APPLICATION OF NANOPARTICLES IN LIVESTOCK MANURE FOR UNDERSTANDING HYDROGEN SULFIDE AND GREENHOUSE GAS REDUCTION MECHANISM

A Dissertation
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Niloy Chandra Sarker

In Partial Fulfillment of the Requirements
for the Degree of
DOCTOR OF PHILOSOPHY

Major Department:
Agricultural and Biosystems Engineering

March 2018

Fargo, North Dakota

North Dakota State University

Graduate School

Title

APPLICATION OF NANOPARTICLES IN LIVESTOCK MANURE FOR UNDERSTANDING HYDROGEN SULFIDE AND GREENHOUSE GAS REDUCTION MECHANISM

By

Niloy Chandra Sarker

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

SUPERVISORY COMMITTEE:

Shafiqur Rahman, PhD
Chair

Dean D. Steele, PhD

Mohiuddin Quadir, PhD

Chiwon W. Lee, PhD

Approved:

March 26, 2018
Date

Sreekala Bajwa, PhD
Department Chair

ABSTRACT

The agricultural sector is one of the sources of greenhouse gases (GHGs) emission, especially methane (CH_4), and contributing approximately 250 million metric ton carbon dioxide (CO_2) equivalent emission per year. Almost 70% of CH_4 emission from this sector is enteric fermentation, while 26% is from the livestock manure management. Both rumen and animal manure are the impending sources of carbon (C), sulfur (S), and water (H_2O) and microbial populations utilize these constituents to produce GHGs, and hydrogen sulfide (H_2S). Nanoparticles (NPs) application in manure is a promising treatment option for mitigating GHG and H_2S gases, but limited information is available on how the reduction mechanism occurs. In this study, zinc silica nanogel (ZnSNL), copper silica nanogel (CuSNL), and nano acetyl cysteine (NACL) coated zinc oxide quantum dots (Qdots), zinc oxide (nZnO), and silver (nAg) NPs were tested in manure stored under anaerobic conditions to understand the reduction mechanism of GHG and H_2S resulting from NPs application. Additionally, in vitro study with nZnO and two types of feed (alfalfa and corn silage) were conducted to investigate the efficacy of nZnO in mitigating ruminal gas emission. Methane and CO_2 concentrations were measured using an SRI-8610 gas chromatograph and H2S was measured using a Jerome 631X meter. Microbial populations were characterized using both plate counts and quantitative real-time polymerase chain reaction (qRT-PCR). Application of NPs reduced gas volumes ranging 16 to 99%, and concentrations reduced by 49 to ~100% for H_2S, and 20.24 to ~100% for GHGs. Application of NPs reduced 38.49 to 94.32% aerobic- and 7.43% to 82.04% anaerobic-microbial populations. Furthermore, the qRT-PCR analysis showed that reduction of gases was due to the inhibition of gas specific microbial population. Overall, nZnO based treatments reduced 8.80 to 55.64%

methyl coenzyme M reductase (mcrA) gene copies and 0.74 to 25.16% *dissimilatory sulfide reductase (DSR)*.

Contrariwise, compared to the control treatment, in vitro study demonstrated 4.89 to 53.65% H_2S and GHGs concentration reduction with the applied nZnO inclusion rates. Additionally, alfalfa as feed exhibited 37 to 45% cumulative gas reduction than corn silage but increased GHGs generation 2.17 to 23.17% and ~60% H_2S concentration.

ACKNOWLEDGEMENTS

The USDA National Institute of Food and Agriculture award #2015-67022-22996 supported part of my research work. This research was also supported by the USDA National Institute of Food and Agriculture, Hatch project number# ND01477.

I would like to express my sincere gratitude to my supervisor Dr. Shafiqur Rahman for his valuable advice, guidance, positive criticism, and encouragement. His inspiration and supervision throughout my research period allowed me to work independently towards achieving the project goal within the deadlines. It is his cooperation and support that made possible to achieve this degree.

I am thankful to my advisory committee members Dr. Dean D. Steele, Dr. Mohiuddin Quadir, and Dr. Chiwon W. Lee for their very insightful suggestions and constructive comments which helped me to conclude this work.

I would like to offer special thanks and acknowledge Dr. Md. Saidul Borhan and Dr. Ann-Marie Fortuna for their guidance, valuable advice, time and supports throughout this research both as a collaborator of the project and as a mentor.

I am heartily thankful to Dr. Tao Wang, Dr. Shiny Mathews, James A. Moose, Joel Bell for their support at different stages of my research.

I would also like to thank my colleagues, Suresh Niraula, Dhan Prashad Gautam, and Adimula Ajyai Banji for their help and encouragement at each step of this degree.

Finally, I would like to express my heartiest thanks and appreciation to my parents, my son, my wife, and family members for their encouragement and invaluable supports for such a prolonged period.

DEDICATION

This dissertation is dedicated

To my Parents:

Nitai Chandra Sarker & Dipali Rani Sarker

To my Wife & Son:

Shrabani Roy & Nilanjan Sarker (Niraj)

To My Grandparents:

Avay Lal Sarker & Jushna Rani Sarker

Late Shatish Sarker and Nihar Bala Sarker

To my Brother, Sister-in-law, & Nephew:

Nishith Sarker, Poly Rani Sarker, & Niladri Sarker

To my Parent-in-laws Family:

Shribash Roy, Sabitri Roy, Shitangshu Roy, & Madhuri Das

To all of you who were with me during this journey!

TABLE OF CONTENTS

ABSTRACT iii

ACKNOWLEDGEMENTS v

DEDICATION vi

LIST OF TABLES xii

LIST OF FIGURES xiii

GENERAL INTRODUCTION 1

References 4

LITERATURE REVIEW 8

The Importance of Livestock Management 9

Greenhouse Gas Emission Sources 11

Factors Affecting Gaseous Emission 14

Nourishing Practice of the Animal 14

Housing Types of the Animals 15

Management System of the Manure 16

Environmental Factors (pH and temperature) 17

Greenhouse Gas Emission from Livestock Facilities 18

Methane Emission 20

Carbon Dioxide Emission 22

Nitrous Oxide Emission 23

Hydrogen Sulfide Emission 25

Greenhouse Gaseous Emission Reduction Procedures 26

Overview of Nanotechnology and its Application 30

Application of Zinc Oxide Nanoparticle 33

Application of Silver Nanoparticle 35

Characterization of the Applied Nanoparticles Effect 37
Characterization of NPs Effect by Real-Time Polymerase Chain Reaction 37
Research Objectives 40
Objective 1 40
Objective 2 40
Objective 3 40
References 41
PAPER 1: NANOPARTICLES IN MITIGATING GASEOUS EMISSIONS FROM LIQUID DAIRY MANURE STORED UNDER ANAEROBIC CONDITION 68
Abstract 68
Introduction 68
Materials and Methods 71
Manure Collection and Characterization 71
Nanogel Synthesization 72
Preparation of Copper and Zinc Silica Nanogel 72
Preparation of N-acetyl Cysteine Coated Zinc Oxide q-dot Nanogel 73
Experimental Setup and Gas Sampling 73
Measurement of Gas Volume, GHGs, and Hydrogen Sulfide Concentration 74
Microbial Population Density Analysis 75
Bacterial Cultivation and Quantification 75
Quantitative Real-Time Polymerase Chain Reaction Analysis 76
Statistical Analysis 78
Results and Discussions 78
Effects of Nanoparticles on Manure Properties 78
Effects of Nanoparticles on Volatile Fatty Acids 80
Effect of Nanoparticles on Gas Production 82

Effects of Nanoparticles on Methane Concentration 84
Effects of Nanoparticles on Carbon Dioxide Concentration 85
Effect of Nanoparticles on Hydrogen Sulfide Concentration 87
Effect of Nanoparticles on Bacterial Population 89
Effect of Nanoparticles on Methanogen Population 90
Possibilities and/or Difficulties of the Applied Method 92
Conclusions 93
Acknowledgements 94
References 94
PAPER 2: IN VITRO EVALUATION OF NANO ZINC OXIDE ON MITIGATION OF GASEOUS EMISSIONS 102
Abstract 102
Introduction 103
Materials and Methods 106
Rumen Fluid Collection, Processing and Experimental Set Up 106
Determination of Rumen Fluid pH and Redox 108
Volatile Fatty Acids Analysis 108
Gas Production Measurement and Monitoring System 108
Analysis of Microbial Populations 110
Statistical Analysis 111
Results 112
Effect of nZnO Application Levels on in Vitro pH and Redox 112
Effect of nZnO Application Levels on in Vitro VFA Production 113
Effect of nZnO Application Levels on in Vitro Gaseous Emission and CH_4, CO_2, and H_2S Concentrations 115
Effect of nZnO Application Doses on in Vitro Microbial Populations 119

Discussion 121

Conclusions 125

Acknowledgments 125

References 125

PAPER 3: UNDERSTANDING GASEOUS REDUCTION MECHANISMS IN SWINE MANURE RESULTING FROM NANO-PARTICLE TREATMENTS UNDER ANAEROBIC STORAGE CONDITIONS 133

Abstract 133

Introduction 134

Materials and Methods 138

Manure Collection 138

Characterization of the Manure 138

Nanoparticles Used and Their Application Methods 139

Direct Application Method 140

Indirect Application of Nanoparticles 140

Preparation of Sodium Alginate Beads 140

Experimental Setup 141

Coliform Bacterial Population Determination Using M Endo Broth 143

Total Bacterial Population Determination 144

DNA Isolation and Preparation for q-RT-PCR 145

Inductively Coupled Plasma-Optical Emission Spectroscopy for Manure Mineral Analysis 146

Statistical analysis 147

Results and Discussion 147

Effect of Nanoparticles on Manure Properties 147

Effect of Nanoparticles on Manure VFAs 149

Effect of Nanoparticles on Manure Redox Potential 150
Effect of Nanoparticles on Cumulative Gas Production 152
Effects of Nanoparticles on Carbon dioxide Concentration 153
Effects of Nanoparticles on Methane Concentration 155
Effects of Nanoparticles on Hydrogen Sulfide Concentration 157
Effects of Nanoparticles on Total Coliform Bacteria 159
Effects of Nanoparticles on Anaerobic Bacteria 160
Effects of Nanoparticles on Methanogenic Archaea 162
Effects of Nanoparticles on DSR Gene of SRB 163
Effect of Nanoparticles on Manure Mineral Contents 164
Conclusions 166
References 166
GENERAL CONCLUSIONS 172
RECOMMENDATIONS FOR FUTURE WORKS 173

LIST OF TABLES

Table		Page
1.	Increasing trend of livestock population around the world (Ali, 2015)	9
2.	Effect of flooring on the overall gaseous emission from livestock facility (Source: (Hartung & Phillips, 1994)	27
3.	Potential mitigation methods for N_2O and CH_4 (Source: (Chadwick et al., 2011)	29
4.	Protocols followed to determine manure properties	72
5.	Properties of pre and post treated liquid dairy manure.	80
6.	Volatile Fatty Acids from liquid dairy manure exposed and not exposed to nanoparticle treatments	82
7.	Composition of the feeds (dry matter basis)	107
8.	Effect of different application levels of nZnO (mean ± SEM) on in vitro pH and redox (after 72 hours of incubation).	112
9.	Effect of different application levels of nZnO (mean ± SEM) on the combination of alfalfa and rumen fluid VFA (n = 4 observations/treatment)	114
10.	Effect of different application levels of nZnO (mean ± SEM) on the combination of corn silage and rumen fluid VFA (n = 4 observations/treatment)	115
11.	Protocols followed to determine manure properties.	139
12.	Properties of the Nanoparticles	140
13.	Properties of pre and post treated liquid swine manure	148
14.	Effects of different NP treatments on liquid swine manure VFAs	150
15.	Effects of different NP treatments on pre and post-treated liquid swine manure Redox	152
16.	Effects of different NP treatments on liquid swine manure mineral contents	165

LIST OF FIGURES

Figure		Page
1.	Eighteen top ranked cattle producing countries around the world	9
2.	Sources of emission in the animal housing	10
3.	(a & b) Greenhouse emission sources globally and in the US, (c) Global Greenhouse gas emission by gas, (d) CO_2 emissions from different region all over the world.	12
4.	Gaseous emission from livestock sector	13
5.	Periods and places for gaseous emission from manure management system	19
6.	Degradation of organic material present in manure by anaerobic digestion process	22
7.	Nitrification and denitrification process	24
8.	The workflow of an RT-PCR	39
9.	Schematic diagram of an experimental setup used in this study	74
10.	Gas production trends from dairy manure treated with different treatments and stored under anaerobic condition. Data presented are the means of three independent replications ± standard deviation. Values of each gas production form manure samples followed by different letters (a, b) above the bars indicate that the data points are significantly different at $P \leq 0.05$.	83
11.	Methane (CH_4) production trends from dairy manure treated with different treatments and stored under anaerobic condition. Data presented are the means of three independent replications ± standard deviation. Values of each CH_4 concentrations form manure samples followed by different letters (a, b) above the bars indicate that the data points are significantly different at $P \leq 0.05$.	85
12.	Carbon dioxide (CO_2) production trends from dairy manure treated with different treatments and stored under anaerobic condition. Data presented are the means of three independent replications ± standard deviation. Values of each CO_2 concentrations form manure samples followed by different letters (a, b) above the bars indicate that the data points are significantly different at $P \leq 0.05$.	87
13.	Hydrogen sulfide (H_2S) production trends from dairy manure treated with different treatments and stored under anaerobic condition. Data presented are the means of three independent replications ± standard deviation. Values of each H_2S concentrations form manure samples followed by different letters (a, b) above the bars indicate that the data points are significantly different at $P \leq 0.05$.	88

14. Effect of nanoparticles (NPs) on the colony forming units (CFU) of coliform bacteria in liquid dairy manure treated with three NPs. (N=3; different letters (a, b) above bar indicate treatments are significantly different ($P \leq 0.05$) compared to control. 90

15. Standard curve for qRT-PCR amplification of five ten-fold serial dilutions of pure culture *Methanobacterium formicicum Schnellen* extracted c-DNA samples. 90

16. Melt curve for for qRT-PCR amplification of five ten-fold serial dilutions of pure culture *Methanobacterium formicicum Schnellen* (at ~82˚C) and extracted c-DNA samples (before 82˚C). 92

17. Effects of nZnO application levels and feed types on in vitro gas production. The different lowercase letter indicates significant differences among the treatments with alfalfa feed and the different uppercase letter indicates significant differences among the treatments with corn silage feed at $P \leq 0.05$ significance level. 116

18. Effects of nZnO application levels and feed types on in vitro methane (CH_4) concentration. The different lowercase letter indicates significant differences among the treatments with alfalfa feed and the different uppercase letter indicates significant differences among the treatments with corn silage feed at $P \leq 0.05$ significance level. 117

19. Effects of nZnO application levels and feed types on in vitro carbon dioxide (CO_2) concentration. The different lowercase letter indicates significant differences among the treatments with alfalfa feed and the different uppercase letter indicates significant differences among the treatments with corn silage feed at $P \leq 0.05$ significance level. 118

20. Effects of nZnO application levels and feed types on in vitro hydrogen sulfide (H_2S) concentration. The different lowercase letter indicates significant differences among the treatments with alfalfa feed and the different uppercase letter indicates significant differences among the treatments with corn silage feed at $P \leq 0.05$ significance level. 119

21. Effects of nZnO application levels and feed types on the initial in vitro microbial populations. For both of the individual feed situations, treatments are not significantly different at $P > 0.05$ significance level. 120

22. Effects of nZnO application levels and feed types on the final in vitro microbial populations. The different lowercase letter indicates significant differences among the treatments with alfalfa feed and the different uppercase letter indicates significant differences among the treatments with silage feed at $P \leq 0.05$ significance level. 121

23. Schematic diagram of an incubation vessel 142

24. Biogas production comparison at each sampling event from liquid swine manure treated with and without NPs. The same letter above a bar graph within a sampling day is not significantly different at $P \leq 0.05$. 153

25. Carbon dioxide (CO_2) concentration trends from liquid swine manure treated with and without NPs. The same letter above a bar graph within a sampling day is not significantly different at $P \leq 0.05$. 155

26. Methane (CH_4) concentration trends from liquid swine manure treated with and without NPs. The same letter above a bar graph within a sampling day is not significantly different at $P \leq 0.05$. 157

27. Hydrogen sulfide (H_2S) concentration trends from liquid swine manure treated with and without NPs. The same letter above a bar graph within a sampling day is not significantly different at $P \leq 0.05$. 159

28. Coliform bacterial (aerobic) population counts trends from liquid swine manure treated with and without NPs. The same letter above a bar graph for different treatments is not significantly different at $P \leq 0.05$. 160

29. Anaerobic bacterial population counts trend trends from liquid swine manure treated with and without NPs. The same letter above a bar graph for different treatments is not significantly different at $P \leq 0.05$. 162

30. Trends in *mcrA* gene copies in liquid swine manure treated with and without NPs. The same letter above a bar graph for different treatments is not significantly different at $P \leq 0.05$. 163

31. Trends in SRB, DSR gene copies in liquid swine manure treated with and without NPs. The same letter above a bar graph for different treatments is not significantly different at $P \leq 0.05$. 164

GENERAL INTRODUCTION

Domestication of flora and fauna is one the most significant improvements in the past 13,000 years of anthropological history. However, it is established that floras were domesticated before faunas. Domestication was not only the prerequisite for the rise of the human civilization but also in the center of interest of all scientists and non-scientists alike because most of our food supplies emanate from domestication (Diamond, 2002). People have domesticated different animals for different purposes. Domesticated animals for last 10000 years not only for food supplies but also for labor and fibers are acknowledged (Clutton-Brock, 1999). Domestication of sheep, beef, goat, cattle, and pig was found within the time region of 9000 B.C. to 4000 B.C. (Perkins, 1973). Dairy cattle, beef cattle, pig, goat, water buffalo, sheep, horse, donkey, mule, water buffalo, poultry, and turkey are some of the common domestic animals, and they are commonly called livestock.

It is reported that 26% of the earth is occupied by the livestock systems and livestock industry and their demands are growing globally. The rapid growth of livestock industry all over the world is due to the urbanization, increase income, and increasing population (Delgado, 2005; Thornton, 2010) as well as changes in food habits. Livestock is contributing to the global economy with a noteworthy value of more than $1.4 trillion (Steinfeld et al., 2006), at the same time this industry is contributing to environmental concern.

Modern livestock is raised in confined facilities with a smaller footprint and producing a large amount of manure, which is rich in nutrients. If they are utilized properly, commercial fertilizer can be replaced with manure and at the same time improving soil organic matter. On the other hand, improper management of the manure and incomplete enteric fermentation can lead to huge amount of gaseous emission as well. Besides these, manure can also be a source of

nutrient runoff, pollutant gasses, and greenhouse gasses emission. About 80% of the total agricultural Greenhouse Gas (GHG) emissions are reported from the livestock sector. Moreover, scientists also reported that enteric fermentation and manure management are two sources those responsible for this 80% emission (Hoff et al., 2006; Kristensen et al., 2011; Olesen et al., 2006).

Greenhouse gasses (GHGs) such as methane - CH_4, carbon dioxide - CO_2, nitrous oxide - N_2O, and pollutant gas like hydrogen sulfide – H_2S emission from animal agriculture production operations are identified as an important air quality issue because those emissions have shown global significance (Cole et al., 1997). The authors also reported that almost 20% of the energy change in the atmosphere (i.e. radiative forcing) that responsible for climate change is caused by this gaseous emission from the agricultural sector. Within the agricultural sector, livestock facilities are the major contributor to the H_2S and GHGs emission (Johnson et al., 2007). Scientists also reported that livestock manure is the foremost source of H_2S and GHGs emission. However, Moss et al. (2000) have reported both manure and enteric fermentation are the contributory sources for the gaseous emission from the livestock sector. Based on the available data from the livestock facilities it is reported that the GHGs emission can become a limiting factor for the rapid growth of livestock farming all over the world (Su et al., 2003). Therefore, mitigation of GHGs emission from the livestock facility is a prime concern both from the environmental and economic point of view.

Among the gaseous emission, N_2O is produced by the conversion of the N excreted by livestock (Amon et al., 2006) during the nitrification and denitrification processes. Methane (CH_4) can be generated by enteric fermentation of the organic constituents of the feed and anaerobic decomposition of organic matter present in the manure (Johnson & Johnson, 1995; Steed & Hashimoto, 1994). Furthermore, organic matters present in the feed and manure can be

biologically degraded anaerobically to produce CO_2 and H_2S as well (Drewnoski et al., 2011; Stafford et al., 1981). Besides the emission of GHGs and H_2S, zoonotic pathogens, antibiotic and hormone residues, odors, and dust are the potential concerns from livestock facilities and from manure usage (Massé et al., 2011).

All of these emissions both from enteric fermentation and anaerobic storage are not only environmental concerns but also perilous for animals and personnel working in the confined facilities. For example, Donham (1993) has reported that those who are working more than 2 hours per day for a period of 6 years or more are likely to show lung related diseases or another health risk. Therefore, gaseous emission must need to minimize or control for the betterment of human welfare, safety, and to protect the environment within and around the livestock facilities. However, very limited research has done on the mitigation of GHG and other pollutant gases. Therefore, reduction strategies of these emitted gasses are critically needed.

Since both enteric fermentation and anaerobic storage of manure are the major sources of gaseous emission, hence, emission mitigation strategies must begin with both of these. Diet modifications, the addition of additives, application of antibiotics are few of the common practices towards the mitigation of the GHGs from enteric fermentation of the ruminal animal (Martin et al., 2010). Whereas, the addition of chemicals, enzymes, biofilters, and air scrubbers are some of the common approaches of emission reduction from manure management. Mechanisms like entrapment of the emitted gas or application of chemical treatment within the manure storage system to minimize the gas production can be two viable options (Burton & Turner, 2003). Asis (2008) and Gautam et al. (2013) have already reported about the application of nanoparticles within the manure management system to mitigate the GHGs and H_2S emission. Gautam et al. (2016a & 2016b) evaluated and characterized zinc oxide nanoparticles (nZnO)

alginate beads in reducing gaseous emission from dairy and swine manure. The researcher found a significant reduction of H_2S due to chemical conversion and microbial inhibition. However, no in-depth study was conducted on the characterization of reduction mechanism, as well as the fate and transportation of nanoparticles. Moreover, application of the nanoparticle to reduce the enteric emission were not studied. Hence, the research objectives were to find the gaseous emission mitigation potential of different nanoparticles both in the rumen and in manure. The specific objective was to characterize the gaseous reduction mechanism.

References

Asis, D. A. (2008). Investigation of potential application of nanoparticles in reducing gas and odour emission from swine manure slurry. (Doctoral dissertation). Saskatoon, Canada, University of Saskatchewan, Department of Agricultural and Bioresource Engineering.

Amon, B., Kryvoruchko, V., Amon, T., & Zechmeister-Boltenstern, S. (2006). Methane, nitrous oxide and ammonia emissions during storage and after application of dairy cattle slurry and influence of slurry treatment. *Agriculture, Ecosystems & Environment, 112*(2), 153-162.

Burton, C. H. & Turner, C. (2003). Manure management: Treatment strategies for sustainable agriculture. Silsoe Research Institute, UK.

Cole, C., Duxbury, J., Freney, J., Heinemeyer, O., Minami, K., Mosier, A., . . . Sauerbeck, D. (1997). Global estimates of potential mitigation of greenhouse gas emissions by agriculture. *Nutrient Cycling in Agroecosystems, 49*(1-3), 221-228.

Clutton-Brock, J. (1999). A natural history of domesticated mammals. *Cambridge University Press,* Cambridge, England.

Diamond, J. (2002). Evolution, consequences and future of plant and animal domestication. *Nature, 418*(6898), 700-707.

Donham, K. J. (1993). Respiratory disease hazards to workers in livestock and poultry confinement structures. *Seminars in Respiratory Medicine*, *14*(1), 49-59.

Delgado, C. (2005). Rising demand for meat and milk in developing countries: implications for grasslands-based livestock production. *Grassland: A Global Resource,* 29-39.

Drewnoski, M., Beitz, D. C., Loy, D. D., Hansen, S. L., & Ensley, S. M. (2011). Factors affecting ruminal hydrogen sulfide concentration of cattle. *Animal Industry Report, 657*(1), 11.

Gautam, D., Rahman, S., Borhan, M., & Bezbaruah, A. (2013). Applications of nanoparticles (NPs) in livestock manure and their effects on air emissions. Paper presented at the *Intl. Symp. Animal Environ. Welfare. Chongqing, China.*

Gautam, D. P., Rahman, S., Bezbaruah, A. N., & Borhan, M. S. (2016a). Evaluation of Calcium Alginate Entrapped Nano Zinc Oxide to Reduce Gaseous Emissions from Liquid Dairy Manure. *Applied Engineering in Agriculture, 32*(1), 89-102.

Gautam, D. P., Rahman, S., Fortuna, A.-M., Borhan, M. S., Saini-Eidukat, B., & Bezbaruah, A. N. (2016b). Characterization of zinc oxide nanoparticle (nZnO) alginate beads in reducing gaseous emission from swine manure. *Environmental Technology,* 1-14.

Hoff, S. J., Bundy, D. S., Nelson, M. A., Zelle, B. C., Jacobson, L. D., Heber, A. J., . . . Beasley, D. B. (2006). Emissions of ammonia, hydrogen sulfide, and odor before, during, and after slurry removal from a deep-pit swine finisher. *Journal of the Air & Waste Management Association, 56*(5), 581-590.

Johnson, J. M.-F., Franzluebbers, A. J., Weyers, S. L., & Reicosky, D. C. (2007). Agricultural opportunities to mitigate greenhouse gas emissions. *Environmental Pollution, 150*(1), 107-124.

Johnson, K. A. & Johnson, D. E. (1995). Methane emissions from cattle. *Journal of Animal Science, 73*(8), 2483-2492.

Kristensen, T., Mogensen, L., Knudsen, M. T., & Hermansen, J. E. (2011). Effect of production system and farming strategy on greenhouse gas emissions from commercial dairy farms in a life cycle approach. *Livestock Science, 140*(1), 136-148.

Martin, C., Morgavi, D., & Doreau, M. (2010). Methane mitigation in ruminants: from microbe to the farm scale. *Animal, 4*(03), 351-365.

Massé, D., Talbot, G., & Gilbert, Y. (2011). On farm biogas production: A method to reduce GHG emissions and develop more sustainable livestock operations. *Animal Feed Science and Technology, 166*, 436-445.

Moss, A. R., Jouany, J.-P., & Newbold, J. (2000). Methane production by ruminants: its contribution to global warming. *In Annales de Zootechnie, 49*(3), 231-253. EDP Sciences.

Perkins, D. (1973). The beginnings of animal domestication in the Near East. *American Journal of Archaeology, 77*(3), 279-282.

Olesen, J. E., Schelde, K., Weiske, A., Weisbjerg, M. R., Asman, W. A., & Djurhuus, J. (2006). Modelling greenhouse gas emissions from European conventional and organic dairy farms. *Agriculture, Ecosystems & Environment, 112*(2), 207-220.

Stafford, D. A., Hawkes, D. L., & Horton, R. (1981). Methane production from waste organic matter. *CRC Press*, United States.

Steed, J. & Hashimoto, A. G. (1994). Methane emissions from typical manure management systems. *Bioresource Technology, 50*(2), 123-130.

Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., & de Haan, C. (2006). Livestock's long shadow: environmental issues and options. *Food & Agriculture Organization of the United Nations (FAO).*

Su, J.-J., Liu, B.-Y., & Chang, Y.-C. (2003). Emission of greenhouse gas from livestock waste and wastewater treatment in Taiwan. *Agriculture, Ecosystems & Environment, 95*(1), 253-263.

Thornton, P. K. (2010). Livestock production: recent trends, future prospects. *Philosophical Transactions of the Royal Society B: Biological Sciences, 365*(1554), 2853-2867.

LITERATURE REVIEW

According to the statistics from the UN's Food and Agricultural Organization (FAO), among the livestock, 19 billion are chickens, cattle are about 1.4 billion, while sheep and pigs are around 1 billion each. With a vast population, China is the world's leader in the number of pig, chicken, and sheep production. On the other hand, Brazil has had the largest amount of cattle closely followed by India (EPA, 2011). And, United States is in the second highest position with a huge population of chicken and pig, whereas the country has a third highest population of cattle. Figure 1 shows estimated top cattle producing countries in the world in 2017. Rising income and urbanization are the leading causes of increased demand for meat-based protein, thus driving the livestock production. Due to intensive farming, most of the cattle and pigs are raised in confined feeding operation and they are producing a large amount of manure in a smaller footprint. Table 1 shows the livestock population trends of last decade. However, due to increasing feed costs and environmental concerns (e.g., air, water, and soil pollution), livestock production facilities are under pressure to reduce environmental impact from animal agriculture. Therefore, proper management of livestock animals, livestock facilities, and manure management can play a significant role in mitigating pollutant gasses and GHG emission.

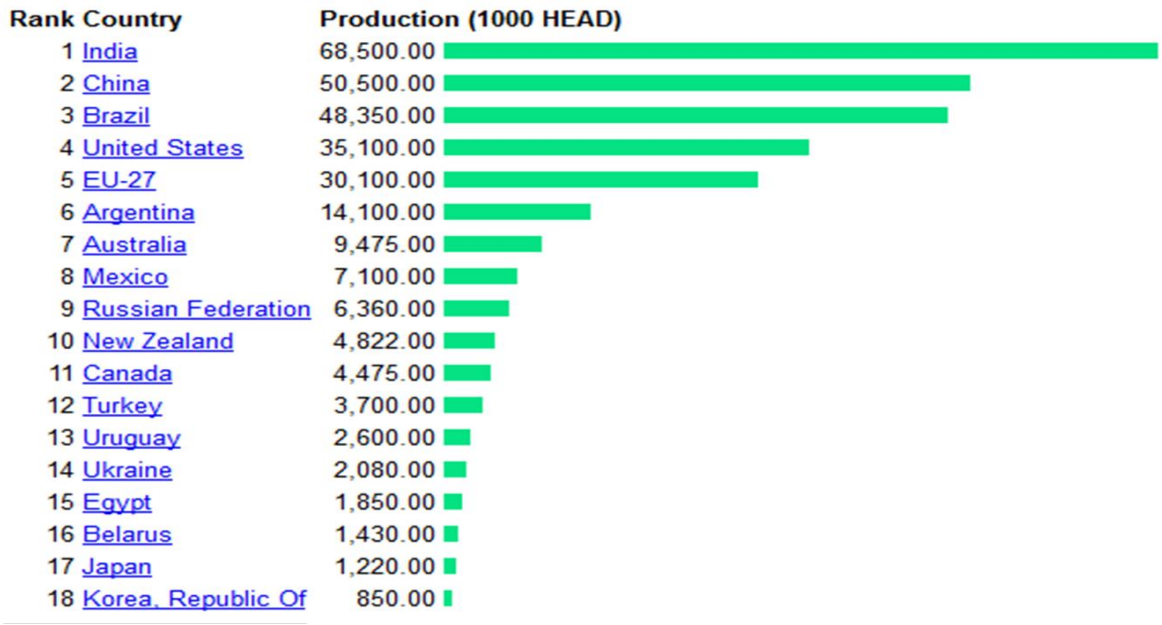

Figure 1. Eighteen top ranked cattle producing countries around the world (USDA, 2017)

Table 1. Increasing trend of livestock population around the world (Ali, 2015)

LIVESTOCK POPULATION 2013-14*			
Species	**2011-12**	**2012-13**	**2013-14**
Cattle	36.9	28.3	39.7
Buffalo	32.7	33.7	34.6
Sheep	28.4	28.8	29.1
Goat	63.1	64.9	66.6
Camels	1.0	1.0	1.0
Horses	0.4	0.4	0.4
Asses	4.8	4.9	4.9
Mules	0.2	0.2	0.2

*Estimated Figures based on inter census growth rate of Livestock Census 1996 & 2006

The Importance of Livestock Management

Substantial public debate and perceptible blame on the livestock production system exists. Most of those debates and blame are due to the utilization of natural resources as food supply and to the noteworthy volume of gaseous emanation during the progression of these collective benefit (Herrero et al., 2009). In most of the cases, enteric fermentation and livestock manure are treated as the reasons of impending emissions (Asis, 2008). Nevertheless, animal manure has the progressive contribution towards the supply of soil nutrients, enhancement of soil

structure, and reduction of attrition potential of the soil by vegetative concealment. Simultaneously, enteric fermentation is the unique digestive system for the livestock animals, to supply food into their bloodstream. However, probable risk of ecological pollution through excessive crop nutrient application and gaseous emission from both of these two process are also perceptible (Broucek, 2014; Knowlton et al., 2004; Leytem et al., 2011). Knowlton et al. (2004) have reported animal manure as an impending source of the nitrogenous and phosphorus contamination towards the deterioration of surface water quality in the United States. Simultaneously, negative impacts like as global warming, ozone layer depletion, acid rain, human and animal health concerns due to the gaseous emission of GHG and pollutant gases have become one of the major concern of the livestock industry (Asis, 2008). Figure 2 shows the most likely sources of gaseous emission within the animal housing and production system.

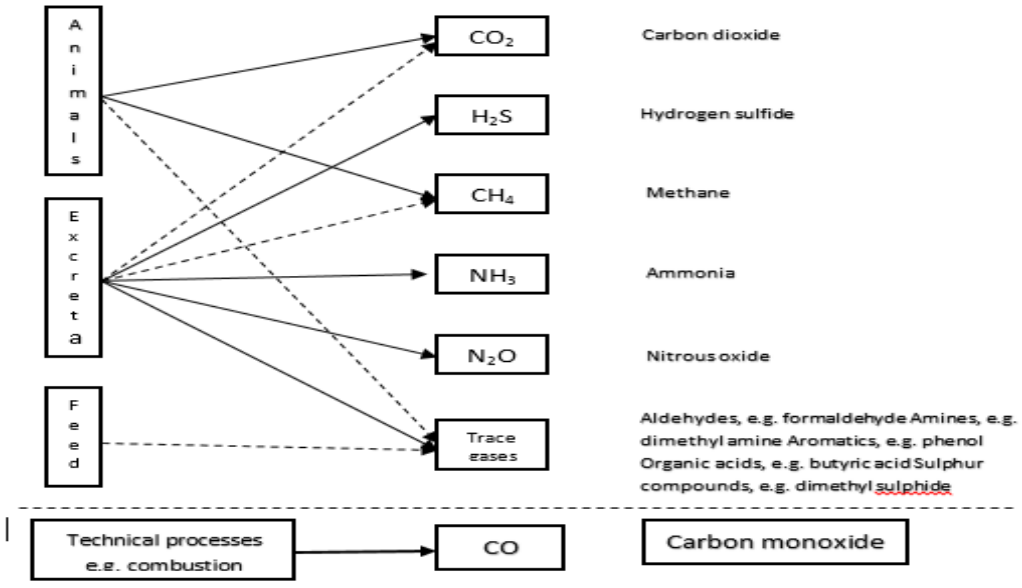

Figure 2. Sources of emission in the animal housing (Hartung & Phillips, 1994)

Casey et al. (2006) reported human and animal health concern due to the long time exposure to pollutant gasses within the livestock facility. It is also understandable that, not only

the human and animals within the facility, but also the vicinity of the facility have the effect as well. For example, odor from livestock production facility is a public nuisance and impediment to expanding an existing operation or establishing a new operation next to a neighbor. At the same time, property values also affected by the odor nuisance (Schiffman et al., 1995). Therefore, the proper management of a livestock production activity and manure management are key for sustainable livestock production system.

Greenhouse Gas Emission Sources

Tim Herzog (2009) has reported that "almost every major human industry and activities are directly or indirectly responsible for greenhouse gas emission". Environmental Protection Agency (EPA) has reported that electricity, heat production, industry, transportation, agricultural, forestry, and other land uses are the foremost sources of the greenhouse gas emissions. According to IPCC (2014) Figure 3 (a) shows electricity and heat production subsectors are the major contributors and are closely followed by agricultural and industrial sectors. Whereas, the other sectors contribution is almost half of any one of these or less than that. Of the total GHG contribution, 76% is CO_2 emission, followed by the CH_4 (16%) and NO_2 (6%) emission (Figure 3c). Boden, Marland, & Andres (2011) has reported the global distribution of the GHG emission and they found that China is the major GHG contributor followed by the United States. Figure 3 (d) shows that 28 and 16% of the world's total GHG emission is occurring from China and USA, respectively. Additionally, global GHGs emission by sectors shows that the agricultural sector contributes 24%, whereas the highest emission occurs from the electricity and heat production sector (25%) (Figure 3a). Although, the GHG contribution from agriculture in the United States is about 9% of the total GHGs emission but this amount is closely followed by the emission from commercial and residential sectors

emission (12%) (Figure 3b). From the latest demonstrated report, it has been reported that the agricultural sector, especially, livestock facilities is becoming the main source for CH_4 emission (Bennetzen et al., 2016).

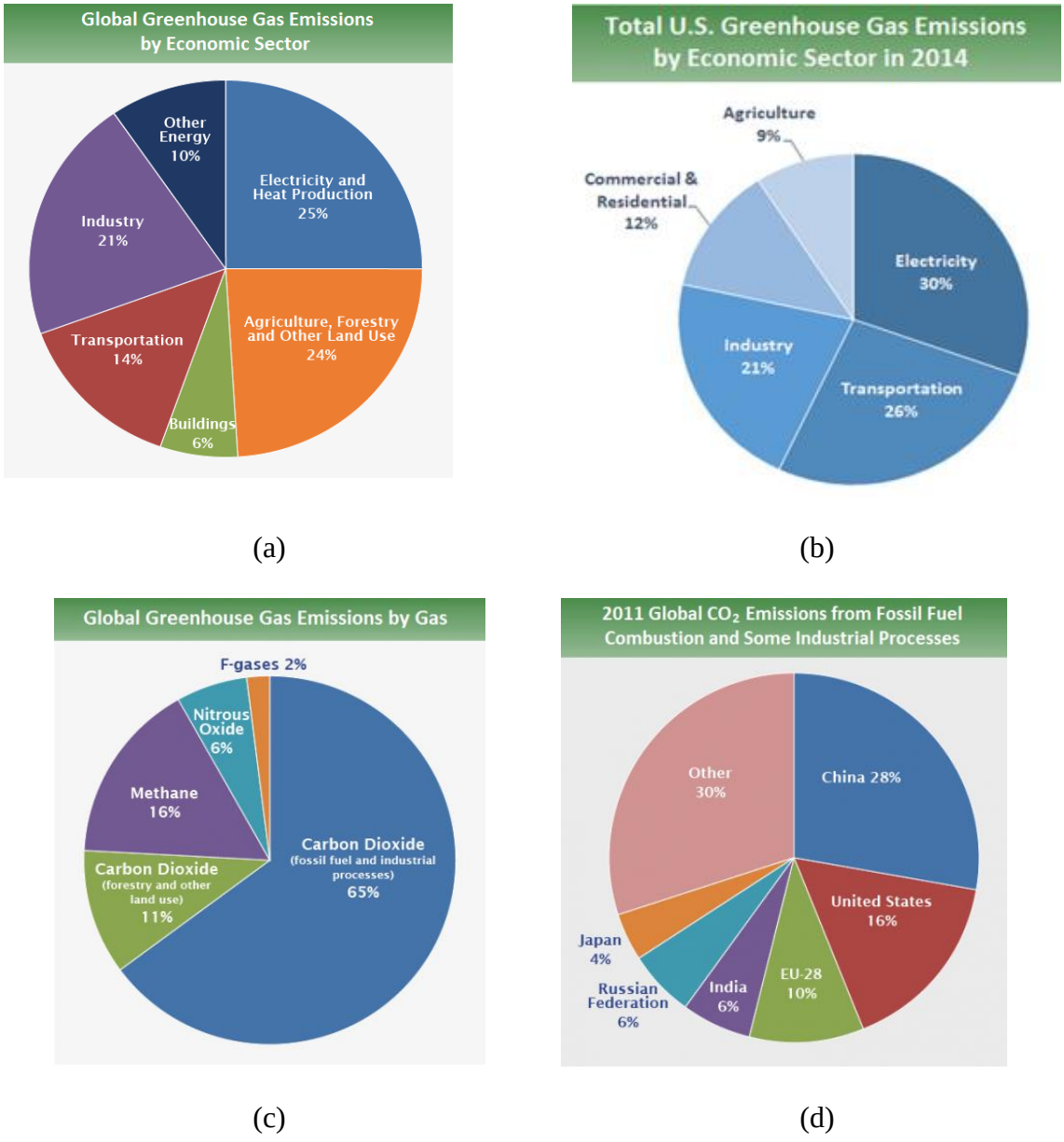

Figure 3. (a & b) Greenhouse emission sources globally and in the US, (c) Global Greenhouse gas emission by gas, (d) CO_2 emissions from different region all over the world. (EPA, 2014; IPCC, 2014)

Of the total GHG contribution of the agricultural sector, Cerri et al. (1996) has reported approximately 50% CH_4, 70% NO_2, and 20% CO_2 emissions occur from the agricultural sector. Within the agricultural sector, deforestation, land degradation, use of fossil-based fuel, soil carbon tillage, application of fertilizer, livestock feeding, residue management are the reason behind gaseous emission (Lesschen et al., 2011; Watson et al., 1992). Moreover, agriculture, forestry and other land use contribute 24% of total GHG emission globally. Additionally, livestock facilities are a major contributor to total agricultural sector's GHGs contribution. The amount is almost similar to that of industrial sectors emission and greater than that of transport (McMichael et al., 2007). Within the livestock facilities, animal production operations are the typical contributor of the airborne contaminants including dust, and microorganism along with GHG and odor (Casey et al., 2006). Major CH_4 emission contribution is the enteric fermentation and anaerobic storage of manure (Asis, 2008; Broucek, 2014). The whole process of the greenhouse gas emission from the livestock facilities is shown in Figure 4:

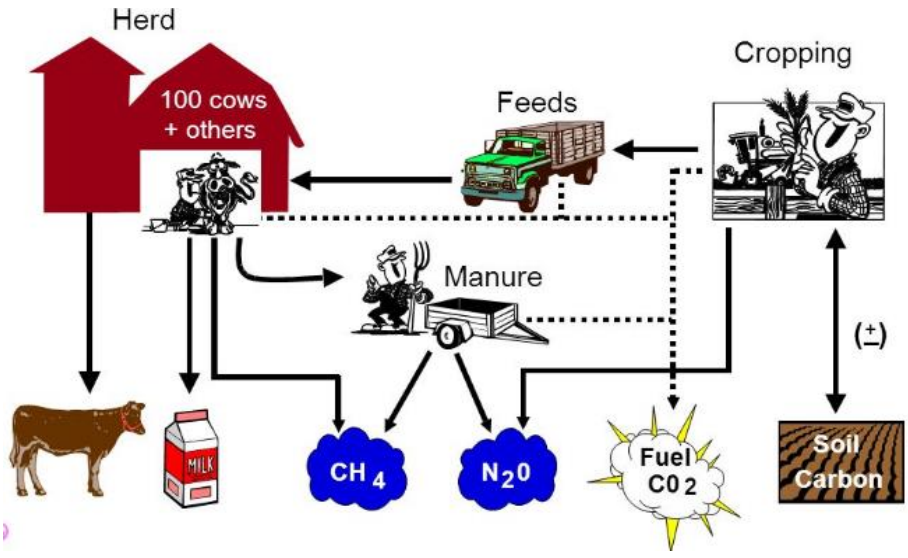

Figure 4. Gaseous emission from livestock sector (Source: Phetteplace et al., 2001)

Factors Affecting Gaseous Emission

Different factors like animal type, population density, diet type and composition, ambient and indoor temperature of livestock operations, manure type, manure management and storage system, and pH of manure determine the gaseous emission from manure (Borhan et al., 2013). Broadly few of these factors are described as follows:

Nourishing Practice of the Animal

Rumen chemistry of the ruminant animals highly depends on the chemical compositions present in the animal diet. As a whole, if carbon content is high on a diet then there is a possibility of a higher amount of methane and carbon dioxide emission due to animal exhalation and from the anaerobic decomposition of manure as well. Then again, if the diet contains a higher amount of sulfur compound or nitrogenous compound, then there is a possibility of a higher amount of H_2S, N_2O, and ammonia (NH_3) emissions (Martin et al., 2008). Borhan et al. (2013) reported the presence of carbohydrate sources within the animal diet can be a probable cause of volatile fatty acid (VFA) emission by altering the microbial fermentation process in the rumen. Furthermore, a diet containing a higher amount of starch content and low fiber diet can be effective in lowering the CH_4 emission both from rumen and manure. These can also reduce the acetate production and hence the reduction of enteric methane production (Beauchemin et al., 2008; Osada et al., 2011). Mills et al. (2001) and Ellis et al. (2007) have reported about the replacement of lignin, neutral detergent fiber, acid detergent fiber containing diets by starch to reduce acetate production and to increase propionate production. They also reported that it is possible to shift the fiber-digesting bacteria by replacing this two lignin and fiber-based diet and hence to minimize methane emission from enteric fermentation. Additionally, scientists also reported about changing diets to reduce the methane emission from the manure management

system as well (Beauchemin et al., 2008; Eckard et al., 2010; Kebreab et al., 2001). Moreover, Kebreab et al. (2006) have reported about lipid-based diets, which can minimize the hydrogen production to a lesser extent and hence methane.

Nitrous oxide (N_2O) emission from livestock animals is mainly from urine and animal feces (Dijkstra et al., 2013). Nampoothiri et al. (2015) have reported about the presence of nitrogen and crude protein (CP) in the animal diet as the reason behind the nitrogenous gaseous emission from livestock. The presence of protein content in a diet is also reported as the responsible reason behind unpredictable N_2O emission and which can also vary based on storage type (Külling et al., 2003). Changing the feed composition such as replacing protein content by some non-protein diets can be a viable alternative to reduce the overall nitrogenous emission from livestock and hence N_2O emission. Dijkstra et al. (2011), Tas et al. (2006), Edwards et al. (2007) and Kingston et al. (2010) have reported that replacing protein diet by carbohydrate diet minimize the amount of protein degradation in the animal rumen, thus minimize N_2O emission. The interaction between different types of carbohydrate and protein with the grass and fiber have also reported by Ellis et al. (2011) and they observed the reduction of nitrogenous emission in both cases. However, replacement of protein by grass fed carbohydrate showed less nitrogenous emission than that of fiber addition.

Housing Types of the Animals

Livestock buildings and anaerobic storage of animal manure are the two major sources of gaseous emission from any livestock production facility. Hartung & Phillips (1994) have reported more than 136 odorous compound emission from a livestock facility. Odor generated in livestock housing can exit the source and make its way to downwind neighbors. Odorous compounds consist of volatile fatty acids, aromatic compounds, volatile nitrogen containing the

compound, sulfur containing compounds, etc. (Rahman & Borhan, 2012). Besides odorous compound, livestock also generates GHGs, particulate matters, ammonia etc. (Seiler & Conrad, 1987). Among the gaseous emission from livestock compound, H_2S has the potential of causing severe health concern including death (Hilliger et al., 1984; Kaesebieter et al., 1985). Housing system consisting of the floor system, manure collection, and manure removal system are the determining factors of gaseous and other emissions (Borhan et al., 2012). Concrete flooring with a smooth surface having a gutter and drain can reduce gaseous emission from the housing (Zhang et al., 2005). Additionally, frequent removal of the manure, flushing liquid, aeration, pH and temperature are the principle determining factors for the emissions from confined animal housing facilities (Melse et al., 2009). Melse et al. (2009) have also reported about the possibility of reduction of gaseous emission by 30-80% by developing all of the above determining factors in a confined animal housing.

Management System of the Manure

Collection, storage, processing and treatment, disposal and field application of manure falls under the manure management system (De Vries et al., 2015). Aerobic and anaerobic are the two types of manure decomposition processes, those are practiced all over the world. In the open grazing system decomposition of manure occurs aerobically, resulting in higher amounts of CO_2 production. Whereas, anaerobic decomposition of manure is predominant in the confined manure management system and mostly leads to CH_4 production. Besides these two gasses, N_2O, H_2S, and NH_3 are some other gases that also generate from the manure decomposition system. Bacteria, archaea, and other microbial populations are involved in the decomposition process and in the production of GHGs and H_2S (Sejian et al., 2015). Chadwick et al. (2011) have reported about two principle steps for gaseous emission from manure. Conversion of the organic

compound present in the manure is one of them and volatilization of the new compound to the open air is the other one. They reported three processes for the conversion and volatilization processes: 1. Conversion of ammonium (NH_4^+) into NH_3 and volatilization of NH_3, 2. Conversion of organic matter into CH_4 and CO_2 and volatilization of both of them, and 3. Conversion of NH_4^+ and NO_3^- into N_2 and finally into N_2O and NO and then volatilization. Many technologies exist to minimize the gaseous emission, but they are specific to a target gas and they are not well established (Groenestein, 2006; Jarvis & Menzi, 2004). Additionally, they may be effective in reducing one gas, but likely to increase the emission of other gases (Berg et al., 2006; Velthof & Mosquera, 2011). Therefore, new technologies need to be developed to reduce overall gaseous emission hence to minimize emission from livestock production facility as well as anaerobic storage of manure.

Environmental Factors (pH and temperature)

Temperature effect on the solubility of oxygen and hence on the metabolic microbial activity is well established. The relationship between temperature and anaerobic digestion is reported by a many of the previous researchers. Angelidaki & Ahring (1994), Hansen et al. (1998) have reported a correlation between CH_4 emission and temperature range (25-44˚C). They reported that lower CH_4 emission with lower ambient temperature and vice versa. Hashimoto et al., (1981) have observed that degradation of the manure organic compounds is higher at a higher temperature, thus may generate higher volatilization. Biotransformation of the organic compounds under anaerobic condition mainly depends on the temperature and pH of the digestion system and transformation of the organic compounds is directly related to the gaseous emission from those (Hahne et al., 1992). Hydrolysis, acidification, and methanogenesis are the reactions essentially controlled by the temperature and these are the three main steps towards

methane production from carbohydrate, fat, and protein (Li et al., 2011). Hastening of the nitrogen and phosphorus alteration is reported due to the escalation of these reactions by intensified temperature. In addition, the upsurge of organic matter hydrolysis is also reported in low pH. Although, biotransformation of nitrogen and phosphorus is also reported at high pH (Angelidaki & Ahring, 1994).

Greenhouse Gas Emission from Livestock Facilities

Although the livestock is providing food and nutrients for the humankind, the relations between the livestock's and the environment have become complex and multifaceted. In addition to that, it is raising severe trepidations all over the world (McMichael et al., 2007). Within the livestock facility, manure is an impending source of inorganic nitrogen, phosphorus, sulfur, carbon, and water, hence it has vast use as plant and soil nutrient. Nevertheless, carbon, nitrogen, and water can serve as an essential substrate of the microbial population present in the manure and they can be responsible for the production of N_2O, CO_2, and CH_4 (Chadwick et al., 2011; Defra, 2010; Møller et al., 2004; Paul et al., 1993). The sulfur-containing organic compound can be converted into H_2S (Arogo et al., 2000). Besides the gaseous emission from enteric fermentation, generation of gasses from the livestock facilities can be divided into three sources (Figure 5): (1) Just after the excretion, (2) during the storage and treatment, and (3) during and following the land or field application of manure (Casey et al., 2006; Chadwick et al., 2011).

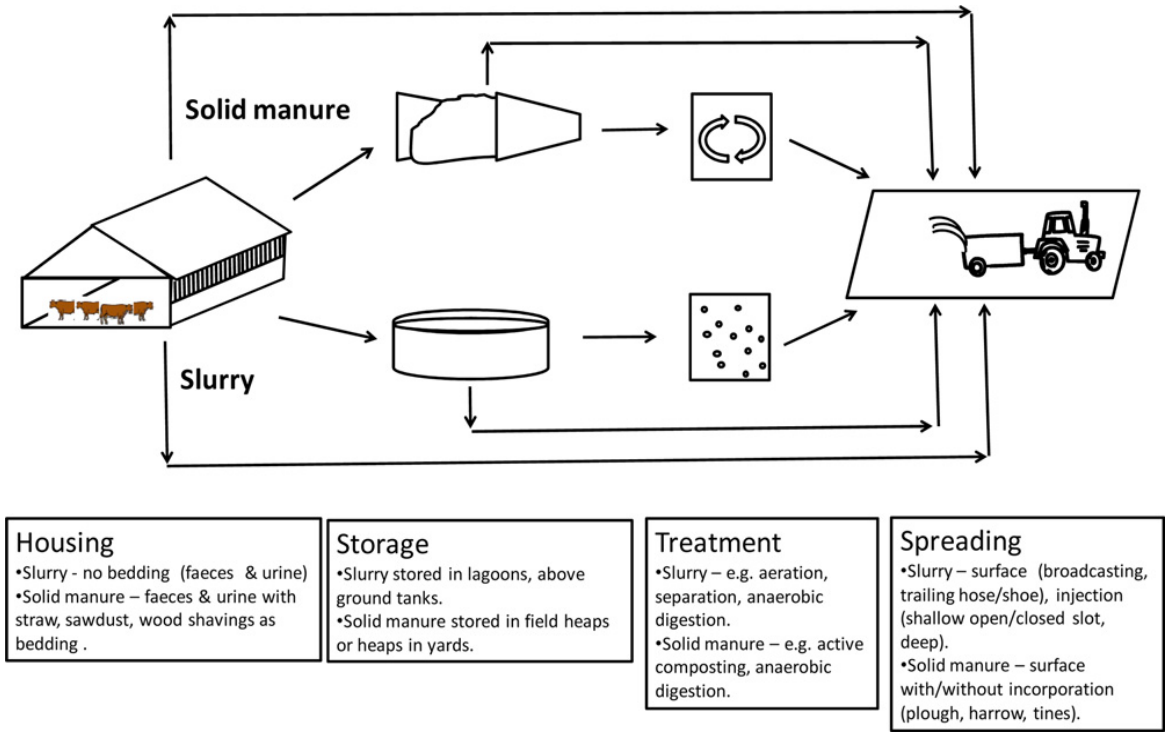

Figure 5. Periods and places for gaseous emission from manure management system (Chadwick et al., 2011)

Foster et al. (2007) and Berlin & Uhlin (2004) have reported the maximum impacts occur during the livestock production system (e.g., manure production, collection and storage, and land application) compare to other food chain system. Enteric fermentation and manure management system is roughly contributing 37% of the global CH_4 emission and around 42 % of the global N_2O emission (Karakurt et al., 2012; Steinfeld et al., 2006). From the biogeochemistry point of view, since manure is a complex compound mostly with organic substances with a few minerals in it, it undergoes a series of reactions during the decomposition process. Among them, hydrolysis, ammonia volatilization, nitrification, denitrification, fermentation etc. are few which are responsible for the emission of CO_2, N_2O, CH_4 and ammonia (NH_3) from manure (Li et al., 2012). Furthermore, anaerobic bacterial degradation of the organic substances in manure is also responsible for gaseous emission, especially, emission of CH_4 and CO_2 (Steed & Hashimoto,

1994). Besides anaerobic degradation process, aerobic degradation also leads to a significant amount of CO_2 production (Møller et al., 2004).

Methane Emission

In comparison with other GHGs, CH_4 is the most important constituent emitting from manure (Kemfert & Schill, 2009). Although CH_4 has a relatively short atmospheric lifespan of twelve years, it has almost 25 times more global warming potential than CO_2 as a GHG (Solomon, 2007). Pearman et al. (1986) and Harriss (1989) have reported that the atmospheric CH_4 concentration has become double within last 200 years. Usually, CH_4 is generated from the decomposition of the organic material in absence of oxygen (anaerobic decomposition) and its contribution is almost 16% of the total greenhouse gaseous emission. Ruminant animals, manure, manure management systems and other agricultural sources are the sources of anthropogenic methane production as well (Bauer et al., 2009; Dong et al., 2011; Kemfert & Schill, 2009; Park et al., 2011; Wang. S. et al., 2009).

Methane emission from livestock is mostly from the domesticated ruminant animals and from the animal manure (Karakurt et al., 2012). 18 % of the global GHGs emission is from manure management and 35-40 % of this emission are from enteric fermentation CH_4 stands for (McMichael et al., 2007)**.** Since most of the feed intake of the animals is organic and cellulosic materials, hence their digestion process results in CH_4 emission. Moreover, fermentation process called enteric fermentation within the rumen of the ruminant animal is reported for a significant amount of CH_4 emission. Alemu (2011), Sejian et al. (2011); and Grainger & Beauchemin (2011) have described the enteric fermentation and reported about the microbial activity in the animal's gastrointestinal system during the enteric fermentation process those are responsible for the enteric CH_4 emission (Garcia-Apaza et al., 2008; Huarte et al., 2010; Jones et al., 2011).

Essentially, the active presence of microbial community such as methanogenic bacteria break down the carbohydrates present in the animal feed and results in methane production, and hence exhalation of CH_4 as a byproduct (Lassey et al., 1997). *Methanobrevibacter ruminantium, methanosphaera stadtmanae, methanomicrobium* are the few methanogenic bacteria responsible for CH_4 production in the animal digestive system (Jarvis et al., 2000).

Simultaneously, to support the CH_4 emission from manure, Johnson and Ward (1996) has reported about approximately 10-14 Tg global CH_4 emission from manure discarding system. In addition, Environmental Protection Agency (EPA) (2011) has reported about only 1% increase of CH_4 emission from 1990 to 2000 and predicting a stiff increase of 12% within next 10 years. Møller et al. (2004) have reported about 90% of the biodegradable organic fraction of the manure and can convert into CH_4 gas. Throughout the process, a complex mixture of the microbial population in absence of oxygen converts the organic materials mostly into a mixture of CH_4 (60%) and carbon dioxide (40%) alongside a small amount of water and hydrogen sulfide (Wilkie, 2005). As mentioned previously four reaction steps (hydrolysis, fermentation, methanogenesis and acetogenesis) are involved in the overall anaerobic digestion process. Among the reactions, hydrolysis is involved in the conversion of carbohydrates, proteins, and fats present in the manure towards sugar, amino acids, and fatty acids. Hydrolysis process is further followed by the fermentation process and later on acetogenesis and methanogenesis. Figure 6 shows the reaction processes and the products in each step. During the course of anaerobic digestion processes sugar, amino acids and fatty acids are converted into volatile fatty acids and then acetic acid, hydrogen (H_2), and CO_2 to give the final product of CH_4 and CO_2 (Li et al., 2011).

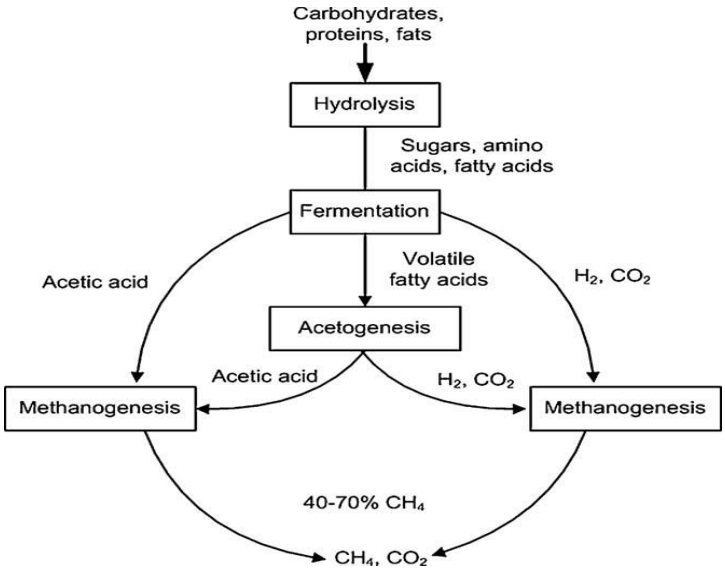

Figure 6. Degradation of organic material present in manure by anaerobic digestion process. (Li et al., 2011)

Carbon Dioxide Emission

Carbon dioxide (CO_2) gas is one of the most common GHG gasses within the livestock facilities, since it is produced due to exhalation and from the decomposition of manure (Ni et al., 1999). Fermentation of carbohydrates and cellulosic materials in the rumen is the source of CO_2 generation in the rumen. However, fermentation and neutralization of hydrogen ion (H^+) and bicarbonate ion (HCO^{3-}) entering the rumen in saliva and across the ruminal wall during VFA absorption are one of the main reason for CO_2 generation (Dehority, 2003; Hristov et al., 2013). In addition to that, there are two reported ways of carbon dioxide generation from animal manure: 1) Animal urea can be easily hydrolyzed and thereafter catalyzed to produce CO_2 and NH_3; and 2) Anaerobic decomposition of the organic components present in the manure can be another way of CO_2 production from manure and manure management system (Aarnink et al., 1995). Again, manure storage in presence of oxygen (aerobic) allows the bacterial population to continue their activity and allows them to produce a higher amount of CO_2 then the anaerobic storage in absence of oxygen (USDA, 2007). It is also reported by Ni et al. (1999) that, faster

ammonia gas generation within the pig house is related to CO_2 generation from manure. Hence, not only the production of CO_2 itself is a problem, but also it can cause some other problems as well.

Nitrous Oxide Emission

As a GHG, N_2O has the most detrimental effect on stratospheric ozone (Crutzen, 1974; Lashof & Ahuja, 1990; Yung et al., 1976). The agricultural sector is the biggest source of N_2O emission and responsible for 67% of the nitrous oxide emission (Denman et al., 2007; IPCC, 2007). IPCC (2007) also reported about 70-90% ingested nitrogen in the animal feed is released via animal waste and contributes to environmental N_2O. Fertilized agricultural soil, livestock manure storage, and manure handling stand for 42% of this direct agricultural emission. While surface runoff and leaching of fertilizer are responsible for 25% emission. Water from rainfall or from irrigation in the cultivated land can leach out some of the nitrogen fertilizer towards drainage or groundwater and eventually break down into the excessive nitrogenous compound in that area in presence of microbes and lead to N_2O emission (Denman et al., 2007; Ussiri & Lal, 2013).

Furthermore, application of manure to the soil and urine deposition by the grazing animals are reported as one of the major contributory sources of N_2O (Brown et al., 2001). Kroze et al. (1999) have reported livestock waste is contributing 35% of the direct global N_2O emission. By applying the animal manure in the soil as fertilizer, it increases the mineral N in the soil and hence leads to the higher emission of N_2O (Velthof et al., 2003). Fertilized soil with animal wastes is estimated at 0.6 (range: 0.12-1.1) Tg N $year^{-1}$ of direct N_2O emission globally (Mosier et al., 1998). Production of N_2O in the soil from animal manure depends upon the microbial nitrification and denitrification process. Moreover, this complex process controls

several factors such as soil mineral N, available C, O_2, and soil temperature (Granli & Bøckman, 1994). Again, degradation of the organic matter by the microbial population present in the animal manure itself is one of the simplest manure management process called composting (Bernal et al., 2009). The organic nitrogen present in the fresh animal manure can be used as an energy source for the microbial community including bacteria and fungi and can be degraded into ammonium. Since it's an exothermic process, part of the produced ammonia can be lost by volatilization or conversion into N_2O or N_2 through nitrification/denitrification process (Maeda et al., 2011). Both in the case of manure in the soil or in the form of compost, nitrification occurs followed by denitrification (Figure 7).

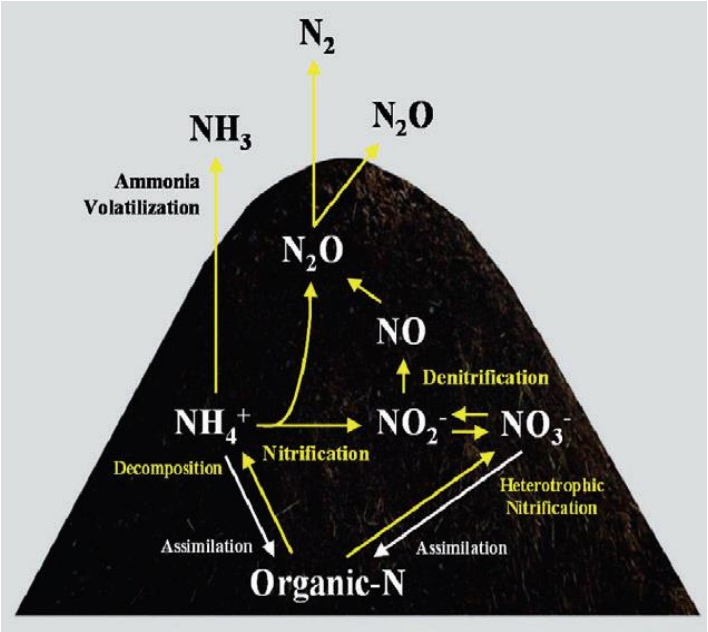

Figure 7. Nitrification and denitrification process (Maeda et al., 2011)

In the nitrification step, the transformation of ammonium to nitrate occurs due to aerobic digestion. The formation of nitrogen gas from nitrate reduction occurs in the denitrification step by anaerobic digestion (Monteny et al., 2006). Ammonia oxidation and nitrite oxidation are two reaction steps involved in the nitrification process (Kowalchuk & Stephen, 2001). Bacteria,

archaea, and fungi are reported for carrying out overall nitrification reaction, but the individual microbial group is responsible for each reaction step of nitrification (Laughlin et al., 2008; Leininger et al., 2006). Moreover, Kowalchuk & Stephen (2001) have reported about ammonia-oxidizing bacteria (AOB) and nitrate-oxidizing bacteria (NOB) for two consecutive reaction steps. As a consequence of hydroxylamine oxidation, nitrous oxide is known to be produced (Brochier et al., 2008).

In the denitrification process, heterotrophic denitrifiers act to reduce nitrate or nitrite in the nitrification step by nitrifiers to produce N_2O and N_2. As shown in Figure 7, nitrate (NO^{3-}) to nitrite (NO^{2-}), nitrite to nitric oxide (NO), nitric oxide to nitrous oxide, nitrous oxide to nitrogen (N_2) are the four chemical reactions involved in the denitrification process (Rudolf & Kroneck, 2005; Tavares et al., 2006). Low organic carbon content, low oxygen pressure, low pH and high nitrogen content are reported as the favorable condition for the denitrification process (McGinn & Beauchemin, 2012).

Hydrogen Sulfide Emission

The increase in hydrogen sulfide (H_2S) emission with intensive livestock production is one of the major concerns in today's world (Yokoyama et al., 2016). Both for odor nuisance and health hazard, H_2S is treated as one of the most harmful gasses associated with animal manure. Bacterial reduction of sulfate and anaerobic bacterial decomposition of the sulfur-containing organic materials present in the manure results in the hydrogen sulfide emission from animal manure and manure management system (Arogo et al., 2000; Hooser et al., 2000). *Pseudomonas, Citrobacter, Aeromonas, Salmonella and Escherichia coli* are some of the bacteria widely known as H_2S producing bacteria.

Almost one-half of the offensive odorants from swine manure is reported as due to H_2S and other sulfur compounds (Clark et al., 2005; Trabue et al., 2011). With the odor of rotten eggs at low concentration (<1 ppm), it can act as an irritant of the eye and at high concentration, it can be fatal both for human and animal (Donham et al., 2006; Hooser et al., 2000; Hays et al., 1972). Most of the human and animal mortalities are reported due to H_2S production (Curtis, 1983). It has also a corrosive nature, which can cause corrosion and deterioration of concrete structures of livestock buildings and equipment (Assaad et al., 2003).

Greenhouse Gaseous Emission Reduction Procedures

Since the gaseous emission from the livestock sector is increasing day by day with the increasing number of livestock all over the world, hence newer mitigation strategies are also needed to minimize GHG contribution from livestock production facilities and manure storage systems. Application of masking agents, enzymes and bacterial preparations, feed additives, chemicals, air scrubbers, biofilters and new ventilation systems are the few processes which have been already studied to minimize the emission from the livestock housing systems (Sutton et al., 1999). Based on the available literature, technologies associated with buildings, technologies used for manure storage, feed modifications, and land application are the four existing gaseous emission reduction approaches. Animals' excreta, deposited or stored manure and urine are the main sources of gaseous emission from an animal husbandry. Table 2 shows the possible alternatives and their relative ammonia emissions within the animal housing. It also showed that application of bedding is one of the best approaches. In addition to that as shown in Table 2 Danish system, fully slatted floor and liquid manure are the possible alternatives closely followed by each other in terms of reducing emission and the partly slatted floor is the worst one among these five (Hartung & Phillips, 1994).

Table 2. Effect of flooring on the overall gaseous emission from livestock facility (Source: (Hartung & Phillips, 1994)

Animal species	Keeping system	Emission (Kg/LU)
Pig	Danish System	11.7
	Fully slatted floor	12.0
	Partly slatted floor	21.7
	Liquid manure	7.5
	Bedding	1.7

LU: Livestock Unit

To show the diet modification effect on the gaseous emission, Ball & Möhn (2003) have reported about 25-30% CH_4 emission reduction from the growing pigs and 10-15% emission reduction from the cows by introducing low protein diet. Again Atakora et al. (2003) has reported about 5% CO_2 emission reduction by changing the barley-based diet into corn-based diets. They also reported about a comparative study by reducing the protein content both in barley and corn based diet and end up with 57% CH_4 emission reduction with barley-based diet.

However, management of feeding strategy includes management of nutrition and manipulation of rumen digestion. Researchers already reported that fermentation of carbohydrate is responsible for VFA generation and eventually CH_4. Among the carbohydrates, cell wall carbohydrate and a roughage-based diet have a higher potential towards CH_4 generation compared to a diet that rich in starch and favor propionate production. Additionally, change in the forage species, good forage processing, reduction of forage maturity, and increased feeding frequency are also noteworthy few strategies those fall under the mitigation strategies of management of feeding strategy (Boadi et al., 2004). However, Benchaar et al. (2001) have reported about the changing the forage species into alfalfa from timothy hay reduced the gaseous emission. Again, Robertson & Waghorn (2002) have reported about the reduction of ruminant gaseous emission by changing the feed into one that stored for a short period of time compared to that of long-term. Furthermore, scientists have also reported about the addition of fats can also

reduce the enteric gas production. Simultaneously, scientists also reported about the addition of low chain fatty acids can reduce the higher amount of gaseous emission compared to another fatty acid (Dohme et al., 2000; Dong et al., 1997; Machmüller & Kreuzer, 1999). But all of these approaches resulted in the very small amount of gaseous emission reduction and most of the cases the mitigation strategy is focused on the reduction of methane. So, better reduction strategy focusing all of the GHGs and other pollutant gasses need to be developed.

Furthermore, since all of the ruminant gasses production is related to the microbial population within the rumen, hence control of the microbial population by direct or indirect immunization are two of the practiced method. Application of the vaccine against three selected methanogen bacteria to reduce methane production was reported by Martin et al. (2010) and Wright et al. (2004) but they have reported about the probable limitation of the vaccines depending on geographical regions. Likewise, reduction of ruminant methane by the application of chloroform is possible but not suitable for practice (Bauchop, 1967; Clapperton, 1974). Additionally, conversion of chloral hydrate into chloroform can also lead to a reduction of methane in the rumen, but there is a possibility of damage to the animal liver and death of the animal for a prolonged period of feeding is also possible (Prins, 1965; Quaghebeur & Oyaert, 1971). Application of amichloral, tri chloroacetamide, and trichloroethylene adipate are few, although in every case there is a possibility of negative impact on the animal for a prolonged period of feeding (Clapperton, 1974, 1977; Trei et al., 1971; Trei et al., 1972). So, by taking all of the issues into consideration it is important to develop a new sustainable technique that can reduce the gaseous emission without obstructing animal health.

Moreover, since the storage of liquid manure is one of the main reason for gaseous emission, hence to minimize the emission from the stored manure are in major concern.

VanderZaag et al. (2008) have reported that the floating cover made from natural resources, from the synthetic origin and a combination of both of these are able to reduce pollutant gas emission. The authors reported consisted reduction of significant amount H_2S and about 70 % NH_3 reduction, ~40 % to ~90 % odor reduction within a time period of less than two weeks of their study. Moreover, Chadwick et al. (2011) have reported about three different alternatives including animal housing, manure storage and land spreading of manure to minimize the nitrous oxide and methane emission (Table 3).

Table 3. Potential mitigation methods for N_2O and CH_4 (Source: (Chadwick et al., 2011)

	Nitrous oxide	Methane
Animal house	• Modify feeding strategy • Adopt a slurry based system compared to a straw or deep litter based system	• Modify feeding strategy • Removal of slurry from beneath the house • Cooling slurry. E.g. below the slatted floor
Manure stores	• Modify feeding strategy • Keep anaerobic (e.g. cover and compact) • Adopt a slurry based system compared to a straw of deep litter based system • Add additional straw to immobilize ammonium-N	• Modify feeding strategy • Removal of slurry from the slurry store • Minimizing slurry volume stored in summer months
Land spreading	• Modify feeding strategy • Nitrification inhibition • Spring application of slurry • Integrate manure N with fertilizer N • Slurry separation? • Solid manure incorporation?	• Modify feeding strategy

However, all of the above approaches including all other widely used in today's world are time overwhelming, laborious and required skills, are effective for a short period of time and can be targeted for one or two gaseous emissions rather than targeting as a whole. Therefore, scientists are still looking for new and ground-breaking technologies to minimize all of the issues

with current approaches and by targeting multiple gaseous emission reductions by using one approach. Nanotechnology has shown its potentiality in a versatile field of application including electronics, construction management, and medical but still it has very limited use in agriculture and in manure management system. Hence, it might be a viable alternative to minimize the existing issues with manure management in terms of reduction of gaseous emission.

Overview of Nanotechnology and its Application

The word 'Nano' originated from the Greek word "nanos" meaning a dwarf. The study of materials within the size range of 1-100 nm is termed as nanoscience (Rotello, 2004). Technology based on nanoparticle termed as nanotechnology is now frequently considered as an "enabling technology" (Mann, 2006). Developing fields of scientific interest all over the world including research and development in Europe and North America are now based on nanoscience and technology (Fernandez & Hullmann, 2007; Zweck et al., 2008). The technology now includes a huge field of application starting from daily life towards industrial sector. It has been reported that more than 800 nanotechnology-based products are in use every day and more products are expected to appear within a few years (Maynard et al., 2006; Rejeski & Lekas, 2008). Dawson (2008) reported about more than 15 % of the global production will be derived by the incorporation of the nanotechnology within a decade.

Nanoparticles have versatile and safe use in various fields such as in wastewater treatment, environmental sciences, drug delivery, food and bioprocessing industries, electrochemical and sensors and biosensors. In the field of food and bioprocessing industry besides smart food packaging and nano capsulation of bioactive food compounds, nanotechnology has already its active participation in the application of biosensors to identify specific bacterial population and to monitor food quality (Neethirajan & Jayas, 2011). Like in

electrochemical and biosensors, nanoparticles already showed their tremendous potential on designing new and improved sensing devices. Due to their unique physical and chemical properties, nanoparticles such as metal, oxide and semiconductor nanoparticles can play a different role in different sensing systems. Immobilization of biomolecules, catalysis of electrochemical reactions, and the electron transfer between electrode surfaces and proteins, labeling of biomolecules and acting as a reactant are a few of the developed application of nanoparticles (Luo et al., 2006).

Though there is the huge application of nanoparticles and nanotechnology have already been reported in many other fields, but until now the application of nanotechnology in pollution control is far behind those of others. Ground water remediation, sustainable water supply, agricultural pollution and degradation sensing, management of insect pests are a few applications of nanoparticle in the field of pollution control (Baruah & Dutta, 2009; Mueller et al., 2012; Qu et al., 2012; Rai & Ingle, 2012).

Among the wide variety of existing nanoparticles for pollution control, titanium oxide nanoparticle (nTiO2), nano scale zero valent iron (NZVI), carbon nanotube, copper oxide nanoparticle (nCuO), silver nanoparticle (nAg), Zinc Oxide nanoparticle (nZnO) are a few with a wide range of applications (Arogo et al., 2000; Kanel et al., 2005; Kong et al., 2010; Masciangioli & Zhang, 2003; Ren et al., 2009; Thompson et al., 2010). Most of these NPs have a wide variety of uses due to their unique individual properties. Due to its self-cleaning and bactericidal properties, nTiO2 has the potential as an antimicrobial agent and is widely used in gas sensing devices (Kong et al., 2010). The NZVI has application for contamination removal both from soil and water (Thompson et al., 2010). Probable effective application of nCuO against certain pathogens is reported by (Ren et al., 2009). However, both nAg and nZnO have a

wide range of applications over any of these NPs. most extensive use of silver compound for fighting infections and controlling spoilage is also reported. With the broad spectrum of antibacterial agent, nAg is one of the most commercialized NP and extensively used for consumer and medical products (Pal et al., 2007; Xiu et al., 2012). Environmentally friendly nature with easy fabrication and non-toxic synthesis route introduced nZnO as a better option in environmental, biological and industrial application areas (Vaseem et al., 2010). But, both of these has very limited application knowledge in GHGs and H_2S gas emission mitigation from manure management. So, like other fields application of nAg and nZnO in manure management system needs to be explored.

Very limited application of nanotechnology in the mitigation of greenhouse gaseous emission and especially on the mitigation of the enteric fermentation and manure management have been reported. Although Asis (2008) and Gautam et al. (2016a) have reported the application of ZnO nanoparticle to mitigate odor and gaseous emission from the swine and dairy manure, none of them have characterized the mitigation option clearly, such as whether the mitigation is by the absorption of the gaseous emission into the nanoparticle surface or by the reduction of the microbial population. Gautam et al. (2016b) characterized the nZnO in reducing gaseous emission from swine manure and found that reduction of H_2S is likely due to chemical conversion. However, additional studies are needed to characterize the reduction mechanism. Moreover, recovery and reuse of the applied nanoparticle should be the prime concern, since the use of nanoparticles may have some other environmental concerns over the years. Therefore, it is critically important to study gaseous reduction mechanism as well as the fate and transport of the applied nanoparticle.

Application of Zinc Oxide Nanoparticle

The introduction of metal oxide nanoparticle brought a new class of important materials and was developed mainly for their use in research and health-related applications. Besides their wide variety of physical and chemical properties, highly ionic metal oxides came into the spotlight of research utilization due to their highly antibacterial activity. The application of conventional metal oxides is widespread compared to their counterpart as nanoparticle metal oxide.

Zinc oxide (ZnO) is being used worldwide in consumer products and industrial applications (Sharma et al., 2011). Unique photocatalytic, electrical, electronic, optical, dermatological and antibacterial properties of nZnO have introduced numerous application (Arnold et al., 2003; Becheri et al., 2008; Li et al., 2006; Pan et al., 2001; Turkoglu & Yener, 1997; Xiong et al., 2003). Synthesis of nZnO have been reported from the 1960s as thin films and from then until now nanostructured ZnO materials are in broad attention due to their extensive usage in sensors, transducers, optics, photonics and as catalysts. With a diverse group of growth morphologies, such as Nano combs, Nano rings, Nano helixes, Nanobelts, nanowires and Nanocages nZnO is known as a versatile functional material. Application of ZnO in mechanical actuators and in piezoelectric sensors is due to the lacking of the center of symmetry in wurtzite, combined with a large electrochemical coupling which in turn introduced its strong piezoelectric and pyroelectric properties. Furthermore, with a wide bandgap of 3.37 eV and high exciting binding energy of 60 meV, ZnO is suitable for short optoelectronic applications and can confirm efficient excitonic emission at room temperature and room temperature ultraviolet (UV) (Wang, 2004).

Metallic zinc oxide has already proven its antibacterial properties in In-Vitro but nZnO do not have any such proven application for In-Vitro. Although, initial studies and preliminary growth analysis data demonstrated that nZnO have a wide range of antibacterial properties and in some cases, the antibacterial is five times more than that of other metal oxide nanoparticles. The antibacterial properties of the nZnO, however, is somewhat dependent on their size and exposure to normal visible light (Jones et al., 2008).

Nano zinc oxide has also its footprint in mitigating GHGs and other pollutant gasses from the waste management field. Hernández et al. (2011) and Abatzoglou & Boivin (2009) have reported the removal of H_2S gas during purification of biogas and throughout the drilling work for oil gas. They also reported about the high reactivity of nZnO towards sulfur compound and desulfurization of sulfur compounds in presence of nZnO and formation of ZnS during the course of the reaction. However, there is a high probability of H_2S reduction due to chemical interaction with the substrates and hence reduction possibility of the microbial population also exists. Besides H_2S, nZnO has also an affinity to react with NH_3 gas as well. However, it is likely that nZnO will react more with H_2S than the NH_3, if the system contains both of these gasses (Chung et al., 2005). Recently, application of nZnO in both swine and dairy manure to mitigate H_2S, CH_4, CO_2 gaseous emission and odorous emission have also been reported (Gautam et al., 2016a; Predicala et al., 2012). Nevertheless, none of the published articles reported the detail reduction mechanism of all of this emission. Therefore, working principle of the nZnO within the manure management towards GHGs and another pollutant gaseous emission reduction needs to be explored.

Application of Silver Nanoparticle

Application of silver to make water potable is notable from 1000 B.C. and since then it has been used for centuries for the treatment of burns and chronic wounds (Castellano et al., 2007). Ions, nanoparticles, and compounds are the three most common forms of silver that have been used for industrial and consumer products (Nowack, 2010). Metallic silver, silver nitrate, and silver sulfadiazine are the common forms of silver compound which have been used for a long time. Besides the application of silver in burns, wounds and bacterial infections, silver is also well-known due to its use as an active catalyst towards the preparation of formaldehyde from methanol and ethylene oxide from ethanol. Colloidal silver has good conductive, chemical stability, catalytic, antibacterial and some other distinct properties as well. Application of silver as preservatives in the form of inorganic composites with a slow release of silver and silver thiosulfate complex within a new compound of silica gel microspheres and its use in plastic material for the long-lasting antibacterial property is also noteworthy (Sharma et al., 2009). The introduction of new antibiotics limited the use of silver as an antibacterial agent. drastic change in the chemical, physical and optical properties of metallic silver in the direction of silver nanoparticle formation made a remarkable comeback of the silver as an antibacterial agent. Silver nanoparticle also proved its usefulness against the pathogenic bacteria which are resistant to certain antibiotics. Therefore, silver nanoparticle appeared with its diverse application in the medical field as well (Frattini et al., 2005; Nagy & Mestl, 1999; Rai et al., 2009; Sharma et al., 2009).

In most of the cases, the bactericidal effect of silver as a silver ion is well acknowledged; although, the way of action in the direction of this effect is only partially understood to some extent only (Morones et al., 2005). The application rate of silver and release of silver ion is the

main driving force behind the higher antibacterial properties of silver. In the metallic state silver is inert, however, it is highly reactive in its ionic state. In its ionic state silver can bind with the tissue protein and can change the structure of the bacterial cell and nuclear membrane which can lead to cell distortion and eventually death (Castellano et al., 2007; Richard et al., 2002). Active interaction of the ionic silver with the thiol group of vital enzymes to deactivate them and decreasing replication ability of bacterial DNA treated with silver ion are two of the experimental findings (Feng et al., 2000; Gupta et al., 1998; Matsumura et al., 2003). Above and beyond of these two, structural changes in the cell membrane and electron-dense granule formation have been observed (Feng et al., 2000; Nover et al., 1983).

Recent studies by Eckelman & Graedel (2007) reported that the environment in North America are receiving about 2200 metric tons of Ag/year, of which 55 % ends up in the landfills. Mueller & Nowack (2008) have predicted about 4.77 tons of nAg NP is also dumped into landfills each year. Toxicity studies of the nAg on aquatic life showed that nAg is toxic to zebrafish, Daphnis, and algae even with a low concentration 40 µg/L (Chen et al., 2004; Navarro et al., 2008). It is of prime importance to know the effect of silver on the environment from different applications.

Most of the reported studies with nAg were done under aerobic conditions and many of them showed the release of silver ion due to oxidative silver dissolution (Liu & Hurt, 2010). Application of nAg in anaerobic digestion conditions is rare compared with the aerobic conditions digestion. Additionally, the impact of nAg on anaerobic digestion is not well understood (Reinhart et al., 2010). Yang et al. (2012) have reported about the application of nAg in an anaerobic digestion for 256 days and reported the reduction of biogas production.

Furthermore, they also reported the reduction of methanogenic bacterial population, VFAs, soluble COD, and pH.

There is no or limited information on the application of nAg in livestock manure and its efficacy in mitigating gaseous emission or application of NP treated manure to the environment. Therefore, there is a critical need to study the effect of nAg to manure stored under the anaerobic condition as well as its fate and transport.

Characterization of the Applied Nanoparticles Effect

Physical and chemical properties of nanoparticles are related to their chemical composition and surface structural characteristics. Modification of the NPs size can change their surface structural characteristics. However, neither the laws of quantum chemistry nor classical physics can fit NPs. They have their unique mechanical, magnetic, electrical, optical and biological features and all of these features made its wide range of use in industrial, medical, physical and chemical sectors. Hence, to maximize our benefit from NPs, we need to have robust characterizing techniques of the applied NPs (Bustos et al., 2013).

Characterization of NPs Effect by Real-Time Polymerase Chain Reaction

Detection as well as quantification of a targeted DNA molecule with a specific sequence in a sample relative to a standard DNA culture can be done by using a molecular biological laboratory technique called real-time polymerase chain reaction (RT-PCR). The simplicity of the RT-PCR in the direction of its specificity and sensitivity along with its potential for high throughput and continuous improvement including new chemistries to detect and compare even in RNA levels introduced it as a benchmark technology (Bustin et al., 2005). This is a new technology and gained special attention and became a precious tool for researchers from various disciplines. It came with a significant improvement in the quantification of nucleic acid with a

wide dynamic range (7–8 logarithmic decades). As the name suggests, proteins identified as polymerases and enzymes cord together the discrete DNA building block to form long strands by a continuous doubling. To perform these continuous doubling towards the long strand formation, polymerases need to supply the nucleotides consisting of four bases termed as adenine (A), thymine (T), cytosine (C) and guanine (G) and a small fragment of DNA known as a primer. Within the primer, the nucleotides and a longer DNA molecule attach together to serve as template towards a new strand construction. Enzymes can construct exact copies of the template if all of these three ingredients are supplied. Total reaction process consists of three major steps: denaturation, annealing, and extension. The first step is the DNA denatured at 90 - 97˚C, followed by the annealing of the primers to DNA template strands in the direction of principle extension. The annealing step occurs at a lower temperature than that of denaturation step and the usual temperature for this step is 50 – 60˚C. In addition, it also requires around 40 repetitive cycles to complete the reaction. At the end of the annealed primers at step two, complimentary copy strand of DNA formed in step three. In this step, the preferred temperature approximately is 72˚C for 2-5 minutes (Joshi & Deshpande, 2011). The complete workflow of an RT-PCR is shown in Figure 8.

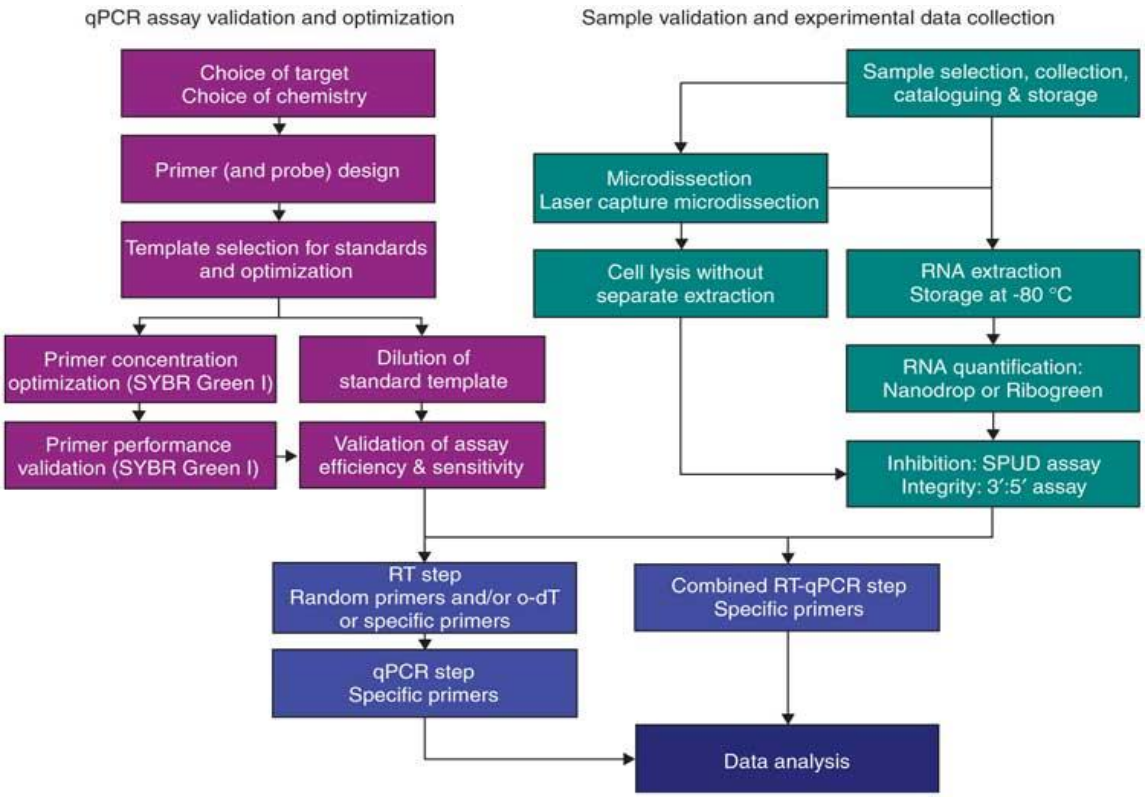

Figure 8. The workflow of an RT-PCR (Nolan et al., 2006)

Clinical microbiology, food microbiology, veterinary microbiology, and clinical oncology have a vast application of real-time PCR (Klein, 2002). Quantification of mRNA expression level, DNA copy number, transgene copy number and expression analysis, allelic discrimination, and measuring viral titers are the common applications of an RT-PCR (Ginzinger, 2002). In the case of the livestock sector, RT-PCR uses have been focused on the rumen. Moreover, application of this precious tool is limited to molecular analysis to identify the bacterial populations present in the manure. Furthermore, identification of specific microbes in the manure which are responsible for greenhouse gaseous emission from manure by using real-time PCR is very limited (Hill et al., 2005; Spence, Whitehead & Cotta, 2008; Tajima et al., 2007). However, three major groups of sulfate-reducing bacteria in swine manure with similarity to *Desulfobulbus*- and *Desulfovibrio*-like species has identified. Among these three groups,

group one and group three *dsrA* sequences are grouped closely with four known species. Whereas, group two *dsrA* sequences do not group closely with any known species, although they fall within a known lineage (Cook et al., 2008; Spence et al., 2008). Nevertheless, none of them or anyone else has identified the bacterial population in the swine manure following treatment with NPs. Therefore, in our present study, we wanted to explore the effect of the NPs application on the microbial population, especially targeted bacterial population responsible for producing methane and hydrogen sulfide.

Research Objectives

The overall objectives of this research were to determine the efficacy of different NPs and their different application methods to reduce GHG and H_2S emissions both from rumen fluid and manure under anaerobic storage conditions. The specific objectives were as follows:

Objective 1

Comparison of different NPs (namely, zinc silica nanogel, copper silica nanogel and NAC coated zinc oxide Qdots) effectiveness in minimizing GHG (e.g. CO_2, CH_4) and H_2S emissions from manure stored under anaerobic conditions. Additionally, changes in the manure properties and gaseous reduction mechanisms were investigated.

Objective 2

To investigate the efficacy of different application levels of nZnO and two types of feed (alfalfa and corn silage) in vitro rumen study in mitigating ruminal gas emission.

Objective 3

To understand reduction mechanisms of hydrogen sulfide and GHG in manure resulting from NPs application.

References

Aarnink, A., Keen, A., Metz, J., Speelman, L., & Verstegen, M. (1995). Ammonia emission patterns during the growing periods of pigs housed on partially slatted floors. *Journal of Agricultural Engineering Research, 62*(2), 105-116.

Abatzoglou, N., & Boivin, S. (2009). A review of biogas purification processes. *Biofuels, Bioproducts and Biorefining, 3*(1), 42-71.

Alemu, A. W., Dijkstra, J., Bannink, A., France, J., & Kebreab, E. (2011). Rumen stoichiometric models and their contribution and challenges in predicting enteric methane production. *Animal Feed Science and Technology, 166*, 761-778.

Ali, Q. (Producer). (2015). Federal Government Initiatives in Livestock and Dairy Development (An overview). *Food & Agriculture Organization of the United Nations (FAO)*.

Angelidaki, I., & Ahring, B. (1994). Anaerobic thermophilic digestion of manure at different ammonia loads: effect of temperature. *Water Research, 28*(3), 727-731.

Arnold, M. S., Avouris, P., Pan, Z. W., & Wang, Z. L. (2003). Field-effect transistors based on single semiconducting oxide nanobelts. *The Journal of Physical Chemistry B, 107*(3), 659-663.

Arogo, J., Zhang, R., Riskowski, G., & Day, D. (2000). Hydrogen sulfide production from stored liquid swine manure: a laboratory study. *Transactions of the ASAE, 43*(5), 1241.

Asis, D. A. (2008). Investigation of potential application of nanoparticles in reducing gas and odour emission from swine manure slurry. (Doctoral dissertation). Saskatoon, Canada, University of Saskatchewan, Department of Agricultural and Bioresource Engineering.

Assaad, V. F., Jofriet, J. C., Negi, S. C., & Hayward, G. L. (2003). Sulfide and sulfate attack on reinforced concrete of livestock buildings. Paper presented at the 2003 ASAE Annual Meeting.

Atakora, J. K., Möhn, S., & Ball, R. O. (2003). Low protein diets for sows reduce greenhouse gas production. *Adv. Pork Prod. 14, Abstract, 16*.

Ball, R. O., & Möhn, S. (2003). Feeding strategies to reduce greenhouse gas emissions from pigs. *Adv. Pork Prod, 14*, 301-311.

Baruah, S., & Dutta, J. (2009). Nanotechnology applications in pollution sensing and degradation in agriculture: a review. *Environmental Chemistry Letters, 7*(3), 191-204.

Bauchop, T. (1967). Inhibition of rumen methanogenesis by methane analogues. *Journal of Bacteriology, 94*(1), 171-175.

Bauer, A., Bösch, P., Friedl, A., & Amon, T. (2009). Analysis of methane potentials of steam-exploded wheat straw and estimation of energy yields of combined ethanol and methane production. *Journal of Biotechnology, 142*(1), 50-55.

Beauchemin, K., Kreuzer, M., O'mara, F., & McAllister, T. (2008). Nutritional management for enteric methane abatement: a review. *Animal Production Science, 48*(2), 21-27.

Becheri, A., Dürr, M., Nostro, P. L., & Baglioni, P. (2008). Synthesis and characterization of zinc oxide nanoparticles: application to textiles as UV-absorbers. *Journal of Nanoparticle Research, 10*(4), 679-689.

Benchaar, C., Pomar, C., & Chiquette, J. (2001). Evaluation of dietary strategies to reduce methane production in ruminants: a modelling approach. *Canadian Journal of Animal Science, 81*(4), 563-574.

Bennetzen, E. H., Smith, P., & Porter, J. R. (2016). Agricultural production and greenhouse gas emissions from world regions—The major trends over 40 years. *Global Environmental Change, 37*, 43-55.

Berg, W., Brunsch, R., & Pazsiczki, I. (2006). Greenhouse gas emissions from covered slurry compared with uncovered during storage. *Agriculture, Ecosystems & Environment, 112*(2), 129-134.

Berlin, D. & Uhlin, H.-E. (2004). Opportunity cost principles for life cycle assessment: toward strategic decision making in agriculture. *Progress in Industrial Ecology, An International Journal, 1*(1-3), 187-202.

Bernal, M. P., Alburquerque, J., & Moral, R. (2009). Composting of animal manures and chemical criteria for compost maturity assessment. A review. *Bioresource Technology, 100*(22), 5444-5453.

Boadi, D., Benchaar, C., Chiquette, J., & Massé, D. (2004). Mitigation strategies to reduce enteric methane emissions from dairy cows: update review. *Canadian Journal of Animal Science, 84*(3), 319-335.

Boden, T., Marland, G., & Andres, R. (2011). National CO2 emissions from fossil-fuel burning, cement manufacture, and gas flaring: 1751-2007. Carbon Dioxide Information Analysis Center Oak Ridge National Laboratory.

Boetius, A., Ravenschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Gieseke, A., . . . Pfannkuche, O. (2000). A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature, 407*(6804), 623-626.

Borhan, M., Gautam, D., Engel, C., Anderson, V., & Rahman, S. (2013). Effects of pen bedding and feeding high crude protein diets on manure composition and greenhouse gas

emissions from a feedlot pen surface. *Journal of the Air & Waste Management Association, 63*(12), 1457-1468.

Borhan, M. S., Mukhtar, S., Capareda, S., & Rahman, S. (2012). Greenhouse gas emissions from housing and manure management systems at confined livestock operations. In *Waste Management-An Integrated Vision*. Available at: https://cdn.intechopen.com/pdfs-wm/40530.pdf

Brochier-Armanet, C., Boussau, B., Gribaldo, S., & Forterre, P. (2008). Mesophilic Crenarchaeota: proposal for a third archaeal phylum, the Thaumarchaeota. *Nature Reviews Microbiology, 6*(3), 245-252.

Broucek, J. (2014). Production of methane emissions from ruminant husbandry: a review. *Journal of Environmental Protection, 5*(15), 1482.

Brown, L., Brown, S. A., Jarvis, S., Syed, B., Goulding, K., Phillips, V., . . . Pain, B. (2001). An inventory of nitrous oxide emissions from agriculture in the UK using the IPCC methodology: emission estimate, uncertainty and sensitivity analysis. *Atmospheric Environment, 35*(8), 1439-1449.

Bustin, S., Benes, V., Nolan, T., & Pfaffl, M. (2005). Quantitative real-time RT-PCR–a perspective. *Journal of Molecular Endocrinology, 34*(3), 597-601.

Bustos, A. R. M., Encinar, J. R., & Sanz-Medel, A. (2013). Mass spectrometry for the characterisation of nanoparticles. *Analytical and Bioanalytical Chemistry, 405*(17), 5637-5643.

Casey, K. D., Bicudo, J. R., Schmidt, D. R., Singh, A., Gay, S. W., Gates, R. S., . . . Hoff, S. J. (2006). Air quality and emissions from livestock and poultry production/waste management systems. *Animal Agriculture and the Environment,* 1-40.

Castellano, J. J., Shafii, S. M., Ko, F., Donate, G., Wright, T. E., Mannari, R. J., . . . Robson, M. C. (2007). Comparative evaluation of silver-containing antimicrobial dressings and drugs. *International Wound Journal, 4*(2), 114-122.

Cerri, C., Minami, K., Moiser, A., Rosenberg, N., & Sauerbeck, D. (1996). Agricultural options for mitigation of greenhouse gas emissions. Publisher: *Cambridge University Press*, USA, 745-771.

Chadwick, D., Sommer, S., Thorman, R., Fangueiro, D., Cardenas, L., Amon, B., & Misselbrook, T. (2011). Manure management: implications for greenhouse gas emissions. *Animal Feed Science and Technology, 166*, 514-531.

Chen, L., Bao, J., Gao, C., Huang, S., Liu, C., & Liu, W. (2004). Combinatorial synthesis of insoluble oxide library from ultrafine/nano particle suspension using a drop-on-demand inkjet delivery system. *Journal of Combinatorial Chemistry, 6*(5), 699-702.

Chung, Y.-C., Lin, Y.-Y., & Tseng, C.-P. (2005). Removal of high concentration of NH 3 and coexistent H 2 S by biological activated carbon (BAC) biotrickling filter. *Bioresource Technology, 96*(16), 1812-1820.

Clapperton, J. (1974). The effect of trichloroacetamide, chloroform and linseed oil given into the rumen of sheep on some of the end-products of rumen digestion. *British Journal of Nutrition, 32*(01), 155-161.

Clapperton, J. (1977). The effect of a methane-suppressing compound, trichloroethyl adipate, on rumen fermentation and the growth of sheep. *Animal Production*, *24*(02), 169-181.

Clark, O. G., Morin, B., Zhang, Y., Sauer, W. C., & Feddes, J. J. (2005). Preliminary investigation of air bubbling and dietary sulfur reduction to mitigate hydrogen sulfide and odor from swine waste. *Journal of Environmental Quality, 34*(6), 2018-2023.

Cook, K. L., Whitehead, T. R., Spence, C., & Cotta, M. A. (2008). Evaluation of the sulfate-reducing bacterial population associated with stored swine slurry. *Anaerobe*, *14*(3), 172-180.

Crutzen, P. J. (1974). Estimates of possible variations in total ozone due to natural causes and human activities. *Ambio*, 201-210.

Curtis, S. E. (1983). Environmental management in animal agriculture. *Iowa State University Press*, USA.

Dawson, N. G. (2008). Sweating the small stuff: environmental risk and nanotechnology. *BioScience*, *58*(8), 690-690.

De Boer, W., & Kowalchuk, G. (2001). Nitrification in acid soils: micro-organisms and mechanisms. *Soil Biology and Biochemistry*, *33*(7), 853-866.

De Vries, J., Hoogmoed, W., Groenestein, C., Schröder, J., Sukkel, W., De Boer, I., & Koerkamp, P. G. (2015). Integrated manure management to reduce environmental impact: I. Structured design of strategies. *Agricultural Systems*, *139*, 29-37.

Defra, A. (2010). Fertiliser manual RB209. *London, UK: Department for Environment, Food and Rural Affairs*.

Dehority, B. A. (2003). Rumen microbiology (Vol. 372). *Nottingham University Press*, Nottingham, England.

Denman, K. L., Brasseur, G. P., Chidthaisong, A., Ciais, P., Cox, P. M., Dickinson, R. E., . . . Jacob, D. J. (2007). Couplings between changes in the climate system and biogeochemistry (No. LBNL-464E). Ernest Orlando Lawrence Berkeley National Laboratory, Berkeley, CA (US).

Dijkstra, J., Oenema, O., & Bannink, A. (2011). Dietary strategies to reducing N excretion from cattle: implications for methane emissions. *Current Opinion in Environmental Sustainability, 3*(5), 414-422.

Dijkstra, J., Oenema, O., Van Groenigen, J., Spek, J., Van Vuuren, A., & Bannink, A. (2013). Diet effects on urine composition of cattle and N_2O emissions. *Animal, 7*(s2), 292-302.

Dohme, F., Machmüller, A., Wasserfallen, A., & Kreuzer, M. (2000). Comparative efficiency of various fats rich in medium-chain fatty acids to suppress ruminal methanogenesis as measured with rusitec. *Canadian Journal of Animal Science, 80*(3), 473-484.

Dong, H., Zhu, Z., Zhou, Z., Xin, H., & Chen, Y. (2011). Greenhouse gas emissions from swine manure stored at different stack heights. *Animal Feed Science and Technology, 166*, 557-561.

Dong, Y., Bae, H., McAllister, T., Mathison, G., & Cheng, K. (1997). Lipid-induced depression of methane production and digestibility in the artificial rumen system (RUSITEC). *Canadian Journal of Animal Science, 77*(2), 269-278.

Donham, K., Aherin, R., Baker, D., & Hetzel, G. (2006). Safety in swine production systems. *Factsheets, Pork Information Gateway*.

Eckard, R., Grainger, C., & De Klein, C. (2010). Options for the abatement of methane and nitrous oxide from ruminant production: a review. *Livestock Science, 130*(1), 47-56.

Eckelman, M. J., & Graedel, T. (2007). Silver emissions and their environmental impacts: a multilevel assessment. *Environmental Science & Technology, 41*(17), 6283-6289.

Edwards, G., Parsons, A., Rasmussen, S., & Bryant, R. (2007). High sugar ryegrasses for livestock systems in New Zealand. *In Proceedings of the New Zealand Grassland Association,* 69, 161-171.

Ellis, J., Dijkstra, J., Bannink, A., Parsons, A., Rasmussen, S., Edwards, G., . . . France, J. (2011). The effect of high-sugar grass on predicted nitrogen excretion and milk yield simulated using a dynamic model. *Journal of Dairy Science, 94*(6), 3105-3118.

Ellis, J., Kebreab, E., Odongo, N., McBride, B., Okine, E., & France, J. (2007). Prediction of methane production from dairy and beef cattle. *Journal of Dairy Science, 90*(7), 3456-3466.

EPA. (2011). Inventory of US greenhouse gas emissions and sinks: 1990-2009. *Environmental Protection Agency*.

Feng, Q., Wu, J., Chen, G., Cui, F., Kim, T., & Kim, J. (2000). A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. *Journal of Biomedical Materials Research, 52*(4), 662-668.

Fernandez, M. P. A., & Hullmann, A. (2007). A boost for safer nanotechnology. *Nano Today, 2*(1), 56.

Field, C., Barros, V., Dokken, D., Mach, K., Mastrandrea, M., Bilir, T., . . . Genova, R. (2014). Contribution of working group II to the fifth assessment report of the intergovernmental panel on climate change. *Climate Change*.

Foster, C., Green, K., & Bleda, M. (2007). Environmental impacts of food production and consumption: final report to the Department for Environment Food and Rural Affairs. Available at: http://agris.fao.org/agris-search/search.do?recordID=GB2013202568.

Frattini, A., Pellegri, N., Nicastro, D., & De Sanctis, O. (2005). Effect of amine groups in the synthesis of Ag nanoparticles using aminosilanes. *Materials Chemistry and Physics, 94*(1), 148-152.

Garcia-Apaza, E., Paz, O., & Arana, I. (2008). Greenhouse gas emissions from enteric fermentation of livestock in Bolivia: values for 1990–2000 and future projections. *Animal Production Science, 48*(2), 255-259.

Gautam, D., Rahman, S., Borhan, M., & Bezbaruah, A. (2013). Applications of nanoparticles (NPs) in livestock manure and their effects on air emissions. Paper presented at the *Intl. Symp. Animal Environment*. Welfare. Chongqing, China.

Gautam, D. P., Rahman, S., Bezbaruah, A. N., & Borhan, M. S. (2016a). Evaluation of Calcium Alginate Entrapped Nano Zinc Oxide to Reduce Gaseous Emissions from Liquid Dairy Manure. *Applied Engineering in Agriculture, 32*(1), 89-102.

Gautam, D. P., Rahman, S., Fortuna, A.-M., Borhan, M. S., Saini-Eidukat, B., & Bezbaruah, A. N. (2016b). Characterization of zinc oxide nanoparticle (nZnO) alginate beads in reducing gaseous emission from swine manure. *Environmental Technology*, 1-14.

Germida, J., Wainwright, M., & Gupta, V. (1992). Biochemistry of sulfur cycling in soil. *Soil Biochemistry, 7*, 1-53.

Ghosh, S., Mashayekhi, H., Pan, B., Bhowmik, P., & Xing, B. (2008). Colloidal behavior of aluminum oxide nanoparticles as affected by pH and natural organic matter. *Langmuir, 24*(21), 12385-12391.

Ginzinger, D. G. (2002). Gene quantification using real-time quantitative PCR: an emerging technology hits the mainstream. *Experimental Hematology, 30*(6), 503-512.

Grainger, C. & Beauchemin, K. (2011). Can enteric methane emissions from ruminants be lowered without lowering their production? *Animal Feed Science and Technology, 166*, 308-320.

Granli, T., & Bøckman, O. C. (1994). Nitrous oxide from agriculture. *Norwegian Journal of Agricultural Sciences (Norway)*.

Groenestein, C. M. (2006). Environmental aspects of improving sow welfare with group housing and straw bedding. PhD thesis, Wageningen University, Wageningen, Netherland.

Gupta, A., Maynes, M., & Silver, S. (1998). Effects of halides on plasmid-mediated silver resistance in Escherichia coli. *Applied and Environmental Microbiology, 64*(12), 5042-5045.

Hahne, J., Janssen, J., Schuchardt, F., Sonnenberg, H., Baader, W., & fuer Landwirtschaft, B. (1992). Treatment of liquid manure with nutrient recovery. *Food and Agriculture Organization of United States (FAO)*. Available at: http://agris.fao. org/agris-search/search.do?recordID=XF2016029677

Hansen, K. H., Angelidaki, I., & Ahring, B. K. (1998). Anaerobic digestion of swine manure: inhibition by ammonia. *Water Research, 32*(1), 5-12.

Harriss, R. C. (1989). Historical trends in atmospheric methane concentration and the temperature sensitivity of methane outgassing from boreal and polar regions. *Ozone Depletion, Depletion, Greenhouse Greenhouse Gases, and Climate Change*. National Academy Press, Washington DC, USA.

Hartung, J., & Phillips, V. (1994). Control of gaseous emissions from livestock buildings and manure stores. *Journal of Agricultural Engineering Research, 57*(3), 173-189.

Hashimoto, A. G., VarelA, V. H., & Chen, Y. R. (1981). Ultimate methane yield from beef cattle waste: effects of temperature, reaction constituents, antibiotics and manure. *Agricultural. Wastes, 3*, 241–256.

Hays, F., Goret, E., Johnson, H., & Hahn, L. (1972). Hydrogen sulfide (H2S) exposure in ruminants. *Journal of Animal Science,* 35(1), 189.

Hernández, S. P., Chiappero, M., Russo, N., & Fino, D. (2011). A novel ZnO-based adsorbent for biogas purification in H 2 production systems. *Chemical Engineering Journal, 176,* 272-279.

Herrero, M., Thornton, P. K., Gerber, P., & Reid, R. S. (2009). Livestock, livelihoods and the environment: understanding the trade-offs. *Current Opinion in Environmental Sustainability, 1*(2), 111-120.

Herzog, T. (2009). World greenhouse gas emissions in 2005. *World Resources Institute*. Washington, DC, USA. Available at: http://papierenkarton.nl/wp-content/uploads/2017/02/world_greenhouse _gas_emissions_2005.pdf

Hill, J. E., Hemmingsen, S. M., Goldade, B. G., Dumonceaux, T. J., Klassen, J., Zijlstra, R. T., . . . Van Kessel, A. G. (2005). Comparison of ileum microflora of pigs fed corn-, wheat-, or barley-based diets by chaperonin-60 sequencing and quantitative PCR. *Applied and Environmental Microbiology, 71*(2), 867-875.

Hilliger, H., Aengst, C., & Jellen, E. (1984). Gravimetric measurements of dust in pig and poultry houses. In *Symposium der International Society of Animal Hygiene in Zusammenarbeit mit der Deutschen Veterinaermedizinischen Gesellschaft eV-DVG-, Fachgruppe Hygiene und der Deutschen Tieraerzteschaft eV, Akademie fuer Tieraerztliche Fortbildung*, Hannover (Germany, FR), 13-14 Mar 1984.

Hook, S. E., Wright, A.-D. G., & McBride, B. W. (2010). Methanogens: methane producers of the rumen and mitigation strategies. *Archaea,* Hindawi Publishing Corporation.

Hooser, S. B., Van Alstine, W., Kiupel, M., & Sojka, J. (2000). Acute pit gas (hydrogen sulfide) poisoning in confinement cattle. *Journal of Veterinary Diagnostic Investigation, 12*(3), 272-275.

Hristov, A., Oh, J., Lee, C., Meinen, R., Montes, F., Ott, T., . . . Adesogan, A. (2013). Mitigation of greenhouse gas emissions in livestock production: A review of technical options for non-CO2 emissions. *FAO Animal Production and Health Paper No, 177*, 1-206.

Huarte, A., Cifuentes, V., Gratton, R., & Clausse, A. (2010). Correlation of methane emissions with cattle population in Argentine Pampas. *Atmospheric Environment, 44*(23), 2780-2786.

IPCC. (2007). Intergovernmental Panel on Climate Change. In *World Meteorological Organization*. Available at: http://wmo. insomnation.com/sites/default/files/documents /meetings/session20/doc2. pdf.

IPCC. (2014). Mitigation of climate change. Summary for Policymakers, 10(5.4).

Jarvis, G. N., Strömpl, C., Burgess, D. M., Skillman, L. C., Moore, E. R., & Joblin, K. N. (2000). Isolation and identification of ruminal methanogens from grazing cattle. *Current Microbiology, 40*(5), 327-332.

Jarvis, S., & Menzi, H. (2004). Optimising best practice for N management in livestock systems: meeting production and environmental targets. *Land Use Systems in Grassland Dominated Regions, 9*, 361-372.

Johnson, D. E., & Ward, G. M. (1996). Estimates of animal methane emissions. *Environmental Monitoring and Assessment, 42*(1-2), 133-141.

Jones, F., Phillips, F., Naylor, T., & Mercer, N. (2011). Methane emissions from grazing Angus beef cows selected for divergent residual feed intake. *Animal Feed Science and Technology, 166*, 302-307.

Jones, N., Ray, B., Ranjit, K. T., & Manna, A. C. (2008). Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. *FEMS Microbiology Letters, 279*(1), 71-76.

Joshi, M., & Deshpande, J. (2011). Polymerase chain reaction: methods, principles and application. *International Journal of Biomedical Research, 2*(1), 81-97.

Kaesebieter, J., Bollwahn, W., & Hilliger, H. (1985). Intoxication of suckling pigs by carbon monoxide. *Praktische Tierarzt (Germany, FR)*.

Kanel, S. R., Manning, B., Charlet, L., & Choi, H. (2005). Removal of arsenic (III) from groundwater by nanoscale zero-valent iron. *Environmental Science & Technology, 39*(5), 1291-1298.

Karakurt, I., Aydin, G., & Aydiner, K. (2012). Sources and mitigation of methane emissions by sectors: A critical review. *Renewable Energy, 39*(1), 40-48.

Kebreab, E., Clark, K., Wagner-Riddle, C., & France, J. (2006). Methane and nitrous oxide emissions from Canadian animal agriculture: A review. *Canadian Journal of Animal Science, 86*(2), 135-157.

Kebreab, E., France, J., Beever, D., & Castillo, A. (2001). Nitrogen pollution by dairy cows and its mitigation by dietary manipulation. *Nutrient Cycling in Agroecosystems, 60*(1-3), 275-285.

Kebreab, E., Strathe, A., Fadel, J., Moraes, L., & France, J. (2010). Impact of dietary manipulation on nutrient flows and greenhouse gas emissions in cattle. *Revista Brasileira de Zootecnia, 39*, 458-464.

Kemfert, C., & Schill, W.-P. (2009). Methane: A Neglected Greenhouse Gas. *Weekly Report, 5*(32), 218-223.

Kingston, A. H., Edwards, J. E., Huws, S. A., Kim, E. J., & Abberton, M. (2010). Plant-based strategies towards minimising 'livestock's long shadow'. *Proceedings of the Nutrition Society, 69*(04), 613-620.

Klein, D. (2002). Quantification using real-time PCR technology: applications and limitations. *Trends in Molecular Medicine, 8*(6), 257-260.

Knowlton, K., Radcliffe, J., Novak, C., & Emmerson, D. (2004). Animal management to reduce phosphorus losses to the environment. *Journal of Animal Science, 82*(13_suppl), E173-E195.

Kong, H., Song, J., & Jang, J. (2010). Photocatalytic antibacterial capabilities of TiO2− biocidal polymer nanocomposites synthesized by a surface-initiated photopolymerization. *Environmental Science & Technology, 44*(14), 5672-5676.

Kowalchuk, G. A., & Stephen, J. R. (2001). Ammonia-oxidizing bacteria: a model for molecular microbial ecology. *Annual Reviews in Microbiology, 55*(1), 485-529.

Kroze, C., Mossier, A., & Bouwman, L. (1999). Closing the global N2O budget: a retrospective analysis. *Global Biogeochem. Cycle, 13*, 1-8.

Külling, D., Menzi, H., Sutter, F., Lischer, P., & Kreuzer, M. (2003). Ammonia, nitrous oxide and methane emissions from differently stored dairy manure derived from grass-and hay-based rations. *Nutrient Cycling in Agroecosystems, 65*(1), 13-22.

Lashof, D. A., & Ahuja, D. R. (1990). Relative contributions of greenhouse gas emissions to global warming. *Nature, 344*(6266), 529.

Lassey, K. R., Ulyatt, M. J., Martin, R. J., Walker, C. F., & Shelton, I. D. (1997). Methane emissions measured directly from grazing livestock in New Zealand. *Atmospheric Environment, 31*(18), 2905-2914.

Laughlin, R., Stevens, R., Müller, C., & Watson, C. (2008). Evidence that fungi can oxidize NH4+ to NO3− in a grassland soil. *European Journal of Soil Science, 59*(2), 285-291.

Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G., . . . Schleper, C. (2006). Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature, 442*(7104), 806-809.

Lesschen, J., Van den Berg, M., Westhoek, H., Witzke, H., & Oenema, O. (2011). Greenhouse gas emission profiles of European livestock sectors. *Animal Feed Science and Technology, 166*, 16-28.

Leytem, A. B., Dungan, R. S., Bjorneberg, D. L., & Koehn, A. C. (2011). Emissions of ammonia, methane, carbon dioxide, and nitrous oxide from dairy cattle housing and manure management systems. *Journal of Environmental Quality, 40*(5), 1383-1394.

Li, C., Salas, W., Zhang, R., Krauter, C., Rotz, A., & Mitloehner, F. (2012). Manure-DNDC: a biogeochemical process model for quantifying greenhouse gas and ammonia emissions from livestock manure systems. *Nutrient Cycling in Agroecosystems, 93*(2), 163-200.

Li, Y., Park, S. Y., & Zhu, J. (2011). Solid-state anaerobic digestion for methane production from organic waste. *Renewable and Sustainable Energy Reviews, 15*(1), 821-826.

Li, Y.-Q., Fu, S.-Y., & Mai, Y.-W. (2006). Preparation and characterization of transparent ZnO/epoxy nanocomposites with high-UV shielding efficiency. *Polymer, 47*(6), 2127-2132.

Lin, D., & Xing, B. (2008). Root uptake and phytotoxicity of ZnO nanoparticles. *Environmental Science & Technology, 42*(15), 5580-5585.

Liu, J., & Hurt, R. H. (2010). Ion release kinetics and particle persistence in aqueous nano-silver colloids. *Environmental Science & Technology, 44*(6), 2169-2175.

Luna-delRisco, M., Orupõld, K., & Dubourguier, H.-C. (2011). Particle-size effect of CuO and ZnO on biogas and methane production during anaerobic digestion. *Journal of Hazardous Materials, 189*(1), 603-608.

Luo, X., Morrin, A., Killard, A. J., & Smyth, M. R. (2006). Application of nanoparticles in electrochemical sensors and biosensors. *Electroanalysis, 18*(4), 319-326.

Machmüller, A., & Kreuzer, M. (1999). Methane suppression by coconut oil and associated effects on nutrient and energy balance in sheep. *Canadian Journal of Animal Science, 79*(1), 65-72.

Maeda, K., Hanajima, D., Toyoda, S., Yoshida, N., Morioka, R., & Osada, T. (2011). Microbiology of nitrogen cycle in animal manure compost. *Microbial Biotechnology, 4*(6), 700-709.

Mann, S. (2006). Nanotechnology and Construction. Retrieved from Nanoforum Report.

Martin, C., Morgavi, D., & Doreau, M. (2010). Methane mitigation in ruminants: from microbe to the farm scale. *Animal, 4*(03), 351-365.

Martin, C., Rouel, J., Jouany, J., Doreau, M., & Chilliard, Y. (2008). Methane output and diet digestibility in response to feeding dairy cows crude linseed, extruded linseed, or linseed oil. *Journal of Animal Science, 86*(10), 2642-2650.

Masciangioli, T., & Zhang, W.-X. (2003). Peer reviewed: environmental technologies at the nanoscale, *Environmental Science & Technology*. 102-108.

Matsumura, Y., Yoshikata, K., Kunisaki, S.-i., & Tsuchido, T. (2003). Mode of bactericidal action of silver zeolite and its comparison with that of silver nitrate. *Applied and Environmental Microbiology, 69*(7), 4278-4281.

Maynard, A. D., Aitken, R. J., Butz, T., Colvin, V., Donaldson, K., Oberdörster, G., . . . Stone, V. (2006). Safe handling of nanotechnology. *Nature, 444*(7117), 267-269.

McGinn, S., & Beauchemin, K. (2012). Dairy farm methane emissions using a dispersion model. *Journal of Environmental Quality, 41*(1), 73-79.

McMichael, A. J., Powles, J. W., Butler, C. D., & Uauy, R. (2007). Food, livestock production, energy, climate change, and health. *The Lancet, 370*(9594), 1253-1263.

Melse, R. W., Ogink, N. W., & Rulkens, W. H. (2009). Air treatment techniques for abatement of emissions from intensive livestock production. *The Open Agriculture Journal, 3*, 6-12.

Mills, J., Dijkstra, J., Bannink, A., Cammell, S., Kebreab, E., & France, J. (2001). A mechanistic model of whole-tract digestion and methanogenesis in the lactating dairy cow: model development, evaluation, and application. *Journal of Animal Science, 79*(6), 1584-1597.

Monteny, G.-J., Bannink, A., & Chadwick, D. (2006). Greenhouse gas abatement strategies for animal husbandry. *Agriculture, Ecosystems & Environment, 112*(2), 163-170.

Morones, J. R., Elechiguerra, J. L., Camacho, A., Holt, K., Kouri, J. B., Ramírez, J. T., & Yacaman, M. J. (2005). The bactericidal effect of silver nanoparticles. *Nanotechnology, 16*(10), 2346.

Mosier, A., Kroeze, C., Nevison, C., Oenema, O., Seitzinger, S., & Van Cleemput, O. (1998). Closing the global N2O budget: nitrous oxide emissions through the agricultural nitrogen cycle. *Nutrient Cycling in Agroecosystems, 52*(2-3), 225-248.

Mueller, N. C., Braun, J., Bruns, J., Černík, M., Rissing, P., Rickerby, D., & Nowack, B. (2012). Application of nanoscale zero valent iron (NZVI) for groundwater remediation in Europe. *Environmental Science and Pollution Research, 19*(2), 550-558.

Mueller, N. C., & Nowack, B. (2008). Exposure modeling of engineered nanoparticles in the environment. *Environmental Science & Technology, 42*(12), 4447-4453.

Møller, H. B., Sommer, S. G., & Ahring, B. K. (2004). Methane productivity of manure, straw and solid fractions of manure. *Biomass and Bioenergy, 26*(5), 485-495.

Nagy, A., & Mestl, G. (1999). High temperature partial oxidation reactions over silver catalysts. *Applied Catalysis A: General, 188*(1), 337-353.

Nampoothiri, V. M., Mohini, M., Thakur, S., & Mondal, G. (2015). Influence of Diet on Methane and Nitrous Oxide Emissions from Cattle Manure. *Asian Journal of Atmospheric Environment (AJAE), 9*(3).

Navarro, E., Piccapietra, F., Wagner, B., Marconi, F., Kaegi, R., Odzak, N., . . . Behra, R. (2008). Toxicity of silver nanoparticles to Chlamydomonas reinhardtii. *Environmental Science & Technology, 42*(23), 8959-8964.

Neethirajan, S., & Jayas, D. S. (2011). Nanotechnology for the food and bioprocessing industries. *Food and Bioprocess Technology, 4*(1), 39-47.

Ni, J.-Q., Vinckier, C., Hendriks, J., & Coenegrachts, J. (1999). Production of carbon dioxide in a fattening pig house under field conditions. II. Release from the manure. *Atmospheric Environment, 33*(22), 3697-3703.

Nolan, T., Hands, R. E., & Bustin, S. A. (2006). Quantification of mRNA using real-time RT-PCR. *Nature Protocols, 1*(3), 1559-1582.

Nover, L., Scharf, K., & Neumann, D. (1983). Formation of cytoplasmic heat shock granules in tomato cell cultures and leaves. *Molecular and Cellular Biology, 3*(9), 1648-1655.

Nowack, B. (2010). Nanosilver revisited downstream. *Science, 330*(6007), 1054-1055.

Orphan, V., Hinrichs, K.-U., Ussler, W., Paull, C. K., Taylor, L., Sylva, S. P., . . . DeLong, E. F. (2001). Comparative analysis of methane-oxidizing archaea and sulfate-reducing bacteria in anoxic marine sediments. *Applied and Environmental Microbiology, 67*(4), 1922-1934.

Osada, T., Takada, R., & Shinzato, I. (2011). Potential reduction of greenhouse gas emission from swine manure by using a low-protein diet supplemented with synthetic amino acids. *Animal Feed Science and Technology, 166*, 562-574.

Pal, S., Tak, Y. K., & Song, J. M. (2007). Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli. *Applied and Environmental Microbiology, 73*(6), 1712-1720.

Pan, Z. W., Dai, Z. R., & Wang, Z. L. (2001). Nanobelts of semiconducting oxides. *Science, 291*(5510), 1947-1949.

Park, K.-H., Jeon, J., Jeon, K., Kwag, J., & Choi, D. (2011). Low greenhouse gas emissions during composting of solid swine manure. *Animal Feed Science and Technology, 166*, 550-556.

Paul, J., Beauchamp, E., & Zhang, X. (1993). Nitrous and nitric oxide emissions during nitrification and denitrification from manure-amended soil in the laboratory. *Canadian Journal of Soil Science, 73*(4), 539-553.

Pearman, G., Etheridge, D., De Silva, F., & Fraser, P. (1986). Evidence of changing concentrations of atmospheric CO_2, N_2O and CH_4 from air bubbles in Antarctic ice. *Nature*, 320(6059), 248.

Phetteplace, H. W., Johnson, D. E., & Seidl, A. F. (2001). Greenhouse gas emissions from simulated beef and dairy livestock systems in the United States. *Nutrient Cycling in Agroecosystems, 60*(1-3), 99-102.

Pipan-Tkalec, Ž., Drobne, D., Jemec, A., Romih, T., Zidar, P., & Bele, M. (2010). Zinc bioaccumulation in a terrestrial invertebrate fed a diet treated with particulate ZnO or ZnCl 2 solution. *Toxicology, 269*(2), 198-203.

Pouliquen, F., Blanc, C., Arretz, E., Labat, I., Tournier-Lasserve, J., Ladousse, A., . . . Perrot, J. Hydrogen sulfide.*Ullmann's Encyclopedia of Industrial Chemistry*.

Predicala, B., Alvarado, A., & Asis, D. (2012). Use of Zinc Oxide Nanoparticles to Control Hydrogen Sulphide, Ammonia and Odour Emissions from Pig Barns. Paper presented at *the The Ninth Intl. Livestock Environ. Symp.(ILES IX). Intl. Conf. Agric. Eng.-CIGR-AgEng 2012: Agriculture and Engineering for a Healthier Life*.

Prins, R. (1965). Action of chloral hydrate on rumen microorganisms in vitro. *Journal of Dairy Science, 48*(7), 991-993.

Qu, X., Brame, J., Li, Q., & Alvarez, P. J. (2012). Nanotechnology for a safe and sustainable water supply: enabling integrated water treatment and reuse. *Accounts of Chemical Research, 46*(3), 834-843.

Quaghebeur, D., & Oyaert, W. (1971). Effect of chloral hydrate and related compounds on the activity of several enzymes in extracts of rumen microorganisms. *Transboundary and Emerging Diseases, 18*(5), 417-427.

Rahman, S., & Borhan, M. (2012). Typical odor mitigation technologies for swine production facilities: A review. *Journal of Civil & Environmental Enggineering 2*(4), 117.

Rai, M., & Ingle, A. (2012). Role of nanotechnology in agriculture with special reference to management of insect pests. *Applied Microbiology and Biotechnology, 94*(2), 287-293.

Rai, M., Yadav, A., & Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. *Biotechnology Advances, 27*(1), 76-83.

Rastogi, M., Singh, S., & Pathak, H. (2002). Emission of carbon dioxide from soil. *Current Science, 82*(5), 510-517.

Reinhart, D. R., Berge, N. D., Santra, S., & Bolyard, S. C. (2010). Emerging contaminants: nanomaterial fate in landfills. In: *Pergamon*.

Rejeski, D., & Lekas, D. (2008). Nanotechnology field observations: scouting the new industrial west. *Journal of Cleaner Production, 16*(8), 1014-1017.

Ren, G., Hu, D., Cheng, E. W., Vargas-Reus, M. A., Reip, P., & Allaker, R. P. (2009). Characterisation of copper oxide nanoparticles for antimicrobial applications. *International Journal of Antimicrobial Agents, 33*(6), 587-590.

Richard, J., Spencer, B., McCoy, L., Carina, E., Washington, J., & Edgar, P. (2002). Acticoat versus Silverlon: the truth. *Journal of Burns Surg Wound Care, 1*(1), 11-19.

Robertson, L., & Waghorn, G. (2002). Dairy industry perspectives o methane emissions and production from cattle fed pasture or total mixed rations in New Zealand. Paper presented at *the proceedings-new zealand society of animal production.*

Rotello, V. M. (2004). Nanoparticles: building blocks for nanotechnology: *Springer Science & Business Media.*

Rudolf, M., & Kroneck, P. M. (2005). The nitrogen cycle: its biology. *Metal Ions in Biological Systems, 43*, 75-103.

Schiffman, S. S., Miller, E. A. S., Suggs, M. S., & Graham, B. G. (1995). The effect of environmental odors emanating from commercial swine operations on the mood of nearby residents. *Brain Research Bulletin, 37*(4), 369-375.

Seiler, W., & Conrad, R. (1987). Contribution of tropical ecosystem to the global budget of trace gases, especilly CH_4, H_2, CO and N_2O. *In. The Geophysiology of Amazonia Vegetation and Climate Interactions,* 133-162.

Sejian, V., Lal, R., Lakritz, J., & Ezeji, T. (2011). Measurement and prediction of enteric methane emission. *International Journal of Biometeorology, 55*(1), 1-16.

Sejian, V., Samal, L., Bagath, M., Suganthi, R., Bhatta, R., & Lal, R. (2015). Gaseous Emissions from Manure Management. *Encyclopedia of Soil Science, Second Edition.* Available at: https://www.researchgate.net/profile/Veerasamy_Sejian/publication/280577945_Gaseous_Emissions_from_Manure_Management/links/5683922b08aebccc4e0fc99c/Gaseous-Emissions-from-Manure-Management.pdf.

Sharma, V., Anderson, D., & Dhawan, A. (2011). Zinc oxide nanoparticles induce oxidative stress and genotoxicity in human liver cells (HepG2). *Journal of Biomedical Nanotechnology, 7*(1), 98-99.

Sharma, V. K., Yngard, R. A., & Lin, Y. (2009). Silver nanoparticles: green synthesis and their antimicrobial activities. *Advances in Colloid and Interface Science, 145*(1), 83-96.

Solomon, S. (2007). Climate change 2007-the physical science basis: Working group I contribution to the fourth assessment report of the IPCC (Vol. 4): *Cambridge University Press*. England.

Spence, C., Whitehead, T., & Cotta, M. (2008). Development and comparison of SYBR Green quantitative real-time PCR assays for detection and enumeration of sulfate-reducing bacteria in stored swine manure. *Journal of Applied Microbiology, 105*(6), 2143-2152.

Steed, J., & Hashimoto, A. G. (1994). Methane emissions from typical manure management systems. *Bioresource Technology, 50*(2), 123-130.

Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., & de Haan, C. (2006). Livestock's long shadow: environmental issues and options. *Food & Agriculture Organization of United States (FAO).*

Su, J.-J., Liu, B.-Y., & Chang, Y.-C. (2003). Emission of greenhouse gas from livestock waste and wastewater treatment in Taiwan. *Agriculture, Ecosystems & Environment, 95*(1), 253-263.

Sutton, A., Kephart, K., Verstegen, M., Canh, T., & Hobbs, P. (1999). Potential for reduction of odorous compounds in swine manure through diet modification. *Journal of Animal Science, 77*(2), 430-439.

Tajima, K., Nonaka, I., Higuchi, K., Takusari, N., Kurihara, M., Takenaka, A., . . . Aminov, R. I. (2007). Influence of high temperature and humidity on rumen bacterial diversity in Holstein heifers. *Anaerobe, 13*(2), 57-64.

Tas, B., Taweel, H., Smit, H., Elgersma, A., Dijkstra, J., & Tamminga, S. (2006). Effects of perennial ryegrass cultivars on milk yield and nitrogen utilization in grazing dairy cows. *Journal of Dairy Science, 89*(9), 3494-3500.

Tavares, P., Pereira, A., Moura, J., & Moura, I. (2006). Metalloenzymes of the denitrification pathway. *Journal of Inorganic Biochemistry, 100*(12), 2087-2100.

Thompson, J. M., Chisholm, B. J., & Bezbaruah, A. N. (2010). Reductive dechlorination of chloroacetanilide herbicide (alachlor) using zero-valent iron nanoparticles. *Environmental Engineering Science, 27*(3), 227-232.

Trabue, S., Kerr, B., Bearson, B., & Ziemer, C. (2011). Swine odor analyzed by odor panels and chemical techniques. *Journal of Environmental Quality, 40*(5), 1510-1520.

Trei, J., Parish, R., Singh, Y., & Scott, G. (1971). Effect of methane inhibitors on rumen metabolism and feedlot performance of sheep. *Journal of Dairy Science, 54*(4), 536-540.

Trei, J., Scott, G., & Parish, R. (1972). Influence of methane inhibition on energetic efficiency of lambs. *Journal of Animal Science, 34*(3), 510-515.

Turkoglu, M., & Yener, S. (1997). Design and in vivo evaluation of ultrafine inorganic-oxide-containing-sunscreen formulations. *International Journal of Cosmetic Science, 19*(4), 193-201.

USDA. (2017). Top Ranked Cattle Producing Countries Around the World.

USDA. (2007). Manure Chemistry – Nitrogen, Phosphorus, & Carbon. In *Natural Resources Conservation Service (NRCS).*

Ussiri, D., & Lal, R. (2013). Nitrous oxide sources and mitigation strategies. In Soil Emission of Nitrous Oxide and its Mitigation . *Springer*, 243-275.

van Elsas, J. D., Trevors, J. T., Jansson, J. K., & Nannipieri, P. (2006). Modern Soil Microbiology. *CRc Press.*England.

VanderZaag, A., Gordon, R., Glass, V., & Jamieson, R. (2008). Floating covers to reduce gas emissions from liquid manure storages: a review. *Applied Engineering in Agriculture, 24*(5), 657-671.

Vaseem, M., Umar, A., & Hahn, Y.-B. (2010). ZnO nanoparticles: growth, properties, and applications. *Metal Oxide Nanostructures and their Applications, 5*, 1-36.

Velthof, G., & Mosquera, J. (2011). The impact of slurry application technique on nitrous oxide emission from agricultural soils. *Agriculture, Ecosystems & Environment, 140*(1), 298-308.

Velthof, G. L., Kuikman, P. J., & Oenema, O. (2003). Nitrous oxide emission from animal manures applied to soil under controlled conditions. *Biology and Fertility of Soils, 37*(4), 221-230.

Wang, H., Wick, R. L., & Xing, B. (2009). Toxicity of nanoparticulate and bulk ZnO, Al 2 O 3 and TiO 2 to the nematode Caenorhabditis elegans. *Environmental Pollution, 157*(4), 1171-1177.

Wang, S., Yang, X., Lin, X., Hu, Y., Luo, C., Xu, G., . . . Chao, Z. (2009). Methane emission by plant communities in an alpine meadow on the Qinghai-Tibetan Plateau: a new experimental study of alpine meadows and oat pasture. *Biology Letters, 5*(4), 535-538.

Wang, Z. L. (2004). Zinc oxide nanostructures: growth, properties and applications. *Journal of Physics: Condensed Matter, 16*(25), R829.

Watson, R., Meira Filho, L., Sanhueza, E., & Janetos, A. (1992). Greenhouse gases: sources and sinks. *Climate Change, 92*, 25-46.

Wilkie, A. C. (2005). Anaerobic digestion of dairy manure: Design and process considerations. *Dairy Manure Management: Treatment, Handling, and Community Relations, 301*, 312.

Wright, A., Kennedy, P., O'Neill, C., Toovey, A., Popovski, S., Rea, S., . . . Klein, L. (2004). Reducing methane emissions in sheep by immunization against rumen methanogens. *Vaccine, 22*(29), 3976-3985.

Xiong, M., Gu, G., You, B., & Wu, L. (2003). Preparation and characterization of poly (styrene butylacrylate) latex/nano-ZnO nanocomposites. *Journal of Applied Polymer Science, 90*(7), 1923-1931.

Xiu, Z.-m., Zhang, Q.-b., Puppala, H. L., Colvin, V. L., & Alvarez, P. J. (2012). Negligible particle-specific antibacterial activity of silver nanoparticles. *Nano Letters, 12*(8), 4271-4275.

Yang, Y., Chen, Q., Wall, J. D., & Hu, Z. (2012). Potential nanosilver impact on anaerobic digestion at moderate silver concentrations. *Water Research, 46*(4), 1176-1184.

Yecheskel, Y., Dror, I., & Berkowitz, B. (2016). Transport of engineered nanoparticles in partially saturated sand columns. *Journal of Hazardous Materials, 311*, 254-262.

Yokoyama, M. T., Spence, C., Hengemuehle, S. M., Whitehead, T. R., von Bernuth, R., & Cotta, M. (2016). Sodium Tetraborate Decahydrate Treatment Reduces Hydrogen Sulfide and the Sulfate-Reducing Bacteria Population of Swine Manure. *Journal of Environmental Quality*.

Yung, Y., Wang, W., & Lacis, A. (1976). Greenhouse effect due to atmospheric nitrous oxide. *Geophysical Research Letters, 3*(10), 619-621.

Zhang, G., Strøm, J. S., Li, B., Rom, H. B., Morsing, S., Dahl, P., & Wang, C. (2005). Emission of ammonia and other contaminant gases from naturally ventilated dairy cattle buildings. *Biosystems Engineering, 92*(3), 355-364.

Zweck, A., Bachmann, G., Luther, W., & Ploetz, C. (2008). Nanotechnology in Germany: from forecasting to technological assessment to sustainability studies. *Journal of Cleaner Production, 16*(8), 977-987.

PAPER 1: NANOPARTICLES IN MITIGATING GASEOUS EMISSIONS FROM LIQUID DAIRY MANURE STORED UNDER ANAEROBIC CONDITION[1]

Abstract

A number of mitigation techniques exist to reduce the emissions of pollutant gases and greenhouse gases (GHGs) from anaerobic storage of livestock manure. Nanoparticle (NP) application is a promising mitigating treatment option for pollutant gases, but limited research is available on the mode of NP application and their effectiveness in gaseous emissions reduction. In this study, Zinc Silica Nanogel (ZnSNL), Copper Silica Nanogel (CuSNL), and N-Acetyl Cysteine (NACL) coated Zinc Oxide Quantum Dots (Qdots) NPs were compared to a control lacking NPs. All three NPs tested significantly reduced gas production and concentrations compared to non-treated manure. Overall, cumulative gas volumes were reduced by 81 to 99%, and concentrations reduced by 49 to ~100% for H_2S, and 20.24 to ~100% for GHGs. Thus, application of NPs is a potential treatment option for mitigating pollutant and GHGs emissions from anaerobically stored manure.

Keywords: Nanoparticles, greenhouse gas, hydrogen sulfide, anaerobic storage, reduction

Introduction

Greenhouse gases (GHGs) such as methane (CH_4), carbon dioxide (CO_2), and nitrous oxide (N_2O) emitted from livestock production operations are suspected of contributing to

[1] The material in this chapter was co-authored by Niloy Chandra Sarker, Ali Ozcan, and Shafiqur Rahman, Md. Saidul Borhan, Swadeshmukul Santra, Parthiban Rajasekaran. Niloy Chandra Sarker had primary responsibility for collecting samples and analyzing laboratory data. Niloy Chandra Sarker also drafted and revised all versions of this paper. Shafiqur Rahman, Md. Saidul Borhan, Swadeshmukul Santra, Parthiban Rajasekaran served as proofreader and checked the math in the statistical analysis conducted by Niloy Chandra Sarker. Paper 1 was submitted for review in October 2017 to *Journal of Environmental Sciences* as manuscript number JES_2017_2969_R1. Status: Accepted.

climate change. The United States Agricultural sector contributes about 9% of the total U.S. GHGs emission, and the US livestock sector alone stands for ~28% of total methane emission (USEPA, 2015; Johnson et al., 2007). GHGs are produced due to anaerobic digestion of manure and biomass, municipal solid waste, freshwater biomass, leaves, grasses, woods, weeds, fruit and vegetable solid wastes) (Gunaseelan, 1997; Kinsman et al., 1995). Carbon dioxide is produced due to both aerobic and anaerobic digestion of manure. During the same digestion period process organic nitrogen is converted into ammonia, nitrite (NO_2^-), and nitrate (NO_3^-) through nitrification process. However, NO_3^- converts back to N_2 through denitrification process (Kinsman et al., 1995; Sommer et al., 2007). Additionally, hydrogen sulfide (H_2S) and other volatile organic compounds are also generated during anaerobic decomposition of manure (Abouelenien et al., 2009; Hobbs et al., 2004).

Worldwide, scientists and researchers are trying various treatment options including feed manipulation; implication of lifetime efficacy (Weiske et al., 2006); application of catalytic processes (Centi and Perathoner, 2012); addition of microbial additives (Rahman et al., 2011), anaerobic digestion (Clemens et al., 2006); and application of probiotics, acetogens, bacteriocins, organic acids (Boadi et al., 2004) for mitigating GHG and Volatile Organic Compounds (VOCs) from animal production facilities. Recently, nanoparticle (NP) application has shown promise in mitigating gaseous emissions from both industrial and animal wastes. NPs are used in the industrial sector for removal of trace amount of pollutants (Salata, 2004). Similarly, NP application is expected to bring solutions offering GHG mitigation not possible by using conventional methods such as household environmental mitigation and land application (Chinnamuthu and Boopathi, 2009). Nanoparticles in the agricultural sector (Arivalagan et al., 2011; Chinnamuthu and Boopathi, 2009), especially due to their presumed ability to mitigate

GHG (Gautam et al., 2016a, 2016b, 2016c) became attractive part of research nowadays. Among the few studies performed on GHGs mitigation, zinc oxide nanoparticles (nZnO) and copper oxide nanoparticles (nCuO) reportedly had inhibitory action concerning CH_4 production (Luna-delRisco et al., 2011; Mu et al., 2011). Depending on the nZnO dosage, a 19-77% reduction of CH_4 was reported from waste activated sludge in comparison with a control (Mu et al., 2011). Other metal oxide nanoparticles explored, such as titanium dioxide (TiO_2), silicon dioxide (SiO_2) and aluminum dioxide (Al_2O_3) did not show any effect (Mu et al., 2011).

Mixing of nZnO with swine manure slurry reduced the concentration of CH_4 and H_2S by 54% and 98%, respectively (Gautam et al., 2016a). nZnO used in a filter media reduced CH_4 and CO_2 concentrations by 14% and 18%, respectively, over the control (Asis, 2008). Asis (2008) also found that spraying tungsten oxide (WO_3) into the headspace gas from the manure slurry did not show any noteworthy response. nZnO compared to zirconium oxide NPs ($nZrO_2$) at application rates of 100, 250, 500 mg/L and 3 g/L in swine manure/dairy manure revealed that nZnO is much more effective than $nZrO_2$ in mitigating CH_4 and H_2S when compared to a control sample (Gautam et al., 2013).

In general, NPs are an effective means for mitigating or reducing gaseous emissions either by directly absorbing gases, by killing gas-producing microorganisms, or converting the contaminating chemical through a chemical reaction (Yang et al., 2013; Zhang et al., 2010; Ševců et al., 2011). However, it is not well understood if there are adverse environmental effects from nanoparticles on aquatic ecosystems, plant uptake, and toxicity mechanisms (Fabrega et al., 2011; Ge et al., 2011; Navarro et al., 2008; Nowack and Bucheli, 2007). Therefore, researchers are continuously striving for new environment-friendly engineered nanoparticles with intact active potentiality and minimal adverse environmental impact (Bolyard et al., 2013; Young and

Santra, 2014). The behavior of such nanoparticle types (ZnO, TiO_2, Ag) has been analyzed for landfill leachate (Bolyard et al., 2013), seed germination (Das et al., 2015), and antibacterial efficacy (Young and Santra, 2014). The potential application of these NPs in livestock manure is limited although the research need has been identified (Gautam et al., 2016a). NPs can be applied either as a liquid, gel, or powder depending on the targeted treatment. However, to our knowledge no study has been previously conducted to examine the efficacy of the liquid formulation of different NPs in reducing GHG emission. Therefore, the objective of this study was to compare the effectiveness of three NPs namely, zinc silica nanogel, copper silica nanogel and NAC coated zinc oxide Qdots in minimizing CO_2, CH_4, and H_2S emissions from liquid dairy manure stored under anaerobic conditions. Additionally, changes in the manure properties and gaseous reduction mechanisms in NPs treated manure were characterized.

Materials and Methods

Manure Collection and Characterization

Dairy manure was collected from the dairy research unit of North Dakota State University (NDSU) to evaluate the effectiveness of three engineered NPs on manure properties, gas volume, CO_2, CH_4, and H_2S concentrations. Manure properties such as pH, conductivity, crude protein, ash, total N, ammonia, and volatile fatty acids (VFAs) were determined both before and after the experimental period. Table 4 lists methods used for manure characterization.

Table 4. Protocols followed to determine manure properties

Parameters	Methods/Protocols Used	References
pH	EPA SW-846, Method 9040	Mulkey, 1999
Redox	ASTM D1498-14 Standard Test Method for Oxidation-Reduction Potential	Batley & Simpson, 2016
Conductivity	ASTM D1125-14 Standard Test Methods for Electrical Conductivity	Marr & Heitkamp, 2015
Total Nitrogen	Recommended Methods of Manure Analysis, A3769 Macro-Kjeldahl method	Borhan et al., 2013
Ammonia	Sigma Technical Bulletin #640. Sigma Diagnostics, St. Louis, MO 63178	Gautam et al., 2016b
Crude Protein	Official Methods of Analysis of AOAC International (2005) 18th ED., AOAC International Gaithersburg, MD, USA, Official Method 2001.11 Run on the Kjeltec 2300, Foss NA, Eden Prairie, MN	Latimer, 2012
Ash Content	Official Methods of Analysis of AOAC International (2005) 18th ED., AOAC International Gaithersburg, MD, USA, Official Method 942.05	Latimer, 2012
Volatile Fatty Acids	The method of Goetsch and Galyean, 1983. Agilent 6890N Gas Chromatograph with a FID (flame ionization detector) and the 7683 Series auto-injector and an auto sampler. The column used was the Supelco brand, NUKOL Fused Silica Column, 15 m x 0.53 mm x 0.5 um.	Gautam et al., 2016c

Nanogel Synthesization

Preparation of Copper and Zinc Silica Nanogel

All the materials required for the nanogel synthesization process were purchased and used unmodified from commercial vendors. Copper silica nanogel (CuSNL) and Zinc silica nanogel (ZnSNL) were prepared as previously described (Young & Santra, 2014)**.** Copper (II) sulfate pentahydrate (38.88 g) (CQ Concepts, Ringwood, IL, USA) or zinc sulfate monohydrate (27.5 g) (Fisher Scientific, Waltham, MA) were added to 1.9 mL of 1% hydrochloric acid (Fisher Scientific, Waltham, MA) in 660 mL of deionized water. After magnetic stirring (30 min)

tetraethylorthosilicate (TEOS) (4.6 mL) (Gelest Inc., Morrisville, PA) was added drop-wise and stirred for 24 hours. pH of the final solution was raised to 7.5 with 1 N sodium hydroxide (Fisher Scientific, Waltham, MA) solution and then the nanogel was formed.

Preparation of N-acetyl Cysteine Coated Zinc Oxide q-dot Nanogel

Zinc oxide nanoparticles (nZnO) were synthesized using a modified sol-gel method described previously (Bang et al., 2006). N-acetyl cysteine (NAC) (14.97 g) (Acros Chemicals, Geel, Belgium) was dissolved in 600 mL of 95% ethanol at ~70°C (hot bath) with constant stirring in a glass beaker. Zinc acetate dehydrate (Cas# 5970-45-6, Sigma, St. Louis, MO) (26.84 g) was added to this solution while in the hot-bath and allowed to dissolve completely. After 10 minutes of stirring, the beaker was transferred to an ice bath and cooled to 4-5°C. In a separate flask, 7.33 g of sodium hydroxide was dissolved in 200 mL of 95% ethanol added dropwise at the rate of 2-3 mL/min to the cooled zinc acetate and NAC solution to form the NAC coated ZnO nanoparticles.

Experimental Setup and Gas Sampling

Twenty liters of raw dairy manure samples were kept at laboratory room conditions (T=22 ± 2˚C) for about six hours to acclimate to the experimental unit environment. Later on, raw manure samples were stirred thoroughly for homogeneous mixing before subsampling and initiating treatments with different Nanogels. Four treatments were prepared including Zinc Silica Nanogel Liquid (ZnSNL), Copper Silica Nanogel Liquid (CuSNL), NAC coated Zinc Oxide Qdots Liquid (NACL), and a control (no NPs added). Based on a previous study (Gautam et al., 2013)**,** an application rate of 3 g/L was maintained for three NP based treatments and all four treatments were replicated three times. Thus, a total 12 Erlenmeyer flasks (4 treatments × 3 replicates) were used. All treatments were carried out in 1-L Erlenmeyer flasks with a working

volume of 500 mL fitted with rubber stoppers. One end of a steel tube (6 mm diameter × 500 mm long) was inserted to each flask through the rubber stopper for headspace gas collection into a 500 mL Tedlar bag (SKC Gulf Coast Inc., Texas, USA) using a Teflon tube. Before sealing the flask, residual oxygen in the headspace was driven out by flushing it with nitrogen to create an anaerobic environment (Figure 9). After setting up all experiments, each treatment flask was mixed once again by shaking the flasks manually. The experiment continued until gas production was stopped (when no gas was collected in sampling bag) completely after 56 days.

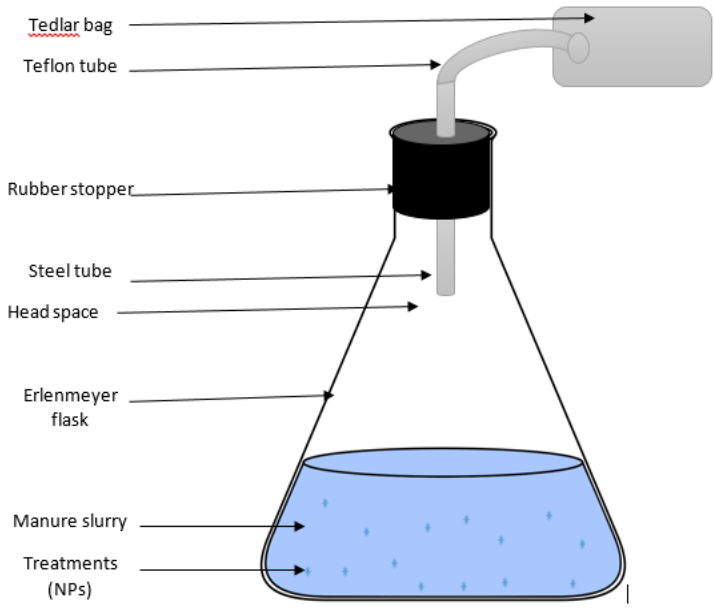

Figure 9. Schematic diagram of an experimental setup used in this study

Measurement of Gas Volume, GHGs, and Hydrogen Sulfide Concentration

Headspace gas accumulated in the Tedlar bags was collected every 2 to14 days during the entire experimental period. Gas was drawn out of the Tedlar bags by a graduated gas-tight syringe (SGE Syringe, 500 MAR-LL-GT, Trajan Scientific Americas Inc, Austin, Texas, USA) for measuring gas volume. A gas-tight syringe (5 mL, Luer-LokTM Tip Syringe, Franklin Lakes, New Jersey, USA) was used to collect headspace gas from Tedlar bags to measure gas

concentration. This sample was diluted with pure nitrogen gas in different Tedlar bags to match the detection limit of the gas analyzer (GC and Jerome meter). This dilution was chosen based on gas concentrations in the headspace. The H_2S gas concentrations were measured with a Jerome meter (Jerome 631X, Arizona Instrument LLC, Arizona, USA). Greenhouse gases (CH_4 and CO_2) were measured using a gas chromatograph (GC, 8610C, SRI instrument, California, USA) equipped with FID and ECD detectors. Based on the pre-scheduled GC event program (method), 1 mL diluted gas mixture was injected into the sample loop. FID and ECD detector temperatures were raised to 300°C and 350°C respectively before the insertion of the gas sample into the GC. Additionally, before each measurement, the GC was calibrated using the research grade standard gasses (5, 10, 100 ppm for CH_4; 500, 1000, 3000 ppm of CO_2) and five to seven replications for each concentration levels were used. Estimated method detection limits (lower) of the GC for CH_4 and CO_2 were 87 and 109 ppm, respectively. Additional calibration and measurement processes are described in (Rahman et al., 2013).

Microbial Population Density Analysis

Bacterial Cultivation and Quantification

Plate counts were done to quantify the effect of different treatments on the aerobic coliform microbial population (e.g., *coliform* and *E.coli*) (Gautam et al., 2016a). Plate counts were carried out before and after the experimental period and reported as Colony Forming Units (CFUs). All experimental plate count preparations of manure and reagents were performed in a sterile fume hood. Growth media for the microbial communities was prepared by placing a sterile membrane filter with an absorbent pad (47 mm diameter, 0.45 μm pore size, WCN type, Whatman Limited, Maidstone, England, UK) in a sterile petri-dish (Sterile Petri dishes, 60 mm diameter and 15 mm height, VWR, Radnor, PA, USA). An M-Endo broth ampule (2 mL)

(HACH LANCH GmbH, Willstatterstrasse 11, Dusseldorf, Germany) was poured evenly over the entire surface of the absorbent pad. Subsequently, 100 µL of the diluted environmental samples was added to the absorbent pad and spread evenly over the pad using a small sterile glass rod. To determine an optimum dilution level for better visibility and CFU counting, five ten-fold serial dilutions (10^3, 10^4, 10^5, 10^6, and 10^7) with duplicates from each treatment were used. Based on the initial test runs, a dilution level of 10^3 was found optimum for each treatment and the study continued using this dilution with three replicates. Petri dishes were incubated (24 h, 35±0.5˚C), CFUs were counted using a manual darkfield colony counter with 1.5× magnification (Reichert, Inc. Depew, NY, USA).

Quantitative Real-Time Polymerase Chain Reaction Analysis

Quantitative real-time polymerase chain reaction (qRT-PCR) analysis was conducted to determine effects of nanoparticle treatment on the CH_4 producing methanogenic microbial community in the treated manure. The α-subunit of the methyl coenzyme M-reductase (mcrA) genes distinctive to methanogenic bacteria was targeted. The DNA copy numbers were used to understand the influence of NPs on methanogenic bacteria (Freitag and Prosser, 2009; Ma et al., 2012).

Ribonucleic acid extraction and cDNA synthesis

To conduct the qRT-PCR analysis towards finding the NPs effect on methanogenic community, ribonucleic acids (RNA) were extracted from 0.25 g of liquid dairy manure samples using the PowerMicrobiome™ RNA isolation kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) and stored at -80°C for further complementary deoxyribonucleic acid (cDNA) synthesis. After RNA extraction and prior to cDNA synthesis, co-isolated or contaminating DNAs were removed from RNA samples using the Ambion DNA-free™ DNase Treatment & Removal Kit (Invitrogen, Carlsbad, CA, USA). Isolated RNA was quantified using a spectrophotometer

(Model: NanoDrop 2000c, ThermoScientific, Waltham, MA, USA). Thereafter, approximately 80 ng of RNA was used to synthesize cDNA using the SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen Carlsbad, CA, USA). The synthesized cDNA was electrophoresed on a 1% agarose gel and was visualized using UV trans-illuminator paired with ethidium bromide dye that fluoresces under UV light to confirm the purity. Furthermore, a nanodrop spectrophotometer was used to quantify the synthesized cDNA. Simultaneously, the purity of RNA and cDNA were assessed based on the spread of the bands on an agarose gel and by measuring absorbance ratios at 260/280 nm and 260/230 nm in a spectrophotometer. Later on, cDNA samples were frozen at -80C° until quantitative polymerase chain reaction (qPCR) analyses were performed.

Quantification of the methanogenic community

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) analysis was carried out in an ABI Prism™ 7500 (Applied Biosystems Inc, Foster City, CA, USA) real-time PCR system. Power SYBER Green PCR Master Mix (Applied Biosystems Inc, Foster City, CA, USA) containing SYBR Green I dye was used for the reaction in a 96-well plate. Forward Primer ((MLF) and reverse primer (mcrA-rev)) specific for mcrA genes were used to amplify genomic DNA and cDNA. MLF GGTGGTGTMGGATTCACACARTAYGCWACAGC 32-base pairs and mcrA-rev CGTTCATBGCGTAGTTVGGRTAGT 24-base pairs were the primer sequence synthesized by Integrated DNA Technologies, Inc. (IDT, Coralville, IA, USA) and were used to enumerate the α-subunit of mcrA gene (Luton et al., 2002; Narihiro and Sekiguchi, 2011). For a target reaction volume of 20 µL in each well in the plate, 10 µL of SYBER Green Master Mix, 1 µL of each template DNA (standard culture DNA and extracted cDNA, respectively), 0.4 µL of each forward and reverse primers when considered 200 nM of primer concentration, and the remaining amount (8.2 µL) was HyPure ™Molecular Biology Grade Water (HyClone Laboratories, Inc., Logan, UT, USA) were used. In this analysis, a total of 54 [{(4 treatments (3

NPs + control) × 3 replications + 6 (5 dilutions of standard DNA + 1 blank)} × 3 replications] wells in a 96 well plate were used. The reactions in denaturing, annealing, and extension phases in the thermocycler were programmed for 10 min initial holding at 95°C; 45 denaturing cycles (30 s, 95°C); annealing (45 s, 72°C) and extension with 45 s at 72°C. The dissociation step at 95ºC for 15 s and 60ºC for 1 min was added at the end to check the specificity of the PCR outputs. Amplifications of five dilutions (10^2, 10^3, 10^4, 10^5, and 10^6) of the standard *Methanobacterium formicicum Schnellen* 1947 (DSM 1535; Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, 38124 Braunschweig, Germany) was plotted against the real-time threshold cycle (CT) to get the standard curve paired with amplification from dairy manure samples.

Statistical Analysis

All treatments were replicated in triplicate and the averages reported. The analysis of variance (ANOVA) test was performed to find out the effect of treatments (e.g., three nanogels) on CH_4, CO_2 and H_2S gas concentrations, pH, conductivity, ash, crude protein, total N, ammonia, VFAs, and microbial population. The averages of each variable among treatments were compared using PROC ANOVA procedure in SAS 9.3 software (SAS Institute Inc., Cary, NC, USA). The null hypothesis was treatments had equal impact on gas concentrations and other parameters at 95% ($P \leq 0.05$) significance level. Then, variables were separated using Duncan's Multiple Range Test if the main effect (NPs dose) using F-test was significant at $P \leq 0.05$.

Results and Discussions

Effects of Nanoparticles on Manure Properties

No significant differences in pH, conductivity, crude protein, total nitrogen, and ash content between pre- and post-treatment were seen in the untreated liquid dairy manure sample

(no NPs). Nanogel treated manure pH values were significantly higher (7.2 to 7.91) than the control (6.47 to 6.64) (Table 5). Near neutral pH is expected for the anaerobic digestion, and higher conversion of VFAs towards methane production is also evidential with neutral pH. In the present study, addition of NPs as treatment has revealed an increase in alkalinity by raising the pH. Higher pH in nanogel treated samples were likely due to use of sodium hydroxide during nanogel formulation and further release of hydroxyl ions in the liquid dairy manure. This increase in pH was likely to lower the VFA accumulation in NP treated manure. Contrariwise, pH below the neutral range may have ended up with higher VFAs in the control treatment (Rea, 2014).

Conductivities for the nanogel treated manures were higher (32 - 52%) compared to the control (final) treatment. The conductivities of the liquid dairy manure treated with three nanogels were significantly different compared with control initial and final manure samples. The presence of metal NPs (zinc and copper) in the synthesized nanogel and their ionic release into the manure might have instigated higher conductivity values (14 to 16 µS/cm). However, no statistically significant difference was observed among the nanogel treated manures. Similar to pH and electrical conductivity, the ash, crude protein, total nitrogen, and ammonia concentrations at the end of the experiment were similar among the nanogel (ZnSNL, CuSNL, and NACL) treated liquid manure, but were statistically significantly higher than those obtained in control manure (control final) except for ash concentrations (Table 5).

No significant difference was observed between ash content levels of ZnSNL and CuSNL treatments. The ash content % of the NACL treatment significantly different from the CuSNL treatments but not the ZnSNL treated manure. Ash content of all treated manure samples was significantly higher than the controls except NACL. Carbonaceous substances in the NPs

formulation could be the probable reason Crude protein and total N % were significantly higher (14.46 to 17.76%) in all treatments compared with the final control. No statistically significant difference was found between the initial control and NPs treatments. Utilization of CP and N by a large microbial population in the control (final) treatment was the probable cause in this case. A lower microbial population in treated samples may result in higher CP and N because of the inhibitory effect of the NPs. Fecal ammonia concentration was significantly lower (23.13 to 53%) in all of the NP treated samples compared with final control (Table 5).

Table 5. Properties of pre and post treated liquid dairy manure.

Treatments	pH	Conductivity (µS/cm)	Ash (%)	Crude Protein (%)	Total N (%)	Ammonia (mM)
Control (Initial)*	6.64^{c}	9.84^{b}	18.47^{c}	15.00^{ab}	2.40^{ab}	96.56^{b}
Control (Final)**	6.47^{c}	10.91^{b}	17.45^{c}	13.33^{b}	2.13^{b}	121.68^{a}
ZnSNL	7.20^{b}	16.58^{a}	22.37^{ab}	15.54^{a}	2.49^{a}	78.42^{bc}
CuSNL	7.42^{b}	16.55^{a}	25.18^{a}	16.06^{a}	2.57^{a}	68.02^{c}
NACL	7.91^{a}	14.35^{a}	19.59^{bc}	16.20^{a}	2.59^{a}	57.59^{c}

N: Nitrogen
Values followed by the same letter in a column are not significantly different at $P \leq 0.05$.
*Control (Initial) means the fresh manure collected from a source before starting the experiment.
**Control (final) means the manure kept in a flask for 56 days without treating with NPs.

Effects of Nanoparticles on Volatile Fatty Acids

Total VFA (TVFA) manure concentrations of controls and NPs treatments ranged between 54.81 to 199.35 mM (Table 6). A significantly higher TVFA concentration was observed with the control (final) compared to the initial control manure and NPs treated manure (Table 6). ZnSNL, and CuSNL NPs treated manure samples showed a significantly lower TVFA concentrations compared to the NACL ($P \leq 0.05$). The VFA concentrations between ZnSNL and CuSNL treated manure were similar ($P \leq 0.05$). Conversely, manure treated with NACL

exhibited a higher amount of acetic acid compared to the other two nanogel treatments. Acetic and propionic acid concentrations in ZnSNL, CuSNL, and NACL treatments were significantly higher in the control samples (initial and final).

Hill & Bolte (1989) and Lahav and Loewenthal (2000) reported that both acetic acid and propionic acid are substrates for bacterial methane production. Hill & Bolte (1989) mentioned acetic acid as a substrate for methanogenic bacteria and reported about 70% of CH_4 emission is from this substrate and bacterial combination under anaerobic storage condition. Hence, lower values of acetic acid from ZnSNL and CuSNL treated manure either revealed lower acetic acid production or conversion of most of the acetic acid to methane. Reduced amount of acetic acid conversion could be either by an inhibition of fermentation or methanogenesis process in the anaerobic digestion pathway. However, a higher amount of acetic acid from the NACL treatment compared with other two NP treatments and lower amount of gas concentration from this treatment was likely to be an indication of reduced bacterial population from this treatment or adverse effect on any of the hydrolysis, acetogenesis or acetogenesis steps towards anaerobic digestion and hence gas production.

Table 6. Volatile Fatty Acids from liquid dairy manure exposed and not exposed to nanoparticle treatments

	Volatile Fatty Acids (mM)						
Treatments	**Acetic acid**	**Propionic acid**	**Isobutyric acid**	**Butyric acid**	**Isovaleric acid**	**Valeric acid**	**Total VFA**
Control (Initial)*	88.36[b]	32.58[a]	11.73[b]	31.98[a]	8.63[b]	2.74[b]	176.00[b]
Control (Final)**	104.47[a]	34.34[a]	14.93[a]	28.92[a]	10.66[a]	6.03[a]	199.35[a]
ZnSNL	39.51[d]	11.51[c]	2.8[d]	7.20[c]	1.32[d]	1.63[c]	63.98[d]
CuSNL	37.02[d]	6.92[d]	1.82[d]	6.44[c]	1.17[d]	1.45[c]	54.81[d]
NACL	80.71[c]	15.8[b]	6.37[c]	16.3[b]	4.36[c]	2.76[b]	126.30[c]

Values followed by the same letter in a column are not significantly different at $P \leq 0.05$.
*Control (Initial) is the initial sample of fresh manure not exposed to nanoparticles (NPs).
**Control (final) manure samples after 56 days incubation without NPs treatment.

Effect of Nanoparticles on Gas Production

The total gas produced during each sampling event from each of the four treatments at the end of 2, 8, 14, 42, and 56 d experimental periods is presented in Figure 10. The cumulative gas production from 500 mL of dairy manure treated with control, ZnSNL, CuSNL, or NACL were approximately 1128, 82, 47, and 60 mL, respectively. The rate of gas production from 500 mL of dairy manure was 20.15, 1.46, 0.83, and 1.07 mL/day for the control, ZnSNL, CuSNL, and NACL treatments, respectively. The experiment demonstrated gas reduction from ZnSNL, CuSNL, and NACL treated manure were approximately 93, 96, and 95%, respectively. Differences in gas production reduction among NP treated manure were not statistically significant ($P \leq 0.05$) but they were significantly lower than the control treatment ($P \leq 0.05$). A 90% reduction of gas production was observed in NP treated manure samples, compared to the control treatment much higher than previously published results where (Gautam et al., 2013) observed 60% total gas reduction using nZnO (10-50 nm size) application. Overall, CuSNL showed the highest total gas reduction potential (96%) among the NPs tested in this study, and

ZnSNL showed the lowest (93%), but the reduction was not statistically significant among the NP treatments. Thus, any of the nanogel treatments were likely to reduce gaseous emissions.

In this study, the NPs eradication or inhibition of microbial growth were the likely contributing factor to overall gas production reduction (Young and Santra, 2014). Additionally, the lower amount of cumulative gaseous emission from the NP treatments might be due to higher pH values in those treatments (Chen et al., 2005; Santra, 2012). Furthermore, absorption of the emitted gases by NPs within the manure slurry may also reduce overall gas production. Therefore, all probable causes for gas reduction need to be determined to get a more detailed understanding of gas reduction chemistry.

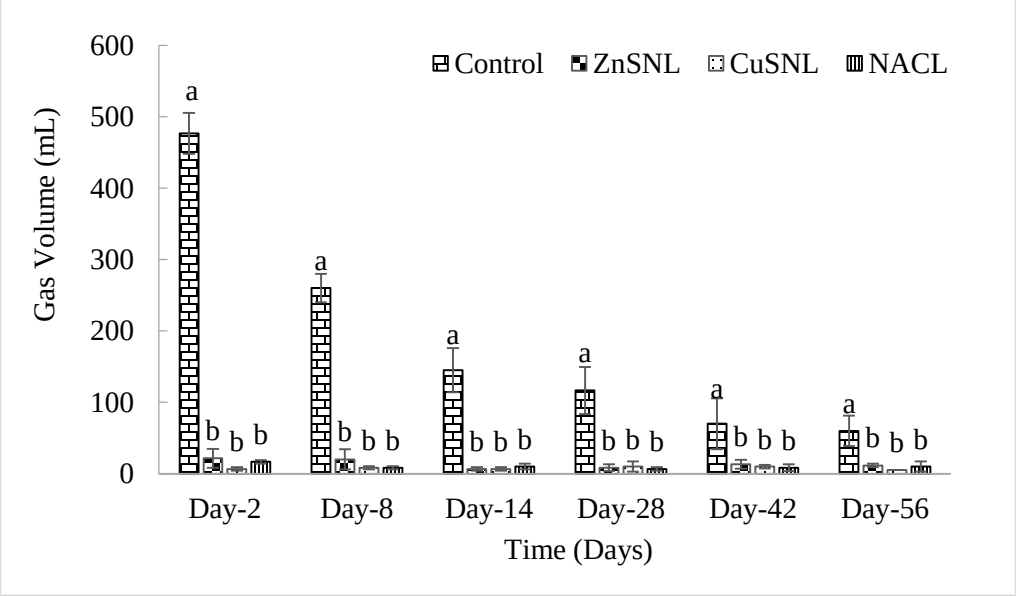

Figure 10. Gas production trends from dairy manure treated with different treatments and stored under anaerobic condition. Data presented are the means of three independent replications ± standard deviation. Values of each gas production from manure samples followed by different letters (a, b) above the bars indicate that the data points are significantly different at $P \leq 0.05$.

Effects of Nanoparticles on Methane Concentration

Methane concentrations at the end of 2, 8, 14, 42, and 56 d are shown in figure 11. No statistically significant differences were observed among NP treatments ($P \leq 0.05$). All NPs treatment had statistically significant less CH_4 compared with the control treatment ($P \leq 0.05$). Average CH_4 concentrations from the control treatment exhibited a sinusoidal pattern throughout the experimental period. Methane concentration increased gradually from 3.12% to 8.64% from 0 to 14 days, then decreased to 4.94% after 42 days, thereafter it increased up to 7.42% until the end of 56 d experimental periods (Figure 11). In contrast, ZnSNL treatment exhibited CH_4 concentration reduction potential consistently. ZnSNL treatment showed an 87.52% CH_4 reduction at the end of day-2 and 98.84% CH_4 reduction at the end of 56 d experimental period. In contrast, the NACL treatment demonstrated a 98.42 to 99.82% reduction of CH_4 concentration compared to the control. This treatment showed a marginally better reduction potential than that of ZnSNL for the entire period of the experiment. Copper Silica Nanogel Liquid (CuSNL) treated samples showed a similar CH_4 reduction trend as NACL and ZnSNL. CH_4 concentration reduction from the CuSNL treated samples were reduced by 97.75% to 99.76% when compared with the control treatment.

The results were compared to a previous study (Gautam et al., 2013), where they observed the effect of nZnO impregnated sodium alginate beads on CH_4 concentration from anaerobic storage of manure and reported about 89% concentration reduction in comparison with the control treatment. All three NPs used in the present study showed a similar or better CH_4 concentration reduction (87.52 to 99.82%) over the entire experimental period. The NACL exhibited the maximum reduction of 99.82% in CH_4 concentration although they are not statistically different. Reduction of CH_4 concentration was likely due to the antagonistic effect of

the NPs on the methanogenic bacterial population because this environment along with the bacterial population is the driving force for methane production (Van Elsas et al., 2006). Additionally, absorption of CH_4 within NPs suspension over time was also likely a contributing factor to CH_4 concentration reduction (Swain et al., 2016).

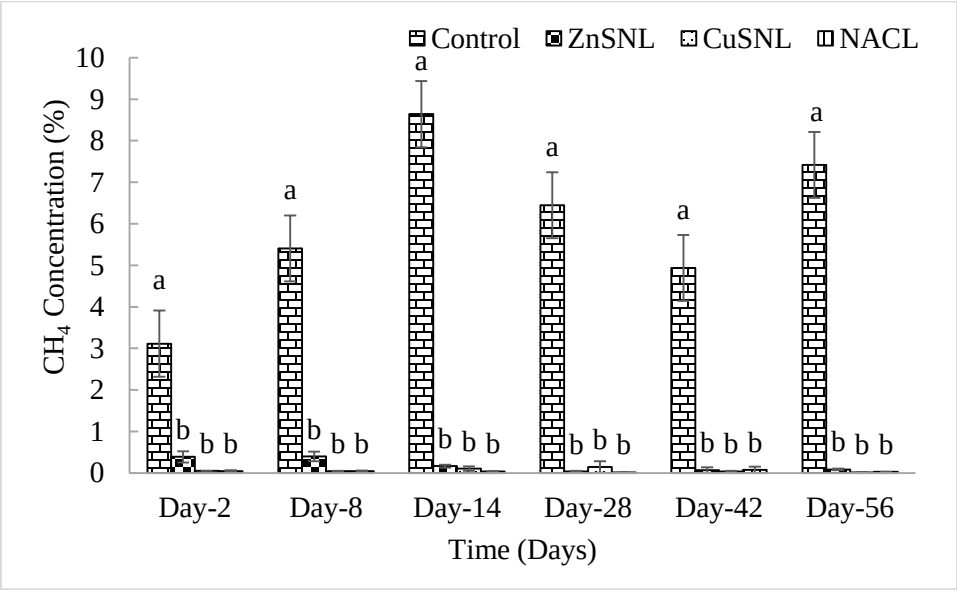

Figure 11. Methane (CH_4) production trends from dairy manure treated with different treatments and stored under anaerobic condition. Data presented are the means of three independent replications ± standard deviation. Values of each CH_4 concentrations form manure samples followed by different letters (a, b) above the bars indicate that the data points are significantly different at $P \leq 0.05$.

Effects of Nanoparticles on Carbon Dioxide Concentration

Carbon dioxide concentrations at the end of 2, 8, 14, 42, and 56 d are shown in figure 12. Carbon dioxide (CO_2) concentrations varied from 19.42 to 44.18%, 4.59 to 23.26%, 1.86 to 10.98%, and 1.52 to 6.87% for control, ZnSNL, CuSNL, and NACL treated samples, respectively (Figure 12). Unlike CH_4 concentration, CO_2 concentration in the control treatment did not have a sinusoidal trend. Instead, it increased from 19.42 to 44.18% up to 28 d of the experimental period and then decreased and remained steady towards the end (29.16 to 31.54%). For all of the three nanogel treatments (ZnSNL, CuSNL, and NACL), the CO_2 concentration was

lower at the beginning of the experimental period and marginally fluctuated up to day 28. Thereafter, CO_2 concentration increased gradually up to 21.28%. However, NACL treated manure showed the lowest CO_2 concentration compared with other two NP treatments and control. In the case of ZnSNL, within 28 days of the experiment CO_2 concentrations ranged 4.59 to 8.05% and then increased towards the end (23.26%). Carbon dioxide reduction from the manure treated with ZnSNL ranged between 20.24 to 85.33% compared with control during this 56 d and showed a maximum reduction at the end of day 28.

Copper silica nanogel liquid manure treatment resulted in 84.42 to 91.32% of CO_2 concentration reduction compared to the control treatment within the first 14 days. The CO_2 reduction was maximum at the end of day 8(91.32%). Thereafter, it showed an increasing trend of CO_2 concentration and ended up with 62.34% reduction compared to the control. In comparison with the control treatment, overall CO_2 concentration reduction by CuSNL was 25.50% more than that the reduction in ZnSNL treatment. In contrast, NACL treatment showed 76.44 to 96.28% reduction in comparison with control. However, compared to the control treatment, both ZnSNL and NACL treatment showed their maximum reduction potentiality by the end of the day 28. Additionally, at the end of the experimental period, NACL treatment exhibited overall 33.66% and 10.96% more CO_2 reduction than the amount reduced by ZnSNL and CuSNL treatments, respectively. Hence, among nanogels, NACL demonstrated the highest CO_2 reduction efficiency during the course of the experiment (Figure 12). Moreover, NACL and CuSNL treatment showed the statistically significant amount of CO_2 reduction at $P \leq 0.05$ compared to other two (control & ZnSNL) treatments but not among them. Contrariwise, no statistically significant differences were found between ZnSNL and control treatments ($P \leq 0.05$).

As with total gas volume and CH_4 production, CO_2 generation is also dependent on the decomposition of organic matter in manure, and reduction in CO_2 generation might be an indication of the reduced amount of organic matter. Consequently, reduced activity of the microbial community due to the application of NPs is also likely to contribute to reduced CO_2 generation. Additionally, conversion of most of the CO_2 to CH_4 through methanogenesis and absorption of CO_2 in the NP suspension are also likely causes towards CO_2 concentration reduction.

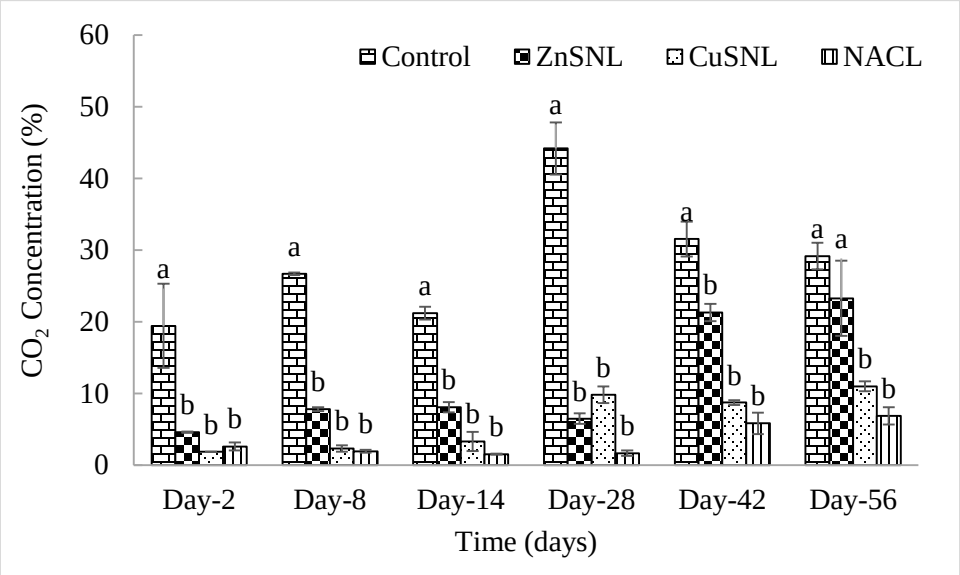

Figure 12. Carbon dioxide (CO_2) production trends from dairy manure treated with different treatments and stored under anaerobic condition. Data presented are the means of three independent replications ± standard deviation. Values of each CO_2 concentrations form manure samples followed by different letters (a, b) above the bars indicate that the data points are significantly different at $P \leq 0.05$.

Effect of Nanoparticles on Hydrogen Sulfide Concentration

Hydrogen sulfide (H_2S) concentrations at the end of 2, 8, 14, 42, and 56 d are shown in figure 13. The H_2S concentration in dairy manure treated with NPs were consistently lower than that of the control (Figure 13). H_2S concentration was 2.45 to 156.00, 0.28 to 1.57, 0.17 to 2.30, and 0.27 to 2.58 ppm for control, ZnSNL, CuSNL, and NACL treatments, respectively. In the

control treatment, apart from day 8, H_2S concentration increased up to day 28 and then decreased gradually. A higher activity of the sulfate reducing bacteria up to day 28 might have contributed towards higher H_2S concentration. In contrast, manure treated with ZnSNL exhibited a continuous reduction in the H_2S concentration until day 28 and then increased slightly. Hydrogen sulfide concentration from the ZnSNL treatment varied between 48.98 to 99.75% throughout the experimental period. The other two NP treatments exhibited a sinusoidal trend of H_2S concentration within this period. No statistically significant differences were observed among the three NP treatments in terms of H_2S concentration reduction ($P \leq 0.05$), at day 42. Hence, H_2S concentration reduction was likely due to the biocidal effect of the NPs on the dissimilatory sulfite reductase (DSR) enzyme as well as reduced amount of substrate in the treatment since anaerobic bacteria use sulfur containing compounds to utilize sulfate as an electron acceptor to produce H_2S (Spence et al., 2008).

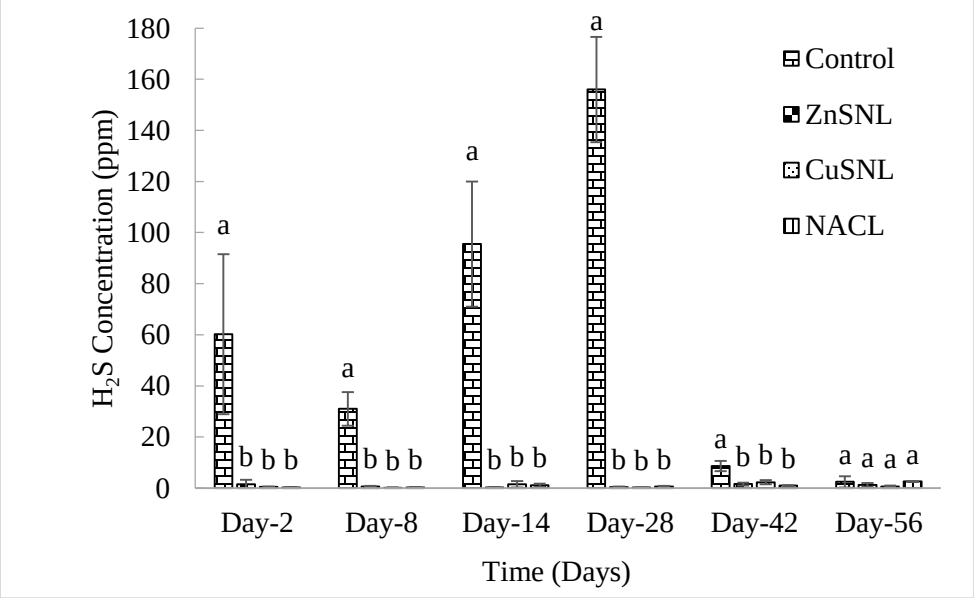

Figure 13. Hydrogen sulfide (H_2S) production trends from dairy manure treated with different treatments and stored under anaerobic condition. Data presented are the means of three independent replications ± standard deviation. Values of each H_2S concentrations form manure samples followed by different letters (a, b) above the bars indicate that the data points are significantly different at $P \leq 0.05$.

Effect of Nanoparticles on Bacterial Population

Total bacterial coliform counts in manure samples were as follows: initial untreated control (7.0×10^4), 56 day untreated control (8.0×10^4), ZnSNL (1.3×10^4), CuSNL 1.1×10^4, NACL (8.0×10^2) CFUs/mL (Figure 14). The coliform counts were validated by similar total coliform bacterial counts of 5.8×10^4, 0.3×10^4, and 3.8×10^4 from untreated control and treated liquid dairy manure with two NP treatments reported by (Gautam et al., 2016a). NACL treated manure exhibited the lowest coliform bacteria CFU/mL, whereas the control treatment showed the highest bacterial count compared to all other treatments. An approximately 15% increase in coliform bacteria count was observed between the initial control and final control treatments during the experimental period.

All three NPs treatments exhibited 81.42% and 90.06% reduction in CFUs compared to the control (initial) and control (final) treatment, respectively ($P \leq 0.05$). CFUs among nanogel treated manure were not statistically significant but they were significantly lower than that with the control treatment ($P \leq 0.05$) (Fig. 14). Bactericidal action of the applied NPs is most likely the cause of the reduced bacterial count. However, the effect of the NPs on individual gas producing bacterial population under anaerobic storage condition needs to be evaluated to get the detailed knowledge on the mechanism and chemistry regulating gas volume and concentrations.

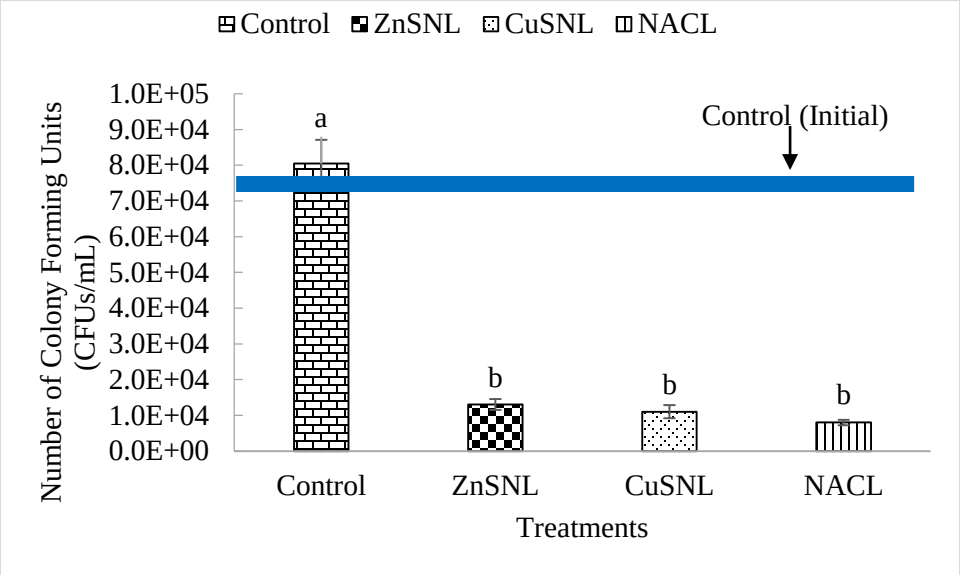

Figure 14. Effect of nanoparticles (NPs) on the colony forming units (CFU) of coliform bacteria in liquid dairy manure treated with three NPs. (N=3; different letters (a, b) above bar indicate treatments are significantly different ($P \leq 0.05$) compared to control.

Effect of Nanoparticles on Methanogen Population

A standard curve representing qRT-PCR amplification of five 10-fold serial dilutions of standard (genomic DNA) from a pure culture of *Methanobacterium formicicum Schnellen* had R^2 > 0.99 with the slope of -4.37 (Figure 15).

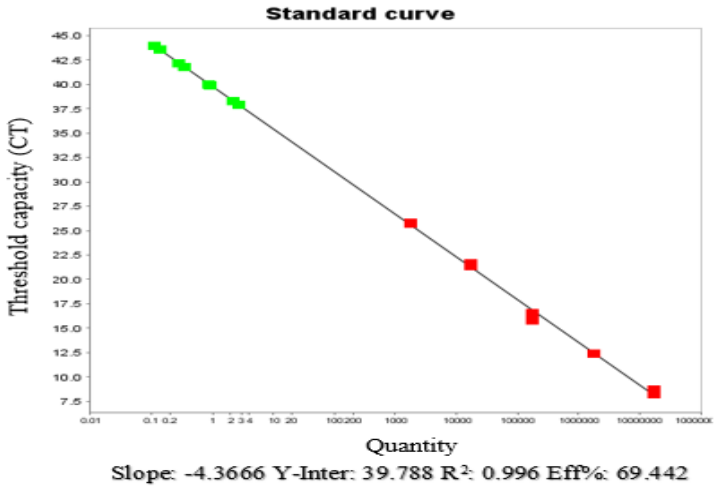

Figure 15. Standard curve for qRT-PCR amplification of five ten-fold serial dilutions of pure culture Methanobacterium formicicum Schnellen extracted c-DNA samples.

A PCR efficiency of >69% across all the standards were found from pre- and post-treated manure for quantification of a mcrA gene, which implied that more than 69% of the target sequences in the template genomic DNA were amplified in every cycle during the reaction process. Thereafter, amplifications of the genomic DNA were further investigated and specificity was found from the melting curve. Specific amplification of the standard mcrA gene was confirmed from the single sharp peak around 78˚C in the melting curve (Figure 16).

The presence of DNA copies (mcrA gene) in the environmental (treated manure) samples were in the same range (below or close to the lowest concentration of the standard chosen) as shown in the standard curves (Figure 15 & 16). None of the mcrA genes from the treated and untreated manure samples were amplified. It is well known that the presence of polymerase inhibitors due to bile salts (cholic and deoxycholic acid) in human feces have a direct effect on the amplification efficiency of PCR with low detection limits and precision of the real-time qPCR quantification (Al-Soud et al., 2005; Lantz et al., 1997). The presence of urea in urine (Khan et al., 1991) and hemoglobin and heparin in a clinical blood samples (Beutler et al., 1990; Brisson-Noel et al. 1991) were also recognized as inhibitors of PCR. Previously, it was also reported that humic and fulvic acid contaminants were extracted along with DNA during the DNA extraction process from soil, manure, and compost samples through inhibition of the amplicon production that limited amplification as PCR progressed (Al-Soud & Rådström, 1998; Fortin et al., 2004; Watson & Blackwell, 2000). In this research, a thorough and repeated optimization process was performed to optimize the PCR reaction parameters (denaturing, annealing, and extension temperatures and events) and concluded that some inhibitory contaminants were extracted along with DNA and were limiting the amplifications of mcrA gene from manure samples. Further studies are needed for a better understanding of the inhibition

process and their removal strategies for getting better amplifications from the target gene sequence. However, a plate count was followed to quantify and to compare the total bacterial count in treated and non-treated samples.

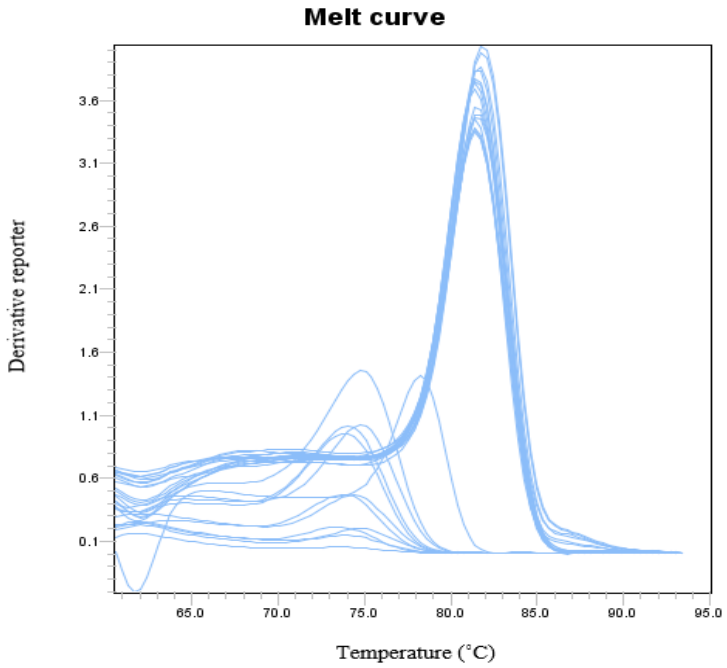

Figure 16. Melt curve for for qRT-PCR amplification of five ten-fold serial dilutions of pure culture *Methanobacterium formicicum Schnellen* (at ~82˚C) and extracted c-DNA samples (before 82˚C).

Possibilities and/or Difficulties of the Applied Method

The present study was a proof of concept study for these applied NPs, and the principle objective of this study was to find the effectiveness of the applied NPs towards GHGs and pollutant gas emission mitigation. Direct Application of such NPs by mixing with manure have revealed 81-99% reduction in cumulative gas volume paired with H_2S and GHGs concentration reduction by 49 to ~100%, and 20 to ~100%, respectively. In contrast, such application method of NPs might have a number of environmental issues coupled with endemic bacterial death of the manure, soil, and neighboring ecosystems. The ultimate application of NP treated manure in the agricultural field presumptively brings, carries, and produces a distress of heavy metal

accumulation in the soil and hence residual toxicity. However, based on the maximum annual pollutant loading rate (140 kg Zn/ha/yr, and 75 kg Cu/ha/yr), and assumption of field application of the NP treated manure initial estimate with the current application rate of 3g Zn/L has exposed with a final concentration of NPs in the soil which is within the acceptable lower limit of 10 ppm for Zinc, 2 ppm for Copper and upper limit 300 ppm for Zinc, 100 ppm for Copper (EPA Region VIII). Furthermore, part of the applied NPs might act as an essential micronutrient and it (NP) may catalytically make fertilizer more readily available for uptake by plants as well. Alternatively, to avoid such environmental consequences, indirect application of NPs such as entrapment of NPs into porous polymer, preparation of biofilters using the NPs can be done for further studies. Although, application of entrapped NPs in manure management systems is very limited, but entrapment of NPs in polymer is widely used in water and solid waste management area. However, indirect application of NPs might have ended up with reduced efficacy of the applied NPs. All of these warrant us a further study for the fate and transport of the applied NPs.

Conclusions

Compared with the control treatment, liquid dairy manure treated with three different NPs have exhibited increase in pH and consequently a decrease in VFA. All three nanogel treatments reduced gaseous volume and gas concentration significantly. CuSNL outperformed other treatment in terms of total gas volume and H_2S concentration reduction. Whereas, NACL treatment outperformed other treatments in terms of CH_4 and CO_2 concentration reduction. Reduction of GHGs and H_2S were likely due to microbial inhibition since NPs treated samples had lower CFUs than the controls. Further studies are needed to understand the amplification of inhibition process since none of the mcrA genes from the treated and untreated manure samples were amplified.

Acknowledgements

This was a collaborative initiation between North Dakota State University (NDSU) and University of Central Florida (UCF). This research was conducted using the corresponding author's discretionary funding. No specific funding was involved. Thanks to Debra Baer, Technical Communication Specialist, Agricultural and Biosystems Engineering Department, NDSU, for reviewing the manuscript.

References

Abouelenien, F., Kitamura, Y., Nishio, N., & Nakashimada, Y. (2009). Dry anaerobic ammonia–methane production from chicken manure. *Applied microbiology and biotechnology, 82*(4), 757-764.

Al-Soud, W. A., Ouis, I.-S., Li, D.-Q., Ljungh, Å., & Wadström, T. (2005). Characterization of the PCR inhibitory effect of bile to optimize real-time PCR detection of Helicobacter species. *FEMS Immunology & Medical Microbiology, 44*(2), 177-182.

Al-Soud, W. A., & Rådström, P. (1998). Capacity of nine thermostable DNA polymerases to mediate DNA amplification in the presence of PCR-inhibiting samples. *Applied and environmental microbiology, 64*(10), 3748-3753.

Arivalagan, K., Ravichandran, S., Rangasamy, K., & Karthikeyan, E. (2011). Nanomaterials and its potential applications. *International Journal of ChemTech Research, 3*(2), 534-538.

Asis, D. A. (2008). Investigation of potential application of nanoparticles in reducing gas and odour emission from swine manure slurry. (Master's thesis) University of Saskatchewan. 211 pgs. Retrieved from eCommons https://ecommons.usask.ca/handle/10388/etd-07082008-121809. Accessed on: 09.19.2017.

Bajpai, P. (2017). Basics of Anaerobic Digestion Process. In *Anaerobic Technology in Pulp and Paper Industry* (pp. 7-12): Springer.

Bang, J., Yang, H., & Holloway, P. H. (2006). Enhanced and stable green emission of ZnO nanoparticles by surface segregation of Mg. *Nanotechnology, 17*(4), 973.

Batley, G. E., & Simpson, S. L. (2016). Sediment sampling, sample preparation and general analysis. *Sediment Quality Assessment: A Practical Handbook. CSIRO Publishing, Canberra, Australia*, 15-46.

Beutler, E., Gelbart, T., & Kuhl, W. (1990). Interference of heparin with the polymerase chain reaction. *Biotechniques, 9*(2).

Boadi, D., Benchaar, C., Chiquette, J., & Massé, D. (2004). Mitigation strategies to reduce enteric methane emissions from dairy cows: update review. *Canadian Journal of Animal Science, 84*(3), 319-335.

Bolyard, S. C., Reinhart, D. R., & Santra, S. (2013). Behavior of engineered nanoparticles in landfill leachate. *Environmental science & technology, 47*(15), 8114-8122.

Borhan, M., Gautam, D., Engel, C., Anderson, V., & Rahman, S. (2013). Effects of pen bedding and feeding high crude protein diets on manure composition and greenhouse gas emissions from a feedlot pen surface. *Journal of the Air & Waste Management Association, 63*(12), 1457-1468.

Brisson-Noel, A., Nguyen, S., Aznar, C., Chureau, C., Garrigue, G., Pierre, C., . . . Gicquel, B. (1991). Diagnosis of tuberculosis by DNA amplification in clinical practice evaluation. *The Lancet, 338*(8763), 364-366.

Centi, G., & Perathoner, S. (2012). Reduction of greenhouse gas emissions by catalytic processes. In *Handbook of Climate Change Mitigation* (pp. 1849-1890): Springer.

Chen, W.-M., Tseng, Z.-J., Lee, K.-S., & Chang, J.-S. (2005). Fermentative hydrogen production with Clostridium butyricum CGS5 isolated from anaerobic sewage sludge. *International Journal of Hydrogen Energy, 30*(10), 1063-1070.

Chinnamuthu, C., & Boopathi, P. M. (2009). Nanotechnology and agroecosystem. *Madras Agricultural Journal, 96*(1-6), 17-31.

Clemens, J., Trimborn, M., Weiland, P., & Amon, B. (2006). Mitigation of greenhouse gas emissions by anaerobic digestion of cattle slurry. *Agriculture, ecosystems & environment, 112*(2), 171-177.

Das, S., Wolfson, B. P., Tetard, L., Tharkur, J., Bazata, J., & Santra, S. (2015). Effect of N-acetyl cysteine coated CdS: Mn/ZnS quantum dots on seed germination and seedling growth of snow pea (Pisum sativum L.): imaging and spectroscopic studies. *Environmental Science: Nano, 2*(2), 203-212.

Fabrega, J., Luoma, S. N., Tyler, C. R., Galloway, T. S., & Lead, J. R. (2011). Silver nanoparticles: behaviour and effects in the aquatic environment. *Environment international, 37*(2), 517-531.

Fortin, N., Beaumier, D., Lee, K., & Greer, C. W. (2004). Soil washing improves the recovery of total community DNA from polluted and high organic content sediments. *Journal of microbiological methods, 56*(2), 181-191.

Freitag, T. E., & Prosser, J. I. (2009). Correlation of methane production and functional gene transcriptional activity in a peat soil. *Applied and Environmental Microbiology, 75*(21), 6679-6687.

Gautam, D., Rahman, S., Borhan, M., & Bezbaruah, A. (2013). Applications of nanoparticles (NPs) in livestock manure and their effects on air emissions. Paper presented at the Intl. Symp. Animal Environ. Welfare. Chongqing, China.

Gautam, D. P., Rahman, S., Bezbaruah, A. N., & Borhan, M. S. (2016a). Evaluation of Calcium Alginate Entrapped Nano Zinc Oxide to Reduce Gaseous Emissions from Liquid Dairy Manure. *Applied Engineering in Agriculture, 32*(1), 89-102.

Gautam, D. P., Rahman, S., Borhan, M. S., & Engel, C. (2016b). The effect of feeding high fat diet to beef cattle on manure composition and gaseous emission from a feedlot pen surface. *Journal of animal science and technology, 58*(1), 22.

Gautam, D. P., Rahman, S., Fortuna, A.-M., Borhan, M. S., Saini-Eidukat, B., & Bezbaruah, A. N. (2016c). Characterization of zinc oxide nanoparticle (nZnO) alginate beads in reducing gaseous emission from swine manure. *Environmental Technology*, 1-14.

Ge, Y., Schimel, J. P., & Holden, P. A. (2011). Evidence for negative effects of TiO2 and ZnO nanoparticles on soil bacterial communities. *Environmental science & technology, 45*(4), 1659-1664.

Gunaseelan, V. N. (1997). Anaerobic digestion of biomass for methane production: a review. *Biomass and bioenergy, 13*(1), 83-114.

Hill, D., & Bolte, J. (1989). Digester stress as related to iso-butyric and iso-valeric acids. *Biological Wastes, 28*(1), 33-37.

Hobbs, P., Webb, J., Mottram, T., Grant, B., & Misselbrook, T. (2004). Emissions of volatile organic compounds originating from UK livestock agriculture. *Journal of the Science of Food and Agriculture, 84*(11), 1414-1420.

Johnson, J. M.-F., Franzluebbers, A. J., Weyers, S. L., & Reicosky, D. C. (2007). Agricultural opportunities to mitigate greenhouse gas emissions. *Environmental pollution, 150*(1), 107-124.

Khan, G., Kangro, H., Coates, P., & Heath, R. (1991). Inhibitory effects of urine on the polymerase chain reaction for cytomegalovirus DNA. *Journal of clinical pathology, 44*(5), 360-365.

Kinsman, R., Sauer, F., Jackson, H., & Wolynetz, M. (1995). Methane and carbon dioxide emissions from dairy cows in full lactation monitored over a six-month period. *Journal of Dairy Science, 78*(12), 2760-2766.

Lahav, O., & Loewenthal, R. (2000). Measurement of VFA in anaerobic digestion: The five-point titration method revisited. *Water Sa-Pretoria, 26*(3), 389-392.

Lantz, P.-G., Matsson, M., Wadström, T., & Rådström, P. (1997). Removal of PCR inhibitors from human faecal samples through the use of an aqueous two-phase system for sample preparation prior to PCR. *Journal of Microbiological Methods, 28*(3), 159-167.

Latimer, G. W. (2012). *Official methods of analysis of AOAC International*: AOAC international.

Luna-delRisco, M., Orupõld, K., & Dubourguier, H.-C. (2011). Particle-size effect of CuO and ZnO on biogas and methane production during anaerobic digestion. *Journal of hazardous materials, 189*(1), 603-608.

Luton, P. E., Wayne, J. M., Sharp, R. J., & Riley, P. W. (2002). The mcrA gene as an alternative to 16S rRNA in the phylogenetic analysis of methanogen populations in landfillb. *Microbiology, 148*(11), 3521-3530.

Ma, K., Conrad, R., & Lu, Y. (2012). Responses of methanogen mcrA genes and their transcripts to an alternate dry/wet cycle of paddy field soil. *Applied and environmental microbiology, 78*(2), 445-454.

Marr, J., & Heitkamp, B. (2015). Minnesota Steel Culvert Pipe Service-Life Map. *Final Report, 31*.

Mu, H., Chen, Y., & Xiao, N. (2011). Effects of metal oxide nanoparticles (TiO 2, Al 2 O 3, SiO 2 and ZnO) on waste activated sludge anaerobic digestion. *Bioresource technology, 102*(22), 10305-10311.

Mulkey, C. (1999). Data Quality Objectives for Regulatory Requirements for Dangerous Waste Sampling and Analysis. LMHC (US). Available at: https://www.osti.gov/scitech/biblio/797557

Narihiro, T., & Sekiguchi, Y. (2011). Oligonucleotide primers, probes and molecular methods for the environmental monitoring of methanogenic archaea. *Microbial biotechnology, 4*(5), 585-602.

Navarro, E., Baun, A., Behra, R., Hartmann, N. B., Filser, J., Miao, A.-J., . . . Sigg, L. (2008). Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. *Ecotoxicology, 17*(5), 372-386.

Nowack, B., & Bucheli, T. D. (2007). Occurrence, behavior and effects of nanoparticles in the environment. *Environmental Pollution, 150*(1), 5-22.

Rahman, S., Borhan, M. S., & Swanson, K. (2013). Greenhouse gas emissions from beef cattle pen surfaces in North Dakota. *Environmental technology, 34*(10), 1239-1246.

Rahman, S., DeSutter, T., & Zhang, Q. (2011). Efficacy of a microbial additive in reducing odor, ammonia, and hydrogen sulfide emissions from farrowing-gestation swine operation. *Agricultural Engineering International: CIGR Journal, 13*(3).

Salata, O.V. 2004. Applications of nanoparticles in biology and medicine. *Journal of Nanobiotechnology*, **2**(1), 3. https://doi.org/10.1186/1477-3155-2-3.

Santra, S. (2012). Silica-based antibacterial and antifungal nanoformulation. In: Google Patents. https://www.google.com/patents/US8221791. Accessed on: 09.20.2017.

Ševců, A., El-Temsah, Y.S., Joner, E.J., Černík, M. 2011. Oxidative stress induced in microorganisms by zero-valent iron nanoparticles. *Microbes and Environments*, *26*(4), 271-281.

Sommer, S. G., Petersen, S. O., Sørensen, P., Poulsen, H. D., & Møller, H. B. (2007). Methane and carbon dioxide emissions and nitrogen turnover during liquid manure storage. *Nutrient Cycling in Agroecosystems, 78*(1), 27-36.

Spence, C., Whitehead, T., & Cotta, M. (2008). Development and comparison of SYBR Green quantitative real-time PCR assays for detection and enumeration of sulfate-reducing bacteria in stored swine manure. *Journal of applied microbiology, 105*(6), 2143-2152.

Swain, P. S., Rao, S. B., Rajendran, D., Dominic, G., & Selvaraju, S. (2016). Nano zinc, an alternative to conventional zinc as animal feed supplement: A review. *Animal Nutrition*, *2*(3), 134-141.

USEPA, E. (2015). Inventory of US Greenhouse Gas Emissions and Sinks: 1990–2013. *Washington, DC, USA, EPA*.

Van Elsas, J. D., Trevors, J. T., Jansson, J. K., & Nannipieri, P. (2006). *Modern soil microbiology*: CRc Press.

Watson, R., & Blackwell, B. (2000). Purification and characterization of a common soil component which inhibits the polymerase chain reaction. *Canadian Journal of Microbiology, 46*(7), 633-642.

Weiske, A., Vabitsch, A., Olesen, J. E., Schelde, K., Michel, J., Friedrich, R., & Kaltschmitt, M. (2006). Mitigation of greenhouse gas emissions in European conventional and organic dairy farming. *Agriculture, ecosystems & environment, 112*(2), 221-232.

Yang, Y., Zhang, C., & Hu, Z. (2013). Impact of metallic and metal oxide nanoparticles on wastewater treatment and anaerobic digestion. *Environmental Science: Processes & Impacts*, *15*(1), 39-48.

Young, M., & Santra, S. (2014). Copper (Cu)–Silica Nanocomposite Containing Valence-Engineered Cu: A New Strategy for Improving the Antimicrobial Efficacy of Cu Biocides. *Journal of agricultural and food chemistry*, *62*(26), 6043-6052.

Zhang, L., Jiang, Y., Ding, Y., Daskalakis, N., Jeuken, L., Povey, M., . . . York, D. W. (2010). Mechanistic investigation into antibacterial behaviour of suspensions of ZnO nanoparticles against E. coli. *Journal of Nanoparticle Research, 12*(5), 1625-1636.

Zhao, Z., Zhang, Y., Chen, S., Quan, X., & Yu, Q. (2014). Bioelectrochemical enhancement of anaerobic methanogenesis for high organic load rate wastewater treatment in a up-flow anaerobic sludge blanket (UASB) reactor. *Scientific reports, 4*, 6658.

PAPER 2: IN VITRO EVALUATION OF NANO ZINC OXIDE ON MITIGATION OF GASEOUS EMISSIONS[2]

Abstract

Enteric methane (CH_4) accounts for about 70% of total CH_4 emissions from the ruminant animal. Researchers are exploring ways to mitigate enteric CH_4 emissions from ruminants. In this study, four levels of nano zinc oxide (nZnO) and two feed types (e.g., alfalfa and corn silage) were mixed with bovine fluid to investigate the efficacy of nZnO and feed in mitigating gaseous production. All experiments were conducted in batches in 250 mL glass bottles with the ANKOM^{RF} gas production monitoring system. Gas production was monitored continuously for 72 h at a constant temperature (39 ± 2°C). Headspace gas samples were analyzed for greenhouse gas (CH_4 and carbon dioxide-CO_2) and hydrogen sulfide-H_2S concentrations. Pre- and post-substrate (i.e. mixed rumen fluid+ NP treatment+ feed composite) samples were collected for bacterial counts, and volatile fatty acids (VFAs) analysis. With the applied nZnO inclusion rates, alfalfa feed exhibited 37 to 45% more cumulative gas reduction than corn silage, but increased GHG concentration by 2.17 to 23.17% and H_2S concentration by 60%. Irrespective of feed types compared to the control treatment, with the different nZnO inclusion levels on an average, H_2S and GHGs concentration reduction varied between 4.89 to 53.65%. Results suggest that both 500 and 1000 μg g^{-1} nZnO application levels have potential to reduce GHG and H_2S concentrations

[2] The material in this chapter was co-authored by Niloy Chandra Sarker, Faithe Keomanivong and Md. Borhan, Shafiqur Rahman, Kendall Swanson. Niloy Chandra Sarker and Faithe Keomanivong had primary responsibility for collecting samples and analyzing laboratory data. Niloy Chandra Sarker also drafted and revised all versions of this paper. Md. Borhan, Shafiqur Rahman, Kendall Swanson served as proofreader and checked the math in the statistical analysis conducted by Niloy Chandra Sarker. Paper 2 was submitted for review in February 2018 to *Grass and Forage Science* (The Journal of the British Grassland Society and the Official Journal of the European Grassland Federation) as manuscript number GFS-2018-0043. Status: Currently under review.

($P \leq 0.05$) during enteric fermentation on the top of feed type. Additionally, feed type and nZnO have impacts on microbial population reduction, which may also contribute to gaseous production.

Keywords: rumen, feed, greenhouse gases, nanoparticle, nan- zinc oxide (nZnO), in vitro

Introduction

The agricultural sector is recognized as one of the sources of methane (CH_4) and other gaseous emissions, and it is contributing approximately 250 million metric ton CO_2 equivalent CH_4 emission per year (EPA 430-P-18-001). Most of the CH_4 emissions from the agricultural sector are from the livestock industry and manure management. Almost 70% of the agricultural sectors CH_4 emission is from enteric fermentation, while 26% is from the livestock manure processing and handling (management) (EIA, 2009). Enteric fermentation includes fermentation in the rumen and hindgut paired with digestive hydrogen (H_2) metabolism by microbial catalyst (Moss et al., 2000). During enteric fermentation, CH_4 and carbon dioxide (CO_2) are the two main greenhouse gases (GHGs) emitted and contribute to global warming (Moss et al., 2000). Hydrogen sulfide (H_2S) is another pollutant gas generated during enteric fermentation, although its amount is not significant compared with CH_4 and CO_2. Hydrogen sulfide might be a potential health hazard to livestock and workers depending on the concentration level (Hughes et al., 2009). Hence, the reduction of these gas emissions without altering animal productivity is a challenge for a healthy environment and sustainable livestock industries.

Fermentation of carbohydrates in the reticulorumen of the ruminant animal occurs for available hydrogen supply towards volatile fatty acid (VFA) production and eventually leads to CH_4 production (Hogan, 1993; Johnson & Johnson, 1995; Bauchop & Mountfort, 1981; Ushida & Jouany, 1996; Wolin & Miller, 1988). Additionally, fermentation and neutralization of

hydrogen ions (H^+), and bicarbonate ions (HCO^{3-}) entering the rumen across the ruminal wall during VFA absorption contribute to CO_2 production in the rumen (Dehority, 2003; Hristov et al., 2013). Similarly, sulfur-containing amino acids and sulfates are the main sources of H_2S within the rumen; H_2S generation depends on the microbial degradation of amino acids and sulfates (Dehority, 2003; Drewnoski, Beitz, Loy, Hansen, & Ensley, 2011; Morine, Drewnoski, & Hansen, 2014).

Since gaseous emissions pose potential environmental and safety concerns, scientists are striving to mitigate the production of these gases. Management of feeding strategy, application of biotechnology, and the introduction of additives are a few of the most common approaches that researchers are working on for abating enteric gaseous emissions (Martin, Morgavi, & Doreau, 2010). Similarly, changes in the forage species, good forage processing, reduction of forage maturity, and increased feeding frequency are a few noteworthy gas mitigation strategies (Boadi, Benchaar, Chiquette, & Massé, 2004). Shifting the forage species from timothy hay to alfalfa (lucerne), changes in feed storage conditions (short vs. long), and the addition of fats into the feeds are a few strategies that have the potential to reduce enteric gaseous emissions (Benchaar, Pomar, & Chiquette, 2001; Robertson & Waghorn, 2002). Simultaneously, the addition of medium chain fatty acids (lipids) have shown the potential in reducing gaseous emission compared to other long chain fatty acids (Dohme, Machmüller, Wasserfallen, & Kreuzer, 2000; Dong, Bae, McAllister, Mathison, & Cheng, 1997; Machmüller & Kreuzer, 1999).

Besides all of these gas mitigation strategies, application of a vaccine to reduce methane production is a prospective method, although inaccessibility of vaccines at different geographical locations limits this approach (Martin et al., 2010; Wright et al., 2004). Reduction of ruminant CH_4 by adding chloroform to the feed is a possible option but not suitable for practice (Bauchop,

1967; Clapperton, 1974). However, application of any of these (vaccine and chloroform) for a prolonged period can damage the animal liver, and death of the animal may occur (Prins, 1965; Quaghebeur & Oyaert, 1971). Similarly, application of amichloral, trichloroacetamide, and trichloroethylene adipate are also possible approaches, but there are possibilities of negative impact on the animal for a prolonged period of feeding (Trei, Parish, Singh, & Scott, 1971; Trei, Scott, & Parish, 1972; Clapperton, 1974, 1977). However, all of these approaches exhibit a very small amount of gaseous emission reduction, and in most of the cases, the mitigation strategy focused on the reduction of CH_4 only. So, it is important to develop a new approach that can reduce multiple gaseous emissions without compromising animal health and productivity.

In recent years, nanotechnology has received attention for improving livestock production (Kuzma & VerHage, 2006). In the U.S., only 26 of 160 agri-food nanotechnology research and development projects were relevant to livestock facilities (Kuzma & VerHage, 2006). Animal health, veterinary medicine, and other animal production facilities are a few of the livestock-related sectors on which nanoparticles (NPs) have their promising footprints (Scott, 2005; Bollo, 2007; Narducci, 2007). For example, silver and zinc NPs have been added to animal feed to control microbial proliferation and promote animal growth, respectively. Similarly, zinc oxide (nZnO) NP is used to enhance growth and feed efficiency in piglets and poultry (Swain, Rao, Rajendran, Dominic, & Selvaraju, 2016). However, application of nanotechnology in mitigating gaseous emissions from livestock facilities is still limited. Swain et al. (2016) reported nZnO changes the rumen fermentation kinetics in ruminants and can alter the volatile fatty acids, therefore it may affect enteric CH_4 production. Similarly, application levels of NPs may also alter the microbial population, thus other gaseous emissions. Among the few studies performed with GHGs mitigation, nZnO were reported to have an inhibitory action

towards CH_4, CO_2 and H_2S from anaerobic storage of manure (Luna-delRisco, Orupõld, & Dubourguier, 2011; Mu, Chen, & Xiao, 2011, Gautam et al., 2016). Therefore, the objective of this study was to evaluate the effectiveness of four different application rates (100, 200, 500, and 1000 µg g^{-1} of feed) of nZnO in mitigating CO_2, CH_4, and H_2S emissions from rumen fluid under anaerobic storage conditions. Other than the application rate of 1000 µg g^{-1}, nZnO application rates were within the general dietary guideline of the maximum tolerable level of Zn mineral concentration provided by the National Academies of Sciences (NAS, 2016). The specific objective was to characterize the changes in the rumen fluid properties and find the gaseous reduction mechanisms such as by bacterial population reduction.

Materials and Methods

Rumen Fluid Collection, Processing and Experimental Set Up

In this in vitro study, ruminal fluid was collected from two ruminally-fistulated mature steers predominately of Angus breeding on a limit-fed grass hay-based diet fed to maintain body weight. Two hours after their morning feeding, approximately one liter of ruminal fluid was collected from each steer. To ensure uniform representation of the liquid and fiber phase, random grab samples were collected both from ventral and dorsal ruminal sacs. Prior to mixing with McDougall's buffer (McDougall, 1948), ruminal fluid from each steer was combined and strained through four layers of cheesecloth to remove the large particulate matter. Five treatments consisting of a control (no nZnO) and four levels of nZnO (100, 200, 500, and 1000 µg g^{-1} of feed), with two different feeds (alfalfa and corn silage; Table 7) were used. Nutrient composition of the two base diets are shown in Table 7. The nZnO (Particle Size = 35-45 nm and 99.5% purity) was purchased from US Research Nanomaterials, Inc., Texas, USA. The nZnO was mixed with two feeds (e.g., alfalfa and corn silage) separately and then a predetermined amount

of rumen fluid was added to each treatment. In each bottle, 1.5 g of ground alfalfa or corn silage (3 to 5 mm size) feed was added. Thereafter, 37.5 mL of the combined rumen fluid and 150 mL of McDougall's buffer were added to each ANKOM^{RF} gas bottle and a sub-sample of the mixed ruminal fluid was stored in the freezer for characterization. Then, each bottle was purged with CO_2 to create an anaerobic environment and sealed with the ANKOM^{RF} pressure monitor cap. Levels of nZnO were selected based on the maximum allowable zinc (Zn) concentration (30 to 500 µg g^{-1}) in feed recommended by the NAS (2016). The 1000 µg g^{-1} of nZnO level was added to investigate the effect of high nZnO application level on ruminal gaseous emission. Thus, in total, twenty (5 treatments × 4 replications) bottles were used for each feed type. The nZnO application levels were weighed on a Sartorius CP2P microbalance (Sartorius Corporation, NY, USA) with an accuracy of 1 µg using small aluminum pans (*DSC Consumables*, Inc., AU, USA).

Table 7. Composition of the feeds (dry matter basis)

	%									
Feeds	**Ash**	**CP†**	**NDF‡**	**ADF§**	**Ca¶**	**Pα**	**Mgβ**	**Kγ**	**Znδ**	**Cuε**
Lucerne	13.16	18.33	60.28	42.59	3.99	0.29	0.39	3.26	0.01	0.06
Corn silage	7.06	6.02	53.65	31.42	0.88	0.26	0.20	1.37	0.01	0.08

†CP = Crude Protein
‡NDF = Neutral Detergent Fiber
§ADF = Acid Detergent Fiber
¶Ca = Calcium
αP = Phosphorus
βMg = Magnesium
γK = Potassium
δZn = Zinc
εCu = Copper

Determination of Rumen Fluid pH and Redox

The pH, and redox of the mixed ruminal fluid were determined before and after the ruminal fluid was mixed with nZnO feed using a HANNA HI 4522 dual channel benchtop meter (VWR, TX, USA). Both probes were calibrated following manufacturer standard protocols. The reading of each probe was also checked with respective standard solutions before each measurement to ensure that the probes were reading correctly. Then, the probes were manually inserted into the mixed rumen fluid and data were recorded when the display was stabilized.

Volatile Fatty Acids Analysis

At the end of the experimental period, Whirl-Pak bags (Nasco, Fort Atkinson, WI and Modesto, CA, USA; 532-mL) were used to collect and store the rumen fluid subsamples at −20°C until further analysis. Thereafter, samples were equally composited using a vortex (Cat: 10153-842, VWR® digital vortex mixer, Radnor, PA, USA) and centrifuged (clinical 100 laboratory centrifuge, VWR, Rndor, PA, USA) at 2000 × g for 20 min. They were filtered through a pore size 0.45 µm to separate out the supernatant and analyzed for VFAs using an Agilent 6890N gas chromatograph (Agilent Technologies, Inc., Wilmington, DE, USA) equipped with an FID and fused silica column (Supleko brand, NUKUL 15 m × 0.53 mm × 0.5 µm, Sigma-Aldrich C., MO, USA), and 7683 series auto-injector following a widely used method (Goetsch & Galyean, 1983).

Gas Production Measurement and Monitoring System

All experiments were conducted using 250 mL ANKOM^{RF} gas glass bottles and under the same conditions. After proper flushing and sealing of bottles, they were placed in a water bath (SWBR17 shaking water bath, Atkinson NH, USA) that oscillated and heated at 125 rpm and 39 ± 2°C, respectively. Once they were placed, a wireless gas production measurement

system **(**ANKOM Technology Corp., Macedon, NY, USA) was used for monitoring and measuring gas production data. An ANKOM^{RF} gas production system consists of 1) sample bottles, 2) pressure sensor modules as bottle caps coupled with wireless communication system which is capable of measuring changes in pressures in real-time relative to the atmospheric pressure, 3) a reference module zero to monitor and record atmospheric pressure, and 4) a base coordinator (communicates with the RF sensor module) interfaced with a computer through operational software. Therefore, RF pressure sensor modules facilitated real-time measurement and monitoring of gas pressure inside the bottle relative to atmospheric pressure as a consequence of the gas produced during fermentation. The ANKOM^{RF} wireless system allows visualizing the pressure data in real-time and exporting data to an Excel file for further analysis. Data obtained from this system were converted from pressure (kPa) units to volume units (mL) using the ideal gas law as follows:

$$n = p\left(\frac{V}{RT}\right) \quad \text{(Eq. 1)}$$

$$Gas\ produced\ (mL) = n \times 22.4 \times 1000 \quad \text{(Eq. 2)}$$

where: n = gas produced in moles (mol), P = pressure in kilopascal (kPa), V = head-space volume in the glass bottle in liters (L), T = temperature in Kelvin (K), and R = gas constant (8.314472 L.kPa.k^{-1}.mol^{-1})

Throughout the experimental period, once gas pressure inside a bottle reached a set-limit in the RF pressure sensor module and recorded by the ANKOM^{RF} system, the headspace gas was released. Each bottle was connected to a Tedlar bag and released gas was collected for further analysis. A typical in vitro study lasts for 24 h, however, in our case it was continued for 72 h to examine the effects of nZnO on long-term in vitro fermentation. After 72 h of the experimental period, gas samples from the Tedlar bags were drawn using a gas-tight syringe (5 mL, Luer-Lok

TM Tip Syringe, Franklin Lakes, NJ, USA) and analyzed for GHGs (CH_4 and CO_2), and H_2S concentrations. Based on previous trials, collected gas was diluted 100 fold in pure nitrogen to keep the concentration in the measurable range of the analytical instruments and two measurements for individual bottles were taken for each of CH_4, CO_2, and H_2S concentration. A Jerome Meter (Jerome 631X, Arizona Instrument LLC, Arizona, USA) was used to measure H_2S concentration and a gas chromatograph (GC, 8610C, SRI instrument, California, USA) equipped with flame ionization detector (FID) and electron capture detector (ECD) detectors were used to measure CH_4 and CO_2 concentrations. Nitrogen at 138 kPa with a flow rate of 250 mL min^{-1} was supplied to the GC as a carrier gas. Additionally, a built-in air compressor and external hydrogen generator were used to supply hydrogen and air to the GC. Temperatures of 300 and 350 °C were maintained respectively on the FID and ECD detectors before insertion of any sample gas into the GC sample loop (Borhan et al., 2011). Calibration gases were used to check the proper functioning of the instruments and blank samples were used to check any contamination within the instruments from previous measurements (Rahman, Lin, & Zhu, 2012).

Analysis of Microbial Populations

Rumen fluid samples (~5 mL) were collected at the beginning (just before the experiment) and at the end of the experiment (after 72 h of the experiment) and they were analyzed for microbial population under aerobic conditions because of a lack of equipment for anaerobic culture. Besides, our main objective was to determine general effects on microbes (aerobic or anaerobic) and potential pathogens. M-Endo agar was used to enumerate coliforms *i.e.,* potential pathogens (particularly *Escherichia coli*) as recommended by the American Public Health Association (APHA) and the Environmental Protection Agency (EPA). Microbial population (potential pathogens) density was analyzed by counting total coliform bacteria in

terms of colony forming units (CFUs) following the plate count method (Gautam, Rahman, Bezbaruah, & Borhan, 2016). All reagents, labware, and Petri dishes used for microbial analysis were handled carefully and the whole experimental preparation was conducted in a sterile environment. One mL of the rumen fluid sample was collected from each treatment and replication, and they were diluted by up to five orders of magnitude (10, 10^2, 10^3, 10^4 and 10^5) to find the optimum dilution for better visibility of the CFUs. Later on, all treatments with three replications for the optimum dilution were established. The growth media used to culture the bacteria in an incubator consisted of a gridded sterile membrane filter attached with absorbent pad (47 mm diameter, 0.45 μm pore size, WCN type, Whatman Limited, Maidstone, England, UK) and placed in a sterile petri-dish (Anaerobic, Sterile Petri dishes, 60 mm diameter and 15 mm height, VWR, Radnor, PA, USA). Then, a 2 mL M-Endo broth ampule (P/N: 23735-50, HACH LANCH GmbH, Willstatterstrasse 11, Dusseldorf, Germany) was poured evenly over the entire surface of the absorbent pad. Subsequently, 100 μL of the diluted rumen fluid sample was added to the absorbent pad and smeared evenly over the pad using a small sterile glass rod. The petri dishes with the growth media and bacterial culture were then incubated for 24 h at 35 ± 0.5˚C in an incubator (Lab Companion IB-01E Incubator, San Diego, CA, USA). After 24 h of incubation, CFUs were counted using a manual dark field colony counter with 1.5X magnification (Reichert, Inc. Depew, NY, USA).

Statistical Analysis

The PROC GLM procedure (SAS 9.3 software, SAS Institute Inc., Cary, NC, USA) was used to investigate the effect of nZnO levels on in vitro pH, redox, VFAs, gas production, H_2S, CH_4, and CO_2 concentrations, and microbial populations. The averages of all dependent variables

for different inclusion levels of nZnO (treatments) and feed were compared using Duncan's Multiple Range Test if F-test were significant at ($P \leq 0.05$).

Results

Effect of nZnO Application Levels on in Vitro pH and Redox

The pH of alfalfa based rumen fluid incubated 72 h with different nZnO levels ranged between 7.20 to 7.25, whereas the pH of corn silage based rumen fluid ranged between 6.92 to 6.96 (Table 8). However, within the same feed type resulted pH was not significantly different for different nZnO levels *(P > 0.05)* (Table 8). Redox potential among the treated rumen fluid and two different feed combinations ranged between -296.50 to -307.23 mV (Table 8), which is the preferred range for producing CH_4 and CO_2 anaerobically (Sigg, 2000). No statistically significant differences *(P > 0.05)* in redox were observed among different nZnO levels within the same feed-based rumen fluid (Table 8). Overall, adding varying amounts of nZnO to rumen fluid and feed mix did not show any effect on pH and redox values.

Table 8. Effect of different application levels of nZnO (mean ± SEM) on in vitro pH and redox (after 72 hours of incubation).

Effects	Feeds	Treatments				
		Control	Treatment 100 µg/g	Treatment 200 µg/g	Treatment 500 µg/g	Treatment 1000 µg/g
pH	Alfalfa	$7.23 \pm 0.05^{a*}$	7.21 ± 0.04^{a}	7.25 ± 0.01^{a}	7.20 ± 0.01^{a}	7.22 ± 0.05^{a}
	Corn Silage	6.96 ± 0.01^{A}	6.93 ± 0.03^{A}	6.92 ± 0.04^{A}	6.94 ± 0.06^{A}	6.95 ± 0.02^{A}
Redox	Alfalfa	-296.53 ± 9.26^{a}	-305.45 ± 6.54^{a}	-297.55 ± 6.51^{a}	-301.90 ± 10.39^{a}	-302.23 ± 7.58^{a}
	Corn Silage	-307.23 ± 6.03^{A}	-297.25 ± 10.2^{A}	-303.4 ± 13.15^{A}	-303.93 ± 10.28^{A}	-296.50 ± 7.48^{A}

*Means in each row followed by the same superscript (lowercase letter for alfalfa and uppercase letter for corn silage) in pH and redox are not significantly different at $P \leq 0.05$.

Effect of nZnO Application Levels on in Vitro VFA Production

Among four applied nZnO levels and the control treatment, the amount of total VFA (TVFA) ranged between 136.52 mM to 194.16 mM for alfalfa-based rumen fluids while the resulted TVFA ranged between 161.36 mM to 192.8 mM for corn silage based rumen fluid (Tables 9 & 10). Irrespective of the feed types, after 72 hours of an experimental period, the control treatments exhibited the highest TVFA (194.16 and 192.8 mM) compared with other treatments (nZnO levels). For the alfalfa-based rumen fluid, compared with the different nZnO treatment levels, the control treatment exhibited 12.36 to 29.67% higher TVFA. In contrast, control treatment TVFA from corn silage based rumen fluid was 6.90 to 16.31% higher than the TVFAs with nZnO treatment levels. Similarly, for both of the feed situations, acetic acid and propionic acid were higher in the respective control treatments than for those treated with different nZnO levels (Tables 9 & 10). Regardless of the treatment types the resulting propionic acid from the silage based rumen fluid was~30% higher than alfalfa. Additionally, propionic acid to acetic acid (P/A) ratio was 15.79-45.45% higher for silage based fermentation than the alfalfa based fermentation. However, for the same feed type the P/A was not significantly different among the treatments *($P > 0.05$).* Alfalfa based fermentation P/A ratio varied from 0.29 to 0.32, whereas this ratio varied from 0.38 to 0.55 for the silage-based fermentation.

Table 9. Effect of different application levels of nZnO (mean ± SEM) on the combination of alfalfa and rumen fluid VFA (n = 4 observations/treatment)

Effects	Treatments				
	Control	Treatment 100 µg/g	Treatment 200 µg/g	Treatment 500 µg/g	Treatment 1000 µg/g
Acetic Acid (mM)	133.53±16.01^{a*}	90.15±12.46^{c}	95.51b±15.00^{c}	109.58±14.06^{bc}	115.63±12.48^{ab}
Propionic Acid (mM)	39.95±4.50^{a}	28.51±2.69^{b}	29.26±4.09^{b}	33.44±3.56^{b}	34.08±5.27^{ab}
P/A ratio†	0.30±0.10^{a}	0.32±0.03^{a}	0.31±0.01^{a}	0.31±0.01^{a}	0.29±0.02^{a}
Isobutyric Acid (mM)	2.58±0.26^{a}	2.19±0.27^{a}	2.21±0.26^{a}	2.42±0.15^{a}	2.52±0.51^{a}
Butyric Acid (mM)	11.78±1.25^{a}	9.96±1.06^{a}	10.27±1.17^{a}	10.93±0.79^{a}	11.78±2.40^{a}
Isovaleric Acid (mM)	4.32±0.43^{a}	3.96±0.78^{a}	4.05±0.35^{a}	4.17±0.31^{a}	4.42±0.98^{a}
Valeric Acid (mM)	2.00±0.20^{a}	1.75±0.30^{a}	1.78±0.12^{a}	1.87±0.07^{a}	2.03±0.44^{a}
Total VFA (mM)	194.16±22.13^{a}	136.52±14.93^{c}	143.08±20.81^{bc}	162.04±18.09^{bc}	170.16±21.04^{ab}

*Means followed by the same superscript (lowercase letter) in each VFA type are not significantly different at $P \leq 0.05$.

† P/A ratio = Propionic acid/Acetic acid ratio

Table 10. Effect of different application levels of nZnO (mean ± SEM) on the combination of corn silage and rumen fluid VFA (n = 4 observations/treatment)

Effects	Treatments				
	Control	Treatment 100 µg/g	Treatment 200 µg/g	Treatment 500 µg/g	Treatment 1000 µg/g
Acetic Acid (mM)	112.33±3.36^{A*}	104.07±6.32^{A}	102.05±25.83^{A}	107.49±7.96^{A}	113.64±15.24^{A}
Propionic Acid (mM)	61.80±1.67^{A}	39.51±13.91^{B}	44.62±15.48^{B}	48.77±1.08^{AB}	52.93±8.26^{AB}
P/A ratio†	0.55±0.001^{A}	0.38±0.13^{A}	0.43±0.09^{A}	0.46±0.02^{A}	0.47±0.03^{A}
Isobutyric Acid (mM)	1.34±0.11^{B}	2.21±0.60^{A}	1.34±0.57^{B}	1.08±0.08^{B}	1.09±0.16^{B}
Butyric Acid (mM)	14.14±0.99^{A}	11.97±1.27^{B}	10.07±1.78^{BC}	9.98±0.68^{BC}	9.33±1.94^{C}
Isovaleric Acid (mM)	1.71±0.15^{B}	3.61±1.32^{A}	1.97±1.22^{B}	1.39±0.15^{B}	1.30±0.29^{B}
Valeric Acid (mM)	1.48±0.08^{AB}	1.61±0.15^{A}	1.30±0.20^{BC}	1.31±0.09^{BC}	1.22±0.24^{C}
Total VFA (mM)	192.8±6.33^{A}	162.99±16.13^{A}	161.36±40.77^{A}	170.01±9.90^{A}	179.49±25.49^{A}

*Means followed by the same superscript (uppercase letter) in each VFA type are not significantly different at $P \leq 0.05$.
† P/A ratio = Propionic acid/Acetic acid ratio

Effect of nZnO Application Levels on in Vitro Gaseous Emission and CH_4, CO_2, and H_2S Concentrations

Figure 17 represents the cumulative gas produced over 72 hours of incubation with different nZnO application level and feed types. The cumulative gas produced from the alfalfa fermentation ranged between 39.86 to 43.90 mL, while it varied from 69.56 to 75.36 mL from the corn silage fermentation. Regardless of the treatments (NP doses), cumulative gas from the corn silage-based fermentation was 37 to 45% higher than the alfalfa-based fermentation. As compared to the respective control treatments, except for the 1000 µg g^{-1} nZnO application level

with the alfalfa feed type and 500 µg g^{-1} nZnO application level with the silage feed, all other nZnO application levels for both of the feed types reduced cumulative gas production by 1.25 to 7.51%. In comparison to their respective control treatment, both 1000 µg g^{-1} and 500 µg g^{-1} application rate with alfalfa and silage, respectively, exhibited 6.74 and 0.20% increase in gas volume. However, for the same feed type no significant difference in terms of cumulative gas production among different applied nZnO levels was found *(P > 0.05)* (Figure 17).

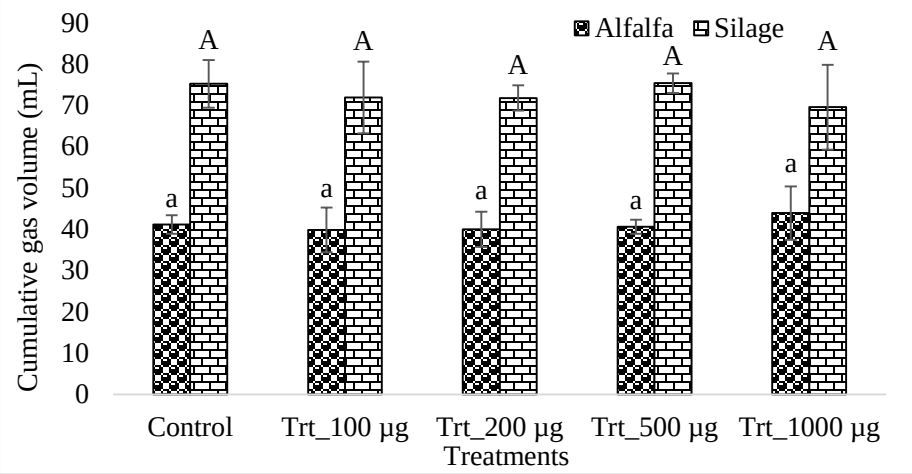

Figure 17. Effects of nZnO application levels and feed types on in vitro gas production. The different lowercase letter indicates significant differences among the treatments with alfalfa feed and the different uppercase letter indicates significant differences among the treatments with corn silage feed at $P \leq 0.05$ significance level.

Although cumulative gas volume measured from the corn silage based rumen fluid was almost two times higher than that of alfalfa based rumen fluid, corn silage based rumen fluid produced lower CH_4, CO_2, and H_2S gas concentrations than that of alfalfa. Irrespective of the feed types as compared to the control treatment, all of the applied nZnO levels showed a similar reduction trend for all CH_4, CO_2, and H_2S concentrations (Figure 18, 19, & 20). Application of nZnO in alfalfa-based rumen fluid reduced CH_4 concentration by 5.30 to 48.41% compared with the respective control treatment, while the reduction varied between 13.21 to 45.09% in the silage-based rumen fluid (Figure 18). It is noteworthy that CH_4 concentrations of alfalfa-based

rumen fluid were 2.17 to 23.17% higher than the corn silage-based rumen fluid. In contrast, compared with the individual control treatments, CO_2 concentration reduction from nZnO treated alfalfa-based rumen fluid ranged between 4.51 to 41.12%, and its concentration reduction from corn silage-based rumen fluid ranged between 5.29 to 44.50%, while both feeds and rumen fluid combinations were treated with four different application levels of nZnO (Figure 19). Overall, CO_2 concentrations from corn silage were 3.20 to 12.66% lower than its counterpart alfalfa.

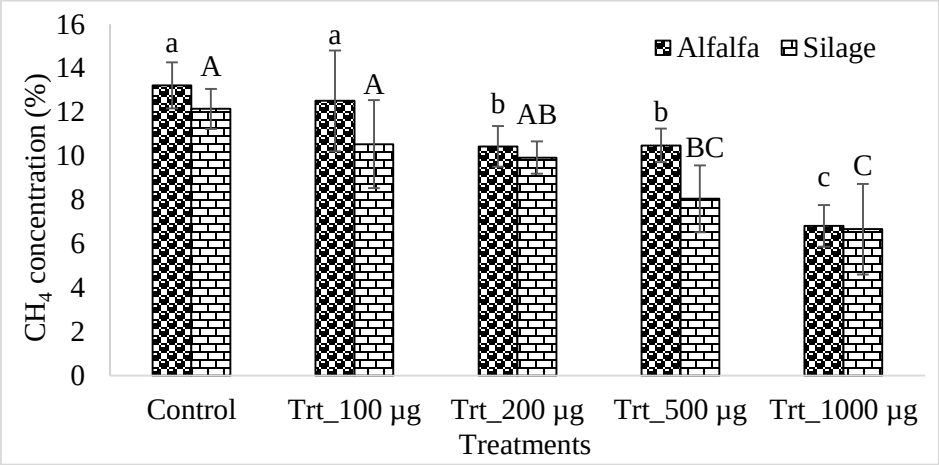

Figure 18. Effects of nZnO application levels and feed types on in vitro methane (CH_4) concentration. The different lowercase letters indicate significant differences among the treatments with alfalfa feed and the different uppercase letters indicates significant differences among the treatments with corn silage feed at $P \leq 0.05$ significance level.

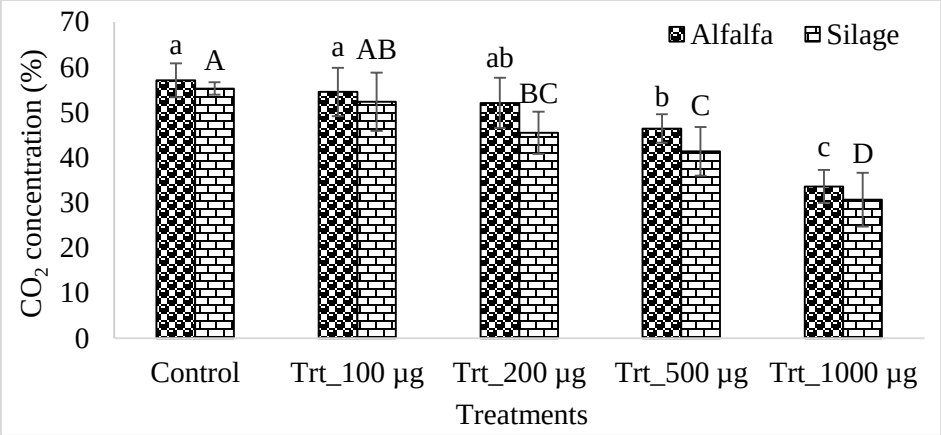

Figure 19. Effects of nZnO application levels and feed types on in vitro carbon dioxide (CO_2) concentration. The different lowercase letters indicate significant differences among the treatments with alfalfa feed and the different uppercase letters indicates significant differences among the treatments with corn silage feed at $P \leq 0.05$ significance level.

The effects of nZnO levels on in vitro H_2S concentration for alfalfa and corn silage are shown in Figure 20. Regardless of the nZnO application levels, H_2S concentration from the corn silage was ~60% less than that of alfalfa. The H_2S concentrations ranged between 2087.5 to 4512.5 ppm and 833.75 to 1787.5 ppm, for alfalfa and silage based rumen fluids, respectively. Regardless of the feed types, nZnO application rates reduced H_2S gas concentrations. As compared to the corresponding control treatments, H_2S concentration reduction ranged between 7.48 to 53.74% for the alfalfa-based rumen fluid while the reduction was 13.99 to 53.36% for the corn silage-based rumen fluid.

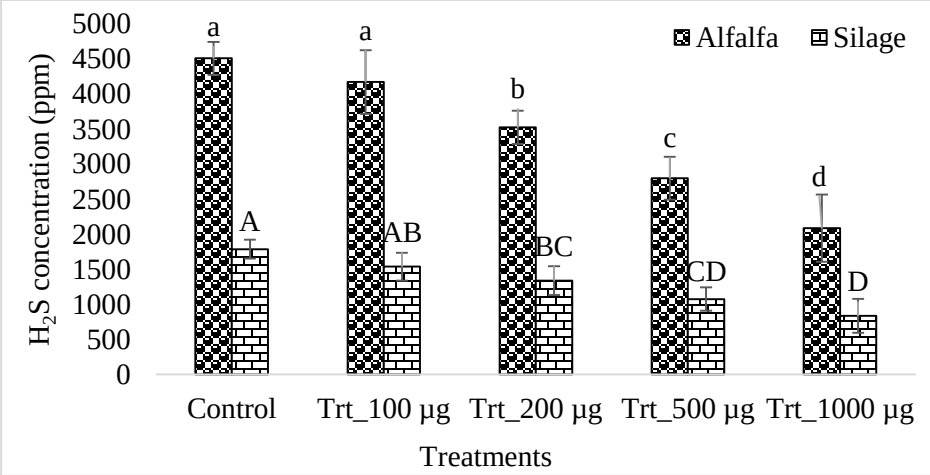

Figure 20. Effects of nZnO application levels and feed types on in vitro hydrogen sulfide (H_2S) concentration. The different lowercase letters indicate significant differences among the treatments with alfalfa feed and the different uppercase letter indicates significant differences among the treatments with corn silage feed at $P \leq 0.05$ significance level.

Overall, for both feeds, CH_4, CO_2, and H_2S concentrations reduction were related to the nZnO application rates. Both 500 and 1000 µg g^{-1} nZnO levels significantly *($P \leq 0.05$)* decreased CH_4, CO_2, and H_2S concentrations compared to other treatments (Figure 18, 19, & 20).

Effect of nZnO Application Doses on in Vitro Microbial Populations

Plate counts were done in terms of CFUs from pre- and post-treated rumen fluid samples to determine the effects of applied nZnO levels on in vitro microbial populations (Figure 21 & 22). The average initial CFUs were 88.4 counts with alfalfa feed based rumen fluid, and it was 85.2 counts with the corn silage feed based rumen. Initial CFUs were similar regardless of feed type or nZnO inclusion levels ($P > 0.05$) (Figure 21). In contrast, final CFU numbers exhibited a different trend than the initial number of CFUs (Figure 22). At the end of the 72 h experimental period, CFU numbers increased by ~98% for all of the treatments including control, and they ended up with an average of 4630, and 5155 counts for alfalfa and corn silage feeds, respectively. Final CFUs ranged between 3750 to 5000 counts for the alfalfa and 4575 to 5700 counts for the corn silage. Compared to the final CFU counts of corresponding control

treatments, ithe nclusion of different nZnO levels reduced CFUs 6.00 to 25.00% and 3.51 to 19.74% CFUs for alfalfa and corn silage based rumen fluids, respectively. Regardless of the nZnO inclusion levels, final CFU counts were 6.80-22.00% higher with the corn silage based rumen fluid compared with the alfalfa based rumen fluid. Overall, lower application levels of nZnO exhibited very small CFU reduction efficiency compared with the higher levels. The greatest reduction ($P \leq 0.05$) in microbial population was observed at the highest nZnO inclusion level (1000 µg g^{-1}).

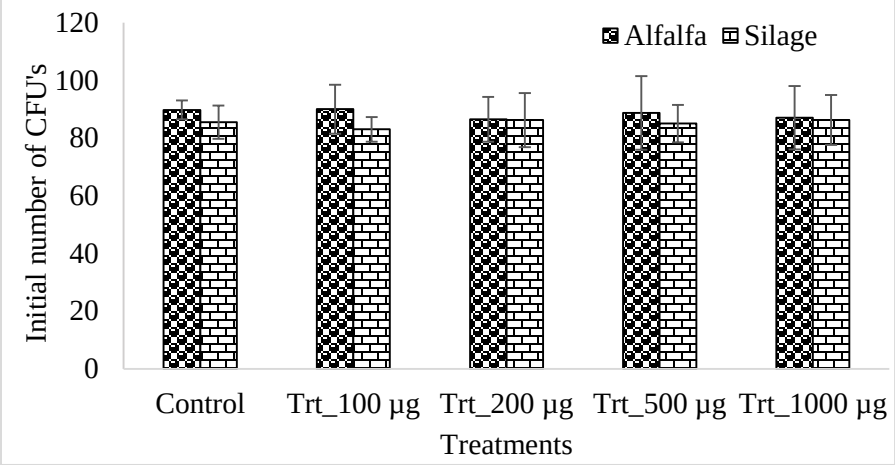

Figure 21. Effects of nZnO application levels and feed types on the initial in vitro microbial populations. For both of the individual feed situations, treatments are not significantly different at $P > 0.05$ significance level.

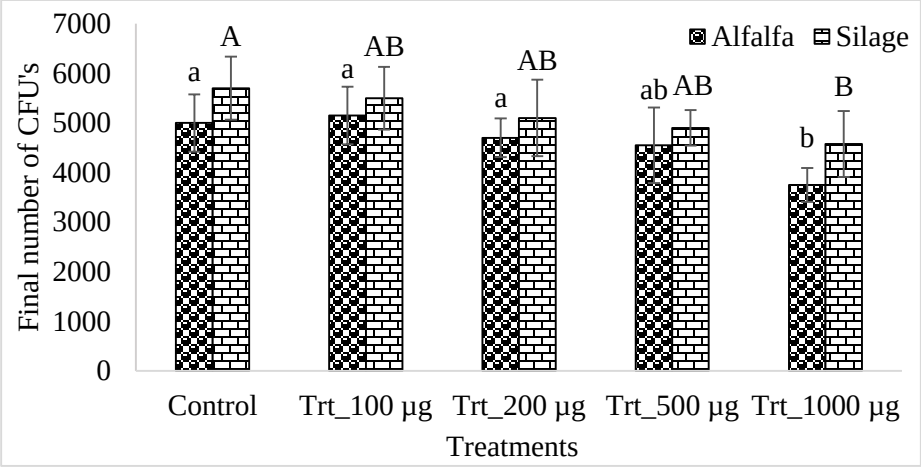

Figure 22. Effects of nZnO application levels and feed types on the final in vitro microbial populations. The different lowercase letter indicates significant differences among the treatments with alfalfa feed and the different uppercase letter indicates significant differences among the treatments with silage feed at $P \leq 0.05$ significance level.

Discussion

Lower pH of the rumen fluid incubated with corn silage based treatments might affect/inhibit acidogenic bacteria those are responsible for anaerobic digestion (Grant & Mertens, 1992; Bhandari, Ominski, Wittenberg, & Plaizier, 2007; Nutrition, 2016). In contrast, higher pH in alfalfa feed based treatments might increase the rate of fermentation, and contribute to the growth of spoilage microbes (Grant & Mertens, 1992; Bhandari et al., 2007; Nutrition, 2016). Moreover, a higher amount of crude protein in the alfalfa feed (Table 7) and the higher pH in the post-treated alfalfa-based rumen fluid would likely produce a higher amount of soluble protein (Wu, Yang, Zhou, & Song, 2009). Hence, higher concentrations of all three gases (CH_4, CO_2, and H_2S) were likely from the alfalfa-based treatments compared with its counterpart. The resulting consistent redox potential among the treatments was preferred for anaerobic fermentation (Blanc & Molof, 1973; Colmenarejo, Sánchez, Bustos, Garcıa, & Borja, 2004; Lee, 2008; Shete & Tomar, 2010). Additionally, redox potential among the treated rumen fluid and

two different feed combinations were in the preferred range for producing CH_4, CO_2, and H_2S anaerobically (Environmental, 2008).

Volatile fatty acids are considered as one of the most important parameters for ensuring anaerobic fermentation. The resulting P/A ratio from the corn silage was 26% higher than the previously reported value, while the P/A ratio for the alfalfa was identical to the reported value (0.3 to 0.4) (Ghimire, 2015). Higher P/A ratio might be an indication of imbalanced anaerobic fermentation with the corn silage-based rumen fluid fermentation (Lee, 2008). Subsequently, a higher amount of gas production from the corn silage-based fermentation was likely (Figure 17). Application of nZnO was presumed to affect either hydrolysis, acetogenesis, fermentation, methanogenesis or a combination of these in the fermentation process. In some cases, the bactericidal action of the applied higher nZnO levels might have killed the higher amount of methanogens, and hence higher amount of unconverted TVFA was likely. Furthermore, increased energy utilization followed by ruminal microbial protein synthesis by the microbes in the early stages of fermentation might have increased the TVFA with the applied higher nZnO levels as indicated by others (Zhisheng, 2011).

Higher gas production from the corn silage fermentation might be due to higher carbohydrate content and subsequent higher fermentability of corn silage compared to alfalfa. Additionally, a higher amount of fiber content in the alfalfa feed (Table 7) may have suppressed the cumulative gas production from the respective treatments. None of the applied nZnO application levels were able to reduce cumulative gas volume significantly, even 1000 $\mu g\ g^{-1}$ of nZnO was not enough to reduce a significant amount of cumulative gas production. Therefore, nZnO at this application rate does not appear to decrease the digestibility of feed by the animal,

and therefore, should not decrease productivity or growth. However, further studies are needed to understand the process and verify productivity is sustained when nZnO is included in the diet.

It is noteworthy that CH_4 concentrations with alfalfa-based treatment were higher than those of corn silage-based treatment (Figure 18), although higher cumulative gas production was observed in corn silage-based fermentation (Figure 17). This was likely due to favorable P/A ratio and subsequent balanced fermentation with alfalfa-based rumen fluid that might prompt higher CH_4, CO_2, and H_2S concentration as well (Lee, 2008). Generally, a group of archaea belonging to the phylum *Euryarcheota*, and collectively known as methanogens are responsible for CH_4 production within the animal rumen and hindgut (Hook, Wright, & McBride, 2010). Reduction of the CH_4 concentration from rumen fluid at the highest application level of nZnO (1000 µg g^{-1}) was likely due to the impact of excessive nZnO application rate (which was almost two-fold of the allowable limit as recommended by NAS as feed) specifically on methanogens (Swain et al., 2016). As mentioned previously, the highest application rate (1000 µg g^{-1}) of nZnO did not affect cumulative gas production, but likely reduced the enteric CH_4 concentration due to inhibitory action on the CH_4-producing methanogenic archaea. Additionally, adsorption of the produced methane on the NPs surface might also contribute to the reduction in CH_4 when nZnO was added to the rumen fluid. This situation warrants further study for investigating the effect of higher levels of zinc as a feed additive on animal growth and productivity.

Regardless of the feed types nZnO application levels, four to five times higher CO_2 concentration than the CH_4 concentration might be an indication of biocidal action of nZnO on methanogen archaea. Nano zinc oxide might leave only a small amount of methanogenic archaea active, and thus a higher amount of unconverted CO_2 was likely. During the anaerobic digestion process, methanogenic archaea utilize CO_2, and H_2 to produce CH_4. Furthermore, CO_2 emission

from rumen is directly related to the degradation of the organic constituents present in the feed, hence the decreasing trend in the CO_2 concentration was likely to indicate lower degradation rate of the organic matter in the rumen. Application of NPs might have an adverse impact on the microbial community and as a consequence lower degradation of organic compounds might occur. However, additional microbial studies are needed to understand the in-depth process.

The higher amount of H_2S concentration from the alfalfa based feed compared with the corn silage (Figure 20) was likely to be an indication of higher activity of the microorganisms. Since, in absence of oxygen (O_2) sulfate-reducing bacteria utilize sulfate to oxidize organic compounds present in the feed and ends up with the H_2S production as a byproduct, hence the reduction trend of H_2S concentration might be due to the reduced activity of the sulfate-reducing bacteria (Pouliquen et al., 1985). However, the concentration reduction mechanism needs to be explored to investigate the adverse effect of the nZnO on the microbial community.

Initial CFUs were measured right after the application of the nZnO in the system, therefore, there was little or no effects of nZnO levels on CFUs (Figure 21). In this circumstance, irrespective of the nZnO application levels, the number of microbial populations was most likely to represent the similar number of the populations present in the rumen fluid. In contrast, the addition of fresh feed was most likely to contribute towards the increasing amount of final CFUs. However, compared with the control (final), lower CFU numbers in nZnO treated samples were most likely due to the biocidal effect of nZnO. An insignificant reduction of CFUs with lower nZnO application levels might be an indication of the insufficient amount of available biocides. In contrast, higher reduction in CFUs was observed with higher application levels of nZnO and the reduction was significant only with 1000 $\mu g\ g^{-1}$ nZnO inclusion level. Furthermore, the presence of higher CFUs in the corn silage based treatments were likely to validate the higher

gas production from those treatments, and vice versa. Additional study at different levels and feed types are needed to understand in depth CFU reduction chemistry of nZnO.

Conclusions

Application of alfalfa feed reduced the cumulative gas volume significantly and increased CH_4, CO_2 and H_2S concentrations. Compared with the control treatment, irrespective of feed type, higher nZnO application rate (500 and 1000 μg g^{-1}) reduced CH_4, CO_2, and H_2S concentrations significantly (ranged from 21.85 to 53.65%). Similarly, the 1000 μg g^{-1} inclusion level reduced the microbial population in both feeds significantly (22.21%) as compared to individual control treatments. Based on this study, application of alfalfa feed and the inclusion of 500 or 1000 μg g^{-1} nZnO may reduce enteric fermentation resulting in lower enteric GHG emission from grass-fed beef. However, additional microbial studies are necessary to determine the mode of action. Additionally, further work is needed to assess the effect of nZnO inclusion on animal performance when cattle are fed ingredients commonly used in beef feedlot diets.

Acknowledgments

Thanks to Debra Baer, Technical Communication Specialist, Agricultural and Biosystems Engineering Department, NDSU, USA, for reviewing the manuscript. This study was conducted using the discretionary funding of the corresponding author. No specific funding was involved.

References

Bauchop, T. (1967). Inhibition of rumen methanogenesis by methane analogues. *Journal of Bacteriology, 94*(1), 171-175.

Bauchop, T., & Mountfort, D. O. (1981). Cellulose fermentation by a rumen anaerobic fungus in both the absence and the presence of rumen methanogens. *Applied and Environmental Microbiology, 42*(6), 1103-1110. doi: 0099-2240/81/121103-08$02.00/0.

Benchaar, C., Pomar, C., & Chiquette, J. (2001). Evaluation of dietary strategies to reduce methane production in ruminants: a modeling approach. *Canadian Journal of Animal Science, 81*(4), 563-574. doi: https://doi.org/10.4141/A00-119.

Bhandari, S., Ominski, K., Wittenberg, K., & Plaizier, J. (2007). Effects of chop length of alfalfa and corn silage on milk production and rumen fermentation of dairy cows. *Journal of Dairy Science, 90*(5), 2355-2366. doi: https://doi.org/10.3168/jds.2006-609.

Blanc, F. C., & Molof, A. H. (1973). Electrode potential monitoring and electrolytic control in anaerobic digestion. *Journal of Water Pollution Control Federation*, 655-667.

Boadi, D., Benchaar, C., Chiquette, J., & Massé, D. (2004). Mitigation strategies to reduce enteric methane emissions from dairy cows: update review. *Canadian Journal of Animal Science, 84*(3), 319-335. doi: https://doi.org/10.4141/A03-109.

Bollo, E. (2007). Nanotechnologies applied to veterinary diagnostics. *Veterinary Research Communications, 31,* 145-147. doi: 10.1007/s11259-007-0080-x.

Borhan, M. S., Capareda, S. C., Mukhtar, S., Faulkner, W. B., McGee, R., & Parnell, C. B. (2011). Greenhouse gas emissions from ground level area sources in dairy and cattle feedyard operations. *Atmosphere, 2*(3), 303-329. doi: 10.3390/atmos2030303.

Clapperton, J. (1974). The effect of trichloroacetamide, chloroform and linseed oil given into the rumen of sheep on some of the end-products of rumen digestion. *British Journal of Nutrition, 32*(01), 155-161. doi: https://doi.org/10.1079/BJN19740065.

Clapperton, J. (1977). The effect of a methane-suppressing compound, trichloroethyl adipate, on rumen fermentation and the growth of sheep. *Animal Production, 24*(02), 169-181. doi: https://doi.org/10.1017/S0003356100011636.

Colmenarejo, M., Sánchez, E., Bustos, A., Garcıa, G., & Borja, R. (2004). A pilot-scale study of total volatile fatty acids production by anaerobic fermentation of sewage in fixed-bed and suspended biomass reactors. *Process Biochemistry, 39*(10), 1257-1267. doi: https://doi.org/10.1016/S0032-9592(03)00253-X.

Dehority, B. A. (2003). Rumen microbiology (Vol. 372): Nottingham University Press, Nottingham, United Kingdom.

Dohme, F., Machmüller, A., Wasserfallen, A., & Kreuzer, M. (2000). Comparative efficiency of various fats rich in medium-chain fatty acids to suppress ruminal methanogenesis as measured with RUSITEC. *Canadian Journal of Animal Science, 80*(3), 473-484. doi: https://doi.org/10.4141/A99-113.

Dong, Y., Bae, H., McAllister, T., Mathison, G., & Cheng, K. (1997). Lipid-induced depression of methane production and digestibility in the artificial rumen system (RUSITEC). *Canadian Journal of Animal Science, 77*(2), 269-278. doi: https://doi.org/10.4141/A96-078.

Drewnoski, M., Beitz, D. C., Loy, D. D., Hansen, S. L., & Ensley, S. M. (2011). Factors affecting ruminal hydrogen sulfide concentration of cattle. *Animal Industry Report, 657*(1), 11.

EIA, 2009. Emissions of greenhouse gases in the U. S. Release date: March 31, 2011. Report number: doe/eia-0573(2009). Available at: https://www.eia.gov/environment/emissions/ghg_report/notes_sources.php

EPA 430-P-18-001, Draft inventory of us greenhouse gas emissions and sinks: 1990-2016. Published on: February 8, 2018. Available at: https://www.epa.gov /sites/ production /files /2018-01/documents/2018_complete_report.pdf.

Environmental, Y. (2008). ORP Management in wastewater as an indicator of process efficiency. *YSI, Yellow Springs, OH http://www. ysi. com/media/pdfs/A567-ORP-Management-in-Wastewater-as-an-Indicator-of-Process-Efficiency. pdf (accessed on 15.08. 13).*

Gautam, D. P., Rahman, S., Bezbaruah, A. N., & Borhan, M. S. (2016). Evaluation of Calcium Alginate Entrapped Nano Zinc Oxide to Reduce Gaseous Emissions from Liquid Dairy Manure. *Transaction of American Society Of Agricultural And Biological Engineers*, 89-102. doi: 10.13031/aea.32.11445.

Ghimire, S. (2015). Volatile Fatty Acid Production in Ruminants, Doctoral dissertation, Virginia Polytechnic Institute and State University, VA, USA.

Grant, R. H. & Mertens, D. R. (1992). Influence of buffer pH and raw corn starch addition on in vitro fiber digestion kinetics. *Journal of Dairy Science*, 75(10), 2762-2768. doi: https://doi.org/10.3168/jds.S0022-0302(92)78039-4.

Goetsch, A. & Galyean, M. (1983). Influence of feeding frequency on passage of fluid and particulate markers in steers fed a concentrate diet. *Canadian Journal of Animal Science, 63*(3), 727-730. doi: https://doi.org/10.4141/cjas83-084.

Hogan, K. B. (1993). Anthropogenic methane emissions in the United States, estimates for 1990. http://www.nal.usda.gov/.

Hook, S. E., Wright, A.-D. G., & McBride, B. W. (2010). Methanogens: methane producers of the rumen and mitigation strategies. *Archaea, 2010*. doi: 10.1155/2010/945785.

Hristov, A., Oh, J., Lee, C., Meinen, R., Montes, F., Ott, T., . . . Adesogan, A. (2013). Mitigation of greenhouse gas emissions in livestock production: A review of technical options for non-CO_2 emissions. *FAO Animal Production and Health Paper No, 177*, 1-206. doi: https://doi.org/10.1017/S1751731113000876.

Hughes, M.N., Centelles, M.N., Moore, K.P. 2009. Making and working with hydrogen sulfide: the chemistry and generation of hydrogen sulfide in vitro and its measurement in vivo: a review. *Free Radical Biology and Medicine*, 47(10), 1346-1353. doi: https://doi.org/10.1016/j.freeradbiomed.2009.09.018.

Johnson, K. A. & Johnson, D. E. (1995). Methane emissions from cattle. *Journal of Animal Science, 73*(8), 2483-2492.

Kuzma, J. & VerHage, P. (2006). Nanotechnology in agriculture and food production: Anticipated applications. Woodrow Wilson International Center for Scholars, *Project on Emerging Nanotechnologies, 44.*

Lee, S. J. (2008). Relationship between oxidation reduction potential (ORP) and volatile fatty acid (VFA) production in the acid-phase anaerobic digestion process. doi: http://hdl.handle.net/10092/1262.

Luna-delRisco, M., Orupõld, K., & Dubourguier, H.-C. (2011). Particle-size effect of CuO and ZnO on biogas and methane production during anaerobic digestion. *Journal of Hazardous Materials, 189*(1), 603-608. doi: https://doi.org/10.1016/j.jhazmat.2011.02.085.

Machmüller, A. & Kreuzer, M. (1999). Methane suppression by coconut oil and associated effects on nutrient and energy balance in sheep. *Canadian Journal of Animal Science, 79*(1), 65-72. doi: https://doi.org/10.4141/A98-079.

Martin, C., Morgavi, D., & Doreau, M. (2010). Methane mitigation in ruminants: from microbe to the farm scale. *Animal, 4*(03), 351-365. doi: https://doi.org/10.1017/S17517311 09990620.

McDougall, E. (1948). Studies on ruminant saliva. 1. The composition and output of sheep's saliva. *Biochemical Journal, 43*(1), 99.

Morine, S., Drewnoski, M., & Hansen, S. (2014). Increasing dietary neutral detergent fiber concentration decreases ruminal hydrogen sulfide concentrations in steers fed high-sulfur diets based on ethanol coproducts. *Journal of Animal Science, 92*(7), 3035-3041. doi: https://doi.org/10.2527/jas.2013-7339.

Moss, A. R., Jouany, J.-P., & Newbold, J. (2000). Methane production by ruminants: its contribution to global warming. *Ann. Zootech. EDP Sciences.* pp. 231-253. doi: https://doi.org/10.1051/animres:2000119.

Mu, H., Chen, Y., & Xiao, N. (2011). Effects of metal oxide nanoparticles (TiO_2, Al_2O_3, SiO_2 and ZnO) on waste activated sludge anaerobic digestion. *Bioresource Technology, 102*(22), 10305-10311. doi: https://doi.org/10.1016/j.biortech.2011.08.100.

National Academies of Sciences (NAS). 2016. Nutrient Requirements of Beef Cattle. Eighth Revised ed. The National Academies Press, Washington, DC.

Narducci, D. (2007). An Introduction to Nanotechnologies: What's in it for Us? *Veterinary Research Communications, 31*, 131-137.

Nutrition, L. A. (2016). Target pH levels in silage. In. Dairy Herd Management: http://www.dairyherd.com/quality-silage/target-ph-levels-silage.

Pouliquen, F., Blanc, C., Arretz, E., Labat, I., Tournier-Lasserve, J., Ladousse, A., . . . Perrot, J. (1985). Ullmann's Encyclopedia of Industrial Chemistry.

Prins, R. (1965). Action of chloral hydrate on rumen microorganisms in vitro. *Journal of Dairy Science, 48*(7), 991-993.

Quaghebeur, D. & Oyaert, W. (1971). Effect of chloral hydrate and related compounds on the activity of several enzymes in extracts of rumen microorganisms. *Transboundary and Emerging Diseases, 18*(5), 417-427. doi: 10.1111/j.1439-0442.1971.tb00595.x.

Rahman, S., Lin, D., & Zhu, J. (2012). Greenhouse gas (GHG) emissions from mechanically ventilated deep pit swine gestation operation. *Journal of Civil and Environmental Engineering 2,* 104. doi: 10.4172/2165-784X.1000104.

Robertson, L. & Waghorn, G. (2002). Dairy industry perspectives o methane emissions and production from cattle fed pasture or total mixed rations in New Zealand. Paper presented at the proceedings-New Zealand society of animal production.

Scott, N. (2005). Nanotechnology and animal health. *Revue Scientifique Et Technique-Office International Des Epizooties, 24*(1), 425.

Shete, S. & Tomar, S. (2010). Ruminating Over Methane Emissions. NISCAIR-CSIR, 31-32. Available at: http://hdl.handle.net/123456789/10197.

Sigg, L. 2000. Redox potential measurements in natural waters: significance, concepts and problems. *Redox, Springer,* 1-12.

Swain, P. S., Rao, S. B., Rajendran, D., Dominic, G., & Selvaraju, S. (2016). Nano zinc, an alternative to conventional zinc as animal feed supplement: A review. *Animal Nutrition, 2*(3), 134-141. doi: https://doi.org/10.1016/j.aninu.2016.06.003.

Trei, J., Parish, R., Singh, Y., & Scott, G. (1971). Effect of methane inhibitors on rumen metabolism and feedlot performance of sheep. *Journal of Dairy Science, 54*(4), 536-540. doi: https://doi.org/10.3168/jds.S0022-0302(71)85882-4.

Trei, J., Scott, G., & Parish, R. (1972). Influence of methane inhibition on energetic efficiency of lambs. *Journal of Animal Science, 34*(3), 510-515.

Ushida, K. & Jouany, J. (1996). Methane production associated with rumen-ciliated protozoa and its effect on protozoan activity. *Letters in Applied Microbiology, 23*(2), 129-132. doi: 10.1111/j.1472-765X.1996.tb00047.x.

Wolin, M. & Miller, T. (1988). Microbe interactions in the rumen microbial ecosystem. *The Rumen Ecosystem (ed. PN Hobson)*, 343-359.

Wright, A., Kennedy, P., O'Neill, C., Toovey, A., Popovski, S., Rea, S., . . . Klein, L. (2004). Reducing methane emissions in sheep by immunization against rumen methanogens. *Vaccine, 22*(29), 3976-3985. doi: https://doi.org/10.1016/j.vaccine.2004.03.053.

Wu, H., Yang, D., Zhou, Q., & Song, Z. (2009). The effect of pH on anaerobic fermentation of primary sludge at room temperature. *Journal of Hazardous Materials, 172*(1), 196-201. doi: https://doi.org/10.1016/j.jhazmat.2009.06.146.

Zhisheng, C. (2011). Effect of nano-zinc oxide supplementation on rumen fermentation in vitro. *Chinese Journal of Animal Nutrition, 8*, 023. doi: 10.14202/vetworld.2015.888-891.

PAPER 3: UNDERSTANDING GASEOUS REDUCTION MECHANISMS IN SWINE MANURE RESULTING FROM NANO-PARTICLE TREATMENTS UNDER ANAEROBIC STORAGE CONDITIONS[3]

Abstract

Manure is an important source of carbon (C), sulfur (S) and water (H_2O). Consequently, microbial populations utilize these constituents to produce methane (CH_4), carbon dioxide (CO_2), greenhouse gases (GHGs), and hydrogen sulfide (H_2S). Application of nanoparticles (NPs) to stored manure is an emerging GHG mitigation technique. In this study, two NPs: nano zinc oxide (nZnO) and nano silver (nAg) were tested in swine manure stored under anaerobic conditions to determine their effectiveness in mitigating gaseous emissions and total gas production. The biological mechanisms of gaseous reduction, *i.e.*, microbial populations were characterized via Quantitative Polymerase Chain Reaction (qPCR) analysis. Each treatment of the experiment was replicated three times in 1-L Erlenmeyer flasks with a working volume of 500 mL of swine manure to which NPs were applied at a dose of 3 g L^{-1} of manure. Headspace gas from all treatment replicates were analyzed for CH_4 and CO_2 gas concentrations using an SRI-8610 Gas Chromatograph and H_2S concentrations were measured using a Jerome 631X meter. Nanoparticles tested in this study reduced the cumulative gas volume by 16 to 79% compared to the control. Among the NPs tested, only nZnO consistently reduced GHG concentrations by 37 to 97%. Reductions in H_2S concentrations ranged from 87 to 97%. Gaseous reductions were

[3] The material in this chapter was co-authored by Niloy Chandra Sarker and Shafiqur Rahman, Md Borhan, Ann-Marie Fortuna. Niloy Chandra Sarker had primary responsibility for collecting samples and analyzing laboratory data. Niloy Chandra Sarker also drafted and revised all versions of this paper. Shafiqur Rahman, Md Borhan, Ann-Marie Fortuna served as proofreader and checked the math in the statistical analysis conducted by Niloy Chandra Sarker. Paper 3 was submitted for review in March 2018 to *Environmental Technology and Innovation* journal as manuscript number ETI_2018_93. Status: Currently under review.

likely due to decreases in the activity and numbers of specific gas producing methanogenic archaea and sulfate reducing bacterial (SRB) species.

Keywords: Swine, Nanoparticles, Greenhouse gas, Methanogen, Sulfate Reducing Bacteria.

Introduction

Animal housing including manure management systems are considered to be a primary source of odorous and hazardous gaseous emission (Zhu et al., 2000). Within the agricultural sector, the livestock subsector is reported as one of the substantial contributors of greenhouse gases (GHG) and hazardous hydrogen sulfide (H_2S) gas emissions. Research has demonstrated that approximately 80% of the agricultural sector's contribution to GHG emissions is from the livestock sector (Friel et al., 2009). Swine production facilities, in particular, are a major agricultural source of all of these gas emissions.

Livestock manure is an important source of carbon (C), sulfur (S) and water (H_2O) that can be used by microbial populations as substrate to produce methane (CH_4), carbon dioxide (CO_2), and H_2S (Arogo et al., 2000; Chadwick et al., 2011; Defra, 2010; Møller et al., 2004; Paul et al., 1993). Rotmans et al. (1992) have reported about 5 to 10% of global CH_4 emissions is from livestock manure. Additionally, 70% of the total CH_4 emitted from a swine farm is reported to be from swine manure (Monteny et al., 2006). The majority of gaseous emissions released from swine manure occurs during storage while both liquid and solid manure await disposal. These emissions are a byproduct of manure decomposition and the redox state (aerobic and anaerobic) of the storage environment determines the types of gases emitted (Kirchmann & Lundvall, 1998).

Among the emitted gases, both CH_4 and H_2S are reported to have greater potential hazard compared to CO_2. Although CH_4 has a short lifespan of 12 years, this gas has 25 times the global

warming potential of CO_2. As a result, this GHG is of major global concern (IPCC, 2007; Kemfert & Schill, 2009). Contrariwise, the presence of H_2S either in animal housings or manure pits is an odor nuisance and health hazard (Arogo et al., 2000). Exposure to high concentration of H_2S (e.g., 200-700 to ppm) may lead to severe health effects on animals and workers within livestock facilities (Hughes et al., 2009; Reiffenstein et al., 1992). Consequently, measures to reduce potential gaseous emissions need to be taken for the betterment of the livestock industry that is experiencing rapid global growth.

This experiment targets methanogenic archaea that utilize CO_2 and H_2 to produce metabolic energy, the byproduct of which is CH_4. Specifically, we measured *Methyl coenzyme Mreductase (Mcr),* an enzyme unique to methanogenic archaea (Van Elsas et al., 2006) via the Quantitative Real-Time Polymerase Chain Reaction (qPCR). Anaerobic decomposition of organic C present in manure produces CO_2 (Aarnink et al., 1995) that is utilized by methanogens. Dissimilatory sulfate reduction is an anaerobic process that utilizes H_2 and produces H_2S. The process is governed by three groups of sulfate reducing bacterial (SRB) species, *Desulfovibrio spp., Desulfomonas spp., and Desulfotomaculum spp* (Germida et al., 1992).

Methanogens and SRB concurrently utilize H_2 as an electron donor to produce CH_4 or H_2S. Consequently, competition between these two groups of microorganisms can occur (Kushkevych et al., 2017). Both methanogens and SRB bacteria coexist within overlapping redox ranges (-200 to -300 mV) (Sigg, 2000). Additionally, simultaneous production of H_2S with that of CH_4 might have an inhibitory or toxic effect on the methanogen community and the process of methanogenesis (Kushkevych et al., 2017). Therefore, the effects of NP applications on microbial communities and the anaerobic processes these microorganisms control should be

monitored in addition to the inorganic mechanisms associated with reductions in gaseous emissions.

Numerous manure management strategies are currently practiced to minimize gaseous emissions that include reducing the temperature (<10 °C) of the stored manure, increasing frequency of manure removal, management of the bedding and manure heaps, use of masking agents to cover up or eliminate odors, enzymes and bacterial preparations, chemicals, oxidation processes, air scrubbers, bio-filters, and new ventilation systems (Monteny et al., 2006; Sommer et al., 2004; Sutton et al., 1999). However, most of these practices are either labor intensive, costly or not entirely efficacious (Sutton et al., 1999). In most instances, the efficacy of the gaseous emission reduction is low or targeted at only one of the emitted gases (Kreuzer & Hindrichsen, 2006; Liao et al., 1995). Therefore, development of a better gaseous emission abatement strategy focusing all of the GHGs and other pollutant gases for a longer period of time is needed. Recently, Yang et al. (2012) and Dankovich and Gray (2011) have reported potential mitigation of CH_4 and H_2S gas emission from solid waste and water treatment subsectors using application of silver (Ag) NPs. Similarly, application of other NPs (*e.g.*, nano copper oxide-nCuO and nano zinc oxide-nZnO) for mitigating gaseous emissions from livestock manure has also been reported by several researchers (Gautam et al., 2016b; Luna-delRisco et al., 2011; Predicala et al., 2012). Versatile use of nanotechnology and the recent application of nanoparticles (NPs) in mitigating potential gaseous emissions from manure (Gautam et al., 2016a) motivated us to continue exploring the potential of NPs applications to reduce pollutant gases and the biological and chemical mechanisms involved, which has not been explored previously.

Most of the previous research conducted with nanoparticles utilized direct application of these materials (in bare-form or as-is without any coating) that resulted in unintended environmental consequences such as endemic bacterial death and residual toxicity. Therefore, utilization of coated and bare NPs for remediation purposes is considered to be an emerging research area requiring further investigation. Entrapment of NPs in polymers is widely used in water and solid waste management but has limited application in manure management. Gautam et al (2016a) entrapped NPs in polymers and conducted comparative studies between bare and entrapped nZnO in livestock manure to control gaseous emissions. Gautam et al. (2016a; 2016b) found that both application methods are effective in mitigating GHG and H_2S. However, bare applications were more effective in reducing gaseous emissions but in terms of recovery, entrapment of NPs in alginate beads is more effective. These studies did not explore the mechanisms that resulted in reductions in H_2S concentration.

There are two possible means (*e.g.*, biological and chemical) of reducing gaseous emissions via application of NPs. Currently, neither reduction mechanism has been properly explored. Based on the antibacterial properties of both nZnO and nAg, it is believed that application of these NPs will reduce biological activity and growth of microbial populations but to what degree requires further investigation. Both aerobic and anaerobic coliform counts and redox conditions in treated versus non-treated treatments is expected to aid in determining the biocidal effects of NPs under aerobic and anaerobic conditions. In addition, advanced methods like qPCR analysis provide a means of identifying the impact of the applied NPs on specific gas-producing microorganisms such as methanogens and SRB. Additionally, Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) may determine the effect of NPs on the manure mineral contents before and after any NP treatment. All of the above analyses provide

quantitative means of measuring how methanogens and SRB are affected by NP treatments. Therefore, the primary objectives of this research were to determine the efficacy of both the direct and indirect application of NPs to reduce GHG emissions from swine manure under anaerobic storage conditions (standard storage conditions for manure). An additional objective of this experiment was to examine the gaseous reduction mechanisms of each pollutant (CO_2, CH_4, and H_2S) treated with NPs.

Materials and Methods

Manure Collection

Swine manure was collected from the North Dakota State University (NDSU) swine research unit and stored for six hours at room temperature (22 ± 2 ˚C) and pressure (760 mm Hg) before use. Before setting up an experiment, manure was subsampled for measuring pH, redox, and conductivity. Along with these parameters, additional parameters such as total solids (TS), crude protein (CP), fecal ammonia (NH_3), and volatile fatty acids (VFAs) were also measured before and after application of the treatments. All experiments in this study were performed at room temperature and atmospheric pressure as mentioned above.

Characterization of the Manure

Liquid swine manures were characterized both before (i.e. initial samples, before adding any treatments) and after the experiment (i.e. at the end of 33 days) to determine the matrix properties of the manure and to categorize the changes in VFAs among the treatments along with initial and control treatments. Methods outlined by the Association of Analytical Communities (AOAC) were used to characterize manure properties listed in Table 11.

Table 11. Protocols followed to determine manure properties.

Parameters	AOAC Protocols used
pH	EPA SW-846, Method 9040
Redox	ASTM D1498-14 Standard Test Method for Oxidation-Reduction Potential
Dry matter (DM)	Official Methods of Analysis of AOAC International (2005) 18th ED., AOAC International Gaithersburg, MD, USA, Official Method 934.01
Total Nitrogen (TN)	Recommended Methods of Manure Analysis, A3769 Macro-Kjeldahl method
Ammonia (NH_3)	Sigma Technical Bulletin #640. Sigma Diagnostics, St. Louis, MO 63178
Crude Protein (CP)	Official Methods of Analysis of AOAC International (2005) 18th ED., AOAC International Gaithersburg, MD, USA, Official Method 2001.11 Run on the Kjeltec 2300, Foss NA, Eden Prairie, MN
Ash Content	Official Methods of Analysis of AOAC International (2005) 18th ED., AOAC International Gaithersburg, MD, USA, Official Method 942.05.
Volatile Fatty Acids (VFAs)	Method of Goetsch and Galyean, 1983. Agilent 6890N Gas Chromatograph with an FID (flame ionization detector) and the 7683 Series auto injector and auto sampler. The column used was the Supelco brand, NUKOL Fused Silica Column, 15 m x 0.53 mm x 0.5 um.

Nanoparticles Used and Their Application Methods

In this study, the nZnO and nAg used were purchased from US Research nano-materials, Inc., TX, USA. Nanoparticle properties pertinent to this experiment are listed in Table 12. NPs were applied to manure both directly (bare-uncoated) and in-directly (coated) at an application rate of 3 g L^{-1}. Rates are based on previously published literature (Gautam et al., 2016b). For indirect applications, nZnO were entrapped in sodium alginate beads, the process of which is provided in the following section.

Table 12. Properties of the Nanoparticles

Nanoparticles	CAS Number	Nominal size, nm	Purity, %	SSA (m^2g^{-1})	Form	Color	Morphology	True density
ZnO	1314-13-2	35-45	99+%	~65	powder	Milky White	Nearly spherical	5.606 gcm^{-3}
Ag	7440-22-4	50-80	99.99%	-	powder	Dark Black	spherical	10.5 gcm^{-3}

SSA: Specific surface area

Direct Application Method

The direct application method consisted of bare nZnO and nAg particles (without any entrapment or coating) added to manure in 1-L Erlenmeyer flasks at an application rate of 3 g L^{-1}. Nanoparticles were weighed using an AB204-S/FACT analytical balance (Mettler-Toledo, LLC Columbus, OH, USA) with a precision of 0.1 mg. Following addition of the nanoparticles (NPs) in manure, vigorous mixing of the NPs with manure was accomplished by hand shaking of flasks.

Indirect Application of Nanoparticles

Preparation of Sodium Alginate Beads

In this study, nZnO (US3580, US Research Nano-materials, Inc., Houston, TX, USA) were entrapped in sodium alginate beads (($C_6H_7O_6Na$)n, S1118, Spectrum, Gardena, Calif. and New Brunswick, NJ, USA) to avoid any diffusion of nZnO to the environment, as well as reuse of them to reduce application cost. Alginate beads were prepared by mixing nZnO solution with sodium alginate powder followed by hardening with calcium chloride solution. Pre-calculated nZnO (6 g L^{-1}) was added to an Erlenmeyer flask containing deionized (DI) water and then stirred at 350 rpm for 10 minutes at 50˚C using a hot plate stirrer (Cat No: N97042-642, 120 v, 1000 w, 10 amp, 50/60 Hz, VWR, Henry Troemner LLC, USA) to homogeneous the mixture. Sodium alginate powder was then added to the nZnO solution at a rate of 15 g L^{-1} and stirred for 48 h at 50˚C by using the magnetic hotplate stirrer. To completely suspend the nZnO and sodium

alginate in solution, 48 h of stirring was followed by 1 h of sonication using a Qsonica Sonicator (ultrasonic processor, Q700, Newtown, CT 06470, USA). Following sonication, the mixture was added into the 3.5% of calcium chloride ($CaCl_2.2H_2O$, BDHO224, VWR International LLC, West Chester, PA, USA) solution dropwise using a variable flow chemical transfer pump (Cat No. : 23609-170, 120 mL to 2.2 L min^{-1}, VWR, USA). Calcium chloride solution acted as a binder and alginate beads formed as drops of the mixture encountered the calcium chloride solution. Beads were left in the solution for 6 to 8 h to allow for complete hardening and to ensure porosity for solute transport. After which, the hardened sodium alginate beads were washed with deionized (DI) water to remove excess amounts of calcium chloride and were stored in fresh DI water until they were used. At the same time, bare sodium alginate beads without nZnO were also prepared by the same procedure.

Experimental Setup

Before experimental set-up, manure samples were thoroughly mixed and homogenized. Following mixing, a known amount of manure (500 mL) was transferred into a 1-L Erlenmeyer flask. In this study, nZnO and nAg were tested and the treatments were: control (without NPs), bare nZnO, nZnO entrapped in sodium alginate bead, bare sodium alginate bead, and nAg. All treatments were replicated three times. Following application of individual treatments, each Erlenmeyer flask was purged with inert nitrogen gas (N_2) to simulate anaerobic conditions before sealing with rubber stoppers. Each rubber stopper was fitted with a steel tube (5 mm diameter and 500 mm length) and inserted into the center of the stopper to collect gas samples from the reactor into a 500 mL Tedlar bag (SKC Gulf Coast Inc., Texas, USA) using a Teflon tube (Figure 23). Figure 23 depicts an experimental set up used in this study. All reactor connections were checked and sealed to avoid any leakage. In this way, a total of 21 Erlenmeyer flasks (7

treatments × 3 replications) were prepared. This study continued for 26 days or until the gas production stopped.

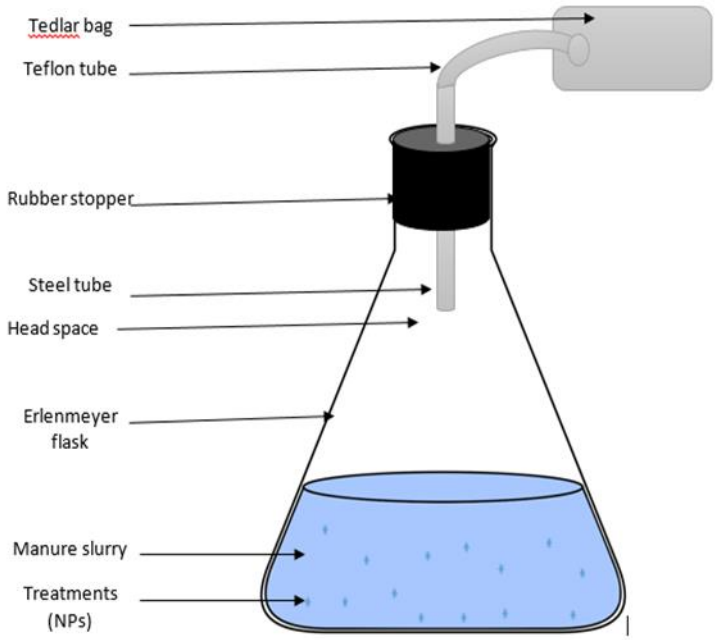

Figure 23. Schematic diagram of an incubation vessel

Throughout the experiment, depending upon the gas produced, headspace gas was collected in Tedlar bags at 2- to 8-day intervals until gas production ended at day 33. Headspace gas volume was measured with a gas-tight syringe (SGE Syringe, 500 MAR-LL-GT, Trajan Scientific Americas Inc, Austin, TX, USA) and diluted 100-fold (based on previous experiments) with N_2 gas in a separate Tedlar bag prior to analyses of gas concentrations. The H_2S gas concentrations were measured with a Jerome meter (Jerome 631X, Arizona Instrument LLC, Arizona, USA) and GHG (*e.g.*, CH_4 and CO_2) were measured using a gas chromatograph (GC, 8610C, SRI instrument, California, USA) equipped with an FID and ECD detector. An air sample from the Tedlar bag was drawn into a 1 mL sample loop of the GC using an inbuilt vacuum pump interfaced with the GC system according to a prescheduled event program. Before drawing any sample into the sample loop, the FID detector temperature was raised to 300˚C and

the ECD detector temperature was raised to 350˚C. The system was operated on a nitrogen carrier at 139 kPa for the ECD hydrogen and air was supplied to the FID/methanizer at 139 kPa. In this system, the ECD detects N_2O, while the FID/methanizer detects both CH_4 and CO_2. A detailed description of the GHG measurement procedure using this GC can be found in Borhan et al. (2011). Before each measurement, the GC was calibrated using calibration quality standard gases (5, 10, 100 ppm for CH_4; 500, 1000, 3000 ppm of CO_2). For each concentration, five to seven replicated measurements were made. Additional calibration and measurement processes are described in Rahman et al. (2013).

Coliform Bacterial Population Determination Using M Endo Broth

To enumerate coliforms, *i.e.,* potential pathogens (particularly *Escherichia coli*) originating in mammalian feces the American Public Health Association (APHA) and the Environmental Protection Agency (EPA) recommend the use of total coliform counts. Specifically, m-Endo agar was used to conduct total coliform counts (bacteria) in terms of Colony Forming Units (CFUs) using the plate count method to investigate the biocidal effects of NPs on coliform populations. CFUs were determined before and after treatment with NPs. All experimental treatments were prepared in a sterile hood (SS-324-PCR, Sentry Air Systems, Cypress, TX, USA). Environmental samples were collected, and each treatment was run in triplicate at five different dilutions (10, 10^2, 10^3, 10^4 and 10^5). The 10^3 dilution was determined to be optimal for plate counting and was thereafter used for coliform enumerations throughout this study. Growth media was prepared by placing a sterile membrane filter with absorbent pad (47 mm diameter, 0.45 μm pore size, WCN type, Whatman Limited, Maidstone, England, UK) in a sterile petri-dish (Anaerobic, Sterile Petri dishes, 60 mm diameter and 15 mm height, VWR, Radnor, Pa.) after which a 2 mL M-Endo broth ampule (23735-50, HACH LANCH GmbH,

Willstatterstrasse 11, Dusseldorf, Germany) was poured evenly over the entire surface of the absorbent pad. Subsequently, 100 µL of each diluted environmental sample was added to an absorbent pad and spread evenly across using a small sterile glass rod. The Petri dishes containing the growth media and bacterial inoculant were then incubated under aerobic conditions for 24 hr at 35±0.5 ˚C and CFUs were counted using a manual Darkfield Colony Counter with 1.5× magnification (Reichert, Inc. Depew, NY, USA).

Total Bacterial Population Determination

Anaerobic bacterial plate counts were conducted using Brain Heart Infusion (BHI) (BactoTM, Becton, Dickinson, and Company; Sparks, MD 21152 USA) and media was prepared for plate counts following the manufacturer's instructions. Specifically, 37 g of the BactoTM BHI powder and 20 g of agarose (IBI Scientific, 9861 Kappa Court, Peosta, IA 52068, USA) were added to 1.00 L of deionized water and dissolved by bringing the media to a boiling point using a hot plate (Cat No: N97042-642, 120 v, 1000 w, 10 amp, 50/60 Hz, VWR, Henry Troemner LLC, USA) and homogenizing using a magnetic stirrer. The mixed solution was then autoclaved at 121°C and 278 kPa for 70 min and cooled to ~50-60˚C. Afterward, approximately 10 mL of the solution media was poured into a petri dish and covered for ~40 minutes to cool it down to room temperature. A set of serial dilutions (10^2, 10^3, 10^4, 10^5 and 10^6) were used to determine the optimum dilution at which to conduct the plate counts. Plates were inoculated with 10 µL of each diluted manure treatment that were run in triplicate. Petri dishes containing diluted manure samples were then placed into an anaerobic chamber, and the ambient air in the headspace of the chamber was replaced by nitrogen gas to maintain an anaerobic environment for bacterial growth. Subsequently, Petri dishes were incubated in the chamber for 24 hr at 30˚C after which bacterial growth was estimated by counting the number of colony forming units

(CFU's) using a manual Darkfield Colony Counter with 1.5× magnification (Reichert, Inc. Depew, NY, USA).

DNA Isolation and Preparation for q-RT-PCR

Genomic DNA (gDNA) was extracted from 0.25 g of liquid swine manure using the Powersoil™ DNA isolation kit (MoBio Laboratories, Inc., Carlsbad, CA, USA). Quantitative polymerase chain reaction assays were conducted to quantify the dissimilatory sulfide reductase (DSR) gene of sulfate reducing bacteria (SRB) commonly found in waste water systems as described by Karunakaran et al., 2016. Copy numbers of the DSR gene represent shifts in SRB population size among treatments and are diagnostic for the approximately one gene copy of the DSR gene per SRB genome (Müller et al., 2015). Primers specific for SRB were used to amplify genomic DNA (gDNA): DSR1F ACSCACTGGAAGCACG and RH3-dsr-R GGTGGAGCCGTGCATGTT (Product size 222 bp fragments). Primers were synthesized by Integrated DNA Technologies, Inc. (IDT) Coralville, IA, USA. Each reaction contained a total volume of 20 µl that included: 10 µL of Power SYBER Green Master Mix (Applied Biosystems Inc., Foster City, CA, USA), 150 nmol (0.3 µL of 10 µM) of each forward and reverse primer, 2 µl of gDNA (environmental samples) and 7.4 uL of HyPure ™Molecular Biology Grade Water (HyClone Laboratories, Inc., Logan, UT, USA). Standard curves were generated, and amplification efficiencies determined using five different gDNA concentrations extracted from a pure culture of *Desulfovibrio Vulgaris* DSM 644 containing 124 ng μL^{-1} of gDNA. Three replicate PCR reactions were run for each environmental sample and genomic DNA concentration that ranged from 1.24 pg (10^2 gene copies) to 12.4 ng (10^7 gene copies) per reaction. Reaction mixtures were amplified and quantified using an ABI Prism™ 7500 (Applied Biosystems Inc, Foster City, CA, USA). The thermocycler conditions used for the primer set

were: 10 min at 95°C; followed by 40 cycles of 15 s at 95°C, and combined annealing and extension of 60 s at 60°C.

Shifts in copy numbers of the α-subunit of the methyl coenzyme M reductase (*mcrA*) gene in methanogens were monitored via qPCR and used as a proxy for changes in methanogen population size. Primers, MLF Forward GTGGTGTMGGATTCACACARTAYGCWACAGC and mcrA-rev Reverse CGTTCATBGCGTAGTTVGGRTAGT were synthesized by Integrated DNA Technologies, Inc. (IDT) Coralville, IA, USA and are diagnostic for the approximately one to two gene copies of the *mcrA* gene. Each reaction contained a total volume of 20 µL: 10 µL of Power SYBR® Green PCR Master Mix (Applied Biosystems, Foster, CA, USA), 200 nmol (0.4 µL of 10 µM) of each forward and the reverse primer, 2 µL of gDNA (environmental samples) and 7.2 uL of HyPure™ Molecular Biology Grade Water (HyClone Laboratories, Inc., Logan, UT, USA). Standard curves were generated, and amplification efficiencies were determined using five different gDNA concentrations extracted from a pure culture of *Methanobacterium Formicicum Schnellen* 1947 (DSM 1535) (Leibniz-Institut DSMZ, Inhoffenstraße 7B, 38124 Braunschweig, Germany) containing 207 ng μL^{-1}. Three replicate PCR reactions were run for each environmental sample and concentration of gDNA that ranged from 10^2 gene copies to 10^7 gene copies per reaction. Reaction mixtures were amplified and quantified using an ABI Prism™ 7500 (Applied Biosystems Inc, Foster City, CA, USA) using the following thermocycler conditions: 10 min at 95°C, followed by 45 cycles of 30 s at 95°C, 45 s at 60°C, and 45 s at 72°C.

Inductively Coupled Plasma-Optical Emission Spectroscopy for Manure Mineral Analysis

For testing mineral contents in the liquid swine manure, both pre- and post-treated manure samples were collected before and after an experiment. Collected samples were stored at

-20°C to avoid microbial/chemical transformation during storage (Peters et al., 2010). Immediately after the conclusion of the experiment, all of the pre- and post-treated manure samples were sent to Agvise Laboratories (Northwood, North Dakota, USA) for Inductively Coupled Plasma (ICP) analysis using microwave digestion in concentrated HNO_3 and H_2O_2 (method A3769) inductively coupled plasma-optical-emission spectrometry (Perkin Elmer Optima 5300 ICP).

Statistical analysis

All treatments were replicated three times and the averages are reported. The analysis of variance (ANOVA) test was performed to find out the effect of treatments on cumulative gas volume, CH_4, CO_2 and H_2S gas concentrations, pH, redox, ash, crude protein, total N, fecal ammonia, VFAs, coliform bacterial population, total bacterial population, *mcrA* gene copies, and DSR gene copies. The averages of each variable among treatments were compared using the PROC ANOVA procedure in SAS 9.3 software (SAS Institute Inc., Cary, NC, USA). The null hypothesis was treatments had equal impact on gas volume, gas concentrations and other parameters at 95% ($P \leq 0.05$) significance level. Then, variables were separated using Duncan's Multiple Range Test if the main effect (NPs dose) using F-test was significant at $P \leq 0.05$.

Results and Discussion

Effect of Nanoparticles on Manure Properties

Manure properties such as total nitrogen (TN), ash, and crude protein (CP) showed little variation among the pre- and post-treated manure samples (Table 13). In contrast, pH, moisture content, ammonia, and dry matter (DM) varied significantly among the treatments (Table 13). The pH values of the treated manure samples varied between 6.95 to 7.15, and none of the treatments were statistically significant $(P \leq 0.05)$ compared to the control treatment except for

the nAg treatment. In general, nZnO treated manure showed significantly lower ($P \leq 0.05$) pH compared to nAg amended swine manure when exposed for 33 days under anaerobic conditions. The pH values between initial swine manure samples and nAg treated manure samples were statistically similar. The DM and fecal ammonia contents were significantly higher *($P > 0.05$)* in the post-treated manure samples compared to that of pre-treated (initial) manure sample. However, as compared to the control treatment, both of the nZnO and nAg based treatments reduced 14 to 19% fecal ammonia and the reduction was significant *($P \leq 0.05$)* (Table 13). The reduction in fecal ammonia content were might be due to the adsorption of NH_3 on applied NP surfaces (Predicala et al., 2012).

Table 13. Properties of pre and post treated liquid swine manure

Parameters	Initial*	Control**	nZnO	Bare Bead	nZnO in Bead	nAg
pH	$7.19^{b} \pm 0.02$	$6.95^{a} \pm 0.02$	$6.94^{a} \pm 0.07$	$6.96^{a} \pm 0.05$	$6.99^{a} \pm 0.04$	$7.15^{b} \pm 0.03$
Moisture (%)	$97.28^{b} \pm 0.66$	$92.81^{a} \pm 0.12$	$92.94^{a} \pm 0.49$	$93.14^{a} \pm 0.43$	$92.28^{a} \pm 0.06$	$92.92^{a} \pm 0.31$
TN (%)	$4.25^{ab} \pm 0.08$	$4.22^{ab} \pm 0.17$	$4.29^{ab} \pm 0.26$	$3.95^{b} \pm 0.17$	$4.37^{a} \pm 0.03$	$4.46^{a} \pm 0.11$
Fecal ammonia (M)	$71^{d} \pm 0.73$	$387^{a} \pm 9.94$	$331^{bc} \pm 23.69$	$344^{b} \pm 1.65$	$311^{c} \pm 4.97$	$330^{bc} \pm 5.12$
DM (%)	$2.48^{c} \pm 0.06$	$7.19^{ab} \pm 0.12$	$7.06^{ab} \pm 0.49$	$6.86^{b} \pm 0.43$	$7.72^{a} \pm 0.06$	$7.08^{ab} \pm 0.31$
Ash (%)	$26.81^{b} \pm 0.35$	$27.55^{b} \pm 0.27$	$28.65^{ab} \pm 2.73$	$31.06^{a} \pm 0.97$	$27.69^{b} \pm 0.2$	$27.60^{b} \pm 0.97$
CP (%)	$27.38^{a} \pm 0.27$	$26.37^{ab} \pm 1.06$	$26.81^{ab} \pm 1.62$	$24.68^{b} \pm 1.07$	$27.32^{a} \pm 0.19$	$27.90^{a} \pm 0.69$

TN: Total Nitrogen
DM: Dry Matter
CP: Crude Protein
Values followed by the same letter in a row are not significantly different at $P \leq 0.05$.
* Initial means the fresh manure collected from a source before starting the experiment.
**Control final means the manure kept in a flask for 33 days without treating with Nanoparticles.

Effect of Nanoparticles on Manure VFAs

Among the treatments, total VFA (TVFA) concentrations ranged between 58.88 to 508.30 mM (Table 14). Compared with the initial sample VFA, all treatments including the control contained higher TVFA by the end of the 33 d experimental period, and the final VFAs were significantly different *($P \leq 0.05$)* than that of initial treatment. Among the treatments, the nZnO treatment exhibited the lowest TVFA (413.57 mM), and the nAg treatment contained the highest amount of TVFA (508.30 mM). The TVFA content, however, among control, nAg, bare bead, and nZnO in beads were not significantly different *($P > 0.05$)*, but were significantly different than that of initial samples. Irrespective of treatments, the resulting TVFA concentration in the treated swine manure samples were 6 to 7 times higher than that of initial samples. The bare nZnO treatment contained 17% lower TVFA compared to the control treatment. Gaseous emissions from anaerobic digestion were highly dependent on the amount of TVFA. Hence, the nZnO treated manure samples containing lower VFA were likely to generate low gaseous emissions.

All individual VFAs except for acetic and propionic acids were lower in both nZnO, and nZnO in bead treated samples compared with the control treatment. A reduction in acetic acid concentrations could be the result of inhibition of SRB metabolism and or methanogenesis both of which require anaerobic metabolic pathways. In contrast, compared with nZnO based treatments, a higher amount of acetic acid in nAg treated samples might be an indication of higher gas production from this treatment. Acetic acid and propionic acid are reported to contribute to CH_4 production (Hill & Bolte, 1989); Lahav & Loewenthal, 2000). Hill & Bolte (1989) reported that 70% of the acetic acid is converted to CH_4 by methanogenic bacteria under

anaerobic storage conditions. Therefore, compared with the control treatment, lower values of acetic acid from nZnO and nZnO in bead treatments means lower CH_4 production and emissions.

Table 14. Effects of different NP treatments on liquid swine manure VFAs

VFAs (mM)	Initial	Control	nZnO	Bare Bead	nZnO in Bead	nAg
Acetic Acid	$35.37^{c} \pm 1.43$	$245^{ab} \pm 15.97$	$216^{b} \pm 24.93$	$227^{ab} \pm 10.42$	$237^{ab} \pm 15.52$	$261^{a} \pm 8.52$
Propionic Acid	$8.96^{c} \pm 0.2$	$65.95^{ab} \pm 4.99$	$55.17^{b} \pm 8.27$	$61.34^{b} \pm 1.63$	$62.59^{ab} \pm 4.91$	$72.91^{a} \pm 5.04$
Isobutyric Acid	$3.17^{d} \pm 0.27$	$31.04^{a} \pm 3.40$	$18.02^{c} \pm 4.14$	$25.52^{b} \pm 0.69$	$20.88^{bc} \pm 0.97$	$31.27^{a} \pm 2.41$
Butyric Acid	$5.43^{b} \pm 0.32$	$100.33^{a} \pm 8.16$	$89.71^{a} \pm 13.26$	$90.18^{a} \pm 2.12$	$84.77^{a} \pm 5.76$	$92.70^{a} \pm 6.28$
Isovaleric Acid	$4.39^{c} \pm 0.16$	$39.27^{a} \pm 3.11$	$24.59^{b} \pm 4.99$	$35.68^{a} \pm 0.94$	$27.7^{b} \pm 1.10$	$34.19^{a} \pm 2.61$
Valeric Acid	$2.75^{d} \pm 0.21$	$13.45^{b} \pm 0.86$	$10.01^{c} \pm 1.98$	$16.46^{a} \pm 0.50$	$13.16^{b} \pm 0.83$	$16.22^{a} \pm 1.18$
Total VFA	$58.88^{c} \pm 0.36$	$495^{a} \pm 36.45$	$413^{b} \pm 57.53$	$456^{ab} \pm 15.54$	$446^{ab} \pm 28.$	$508^{a} \pm 25.74$

VFA: Volatile fatty acid
Values followed by the same letter (superscript) in a row are not significantly different at $P \leq 0.05$.
* Initial means the fresh manure collected from a source before starting the experiment.
**Control means the manure kept in a flask for 33 days without treating with NPs.

Effect of Nanoparticles on Manure Redox Potential

It is well known that the imbalance between total oxidants and reductants may cause the production of reactive oxygen which ultimately either kills or damages the cells of microorganisms. Thus, measurement of redox levels/potentials in the slurry was critical to the present study. In our experiment, initial redox potentials for the treatments varied between -315.67 to -320 mV (Table 15), and represented a favorable range for sulfate reduction, CO_2, and CH_4 generation (Sigg, 2000). Since the initial redox was measured immediately after the addition of treatments, no significant differences *($P > 0.05$)* were observed among the treatments. However, redox potentials changed among the treatments by the end of the 33 day experimental

period. Redox potentials were -251.7, -28.67, -44.67, -229.77, and -173.57 mV for the control, nZnO, nZnO in bead, bare bead, and nAg treatments, respectively. Irrespective of the treatments, redox potentials of the post-treated manure samples were higher *($P \leq 0.05$)* than that of their pretreated counterparts. Among the treatments, nZnO had the highest redox potential, while the control treatment exhibited the lowest redox potential. Both nZnO in bead and nAg exhibited lower redox potentials compared with the nZnO treatment; the redox potential of the coated nZnO was significantly higher *($P \leq 0.05$)* than that of the control and bare bead treatments. The release of Zn^{2+} and Ag^{2+} from the metal nanoparticles and their further oxidation might have caused higher redox potentials in nanoparticle treatments, although they were not measured. In contrast, the absence of metal ions in both control and bare bead treatments resulted in redox potentials that remained low and was statistically similar *($P > 0.05$)*. Moreover, among the treatments, only the control and bare bead maintained favorable redox conditions for sulfate reduction, CO_2 and CH_4 generation throughout the experiment. Manure treated with nAg exhibited somewhat favorable redox conditions. Redox conditions in manure treated with both nZnO and nZnO in bead treatments exhibited redox potentials after 33 days that were sufficiently high to disfavor sulfate reduction, CO_2 and CH_4 generation. Hence, the measured lower emissions of CH_4, CO_2, and H_2S from the nZnO based treatments was expected.

Table 15. Effects of different NP treatments on pre and post-treated liquid swine manure Redox

Redox (mV)	Control	nZnO	Bare Bead	nZnO in Bead	nAg
Initial Redox*	-317.67^{a} ± 4.11	-316.67^{a} ± 10.14	-318.37^{a} ± 7.37	-320^{a} ± 15.94	-315.67^{a} ± 8.73
Final Redox**	-251.70^{c} ± 14.38	-28.67^{a} ± 6.65	-229.77^{c} ± 10.16	-44.67^{a} ± 11.44	-173.57^{b} ± 16.85

Values followed by the same letter (superscript) in a row are not significantly different at $P \leq 0.05$.
* Initial Redox means the redox of the fresh manure collected from a source before starting the experiment.
**Final redox means the redox of the manure incubated in a flask for 33 days within each treatment.

Effect of Nanoparticles on Cumulative Gas Production

Trends in cumulative gas production at each time interval from all seven treatments are presented in Figure 24. Cumulative gas production from the control treatment and manure treated with nZnO, bare beads, nZnO in beads, and nAg were 930.00, 195.00, 868.33, 378.33, and 776.67 mL, respectively. The gas production per day from the respective treatments were 28.18, 5.91, 26.31, 11.46, and 23.54 mL, respectively. Relative to control, the manure treated with bare nZnO had reduced the highest amount of cumulative gas volume (79.03%). Total reduction in gas production after treatment with nZnO in bead was 59.32%, bare bead was 6.63%, and the nAg was 16.49% compared to that of the control treatment. Additionally, in comparison with the control treatment, both nZnO based treatments, and the nAg treatment exhibited a statistically significant reduction in gas production. However, no statistically significant difference was found for the bare bead treatment.

Lower gas production in nZnO treated samples was likely due to a combination of mechanisms that included the production of initial reactive oxygen species (ROS), absorption and biocidal effects and/or chemical transformation. However, application of bare nZnO was more effective than the nZnO in beads. This was likely due to the exposed surface areas and their

adsorption capacity. When nZnO was in a bead, surface area and absorption capacity of the nanoparticle was reduced due to the polymer coating, but the coating on the nZnO renders the beads recoverable allowing for their reuse, which is not possible in the bare nZnO application. Lowered reactive surface area would also reduce ROS production from entrapped nZnO beads. As compared to nZnO, nAg was not very effective in reducing total gas production, which may be due to the larger particle size of the applied nAg and thus reduced bactericidal activity that includes less ROS production compared to that of nZnO. Ultimately, nAg was not very effective in reducing total gas production.

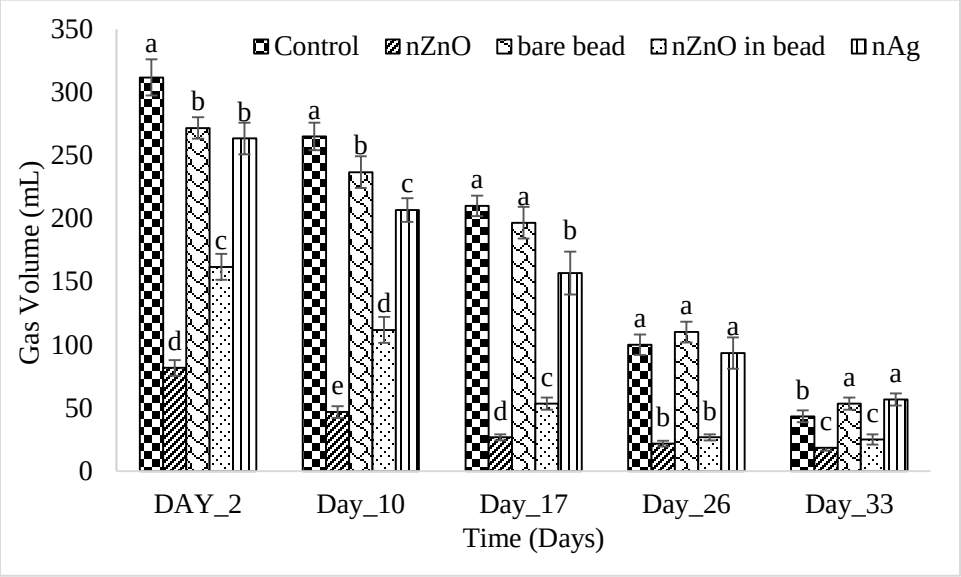

Figure 24. Biogas production comparison at each sampling event from liquid swine manure treated with and without NPs. The same letter above a bar graph within a sampling day is not significantly different at $P \leq 0.05$.

Effects of Nanoparticles on Carbon dioxide Concentration

Carbon dioxide concentration varied from 17.98 to 38.05%, 7.72 to 19.93%, 13.95 to 38.10%, 9.26 to 20.35%, and 19.70 to 46.24% for the control, bare nZnO, bare bead, nZnO in bead, and nAg treatments, respectively (Figure 25). Irrespective of the treatments, CO_2 concentrations exhibited a sinusoidal trend throughout the experimental period. Among the

treatments, control, bare bead, and nAg consistently exhibited higher CO_2 concentrations compared with nZnO based (Bare nZnO, and nZnO in bead) treatments. Compared to the control, both nZnO treatments reduced CO_2 concentrations significantly *($P \leq 0.05$)* during the study period. Additionally, the reduction in CO_2 concentration with the application of bare nZnO relative to the control ranged from 36.92 to 67.93%, while its concentration reduction was 40.36 to 48.52% when manure was treated with nZnO in beads. Initially, the bare bead treatment reduced CO_2 concentrations (0.34 to 22.41%), but by the end of the 33 days this treatment increased CO_2 concentrations by 20.58%. In contrast, nAg exhibited an increase in CO_2 concentration (1.91 to 25.21%) except on day 10, when CO_2 concentrations were reduced although the reduction was not statistically significant relative to the control treatment *($P \geq 0.05$).*

Generally, biological processes such as microbial respiration and non-biological processes such as chemical oxidation are responsible for the generation of CO_2 within an anaerobic digestion system. Therefore a trend of reduced CO_2 generation in the presence of nZnO based treatments was likely due to the adverse effect(s) on one or the other of these two processes or both. The biocidal properties of nZnO were likely to have contributed to reduced CO_2 concentrations (Rastogi et al., 2002). Therefore, a detailed study was obligatory to obtain a complete understanding of the reaction chemistry.

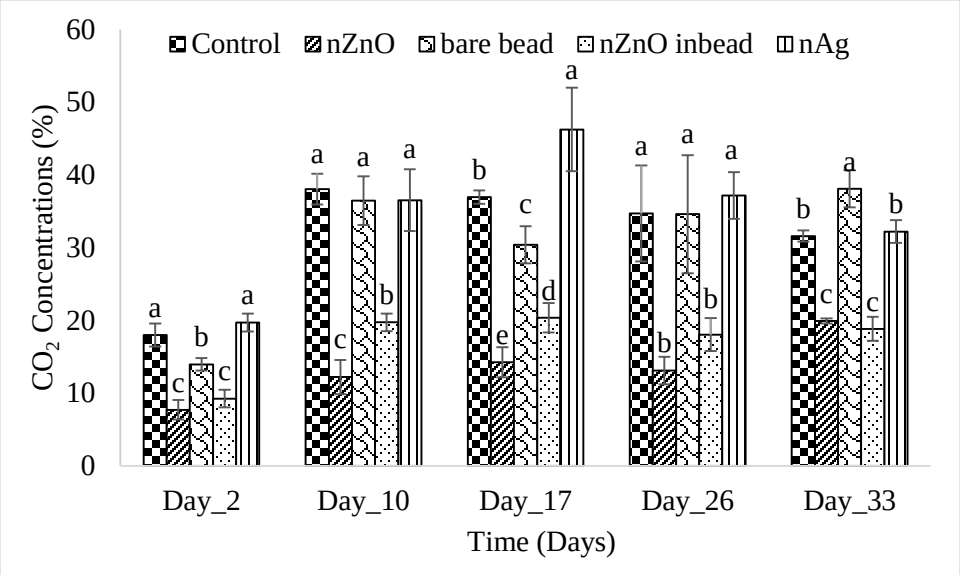

Figure 25. Carbon dioxide (CO_2) concentration trends from liquid swine manure treated with and without NPs. The same letter above a bar graph within a sampling day is not significantly different at $P \leq 0.05$.

Effects of Nanoparticles on Methane Concentration

Methane concentrations ranged from 1.93 to 8.37%, 0.13 to 0.34%, 1.72 to 8.91%, 1.05 to 4.32%, and 3.03 to 12.55% for the control, nZnO, bare bead, nZnO in bead, and nAg treatments, respectively (Figure 26). Irrespective of the treatments, CH_4 concentrations increased after day 2 and remained stable until termination of the experiment. The growth of methanogenic microorganisms is slow requiring nearly 12 days of growth to reach stationary phase. Thus, increased concentrations of CH_4 on day 2 were most likely due to shifts in methanogenic activity rather than growth of methanogens that occurred during the latter part of the study (Kushkevych et al., 2017). Manure treated with nAg showed sudden increases in CH_4 concentration at the end of day 17. Among the applied treatments, only nZnO and nZnO in bead exhibited significant reductions in CH_4 concentrations *($P \leq 0.05$)* compared to the control during the 33 d experimental period. Relative to the control treatment, reductions in CH_4 concentrations resulting from application of bare nZnO and nZnO in bead varied between 93.04 to 97.02% and

45.61 to 48.37%, respectively. Overall, CH_4 concentrations were reduced by 50% in manure treated with nZnO relative to manure treated with nZnO in beads. Reductions in the reactive surface area of polymer impregnated nZnO beads applied to manure may have reduced the biocidal effects of the treatment resulting in increased concentrations of CH_4 compared to the manure treated with bare nZnO. In contrast, nAg treatments were not able to reduce CH_4 concentrations significantly and nAg was also not able to reduce the total gas production as mentioned previously. Methane emissions are controlled by methanogenic archaea (Van Elsas et al., 2006). The biocidal properties of ZnO and Ag based NPs were reported to either destroy or inhibit the activity of methanogens (Gautam et al., 2016b). Therefore, lower CH_4 emissions from the NP based treatments was likely due to the effects of ROS production that include initial effects that disrupt or destroy microbial cells and residual effects that result in elevated redox conditions disfavorable to anaerobic metabolisms. The large particle size and consequently lower surface area of nAg may have reduced its efficacy relative to the nZnO based treatments and in particular the nZnO based treatment. Moreover, the higher density of the nAg may have resulted in precipitation of much of the nAg and hence reduced the amount of NPs in solution further lowering the material efficacy relative to nZnO.

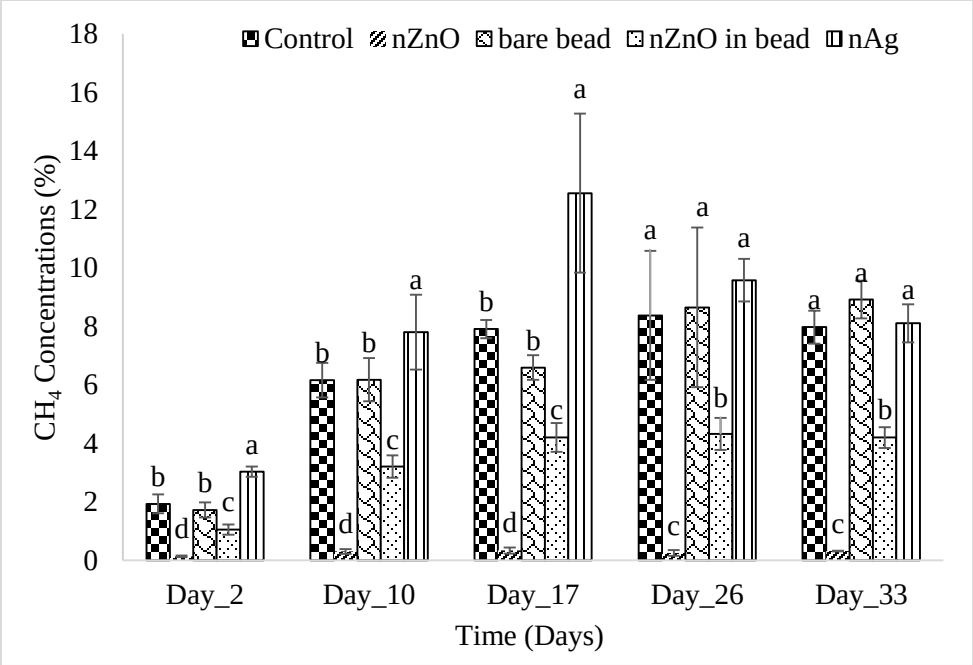

Figure 26. Methane (CH_4) concentration trends from liquid swine manure treated with and without NPs. The same letter above a bar graph within a sampling day is not significantly different at $P \leq 0.05$.

Effects of Nanoparticles on Hydrogen Sulfide Concentration

The trend in H_2S produced was slightly different than that of CO_2 and CH_4. Across all treatments, H_2S concentrations were higher at the beginning of the 33 d experiment and decreased with time. For all treatments, the H_2S concentration was highest at the end of day 10, which was likely due to increased activity and numbers of sulfate-reducing bacteria (Kushkevych et al., 2017). Additionally, the presence of small numbers of methanogenic bacteria during the first 12 days may have enhanced the activity of sulfate reducing bacteria that utilize most of the hydrogen produced as an electron acceptor during the process of dissimilatory sulfate reduction the byproduct of which is H_2S (Kushkevych et al., 2017). Hydrogen sulfide concentrations ranged from 888.33 to 4383.33, 38.17 to 571.67, 866.67 to 4500.00, 113.00 to 1156.67, and 706.67 to 4216.67 ppm for the control, nZnO, bare beads, nZnO in beads, and nAg treatments,

respectively (Figure 27). Like CH_4 and CO_2, H_2S concentrations were lowest in the nZnO treatment, followed by the nZnO in bead treatment.

Reductions in H_2S concentrations varied between 88.18 to 96.51% and 73.61 to 87.28%, respectively, for the manure treated with bare nZnO and nZnO in beads throughout the experimental period. A smaller but statistically significant reduction in H_2S concentration was measured in samples treated with nZnO in bead. This effect was expected due to the reduced surface area of nZnO coated with alginate. In contrast, both the bare bead and nAg treatments exhibited little or no reduction in H_2S concentrations compared to the control treatment and in most instances, concentrations were not significantly different relative to the control treatment at $P > 0.05$. Measured reductions in H_2S during the experimental period may have been due to each type of NPs inhibitory effects on the anaerobic SRB population that are responsible for sulfate reduction and H_2S production. The initial ROS produced by addition of both nZnO treatments would reduce both the activity and numbers of SRB as well as increase redox conditions. Additionally, both assimilatory and dissimilatory processes were likely to be affected by reductions in H_2S.The coexistence of both active methanotrophic archaea and sulfate-reducing bacteria in the same environment has previously been reported to influence production of CH_4 and H_2S by Boetius et al. (2000) and Orphan et al. (2001) and likely to contribute to the reduction of H_2S and CH_4 gases (Germida et al., 1992). Among the NP treatments, nZnO based treatments outperformed the nAg treatment. To conclude, due to comparable H_2S reduction drift among treatments, nZnO in beads is most likely to be the best potential application method in terms of capturing, recovery and regeneration, and repeated use of the applied nanoparticles.

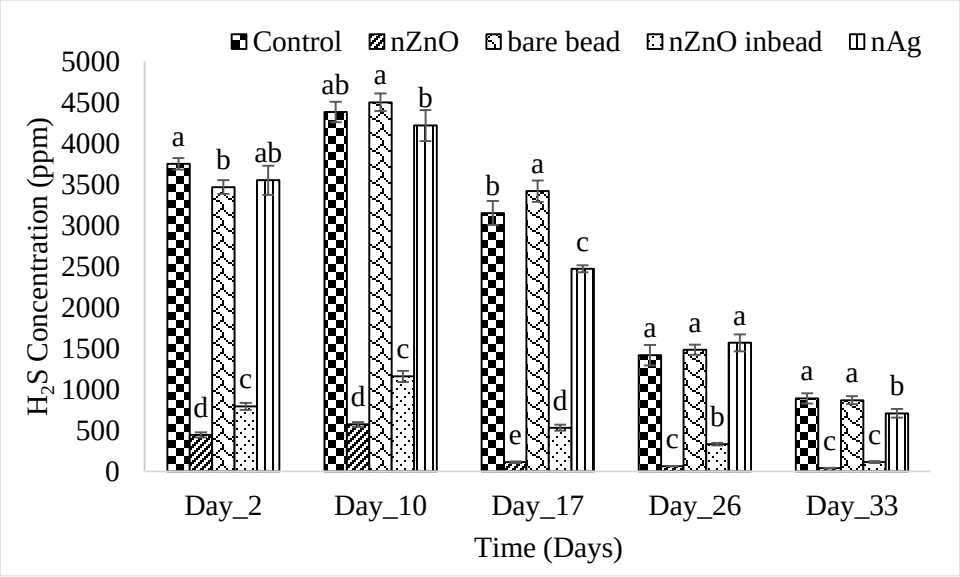

Figure 27. Hydrogen sulfide (H_2S) concentration trends from liquid swine manure treated with and without NPs. The same letter above a bar graph within a sampling day is not significantly different at $P \leq 0.05$.

Effects of Nanoparticles on Total Coliform Bacteria

The number of total coliform bacteria (aerobic) as the average number of CFUs in the initial sample (untreated manure), control treatment, swine treated manure with nZnO, bare bead, nZnO in bead, and nAg are shown in Figure 28. An average number of CFUs/mL of manure were 1.13×10^5, 5.28×10^5, 3.0×10^5, 2.4×10^5, 5.18×10^5, and 3.25×10^5 in the initial, control, nZnO, nZnO in bead, bare bead, and nAg treatments, respectively. Among the treatments, nZnO treated manure contained the fewest number of coliform bacteria, whereas the control treatment contained the highest number. Compared to the initial treatment, the control treatment contained 40.63% more coliform bacteria within the experimental period. In contrast, the biocidal activity of the nZnO was the likely cause of reductions in CFUs. Moreover, the trends in gene copies of methanogens and SRB along with a reduction in CH_4, CO_2, and H_2S concentrations is further evidence of nZnO biocidal activity. Moreover, trends in CH_4, CO_2, and H_2S gas concentrations exhibited a similar reduction in treatments with bare and entrapped nZnO. Compared with the

control treatment, the reduction in CFUs was 94.32% and 54.57% from the swine manure treated with nZnO and nZnO in bead treatments, respectively. Additionally, both nZnO and nZnO in bead treatments were able to reduce the number of bacteria significantly relative to other treatments at $P \leq 0.05$. Manure treated with the bare beads, however, contained almost identical numbers of CFUs as the untreated control treatment indicating little or no effect. In contrast, the nAg treatment was not able to reduce gas concentrations to the levels in the nZnO treatments. Gaseous emissions and CFUs were lower in the nZnO treatments relative to control treatment.

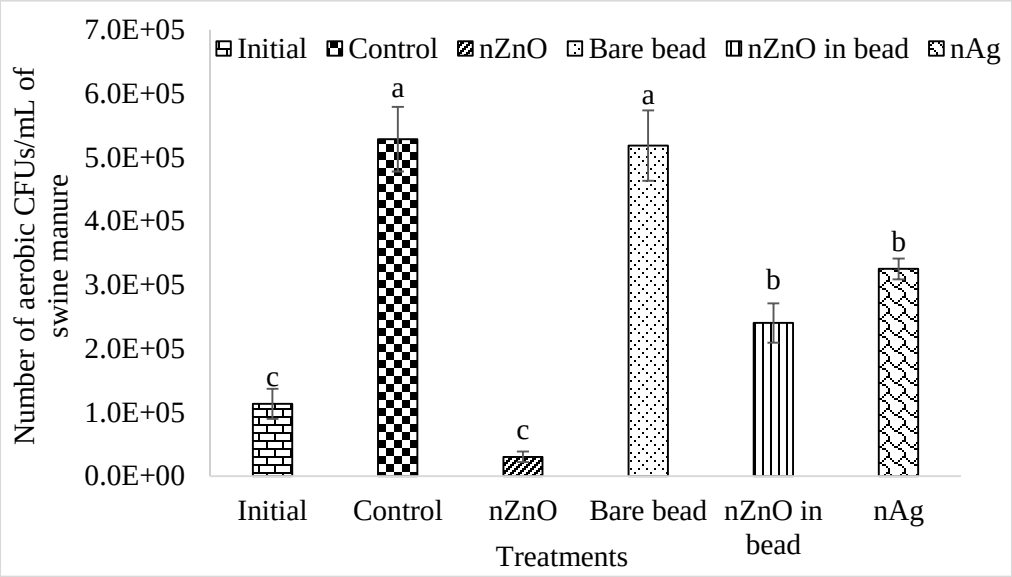

Figure 28. Coliform bacterial (aerobic) population counts trends from liquid swine manure treated with and without NPs. The same letter above a bar graph for different treatments is not significantly different at $P \leq 0.05$.

Effects of Nanoparticles on Anaerobic Bacteria

Average number of CFUs from the liquid swine manure stored under anaerobic storage conditions were 3.5×10^7, 5.3×10^7, 9.6×10^6, 3.1×10^7, 5.1×10^7, and 4.9×10^7 for the initial, control, nZnO, nZnO in bead, bare bead, and nAg treatments, respectively (Figure 29). The biocidal efficacy of the nAg was reduced under anaerobic conditions and the colony counts tended to be higher. Relative to the initial manure sample, the control treatment contained a nearly 5-fold

increase in CFUs relative to the control treatment. In contrast, the nZnO, nZnO in bead, and nAg treatments reduced the number of CFUs relative to the control by 82.04%, 40.87%, and 7.43%. Additionally, there were significant reductions in the CFUs with nanoparticle (nZnO, nZnO in bead, and nAg) additions relative to the control treatment. This can be explained by the fact that these results were likely due to both NP treatments having a biocidal impact on microbial populations. On the other hand, swine manure treated with bare beads did not significantly reduce the amount of CFUs. Therefore, we inferred that the beads had no measurable effect on the microbial populations**.** Irrespective of the treatments the number of CFUs present under anaerobic conditions at the end of the 33-day experimental period was~99% higher than those of aerobic conditions. However, trends in the number of CFUs among treatments under anaerobic and aerobic conditions were similar with the exception of the nAg treatment. No information could be obtained with respect to the effects of applied treatments on specific microbial populations responsible for gas production via the CFU data. Therefore, we conducted qPCR assays to determine the effects of treatments on methane and SRB populations.

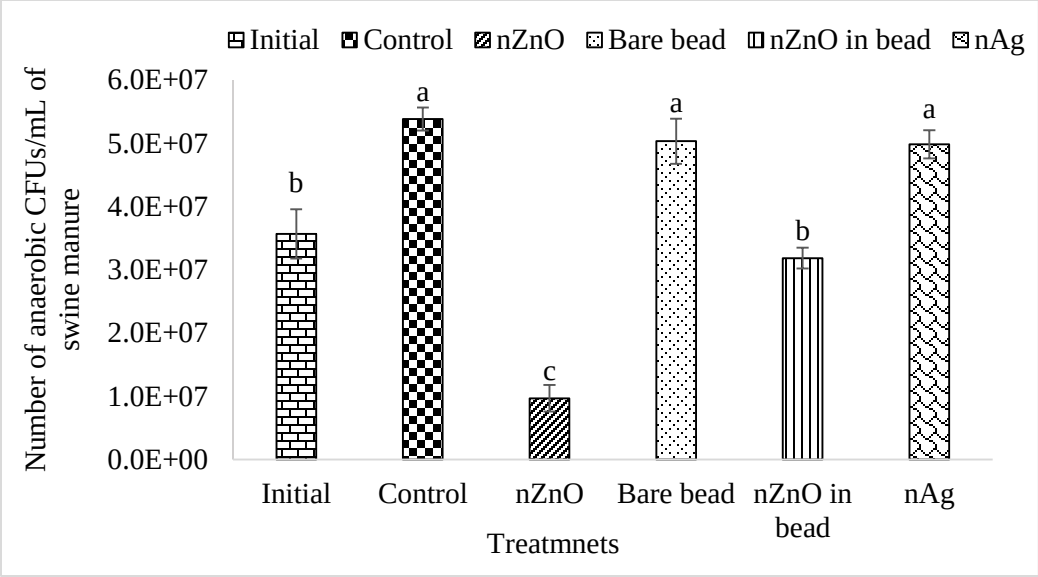

Figure 29. Anaerobic bacterial population counts trend trends from liquid swine manure treated with and without NPs. The same letter above a bar graph for different treatments is not significantly different at $P \leq 0.05$.

Effects of Nanoparticles on Methanogenic Archaea

Figure 30 represents the effect of different treatments on methanogens in swine manure incubated under anaerobic conditions. Average *mcrA* gene copy numbers (an approximate estimate of methanogen numbers) on day 33 were 6.90×10^6, 3.06×10^6, 7.45×10^6, 6.30×10^6, and 7.36×10^6, respectively for the control, nZnO, bare bead, nZnO in bead, and nAg treatments. However, the initial population of methanogens in manure was 3.13×10^6. Hence, there was an increase in the methanogenic community throughout all treatments relative to the initial treatment except for the bare nZnO treatment. Decreased copies of the *mcrA* gene in the nZnO treatment were likely due to the initial reactive oxygen species (ROS) produced (Yu et al. 2013) and as a result the persistence of higher redox conditions relative to the control that were likely maintained throughout the experiment. Relative to the control, *mcrA* gene copies in the nZnO in bead and nZnO treatments were 55.64%, and 8.80% lower, respectively. Manure treated with bare bead, and nAg exhibited a 7.83%, and 6.64% increase in *mcrA* gene copies. Among the

treatments, only manure treated with nZnO exhibited a significant reduction in methanogens estimated via the *mcrA* gene copies ($P \leq 0.05$). There was also a downward trend in *mcrA* gene copies in the nZnO in bead treatment. Furthermore, the trends in gas emissions and in *mcrA* gene copies appear to be similar. Hence, in the case of both nZnO based treatments, the biocidal action of the nZnO was found to reduce the methanogen community. In contrast, although nAg has proven to have biocidal properties in other applications, our data do not reveal such a relationship.

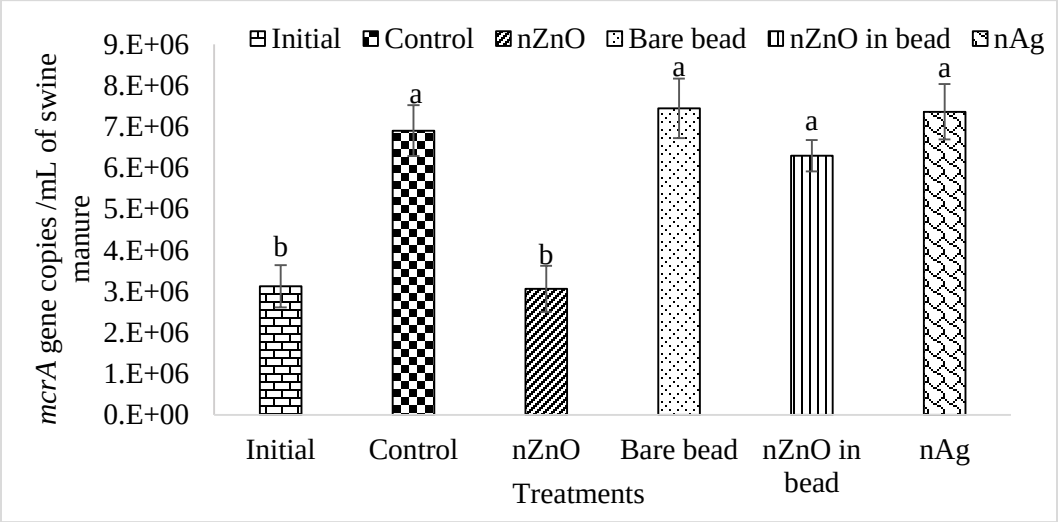

Figure 30. Trends in *mcrA* gene copies in liquid swine manure treated with and without NPs. The same letter above a bar graph for different treatments is not significantly different at $P \leq 0.05$.

Effects of Nanoparticles on DSR Gene of SRB

Average DSR gene copies in SRB populations were 4.50×10^5, 5.01×10^5, 3.75×10^5, 4.98×10^5, 4.93×10^5, and 5.93×10^5 for the initial, control, nZnO, bare bead, nZnO in bead, and nAg treatments respectively (Figure 31). As was the case with the *mcrA* gene copies in the methanogen community, DSR gene copies of SRB decreased in the presence of nZnO relative to the initial manure sample and all other treatments. In contrast, among the treatments, only the nAg treatment exhibited an increase in SRB estimated via DSR gene copies relative to the

control treatment ($P \leq 0.05$). Compared with the control treatment liquid manure treated with nZnO resulted in a reduction in SRB, DSR gene copies of 25.16%, while reductions in SRB DSR gene copies in bare bead, and nZnO in bead treatments were negligible (only 0.736 and 1.499% sulfate reducing population). The CFUs in the nZnO in bead treatment followed a similar pattern. Additionally, adsorption of the sulfide compound on the nZnO, and nZnO in bead surface was also evident in a similar study conducted by some of the same authors (Gautam et al. 2016a).

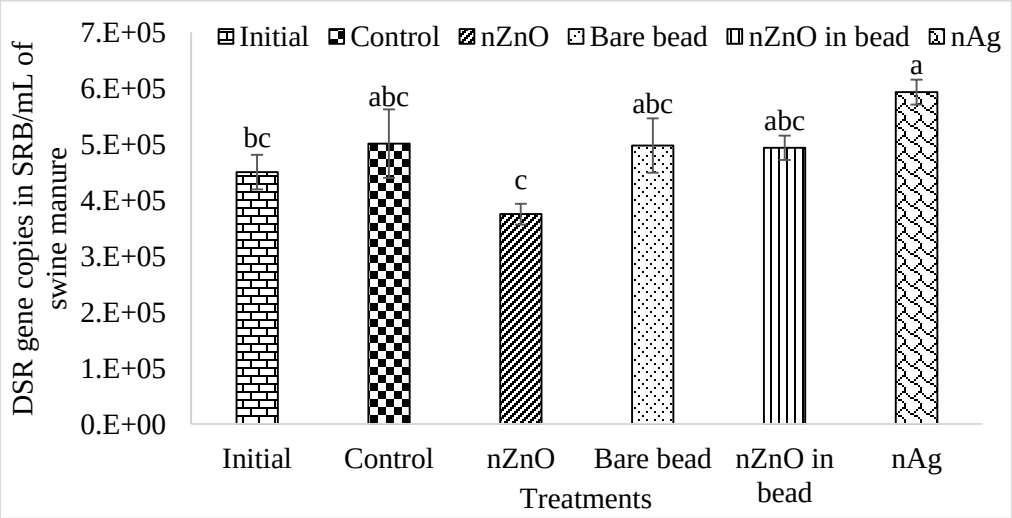

Figure 31. Trends in SRB, DSR gene copies in liquid swine manure treated with and without NPs. The same letter above a bar graph for different treatments is not significantly different at *P ≤ 0.05*.

Effect of Nanoparticles on Manure Mineral Contents

Effects of treatments on manure mineral contents are presented in Table 16. Although, application of bare nZnO exhibited its potential towards higher cumulative gas volume, and reductions in the concentration of gas constituents, the material had no significant effect on mineral contents (the exception being Phosphate and zinc). However, manure treated with nZnO in bead contained significantly less P_2O_5, and significant increases in Mg, Zn, and Cu minerals. As was found in the control treatment, variations in manure mineral content may be the result of precipitation of the manure minerals over the course of the 33-day experiment. Significant

increases in manure Ca resulting from treatment of the manure with both the bare bead, and nZnO in bead were likely due to release of Ca ions bound to the beads. Moreover, significant increases in Zn in the manure treated with both nZnO based amendments were likely due to the addition of nZnO, and the subsequent release of Zn ion. Zinc concentrations in the manure treated with nZnO in bead treatments are indicative of partial release of nZnO from the beads, and hence potential recovery of the nZnO even after 33 days. Similarly, manure treated with nAg exhibited a significantly higher Ag concentration compared to all other treatments and was likely due to the addition of nAg.

Table 16. Effects of different NP treatments on liquid swine manure mineral contents

Treatments	Initial	Control	nZnO	Bare bead	nZnO in bead	nAg
P_2O_5 (%)	$5.13^{c} \pm 0.15$	$5.70^{a} \pm 0.20$	$4.87^{d} \pm 0.21$	$5.30^{bc} \pm 0.00$	$4.33^{e} \pm 0.21$	$5.67^{ab} \pm 0.31$
K_2O (%)	$8.03^{b} \pm 0.35$	$9.45^{a} \pm 0.40$	$7.90^{b} \pm 0.17$	$8.03^{b} \pm 1.08$	$7.63^{b} \pm 0.32$	$7.97^{b} \pm 0.51$
Na (%)	$1.80^{b} \pm 0.10$	$2.17^{a} \pm 0.06$	$1.83^{b} \pm 0.06$	$2.23^{a} \pm 0.29$	$1.77^{b} \pm 0.06$	$1.80^{b} \pm 0.10$
Ca (%)	$2.07^{d} \pm 0.06$	$2.43^{c} \pm 0.06$	$2.03^{d} \pm 0.06$	$3.17^{a} \pm 0.23$	$2.80^{b} \pm 0.20$	$2.23^{cd} \pm 0.06$
Mg (%)	$1.70^{b} \pm 0.00$	$1.97^{a} \pm 0.06$	$1.70^{b} \pm 0.00$	$1.87^{a} \pm 0.06$	$1.87^{a} \pm 0.06$	$1.90^{a} \pm 0.10$
Zn (ppm)	$1193^{c} \pm 26.7$	$1375^{c} \pm 36.2$	$29641^{a} \pm 354$	$1388^{c} \pm 31.3$	$16726^{b} \pm 1361$	$1486^{c} \pm 233$
Fe (ppm)	$1503^{a} \pm 34.1$	$1726^{a} \pm 127$	$1409^{a} \pm 45.6$	$1586^{a} \pm 43$	$1413^{a} \pm 70.8$	$3601^{a} \pm 3540$
Mn (ppm)	$459^{c} \pm 8.96$	$529^{a} \pm 15.2$	$455^{c} \pm 15.6$	$493^{b} \pm 17.5$	$482^{bc} \pm 26.3$	$512^{ab} \pm 17.6$
Cu (ppm)	$146^{cd} \pm 6.24$	$163^{ab} \pm 4.62$	$138^{d} \pm 6.56$	$155^{cb} \pm 3.51$	$170^{a} \pm 10.1$	$160^{ab} \pm 8.74$
S (%)	$1.13^{ab} \pm 0.06$	$1.10^{b} \pm 0.10$	$1.10^{b} \pm 0.00$	$0.967^{c} \pm 0.05$	$1.23^{a} \pm 0.06$	$1.13^{ab} \pm 0.06$
B (ppm)	$75^{b} \pm 3.46$	$83.3^{a} \pm 1.53$	$72^{b} \pm 4.58$	$75^{b} \pm 4.36$	$73^{b} \pm 2.65$	$76.7^{ab} \pm 4.93$
Ag (ppm)	$0.797^{b} \pm 0.12$	$0.857^{b} \pm 0.12$	$0.787^{b} \pm 0.11$	$0.790^{b} \pm 0.16$	$0.827^{b} \pm 0.09$	$5.60^{a} \pm 1.35$

Values followed by the same letter (superscript) in a row are not significantly different at $P \leq 0.05$.
P_2O_5 : Phosphate; K_2O : Potash; Na : Sodium; Ca : Calcium; Mg : magnesium; Zn : Zinc; Fe : Iron; Mn : Manganese; Cu : Copper; S : Sulphur; B : Boron; Ag : Silver.

Conclusions

Both nZnO based treatments reduced gaseous volumes significantly. Only nZnO and nZnO in bead treatments exhibited significant reductions in concentrations of CO_2, CH_4, and H_2S. Gaseous concentration reduction was not significant in manure treated with nAg. Among the treatments, nZnO outperformed other treatments irrespective of the gas volume and concentration reduction. Reduction of GHGs and H_2S were likely due to microbial inhibition since treated samples had lower coliform counts and total bacterial counts relative to the control. Quantitative polymerase chain reaction analysis revealed a similar decrease in both *mcrA* and DSR gene copies with the application of the nZnO and nZnO in bead treatments.

References

Aarnink, A., Keen, A., Metz, J., Speelman, L., & Verstegen, M. (1995). Ammonia emission patterns during the growing periods of pigs housed on partially slatted floors. *Journal of Agricultural Engineering Research, 62*(2), 105-116.

Arogo, J., Zhang, R., Riskowski, G., & Day, D. (2000). Hydrogen sulfide production from stored liquid swine manure: a laboratory study. *Transactions of the ASAE, 43*(5), 1241.

Boetius, A., Ravenschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Gieseke, A., . . . Pfannkuche, O. (2000). A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature, 407*(6804), 623-626.

Borhan, M. S., Capareda, S. C., Mukhtar, S., Faulkner, W. B., McGee, R., & Parnell, C. B. (2011). Greenhouse gas emissions from ground level area sources in dairy and cattle feedyard operations. *Atmosphere, 2*(3), 303-329.

Chadwick, D., Sommer, S., Thorman, R., Fangueiro, D., Cardenas, L., Amon, B., & Misselbrook, T. (2011). Manure management: implications for greenhouse gas emissions. *Animal Feed Science and Technology, 166*, 514-531.

Dankovich, T. A., & Gray, D. G. (2011). Bactericidal paper impregnated with silver nanoparticles for point-of-use water treatment. *Environmental Science & Technology, 45*(5), 1992-1998.

Defra, A. (2010). Fertiliser manual RB209. *London, UK: Department for Environment, Food and Rural Affairs*.

Friel, S., Dangour, A. D., Garnett, T., Lock, K., Chalabi, Z., Roberts, I., . . . McMichael, A. J. (2009). Public health benefits of strategies to reduce greenhouse-gas emissions: food and agriculture. *The Lancet, 374*(9706), 2016-2025.

Gautam, D. P., Rahman, S., Bezbaruah, A. N., & Borhan, M. S. (2016). Evaluation of Calcium Alginate Entrapped Nano Zinc Oxide to Reduce Gaseous Emissions from Liquid Dairy Manure. *Transaction of ASABE*, 89-102. doi: 10.13031/aea.32.11445.

Gautam, D. P., Rahman, S., Fortuna, A.-M., Borhan, M. S., Saini-Eidukat, B., & Bezbaruah, A. N. (2016). Characterization of zinc oxide nanoparticle (nZnO) alginate beads in reducing gaseous emission from swine manure. *Environmental Technology*, 1-14.

Germida, J., Wainwright, M., & Gupta, V. (1992). Biochemistry of sulfur cycling in soil. *Soil Biochemistry, 7*, 1-53.

Hill, D., & Bolte, J. (1989). Digester stress as related to iso-butyric and iso-valeric acids. *Biological Wastes, 28*(1), 33-37.

Hughes, M. N., Centelles, M. N., & Moore, K. P. (2009). Making and working with hydrogen sulfide: the chemistry and generation of hydrogen sulfide in vitro and its measurement in vivo: a review. *Free Radical Biology and Medicine, 47*(10), 1346-1353.

IPCC. (2007). Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Retrieved from [Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Karunakaran, E., Vernon, D., Biggs, C. A., Saul, A., Crawford, D., & Jensen, H. (2016). Enumeration of sulphate-reducing bacteria for assessing potential for hydrogen sulphide production in urban drainage systems. *Water Science and Technology, 73*(12), 3087-3094.

Kemfert, C., & Schill, W.-P. (2009). An analysis of methane mitigation as a response to climate change. *Copenhagen Consensus Center. Denmark. Accessed at: http://fixtheclimate. com/uploads/tx_templavoila/AP_Methane_Kemfert_Schill_v, 5*.

Kirchmann, H., & Lundvall, A. (1998). Treatment of solid animal manures: identification of low NH 3 emission practices. *Nutrient Cycling in Agroecosystems, 51*(1), 65-71.

Kreuzer, M., & Hindrichsen, I. K. (2006). Methane mitigation in ruminants by dietary means: the role of their methane emission from manure. *In International Congress Series, Elsevier*. 1293, 199-208.

Kushkevych, I., Vítězová, M., Vítěz, T., & Bartoš, M. (2017). Production of biogas: relationship between methanogenic and sulfate-reducing microorganisms. *Open Life Sciences, 12*(1), 82-91.

Lahav, O., & Loewenthal, R. (2000). Measurement of VFA in anaerobic digestion: The five-point titration method revisited. *Water sa-pretoria-, 26*(3), 389-392.

Liao, P., Chen, A., & Lo, K. (1995). Removal of nitrogen from swine manure wastewaters by ammonia stripping. *Bioresource Technology, 54*(1), 17-20.

Luna-delRisco, M., Orupõld, K., & Dubourguier, H.-C. (2011). Particle-size effect of CuO and ZnO on biogas and methane production during anaerobic digestion. *Journal of Hazardous Materials, 189*(1), 603-608.

Monteny, G.-J., Bannink, A., & Chadwick, D. (2006). Greenhouse gas abatement strategies for animal husbandry. *Agriculture, Ecosystems & Environment, 112*(2), 163-170.

Møller, H. B., Sommer, S. G., & Ahring, B. K. (2004). Biological degradation and greenhouse gas emissions during pre-storage of liquid animal manure. *Journal of Environmental Quality, 33*(1), 27-36.

Orphan, V., Hinrichs, K.-U., Ussler, W., Paull, C. K., Taylor, L., Sylva, S. P., . . . DeLong, E. F. (2001). Comparative analysis of methane-oxidizing archaea and sulfate-reducing bacteria in anoxic marine sediments. *Applied and Environmental Microbiology, 67*(4), 1922-1934.

Paul, J., Beauchamp, E., & Zhang, X. (1993). Nitrous and nitric oxide emissions during nitrification and denitrification from manure-amended soil in the laboratory. *Canadian Journal of Soil Science, 73*(4), 539-553.

Peters, J., Combs, S., Hoskins, B., Jarman, J., Kovar, J., Watson, M., . . . Wolf, N. (2010). Recommended methods of manure analysis (A3769). The Board of Regents of the University of Wisconsin System, Madison, WI, USA.

Predicala, B. Z., Alvarado, A., & Asis, D. (2012). Use of Zinc Oxide Nanoparticles to Control Hydrogen Sulphide, Ammonia and Odour Emissions from Pig Barns. *American Society of Agricultural and Biological Engineers*, 3.

Rahman, S., Borhan, M. S., & Swanson, K. (2013). Greenhouse gas emissions from beef cattle pen surfaces in North Dakota. *Environmental Technology, 34*(10), 1239-1246.

Rastogi, M., Singh, S., & Pathak, H. (2002). Emission of carbon dioxide from soil. *Current Science, 82*(5), 510-517.

Reiffenstein, R., Hulbert, W. C., & Roth, S. H. (1992). Toxicology of hydrogen sulfide. *Annual Review of Pharmacology and Toxicology, 32*(1), 109-134.

Rotmans, J., Den Elzen, M., Krol, M., Swart, R., & Van Der Woerd, H. (1992). Stabilizing atmospheric concentrations: towards international methane control. *Ambio*, 404-413.

Sigg, L. (2000). Redox potential measurements in natural waters: significance, concepts and problems. *Springer*, In *Redox* (pp. 1-12).

Sommer, S. G., Petersen, S., & Møller, H. (2004). Algorithms for calculating methane and nitrous oxide emissions from manure management. *Nutrient Cycling in Agroecosystems, 69*(2), 143-154.

Sutton, A., Kephart, K., Verstegen, M., Canh, T., & Hobbs, P. (1999). Potential for reduction of odorous compounds in swine manure through diet modification. *Journal of Animal Science, 77*(2), 430-439.

Van Elsas, J. D., Trevors, J. T., Jansson, J. K., & Nannipieri, P. (2006). *Modern Soil Microbiology*: CRc Press.

Yang, Y., Xu, M., Wall, J. D., & Hu, Z. (2012). Nanosilver impact on methanogenesis and biogas production from municipal solid waste. *Waste Management, 32*(5), 816-825.

Zhu, J., Jacobson, L., Schmidt, D., & Nicolai, R. (2000). Daily variations in odor and gas emissions from animal facilities. *Applied Engineering in Agriculture, 16*(2), 153-158.

GENERAL CONCLUSIONS

Regardless of manure types (dairy and swine) as compared to the respective control treatments, all of the NP treatments (nanogels-ZnSNL, CuSNL, NACL: NPs- nZnO, and nZnO in bead) but not nAg reduced cumulative gas volume and gas concentrations significantly from manure stored anaerobically. Among the nanogel treatments, CuSNL outperformed other treatments in terms of total gas volume and H_2S concentration reduction, whereas, NACL treatment outperformed other treatments in terms of CH_4 and CO_2 concentrations reduction. Between the nZnO and nAg based treatments, bare nZnO outperformed other treatments regardless of the gas volume and concentrations reduction. Irrespective of manure and NP types, reduction of GHGs and H_2S were due to microbial inhibition since treated samples had lower bacterial counts (CFUs) relative to the respective control treatments. Additionally, quantitative polymerase chain reaction analysis revealed a similar decrease in both *mcrA* and DSR gene copies with the application of the nZnO and nZnO in bead treatments.

Subsequently, in vitro study with four nZnO inclusion levels and two types of feed (alfalfa and corn silage) revealed that alfalfa feed reduced the in vitro gas production significantly but increased CH_4, CO_2 and H_2S concentrations compared to corn silage feed. Irrespective of feed types as compared to the respective control treatments, higher nZnO inclusion rate (500 and 1000 μg g^{-1}) reduced a significant amount of CH_4, CO_2, and H_2S concentrations. Moreover, the 1000 μg g^{-1} nZnO inclusion level reduced the microbial populations significantly. Based on this study, on the top of feed type, the inclusion of 500 or 1000 μg g^{-1} nZnO likely to reduce enteric fermentation resulting in lower enteric GHG emission. However, additional in vitro microbial studies are necessary to determine the mode of action of the NP.

RECOMMENDATIONS FOR FUTURE WORKS

- Recapture and reuse mechanism for the liquid NPs need to be evaluated. Further studies are needed to understand the gas reduction mechanisms using ZnSNL, CuSNL, NACL, since none of the *mcrA* genes from the treated and untreated liquid dairy manure samples were amplified.
- New polymers need to be developed to increase the effectiveness of entrapped NPs. Additionally, regeneration mechanism of the applied polymers and reuse potentiality should be future directions of the present work.
- Having both positive and negative outcomes with alfalfa feed warned to have a further detailed study for a prolonged period of time to get complete idea about feed digestion. Additional microbial studies are necessary to determine the mode of action of the applied nZnO application levels. Further work is needed to assess the effect of nZnO inclusion on animal performance when cattle are fed ingredients commonly used in beef feedlot diets.
- Reactivity and life cycle assessment of the applied NPs and polymers need to be performed.
- The lab scale studies need to be upgraded in pilot scale study to find the effectiveness of the NPs towards field application.
- Fate and transport of the applied NPs both in soil and plant tissue need to be evaluated to investigate the effect of applied NPs on the environment.