4$ oxidizing community initially colonizing the porous support medium;

3. Assessed the moisture, temperature, and $NH_3$ sensitivity of $CH_4$ oxidation by the $CH_4$ oxidizing community initially colonizing the porous support medium;

4. Determined in a laboratory setting, the rates of $CH_4$ oxidation by the microbial community colonizing the porous support medium after several months of field exposure.

# CHAPTER 3: EXPERIMENTAL

## 3.1 Description of Study Site

The study site is a CAFO in western Harnett County, NC, southwest of Lillington. The farm manages a 6,000 head feeder-to-finish operation contracted under Prestage Farms. Swine are housed in 8 parlors and waste is managed using regional standard anaerobic lagoon/sprayfield technology. Waste is periodically flushed from the parlors and held in two lagoons, roughly equal in size at 3 m in depth and 0.7 ha in surface area, with a volume of 2.1 x $10^4$ $m^3$. The liquid effluent is land applied using a solid set sprinkler system to bermudagrass in the summer months and to annual cycles of cereal rye, oats, or wheat in the winter months.

## 3.2 Biofilter Support Medium Preparation and Seed Culture Development

Growstones®, an inert, porous, solid material made of recycled glass, were used as the biomass support medium. The Growstones show a bulk density of 0.18 g cm$^{-3}$ and have a porosity of 82%, yielding a high capacity for both moisture retention and gas diffusion. The particle size of the Growstones from the manufacturer generally varies from 15 to 100 mm (Growstone LLC., 2011). We sieved the Growstones through a #4 US Standard sieve, (4.76 mm mesh opening) to eliminate the finer particles. Roughly 2 L of sieved Growstones were dispensed into each of 6 bags, constructed from window screen material. All of the bags were suspended in a ~250 L, open-top polyethylene drum, to which about 4 L of activated sludge from the Orange Water and Sewer Authority of Orange County, NC (OWASA, 2016) and about 1 kg of cover soil from the Orange County Landfill had been added. The activated sludge-cover soil mixture was amended with a basal medium, a trace element solution, and a phosphate buffer, as

is recommended for culturing methanotrophs (Whittenbury et al., 1970). Tap water was added to fill the polyethylene drum halfway and submerge the Growstone-filled bags. The immersed bags were held at an average temperature of 22˚C and subjected to a continuous flow of 100 mL min$^{-1}$ of 10:1 (v/v) ambient air:high purity $CH_4$ through a diffuser placed at the bottom of the drum. The bags were cycled throughout the drum to ensure equal exposure to the diffused gas for each bag. Daily or weekly monitoring showed that the dissolved $CH_4$ in the drum averaged about 17.5μM.

**3.3 Field Methods**

Following a 6 month incubation with continuous monitoring for dissolved $CH_4$, laboratory colonized Growstones were removed from the polyethylene drum, transported in covered containers to the field site lagoon and transferred to floating chambers (Figure 1). Each of the four units consisted of an aluminum base with a removable acrylic top centered in a float constructed of wood and Styrofoam. The base and top both measured 30 cm in length and 30 cm in width. The top had a height of 20 cm and the base had a height of 15 cm. A wire support screen (30 cm length x 30 cm width) was secured horizontally within the chamber base at a

Top View

Side View
Hood Not Installed
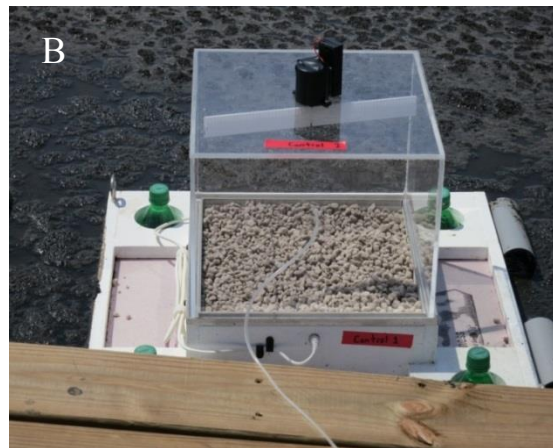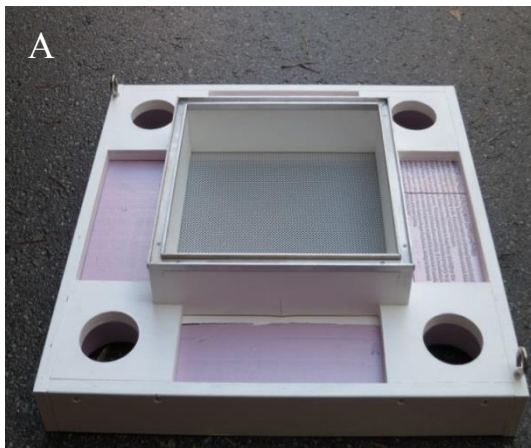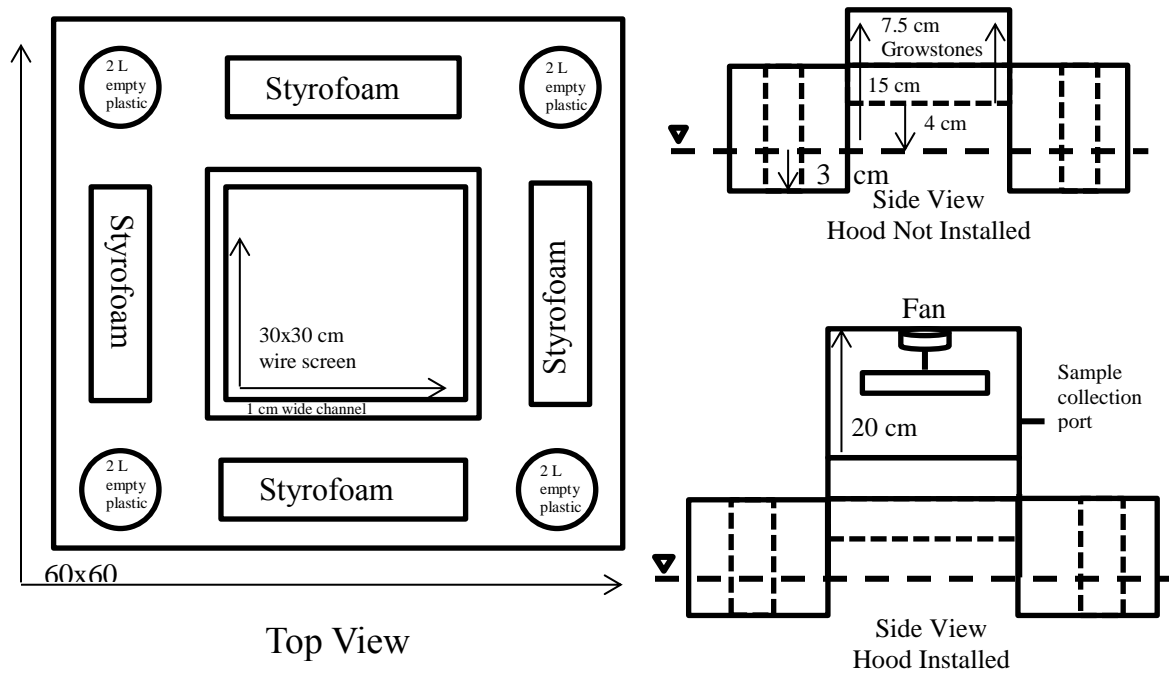
Side View
Hood Installed

Figure 1. Schematic cartoon of floating biofilter system. The top view, seen in the left schematic and photo A, is the chamber base without the removable lid. The schematics on the right and photo B are side views and include the removable chamber lid and the biofilter as it sits typically in the lagoon.

distance of 7.5 cm from the bottom. The outside perimeter of the top of the base was fitted with a 1 cm wide channel to create a seal when the acrylic top was placed on the chamber to measure $CH_4$ flux. A 7.5 cm high layer of laboratory colonized Growstones (biofilter) saturated with growth media was transferred to two of the four floating chamber bases. The two remaining chambers were filled to a similar height with uncolonized, water-saturated Growstones to serve as controls. The mesh suspended the layer of the Growstones in each chamber 4 cm above the lagoon surface, while the bottom of each base was immersed 3 cm into the lagoon to form an air-tight seal with the lagoon surface. Each chamber base was attached by rope to a floating dock and allowed to float freely about the radius of its rope for the duration of the study.

### 3.4 Methane Flux Measurements

Flux was measured using the static chamber technique (Crill, 1991). An acrylic lid was placed over the aluminum base, in the perimeter channel, isolating the headspace gases from the surrounding air. A 1 rpm, 9 V motor was used to power a cardboard paddle with hanging streamers suspended from the interior top of the chamber lid to mix chamber-trapped gases during deployment. The chamber headspace gas was sampled immediately after lid emplacement and at 5 min intervals thereafter to 15 min. Samples were collected into 10 mL syringes via a 2.23 m long section of Tygon tubing (0.23 cm inside diameter) permanently inserted into the side of the acrylic lid. The tubing and the syringe were cleared by filling and expelling their contents three times via a three-way stopcock before a fourth sample was collected for analysis. Once sampling was complete, the lids were removed and the Growstones were allowed to experience ambient conditions until the next sampling session. The samples of the headspace gases were syringe-stored until analysis, within 3 h of collection. Experiments indicated <5% loss of syringe-stored $CH_4$ in 24 h.

### 3.5 Additional Field Measurements

Air and surface lagoon temperature were taken during all sampling sessions with a hand-held thermistor probe. Liquid samples were taken directly from the lagoon surface in 5 mL syringes and immediately transferred to sealed, mason jars (~1 L volume), which had been previously evacuated and filled to 1 atm with ultra-high purity $N_2$. Samples were then immediately acidified with 0.1 mL of 6N HCl and stored at an ambient temperature until analysis, within 5 h. The samples were set on a rotary shaker at 100 rpm for a minimum of 45 min prior to analysis. Experiments indicated <2% loss of $CH_4$ stored in mason jars in 48 h.

In association with each $CH_4$ flux determination, vertically integrated samples of suspended Growstones were collected from the chamber bases using 2.5 cm diameter by 7.5 cm height cylinders fabricated from window screen material. These samples were used to determine moisture content or subjected to experimentation in the lab. Immediately upon collection, the Growstones were transferred to sealed mason jars and kept at the ambient temperature for laboratory transport. The Growstones removed from chamber bases were replaced with a similar volume of laboratory colonized or uncolonized Growstones, as appropriate.

Methane ebullition from the lagoon was measured using the inverted funnel technique (Chanton & Whiting, 2009). A 12.4 cm radius funnel suspended below the lagoon surface from the center of a raft sampled an area of 0.048 $m^2$. The funnel was deployed an average of 3 d before sampling. Ebullitive gas was collected into duplicate 10 mL syringes through the sealing septum at the tip of the funnel. Syringes holding the samples were stored until analyzed for $CH_4$ as previously described for the chamber flux determinations.

**3.6 Laboratory Methods**

Unless otherwise indicated, laboratory experiments were conducted in sealed mason jars (~1 L) to which Growstones (15 to 50 g wet weight, depending on the experiment) were added. O-ring seal fittings attached to chamber lids allowed periodic sampling of headspace gases. Unless specified, headspace $CH_4$ mixing ratios were initially adjusted to uptake-saturated levels (experimentally predetermined) and the time course for $CH_4$ consumption over 1 to 192 h (depending on experiment) was determined. Methane injected at similar mixing ratios into an empty jar showed <2% loss over a period of 48 h. Hereafter, Growstone treatments will be designated as follows: Growstones taken directly from the bioreactor for laboratory manipulation will be referred to as laboratory colonized Growstones (LCG); those initially colonized in the bioreactor, suspended over the lagoon, and retrieved from the field for experimentation will be designated field-exposed laboratory colonized Growstones (FELCG); those not intentionally exposed to microbes in the bioreactor or field will be referred to as uncolonized Growstones (UCG); and those not intentionally exposed to microbes in the bioreactor, but were suspended over the lagoon and retrieved from the field for experimentation will be referred to as field-exposed uncolonized Growstones (FEUCG).

A series of laboratory experiments were designed to describe the physiological characteristics of the $CH_4$ oxidizing community of the LCG. Temperature dependence experiments were conducted by pre-incubating duplicate samples of LCG at temperatures which ranged from 4 to 45˚C. Moisture dependence experiments were performed by air-drying duplicate aliquots of LCG to target % water holding capacities (% WHC) ranging from 0 to 100% WHC. Concentration dependence of $CH_4$ consumption was determined by adjusting triplicate aliquots of LCG to a range of headspace $CH_4$ mixing ratios from <250 $\mu$L L$^{-1}$ to 2,000

$\mu L\ L^{-1}$ and subsequently determining the rate of $CH_4$ consumption. Threshold levels for $CH_4$ consumption were determined by initially exposing triplicates of LCG and UCG to a headspace mixing ratio of ~200 $\mu L\ L^{-1}$. The time course of $CH_4$ consumption in the mason jars was determined and the threshold for $CH_4$ consumption was defined as the point at which the headspace $CH_4$ mixing ratio showed no change with time.

A laboratory experiment was designed to simulate $CH_4$ emission from the lagoon surface and determine the potential rate of consumption of $CH_4$ by the LCG with UCG serving as a control. An acrylic cylinder (inside diameter 10.7 cm; total height of 39.5 cm) was partitioned into a lower chamber of 2 cm height and an upper chamber of 35.5 cm height with a 2 cm thick, porous, polyethylene medium support. Both ends of the acrylic cylinder were sealed and the upper and lower chambers were fitted with o-ring seal fittings for gas introduction (lower chamber) or withdrawal (upper chamber). A 21.6 cm layer of fine-grained, dry sand was placed on the porous support medium. This was covered with a hydrophilic, porous polyethylene disc to which a 7.5 cm layer of LCG or UCG was added. A pulse of $CH_4$ was introduced into the bottom chamber and the time course of $CH_4$ accumulation in the top chamber was determined at 5 min intervals. The height of the sand layer had previously been experimentally determined to give a time-linear rate of $CH_4$ accumulation in the upper chamber equivalent to a $CH_4$ flux of 60 kg ha$^{-1}$ d$^{-1}$, similar to the midrange of emissions previously reported for lagoon surfaces in NC and GA (Sharpe & Harper, 1999). A $CH_4$ flux measurement through UCG was determined and this was immediately followed by a flux determination through LCG.

An experiment was performed to determine the potential influence of $NH_3$ on $CH_4$ consumption by LCG. Triplicate mason jars containing LCG were lined with Teflon tape to prevent $NH_3$ from adhering to the sides and each jar was allowed to acclimate statically for 1 h to

$NH_3$ at levels measured by Harper et al. (2000) at 2.5 m (250 µg m$^{-3}$) and 0.5 m (1250 µg m$^{-3}$) above a lagoon surface. Samples were then amended with $CH_4$ and the time course for $CH_4$ consumption was determined.

FELCG and FEUCG were evaluated for $CH_4$ oxidizing capability by following the time course for $CH_4$ consumption of Growstones in mason jars. Additionally, FELCG and FEUCG were tested for their $NH_3$ oxidizing capabilities following Schmidt and Belser (1994). Briefly, all Growstone samples were immersed in a phosphate buffer solution amended with an $(NH_4)_2SO_4$ substrate and $KClO_4$ in order to inhibit the oxidation of $NO_2^-$ to $NO_3^-$ and the time course of $NO_2^-$ accumulation over 24 h was measured.

### 3.7 Analytical Determinations

Methane determinations were measured by flame ionization detection gas chromatography using a Shimadzu GC-8A instrument with a precision of <1%. Calibration gases were NIST-relatable. The operating conditions were: 1-m molecular sieve 5A column (60/80 mesh), ultra-high purity $N_2$ carrier gas at 33 mL min$^{-1}$, column and injector at temperatures of 70 and 140˚C, respectively.

Nitrite determinations were made colorometrically by diazotization (Tarafder & Rathore, 1988) using a Shimadzu Model 1201 spectrophotometer.

The gravimetric water holding capacity (WHC) of the Growstones was determined from the difference in weight between water saturated and air-dried Growstones. The % WHC of Growstones during experimentation was calculated as a ratio of measured water content to water holding capacity multiplied by 100. The $CH_4$ flux in the static chambers was calculated from the geometry of the chamber and the observed change in $CH_4$ mixing ratio in the headspace. Ebullitive $CH_4$ flux was calculated from the funnel geometry, the $CH_4$ mixing ratio of the funnel-

trapped gas, and total volume of gas collected over the period of deployment. The $CH_4$ dissolved

in liquid samples was calculated by the headspace equilibration technique (Kampbell et al.,

1989) using temperature-corrected solubility constants from Yamamoto et al. (1976). The

saturation index for $CH_4$ in the lagoon water was calculated from the ratio of measured partial

pressure divided by the equilibrium partial pressure of $CH_4$. The rates of $CH_4$ consumption in

laboratory experiments were calculated from the time-linear rate of decrease in $CH_4$ in the mason

jar and were normalized to the dry mass of Growstones ($g_{dw}^{-1}$). A third order polynomial was

fitted to the data for $CH_4$ consumption rate versus temperature or % WHC. The temperature

coefficient ($Q_{10}$) for $CH_4$ oxidation was calculated from the van't Hoff equation (Swan, 1974).

The apparent half-saturation constant for $CH_4$ oxidation ($K_s$) and the maximum rate of $CH_4$

oxidation ($V_{max}$) were estimated by directly fitting a rectangular hyperbola to data for $CH_4$

oxidation rate versus $CH_4$ mixing ratio. The value of $K_s$ was expressed as the aqueous phase

concentration, as calculated above. Student t-tests were used to compare the mean rates of $CH_4$

oxidation between treatments. Simple relationships between variables were assessed by

Pearson's product moment correlation. All statistical analyses were conducted at $\alpha=0.05$.

# CHAPTER 4: RESULTS

Methane flux measurements of all four chambers containing FELCG and FEUCG were conducted on 12 dates. The lagoon surface temperature ranged from 20.4 to 35.2˚C ($\bar{x}$= 29.5˚C), while the air temperature ranged from 20.8 to 35.0˚C ($\bar{x}$= 31.1˚C).

Two patterns of $CH_4$ accumulation in the chambers were observed. A time-linear increase in chamber $CH_4$ (Figure 2A) during the 15 min deployment was associated with a constant, diffusive flux from the lagoon surface, while a spike and subsequent drop in $CH_4$ midway through the chamber deployment (Figure 2B) pointed to ebullition as well as diffusion and inadequate mixing of chamber-trapped gases between sampling intervals. Accordingly, coefficients of variation for $CH_4$ emission in duplicate chambers for each treatment were highly variable, ranging from 3 to 146% for fluxes through FEUCG and 11 to 139% for fluxes through FELCG.  FEUCG showed fluxes that varied over a factor of 45, from 0.2 to 10.0 g $CH_4$ $m^{-2}$ $d^{-1}$. FELCG showed fluxes that varied over a factor of 16, from 0.7 to 11.0 g $CH_4$ $m^{-2}$ $d^{-1}$. The overall mean of 4.3 g $CH_4$ $m^{-2}$ $d^{-1}$ for the FEUCG was not significantly different than the mean of 4.2 g $CH_4$ $m^{-2}$ $d^{-1}$ for the FELCG. The lagoon temperature, air temperature and $CH_4$ mixing ratio immediately above the lagoon surface were not significantly correlated with $CH_4$ emissions for chambers with FELCG, FEUCG, or when the entire data were combined.
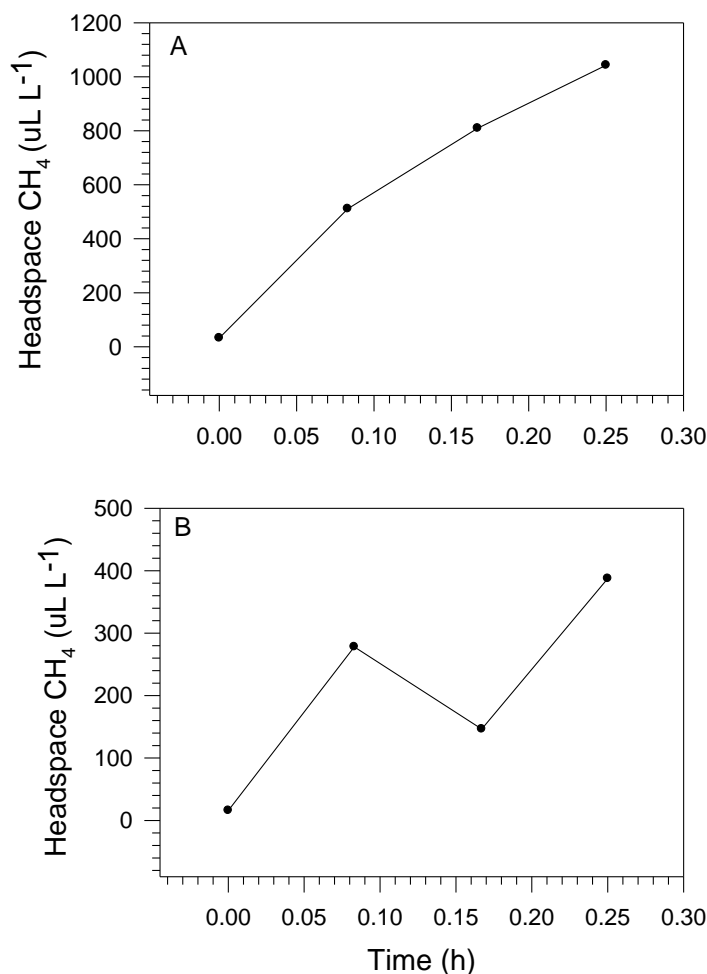
Figure 2: Representative time series for $CH_4$ accumulation in static chambers placed on the surface of an open air lagoon storing liquid hog waste in eastern North Carolina, 21, July 2014. The time-linear increase in (A) is associated with a constant, diffusive flux of $CH_4$ from the lagoon surface and the irregular time course for $CH_4$ accumulation in (B) is indicative of ebullition or unmixed chamber trapped gases.

The % WHC for the FELCG at the time of flux determination ranged over a factor of about 4, from 21.8 to 79.8% WHC ($\bar{x}$= 47.4%), depending on the antecedent weather. FEUCG showed a similar range for % WHC, with values between 20.0 and 74.9% WHC ($\bar{x}$= 47.1%). Differences between treatment means were not significant. Data for % WHC was not significantly correlated with $CH_4$ emissions when the entire data set was analyzed.

Ebullition measurements at the lagoon surface were taken on five dates. The lagoon temperature ranged from 17.8 to 25.2°C. Biogas was comprised (v/v) of 76 to 82% $CH_4$ and the

ebullitive $CH_4$ flux ranged from 0.3 to 1.8 g $m^{-2}$ $d^{-1}$ ($\bar{x}$= 0.9 g $m^{-2}$ $d^{-1}$). The coefficients of

variation for duplicate analyses of ebullitive $CH_4$ ranged from 0.2 to 4.2%. Although data are

not directly comparable due to differing dates of collection, the magnitude of the average

ebullitive $CH_4$ flux was 21% of the overall average total chamber flux of 4.2 g $CH_4$ $m^{-2}$ $d^{-1}$,

suggesting that ebullition is an important component of total $CH_4$ emissions from the surface of

the lagoon. No significant correlation was shown between the temperature of the lagoon and the

ebullitive $CH_4$ flux, the mass of $CH_4$ collected from the trap, or the % $CH_4$ in ebullitive gas.

The level of $CH_4$ saturation in the lagoon was measured on eleven dates. The lagoon

temperature for those dates ranged from 20.4 to 35.2°C and the air temperature ranged from 20.8

to 35.0°C. The concentration of $CH_4$ of the lagoon varied over a factor of 15, ranging from 66 to

973 µmol $L^{-1}$ ($\bar{x}$= 509 µmol $L^{-1}$). The coefficient of variation for duplicate samples on each date

ranged from 0.2 to 33%. The equilibrium dissolved $CH_4$ concentration at 25°C in a clean air

environment is roughly 2.5 nM (Gevantman, 2015). The temperature-corrected saturation factor

ranged from 56000 to 922000 ($\bar{x}$= 554000) indicating supersaturation by a factor of $10^5$ to $10^6$.

No significant correlation was found between the temperature of the lagoon and the dissolved

$CH_4$ concentration. Assuming the average dissolved $CH_4$ concentration of 509 µmol $L^{-1}$ and an

average flux of 4.2 g $CH_4$ $m^{-2}$ $d^{-1}$, the two lagoons store about 342 kg $CH_4$ at capacity and emit

59 kg $CH_4$ $d^{-1}$ or 17% of the $CH_4$ stored in the liquid waste per day.

Physiological characteristics of the $CH_4$ oxidizing community on LCG were assessed.

Triplicate samples with headspace mixing ratios adjusted to about 120 µL $L^{-1}$ $CH_4$ showed an

exponential decline of $CH_4$ over about 100 h to a threshold level ranging from 2 to 6 µL $L^{-1}$ $CH_4$

($\bar{x}$= 4 µL $L^{-1}$ $CH_4$) (Figure 3).
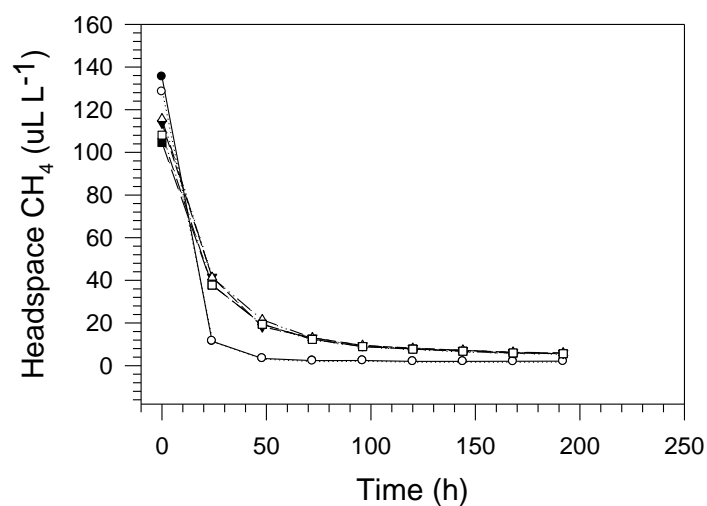
Figure 3: Time course decrease in headspace $CH_4$ mixing ratio in laboratory experiments assessing the threshold of $CH_4$ consumption for laboratory colonized Growstones.

Triplicate experiments assessing the concentration dependence for $CH_4$ oxidation by LCG (Figure 4) showed an average half-saturation constant for $CH_4$ oxidation of 5.0 μmol $L^{-1}$ and an average maximum rate for $CH_4$ oxidation of 1780 nmol $(g_{dw}^{-1})$ $h^{-1}$ (Table 1).



Figure 4: Representative experiment assessing the concentration dependence of $CH_4$ oxidation by laboratory colonized Growstones.

Table 1: Calculated values for the maximum rate of $CH_4$ oxidation ($V_{max}$) and the half-saturation constant for $CH_4$ oxidation ($K_s$) by laboratory colonized Growstones.

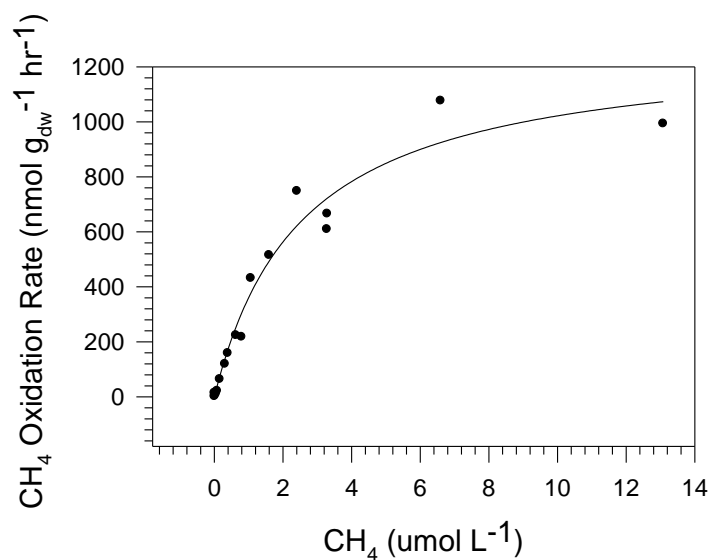| Replicate | $V_{max}$ | 95% CI* | $K_s$ | 95% CI | $R^2$ |
|---|---|---|---|---|---|
| | (nmol $(g_{dw}^{-1})$ $h^{-1}$) | | ($\mu$mol $L^{-1}$) | | |
| 1 | 1520 | 1210-1830 | 3.4 | 1.7-5.0 | 0.95 |
| 2 | 2050 | 1540-2570 | 7.1 | 3.9-10.3 | 0.97 |
| 3 | 1780 | 1550-2020 | 4.7 | 3.5-5.9 | 0.99 |

* 95% CI = 95% Confidence Interval

An experiment assessing the temperature dependence for $CH_4$ oxidation by LCG was best described by a third order polynomial (Figure 5). Between the temperatures of 4 to 45°C, the observed oxidation rates ranged from 260 to 2770 nmol $CH_4$ $(g_{dw}^{-1})$ $h^{-1}$, with a calculated optimum temperature ($T_{opt}$) of 32.7°C. The calculated maximum $CH_4$ oxidation rate at ($T_{opt}$) was 2610 nmol $CH_4$ $(g_{dw}^{-1})$ $h^{-1}$ and the calculated $Q_{10}$ for $CH_4$ oxidation was 2.4 between 12 and 28°C. The air temperatures ranged from 20.8 to 35.0°C during field $CH_4$ flux determinations, indicating that rates of $CH_4$ oxidation rates were 61 to 100% of the maximum ($\bar{x} = 95\%$), when considering the influence of temperature alone on rates of $CH_4$ oxidation.
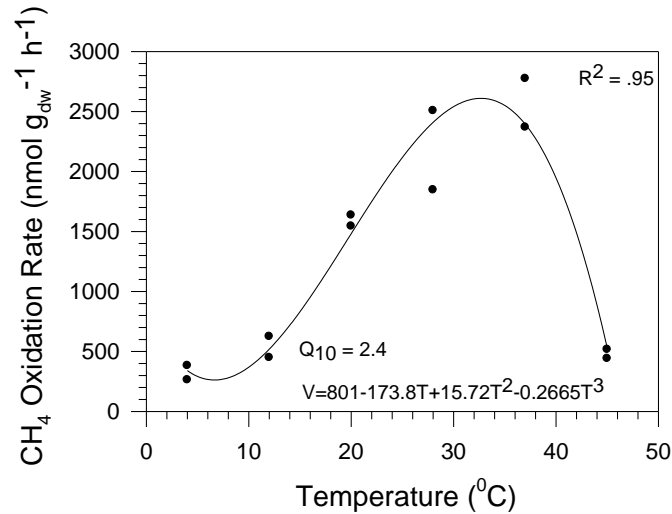


Figure 5: Temperature (T) dependence of $CH_4$ consumption (V) by laboratory colonized Growstones.

The moisture response for $CH_4$ oxidation by LCG was best described by a third order polynomial (Figure 6). Between the values of 0 and 100% WHC, the observed $CH_4$ oxidation rates ranged from 50 to 2510 nmol $CH_4$ $(g_{dw}^{-1})$ $h^{-1}$. The wider range of values relative to the temperature response of $CH_4$ oxidation indicates a greater sensitivity of $CH_4$ oxidation to moisture than temperature. The calculated optimum % WHC was 72%, which gave a calculated maximum $CH_4$ oxidation rate of 2390 nmol $CH_4$ $(g_{dw}^{-1})$ $h^{-1}$ at the laboratory temperature of 20˚C. The observed % WHC of 20 to 80% for FELCG and FEUCG during the $CH_4$ flux determinations, indicates that the rates of $CH_4$ oxidation were 22 to 99% of the maximum ($\bar{x} =$ 68%) when considering the influence of % WHC alone on rates of $CH_4$ oxidation.
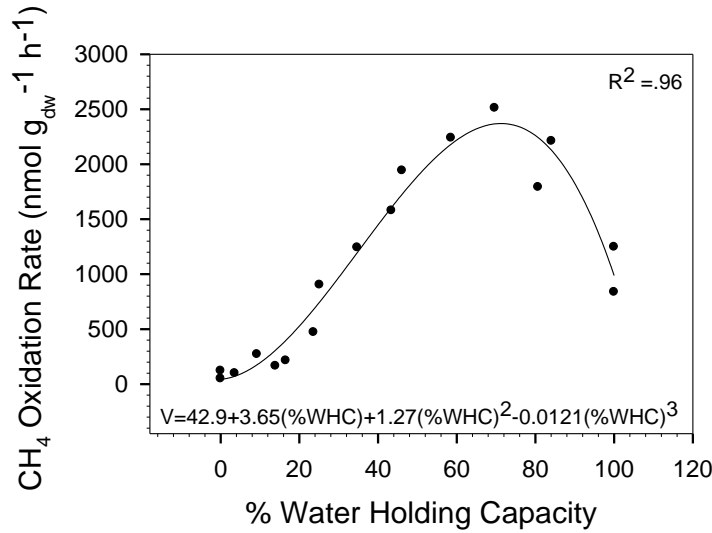


Figure 6: Moisture (%WHC) dependence of $CH_4$ consumption (V) by laboratory colonized Growstones.

Experiments assessing the ability of LCG to oxidize $CH_4$ under simulated rates of exchange of $CH_4$ across the lagoon surface (Figure 7) showed that the time-linear rate of accumulation of $CH_4$ in the chamber headspace ranged from 46 to 51 $CH_4$ µl $L^{-1}$ $min^{-1}$ ($\bar{x} = 48$). In contrast, the time-linear rate of accumulation for UCG, ranged from 63 to 67 $CH_4$ µl $L^{1}$ $min^{-1}$

($\bar{x} = 65$) (Table 2). The mean rate of $CH_4$ accumulation for the LCG was significantly lower than for the UCG. These results indicated that the LCG could potentially oxidize about 25% of the $CH_4$ typically emitted from a lagoon surface under the experimental conditions of 20˚C and 91% WHC.
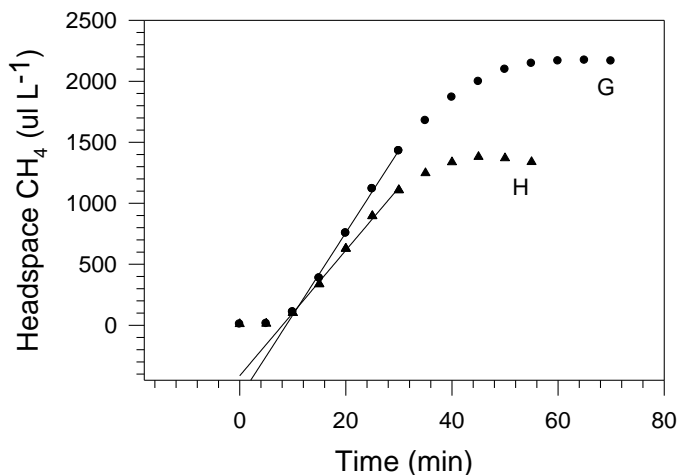


Figure 7: Representative experiment showing the time course for $CH_4$ accumulation in the headspace for a mesocosm packed with uncolonized Growstones (●) or laboratory colonized Growstones (▲). The time-linear segments of the accumulation curves are indicated.

Table 2: Slopes of regression equations describing the time-linear rate of $CH_4$ accumulation in the headspace of a mesocosm packed with uncolonized Growstones or laboratory colonized Growstones.

| Run | Uncolonized | Laboratory Colonized | % Difference |
|---|---|---|---|
| | Slope ($\mu L\ L^{-1}\ min^{-1}$) | | |
| A & B | 63 | 46 | 73 |
| C & D | 62 | 46 | 74 |
| E & F | 66 | 50 | 76 |
| G & H | 67 | 51 | 76 |

Determinations of $CH_4$ oxidation potential for FELCG and FEUCG consistently showed little decrease in headspace $CH_4$ of the mason jars (Figure 8). The rates of $CH_4$ oxidation by the FELCG ranged from 3 to 469 nmol $CH_4$ ($g_{dw}^{-1}$) $h^{-1}$ and averaged 130 nmol $CH_4$ ($g_{dw}^{-1}$) $h^{-1}$, while

the rates of $CH_4$ oxidation by the FEUCG ranged from 7 to 352 nmol $CH_4$ $(g_{dw}^{-1})$ $h^{-1}$ ($\bar{x}=$ 79

nmol $CH_4$ $(g_{dw}^{-1})$ $h^{-1}$) (Table 3). The difference between means was not statistically significant.

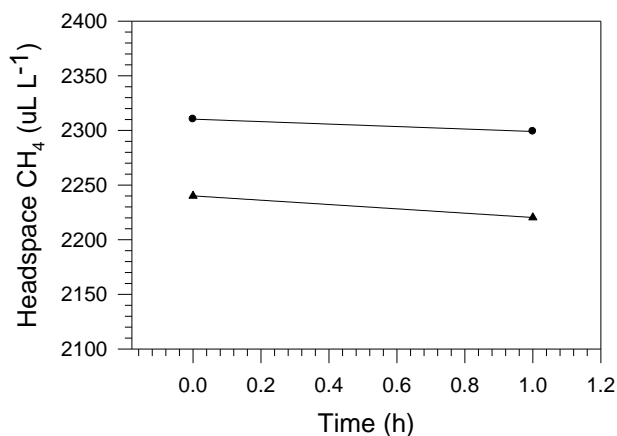

Figure 8: Representative experiment showing the time course for change in headspace $CH_4$ for field-exposed uncolonized Growstones (●) and for field-exposed laboratory colonized Growstones (▲) in jar experiments. Growstones initially suspended above the lagoon surface on 30, June 2014 were returned to the laboratory on 22, August 2014 and exposed to an uptake-saturating concentration of $CH_4$.

Table 3: Potential $CH_4$ oxidation rates measured for field-exposed uncolonized Growstones sampled on 9 dates and field-exposed laboratory colonized Growstones sampled on 14 dates.

|  | Colonized | Uncolonized |
|---|---|---|
|  | (nmol $(g_{dw}^{-1})$ $h^{-1}$) | |
| Range | 3-469 | 7-352 |
| Mean | 130 | 79 |
| SEM | 13 | 10 |

SEM = Standard Error of the Mean

The % WHC of the FELCG returned to the laboratory for $CH_4$ oxidation rate

determinations ranged from 21.8 to 79.8% ($\bar{x} = 47.4\%$). The % WHC of the FEUCG was similar,

ranging from 20.0 to 74.9% ($\bar{x} = 47.1\%$). Results of the moisture dependence experiment (Figure

6) indicate that at 47.4% WHC the rate of $CH_4$ oxidation of the FELCG should be 75% of the

maximum rate under conditions of substrate ($CH_4$) saturation. Similarly, results of the

temperature dependence experiment (Figure 5) indicate that at the laboratory temperature of

20˚C, the $CH_4$ oxidation of the FELCG should be 57% of the maximum rate under conditions of substrate ($CH_4$) saturation. The average observed substrate-saturated $CH_4$ oxidation rate of 130 nmol $CH_4$ $(g_{dw}^{-1})$ $h^{-1}$ for the FELCG was more than an order of magnitude lower than the predicted rates of about 1800 and 1500 nmol $CH_4$ $(g_{dw}^{-1})$ $h^{-1}$ based on the moisture and temperature dependence experiments for the LCG prior to placement above the lagoon surface. Methane oxidation by previously UCG indicates that microbes capable of this process populated the Growstones subsequent to suspension above the lagoon surface.

Using an average dry mass of 22 g for each field-collected sample of FELCG, a mean $CH_4$ oxidation rate of 130 nmol $CH_4$ $(g_{dw}^{-1})$ $h^{-1}$, and a 5 cm diameter for each vertically integrated sample of FELCG, we calculate an average, area-based, daily rate of $CH_4$ oxidation of 51 mg $m^{-2}$. This represents only about 1% of the average daily $CH_4$ emission of 4.2 g $m^{-2}$ from the lagoon surface.

Exposure to $NH_3$ negatively impacted $CH_4$ oxidation by LCG. Exposure to $NH_3$ at a concentration reported 2 m above a swine lagoon surface (250 µg $NH_3$-N $m^{-3}$) immediately reduced $CH_4$ consumption by $34 \pm 1\%$ ($\bar{x} \pm 1$ SEM) (Figure 9). A further reduction in $CH_4$ oxidation of $59 \pm 7\%$ ($\bar{x} \pm 7$ SEM) was observed immediately following exposure to the average $NH_3$-N concentration (1250 µg $NH_3$-N $m^{-3}$) reported just above a swine lagoon surface (Figure 10).

Figure 9: Representative experiment showing the time course for change in headspace $CH_4$ before the addition of $NH_3$ (●) and immediately after the addition of 250 μg $NH_3$-N $m^{-3}$(▲) in jar experiments with laboratory colonized Growstones.



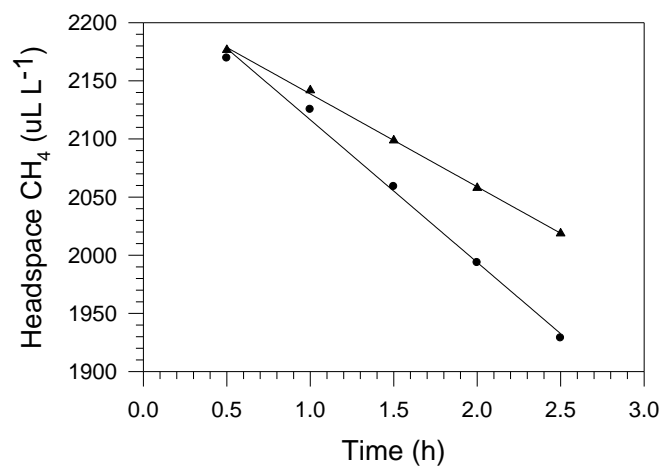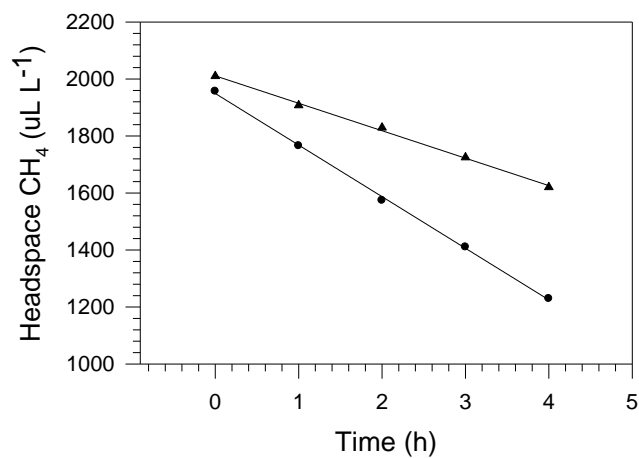Figure 10: Representative experiment showing the time course for change in headspace $CH_4$ before the addition of $NH_3$ (●) and immediately after the addition of 1250 μg $NH_3$-N $m^{-3}$(▲) in jar experiments with laboratory colonized Growstones.

Two experiments assessing the ammonium-oxidizing capability of FEUCG and FELCG

showed no clear evidence that any of the samples were capable of $NH_4^+$ oxidation.

**CHAPTER 5: DISCUSSION**

Three major pathways of gas exchange at the air-liquid interface include diffusive transfer, bubble ebullition, and vascular transport through emergent macrophytes (Macintyre et al., 1995). Aquatic plants are absent in the anaerobic lagoons commonly used in waste management of CAFOs in North Carolina. Accordingly, total $CH_4$ emissions across the lagoon surface can have both diffusive and ebullitive components. Measurement of $CH_4$ by use of floating chambers results in a linear accumulation only when diffusion at the air-liquid interface, a steady stream of microbubbles, or both, are present (Coulthard et al., 2009). Should an episodic release from the sediment and capture of a large bubble occur during deployment of a floating chamber, then the results would manifest as a sudden change in the slope of the mixing ratio of chamber-trapped $CH_4$ (Goodrich et al., 2011). During every sampling session, we observed a continuous stream of small bubbles which remained visible across the entire surface of the lagoon, as well as irregular occurrences of major eruptions of biogas. The presence of both indicated that, in addition to diffusion, both forms of ebullitive flux, continuous and episodic, were active. This is confirmed by the common patterns of $CH_4$ accumulation seen in our floating chamber results (Figure 2). Visual observations of ebullition have also been reported from stored liquid swine (Safley  et al., 1988; Park et al., 2010) and dairy (McGrath & Mason, 2004; VanderZaag et al., 2011) waste.

Comparison of $CH_4$ emission estimates from liquid animal waste are difficult due to the variability in experimental methodology, animal type, waste storage system and reporting units. Many researchers give area based fluxes in terms of $m^3$ $CH_4$, which we have converted to a mass

basis assuming a molar volume of 24.5 L. Our overall mean flux of 4.2 g $CH_4$ m$^{-2}$ d$^{-1}$ (Table 4)

is identical to that measured by Safley & Westerman (1989) using a partial cover over an NC

swine waste lagoon. It is within the range of averages of 0.8 and 6.2 g $CH_4$ m$^{-2}$ d$^{-1}$ determined

using a micrometeorological (gradient) technique for two NC swine waste lagoons (Sharpe et al.,

2002) and is also consistent with a mean of 5.23 g $CH_4$ m$^{-2}$ d$^{-1}$ reported for a GA swine waste

lagoon using similar methodology (Sharpe & Harper, 1999).  However, other reports for $CH_4$

emission from swine and dairy waste stored in lagoons and in tanks in other areas of the US and

elsewhere give means that are up to two orders of magnitude higher (Table 4). Differences in

$CH_4$ emission estimates among studies are not only attributable to differences in experimental

methodology, but are also related to factors contributing to volatile solid loading such as the

number of animals contributing to lagoon effluent and the residence time of waste in animal

houses prior to discharge (Sharpe et al., 2002), as well as the state of digestion of influent waste

as it entered the lagoon (Safley & Westerman, 1989). Methane yields are also influenced by the

species of animal and their diet (McGrath & Mason, 2004).

Table 4: Methane Emissions from Liquid Agricultural Waste Storage Systems

| Liquid Waste Type | Storage System | Location | Measurement Technique | g CH$_4$ m$^{-2}$ d$^{-1}$ range (average) | Authors |
|---|---|---|---|---|---|
| Swine | Lagoon | NC | Floating Chamber | 0.2-11.0 (4.2) | This Study |
| Swine | Lagoon | GA | Micrometerological | 0.1-50 (5.2) | Sharpe & Harper, (1999) |
| Swine | Lagoon | NC | Micrometerological | 2.0-11.5 (6.2)[a] 0.5-1.1 (.8) | Sharpe et al., (2002) |
| Dairy | Lagoon | KS | Micrometerological | 14-102 (40) | Todd et al., (2011) |
| Swine | Tank | Ontario, CA | Micrometerological | 0.4-91 | Park et al., (2006) |
| Swine | Tank | Ontario, CA | Micrometerological, Chamber | 26-518 (142, 205)[b] | Park et al., (2010) |
| Swine | Lagoon | KS | Floating Collection Raft | (60, 210)[c] | DeSutter & Ham, (2005) |
| Swine, Dairy | Lagoon | Australia | Floating Chamber | (346, 15)[d] | Park & Craggs, (2007) |
| Swine, Poultry | Lagoon | NC | Partial Lagoon Cover | (3.2, 4.2)[e] | Safley & Westerman, (1989) |

[a] Ranges and means from two lagoons
[b] Means from micrometeorological and chamber measurements, respectively
[c] Maximum values, depending on location of sampler
[d] Means for swine and dairy lagoons, respectively
[e] Means for swine and poultry lagoons, respectively

Spatial differences in rates of biogas emissions from swine waste storage lagoon on the order of 10's of meters have been attributed to spatial heterogeneity in the vertical dimension in the distribution of organic matter (Safley & Westerman, 1988; Sharpe & Harper, 1999; DeSutter & Ham, 2005). The varying depth of organic matter, however, was likely not the cause of the meter scale variability in CH$_4$ emission across the lagoon surface consistently observed in our sampling sessions, where coefficients of variation between duplicate chambers for a treatment ranged from 3 to 146%. Similarly high episodic variability in repeated 0.5 h measurements of

CH$_4$ flux from a single stationary chamber has been attributed to sporadic eruptions of large bubbles (Park et al., 2010), while stochastic "boiling" resulted in large scale differences in emission from a swine waste lagoon at distances of only 1 to 2 m (Safley & Westerman, 1988). Likewise, random bubble eruptions are the probable cause of meter scale variability in CH$_4$ emission in our study.

Methane emission from lagoons during intensive field studies has frequently been correlated with wind speed, volatile solid or organic loading rate, and temperature of the lagoon or atmosphere (Safley & Westerman, 1988; Sharpe & Harper, 1999; Sharpe et al., 2002; DeSutter & Ham, 2005; Park et al., 2010). Although we did not measure wind speed or C loading rates to the lagoon, we found no correlation between lagoon or air temperature and CH$_4$ flux. It is possible that the unmeasured variables were more important drivers of CH$_4$ emission or that a relationship between flux and lagoon or air temperature would emerge with a more extensive data set covering a broader range of temperatures. It is more likely, however, that the random sediment release of large bubbles into the floating chamber was far more important than temperature as a determinant of CH$_4$ emission.

Floating chamber or micrometeorologically-determined CH$_4$ fluxes from a waste storage lagoon are incapable of partitioning flux between diffusive and ebullitive components. Although our bubble traps were not deployed contemporaneously with our floating chambers, comparison of mean CH$_4$ fluxes using the two techniques suggests that ebullition is important, accounting for roughly 20% of total CH$_4$ emissions. Ebullition is a well-studied and highly important component of CH$_4$ flux in natural wetlands, sometimes accounting for 34 to 80% of total emissions (Chanton et al., 1989; Chanton & Whiting, 2009; Wilson et al., 1989).

Ebullitive fluxes vary widely, but center around 1 g $CH_4$ $m^{-2}$ $d^{-1}$ in wetlands (Goodrich et al. 2011), nearly identical to our average of 0.9 g $CH_4$ $m^{-2}$ $d^{-1}$.  However, the relative contribution of ebullition to total $CH_4$ emissions is less significant in swine lagoons than in wetlands due to differences between the environments with regards to $CH_4$ supersaturation of the liquid surface, an important driver of diffusive $CH_4$ emission (MacIntyre et al. 1995). The average $CH_4$ concentration of surface water in the Amazon floodplain, for instance, was 6.4 μM (Devol et al., 1988), which represents supersaturation by a factor of about $10^3$, while our average lagoon $CH_4$ concentration of 509 μM is supersaturated by a factor in excess of $10^5$.  Other reports of aqueous phase $CH_4$ in swine waste lagoons are lacking, but the measured values of 210 and 490 μM for a slurry storage system in Denmark (Husted, 1993) fall within our measured range of 65 to 973 μM.

The $CH_4$ content (v/v) of ebullitive gas (76 to 82%) in our study was consistent with previous reports for other swine waste lagoons, which vary from 62 to 95% (Safley and Westerman 1988, 1989; DeSutter & Ham, 2005; Park et al. 2007).  Overall, $CH_4$ content of ebullitive gas is high due to high solubility of $CO_2$, the other major end product of decomposition (DeSutter & Ham, 2005).  Variability among studies may be temperature-related; Safley & Westerman (1989) noted that $CH_4$ concentration positively correlated with lagoon temperature, presumably due to increased methanogenic activity (Safley & Westerman, 1989). Variations in composition of bubbles may also be related to the rate of bubble emissions themselves, where slower rates of bubble emissions correspond to lower $CH_4$ content (Keller & Stallard, 1994).

Sites of $CH_4$ oxidation in natural environments can be distinguished by their exposure to either high concentrations of $CH_4$, even if only periodically, or exposure to low (atmospheric) concentrations of $CH_4$. The $K_s$ value and threshold for $CH_4$ oxidation of methanotroph

populations are distinctly different between these two types of environments. The $K_s$ and threshold values for $CH_4$ oxidation associated with high-affinity populations of methanotrophs are characteristic of soils exposed to low, atmospheric mixing ratios of $CH_4$. In contrast, the $K_s$ and $CH_4$ oxidation threshold values associated with low-affinity populations of $CH_4$ oxidizers are reported for lake sediments, landfill cover soils, and other environments that are characterized by higher mixing ratios of $CH_4$ (Bender & Conrad, 1992). Upland soils and other environments where high-affinity populations of $CH_4$ oxidizers are observed have relatively low $CH_4$ thresholds between <0.1 and 0.4 $\mu$L L$^{-1}$ (Bender & Conrad, 1992; Conrad, 1995; King, 1992). The $K_s$ values for these environments range from 0.01 to 0.28 $\mu$M (Bender & Conrad, 1993; Conrad, 1995; 1996; Shukla et al., 2013). Low-affinity methanotrophs have a relatively high threshold for $CH_4$ ranging upwards of 45 $\mu$L L$^{-1}$ (Bender & Conrad, 1992; Conrad, 1996; King, 1992) and $K_s$ values ranging from 0.8 to 66.2 $\mu$M, one to three orders of magnitude higher than high-affinity populations (Dalal et al., 2008; Megraw & Knowles, 1987; Segers, 1998; Shukla et al., 2013). We found a $CH_4$ oxidation threshold between 2 and 6 $\mu$L L$^{-1}$ and $K_s$ values from 3.4 to 7.1 $\mu$M for LCG, consistent with previous reports for low-affinity populations. This is reasonable, given that the Growstone population was cultivated from landfill cover soils and aerated wastewater and was continuously exposed to a high concentration of $CH_4$ (17.5$\mu$M) during culture development.

One indicator of the population size of $CH_4$ oxidizing bacteria in samples taken from the natural environment is $V_{max}$, the maximum rate of oxidation measured in kinetic studies (Whalen, 2005). The variety of units used to express measurements of $V_{max}$ make direct comparisons of $V_{max}$ values across studies difficult, even for measurements taken from the same type of soils (Whalen, 2005). Low-affinity $CH_4$ oxidizing communities have high $V_{max}$ values;

wetland environments show values from 223 nmol $(g_{dw}^{-1})$ $h^{-1}$, while boreal bogs show values from 1000 to 10000 nmol $(g_{dw}^{-1})$ $h^{-1}$, and landfill cover soils have $V_{max}$ values ranging from 40 to 2594 nmol $(g_{dw}^{-1})$ $h^{-1}$ (Czepiel et al., 1995; 1996; Whalen, 2005). In contrast, reported $V_{max}$ values for soils oxidizing $CH_4$ at ambient mixing ratios are lower and range from 0.6 to 7.4 nmol $(g_{dw}^{-1})$ $h^{-1}$ (Bender & Conrad, 1993; Czepiel et al., 1995). Our LCG had $V_{max}$ values between 1510 and 2050 nmol $(g_{dw}^{-1})$ $h^{-1}$, consistent with other reports for low-affinity populations and indicative of a dense population of methanotrophs on the LCG when initially placed above the lagoon surface. Nonetheless, the population size of the methanotrophic community on the LCG was likely less than that of the methanotrophic community colonizing the garden compost and volcanic pumice used in a study assessing the feasibility of using a biofilter to mitigate dairy waste (Pratt et al., 2013), where $CH_4$ oxidation rates in excess of 4000 nmol $(g_{dw}^{-1})$ $h^{-1}$ were reported. Differences between studies may relate to physical differences between support media; garden compost and volcanic pumice may supply greater surface area for colonization and afford more contact time between microbes and $CH_4$.

In low-affinity (high $CH_4$ mixing ratio) environments, the temperature response of $CH_4$ oxidation is frequently described by parabolic or third-order polynomials such as the one shown in Figure 5, indicating that enzymatic processes, rather than substrate ($CH_4$) supply, are rate limiting (Czepiel et al., 1996; King & Adamsen, 1992). The temperature response of $CH_4$ oxidation for LCG gave values for both a $T_{opt}$ and a $Q_{10}$ similar to values for other low-affinity populations of methanotrophs. Landfill cover soils have $Q_{10}$ values ranging from 1.9 to 4.1 and $T_{opt}$ values ranging from 30 to 36°C (Czepiel et al., 1996; Scheutz & Kjeldsen, 2004; Whalen et al., 1990). Wetland soils have somewhat lower values, with $Q_{10}$ values ranging from 1.4 to 2.9

and $T_{opt}$ values ranging from 20 to 25˚C (Chowdhury & Dick, 2013; Dunfield et al., 1993; Whalen, 2005).

Moisture content of support media for methanotroph communities regulates the diffusion of gaseous substrates ($CH_4$ and $O_2$) to the community, impacting the $CH_4$ oxidation rate. Waterlogged environments slow diffusion, while dry environments enhance diffusion, but induce water stress  (Boeckx et al., 1996; Boeckx & Van Cleemput, 1996). At 72% WHC, our LCG supported rapid gas-phase molecular diffusion of $CH_4$ to a maximum area of cell surface. Oxidation rates decreased at lower values of % WHC, when the bacteria experienced water stress, and at higher values of % WHC, when gas phase molecular diffusion of $CH_4$ to the cell surface transitioned to aqueous diffusion, which can be $10^4$-fold slower (Hanson & Hanson, 1996; Whalen et al., 1990). Cross-study comparisons of results of studies assessing the moisture dependence of $CH_4$ oxidation are difficult due to differences in reporting units. Irrespective of reporting units, investigations of the moisture response of $CH_4$ oxidation *at uptake-saturating CH4 mixing ratios* eliminate results affected by interactions between suboptimal $CH_4$ mixing ratios and moisture content. Accordingly, our moisture response experiments with LCG were most comparable with studies that isolated the moisture response by exposing samples to uptake-saturating mixing ratios. Studies assessing the moisture response of $CH_4$ oxidation at uptake-saturating $CH_4$ mixing ratios give optima ranging from 20 to 60% WHC for upland forest soils (Reay et al., 2001; Whalen & Reeburgh, 1996) and a value of 53% WHC for samples from a bog site (Whalen & Reeburgh, 1996). The somewhat higher optimum % WHC that we observed, relative to other studies, is likely due to the high air-filled pore space of a loosely packed and porous medium, which, compared to densely packed, natural media, enhances diffusion. Thus, higher moisture content is needed to increase contact time between $CH_4$ and microbes.

At 91% WHC and 20°C, our LCG were able to oxidize 25% of the $CH_4$ that passed through a column at a rate similar to the reported $CH_4$ flux across the surface of NC hog lagoons (Sharpe & Harper, 1999). Reeburgh (2003) estimated that over half of global $CH_4$ produced annually is oxidized by microbes before being emitted to the atmosphere using oxidation rates from environments that represent the main source terms in the atmospheric $CH_4$ budget. However, logistical constraints restrict the number of plot or mesocosm-scale studies that simultaneously measure rates of $CH_4$ production and oxidation. Nonetheless, studies of freshwater lake environments show 36 to 94% of $CH_4$ produced is oxidized (Frenzel et al., 1992; Hanson & Hanson, 1996; Kankaala et al., 2006; Rudd & Hamilton, 1978; Utsumi et al., 1998). Similarly, studies of temperate wetlands report that 24 to 91% of $CH_4$ produced is oxidized (Bosse et al., 1993; Hanson & Hanson, 1996; Whalen, 2005). Studies of landfills estimate 50% of $CH_4$ produced is oxidized (Hanson & Hanson, 1996; Whalen et al., 1990). Our value falls toward the lower end of these studies, most likely because of the thin (7.5 cm), loosely packed layer of LCG used in the laboratory experiments afforded little contact time between rapidly diffusing $CH_4$ and the methanotroph community.

My LCG were subjected to a number of experiments under simulated field conditions and showed a high capacity for $CH_4$ oxidation. FELCG, however, were only able to oxidize about 1% of the average measured daily rate of $CH_4$ emission from the lagoon. Moisture and temperature limitation cannot be responsible for this exceptionally low rate because the average observed % WHC of the FELCG and the average field temperature were capable of supporting rates of $CH_4$ oxidation that were 75 and 57% of the maximum observed in laboratory manipulations, respectively. It is highly likely that the low rate of $CH_4$ oxidation was due at least in part to $NH_3$ inhibition. Although the mechanisms are not fully understood and not all

environments subjected to exposure to $NH_3$ are negatively impacted, $NH_3$ is a known inhibitor of $CH_4$ oxidation in lowland soils, upland soils, and sediments (Bodelier & Laanbroek, 2004). Experiments with our LCG under favorable conditions of temperature and % WHC showed an immediate 34 to 59% decline in $CH_4$ oxidation rates after static exposure to $NH_3$ at levels reported for measurements taken above the surface of NC hog waste storage lagoons (Harper et al., 2000). FELCG retrieved after several months of suspension above the lagoon surface showed even lower rates of $CH_4$ oxidation, suggesting that long term exposure to $NH_3$ further reduced $CH_4$ oxidation rates.

The low $CH_4$ oxidation rates observed for FELCG could also be explained, in part, by a decline in the methanotroph population or replacement with a community physiologically less capable of oxidizing $CH_4$. Methane oxidation by FEUCG at a low, but similar rate to FELCG after several months indicates colonization of previously uncolonized media with microbes capable of oxidizing $CH_4$ and suggests that the microbial community of FELCG transformed into a similar community. We cannot discount that the microbial communities on FELCG and FEUCG were predominantly $NH_3$-oxidzing bacteria. Methane does not support the growth of $NH_3$ oxidizers, but this microbial group is capable of oxidizing $CH_4$ at rates considerably lower than methanotrophs (Bedard & Knowles, 1989). Although we found no evidence that a vigorous population of $NH_3$ oxidizers had become established on FELCG or FEUCG in experiments directly assessing the potential for $NH_3$ oxidation, these experiments may have been compromised by turbidity introduced by the disintegration of Growstones following immersion into the liquid medium. Hence, the role of $NH_3$ oxidizers in consuming $CH_4$ in FELCG or FEUCG remains uncertain.

# CHAPTER 6: SUMMARY AND CONCLUSIONS

Passive biofiltration is a potentially cost effective strategy to mitigating $CH_4$ emissions from a point source of high rates of release of this radiatively and chemically important trace gas. This approach successfully consumed up to 98% of emitted $CH_4$ from artificial dairy waste in a NZ laboratory study (Pratt et al., 2013). We extended this concept from a laboratory to a field environment to evaluate whether passive biofiltration is a viable method of mitigating $CH_4$ emission from anaerobic lagoons that commonly store swine waste on North Carolina CAFOs. We augmented field studies with laboratory experiments to gain further insights into environmental influences on rates of $CH_4$ consumption.

Field measurements indicated that total $CH_4$ emission from the lagoon averaged 4.2 g $CH_4$ $m^{-2}$ $d^{-1}$. Laboratory experiments showed that the community of methanotrophs initially colonizing the Growstone support medium was capable of oxidizing 25% of the field emitted $CH_4$ under moisture and temperature conditions experienced *in situ*. Field trials, however, showed that less than 1% of the emitted $CH_4$ from the lagoon was oxidized by the LCG after field deployment. Laboratory studies indicated a high sensitivity of the methanotroph community initially colonizing the Growstones to static exposure to $NH_3$ at levels reported above the surface of NC swine waste lagoons. This suggests that poor field performance is likely due, at least in part, to $NH_3$ inhibition. Ammonia is universally emitted in open-air swine waste lagoons in NC, suggesting that a passive biofilter approach is not viable for mitigating $CH_4$ emissions from this regional point source. Although the laboratory study of a dairy biofilter that prompted our investigation showed high and sustained oxidation of $CH_4$, the simulated effluent consisted of

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water through which a 20% $CO_2$/80% $CH_4$ mixture was diffused. Our study suggests that the performance of this biofilter would decline if $NH_3$ was simultaneously emitted with $CH_4$.

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