EVALUATION OF DIFFERENT TECHNIQUES TO CONTROL HYDROGEN SULFIDE AND GREENHOUSE GASES FROM ANIMAL PRODUCTION SYSTEMS

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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

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ABSTRACT

The livestock manure management sector is one of the prime sources for the emission of greenhouse gases (GHGs) and other pollutant gases such as ammonia (NH_3) and hydrogen sulfide (H_2S), which may affect the human health, animal welfare, and the environment. So, worldwide investigations are going on to mitigate these gaseous emissions. The overall objective of this research was to investigate different approaches (dietary manipulation and nanotechnology) for mitigating the gaseous emissions from livestock manure system.

A field study was conducted to investigate the effect of different levels of dietary proteins (12 and 16%) and fat levels (3 to 5.5%) fed to beef cattle on gaseous emission (methane-CH_4, nitrous oxide-N_2O, carbon dioxide-CO_2 and hydrogen sulfide-H_2S) from the pen surface. To evaluate the effects of different nanoparticles (zinc oxide-nZnO; and zirconium-$nZrO_2$) on these gaseous emissions from livestock manure stored under anaerobic conditions, laboratory studies were conducted with different treatments (control, bare NPs, NPs entrapped alginate beads applying freely and keeping in bags, and used NPs entrapped alginate beads). Field studies showed no significant differences in the GHG and H_2S emissions from the manure pen surface.

Between nZnO and $nZrO_2$, nZnO outperformed the $nZrO_2$ in terms of gases production and concentration reduction from both swine and dairy liquid manure. Application of nZnO at a rate of 3 g L^{-1} showed up to 82, 78, 40 and 99% reduction on total gas production, CH_4, CO_2 and H_2S concentrations, respectively. The effectiveness of nZnO entrapped alginate (alginate-nZnO) beads was statistically lower than the bare nZnO, but both of them were very effective in reducing gas production and concentrations. These gaseous reductions were likely due to combination of microbial inhibition of microorganisms and chemical conversion during the treatment, which was confirmed by microbial plate count, SEM-EDS, and XPS analysis.

However, further research are needed to understand the reduction mechanism and to transfer the technology in a real life application.

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GENERAL INTRODUCTION

The major pollutant gases emitted from livestock production facilities are greenhouse gases (GHGs) like methane-CH_4, carbon-dioxide-CO_2 and nitrous-oxide-N_2O as well as ammonia-NH_3, hydrogen sulfide-H_2S (Hou et al., 2015; Wang et al., 2014), and trace amount of other odorous compounds (aldehydes, amines, organic acids, aromatic and sulfur compounds) (Hartung and Phillips, 1994). The emissions occur from both livestock and the livestock production facilities, such as confined housing, manure storage, manure treatment, and land application of manure. Not only these emissions create environmental pollution and impact on human health, but also cause economic loss. For example, NH_3 and N_2O emissions represent a considerable loss of valuable nitrogen fertilizer (Pain et al., 1989; Xiao et al., 2005). Therefore, the emission of GHGs and other pollutant gases from the livestock production system is an environmental, health and economic concern and needs an intensive study to mitigate them.

In the United States; dairy, beef, swine, poultry, turkey, sheep, horse and rabbit are the common livestock and USA produced the major share of dairy, beef, swine and poultry in the world (Spellman and Whiting, 2010). The USA is the number one producer of beef cattle in the world, and beef cattle are the main livestock in animal agriculture in North Dakota. However, limited research has been carried out to determine the gaseous emission from a beef cattle feedlot, especially from feedlot manure management. Therefore, in this study gaseous emission from feedlot pen surfaces has been studied under different dietary and floor conditions.

Reductions of GHG from livestock production facilities are important to reduce the GHG contribution from livestock and animal agriculture to the environment. Similarly, reduction of H_2S is necessary for the safety of workers and the working environment of the facilities. A lot of researches have been conducted for the mitigation of pollutant gas resulting from animal

(Basarab et al., 2003; Buddle et al., 2011; Waghorn et al., 2006) and feedstock (Hao et al., 2005; Novak and Fiorelli, 2010; Waghorn et al., 2006). However, comparatively there is little research about the emissions of pollutant gases from the manure management system, especially from feedlot surfaces. Though some of the research has investigated the mitigation of NH_3 and H_2S (Lee et al., 2007; Luo et al., 2004; Park et al., 2006), but limited research have been conducted on the mitigation techniques for GHG emissions from livestock manure.

Pollutant gases and GHGs are produced at different stages of the livestock production system. As NH_3 is produced from excreta, during storage and land application. Methane is produced from rumens through the digestive process (enteric fermentation) and manure storage. Similarly, H_2S is produced from the anaerobic storage system, and N_2O is produced during storage and land application of manure (USEPA, 2014). In livestock production system, enteric CH_4 accounts a major portion (one-third) of the CH_4 emission (USEPA, 2014); thus the researcher developed different options or technologies to inhibit enteric fermentation in the rumen. The most prominent approach involves the manipulation of livestock diet, which showed effective results in reducing enteric CH_4 production (Greger and Koneswaran, 2010; Mitloehner and Schenker, 2007). In the other hand, the manipulation of livestock diet with or without feed additives also effect on manure pH, concentration and solubility of carbon and nitrogen in manure, which are pertinent to CH_4 and N_2O emission from manure (Hribar and Schultz, 2010; Mirabelli et al., 2006). Therefore, the dietary manipulation could be an effective tool in reducing nutrient/mineral pollution, and odorous and gaseous emissions from manure (Bowman et al., 2000; Mirabelli et al., 2006). On the other hand, manure stored under the anaerobic condition is a major source of pollutant gases and GHG emissions, so an effective mitigation approach in this sector is also crucial.

Two mechanisms are assumed to be effective for inhibiting the gaseous emission from manure: one is the entrapment of the produced gases and stopping them from releasing into the environment, and the other one is the suppression of gas generation or modification on the system involve in gas production. In the environment, the mechanisms for the mitigation of gas emission are the sorption or capture of emitted gases and adoption of chemical or biological treatment in the system to suppress gas emissions. Many technologies have been developed to mitigate H_2S and GHGs, however, some of them are effective and some of them are not. Nanotechnology is an emerging technology and has been used in other sectors, but not in livestock agriculture, especially in treating manure. Nanoparticles can be an effective substance for absorbing/adsorbing the emitted gases and performing biocide effect of the microorganism responsible for those gases emission. Substance on its nano-scale can be more reactive and can exhibit effective and distinct physical, chemical and biological properties compared to its bulk form (Bergeson and Dassa, 2007). Because of size reduction, a small amount of nanomaterial could be sufficiently effective for reacting with a bulky mass of substrates while applying in chemical or biological reaction. Nanoparticles (NPs) have already proved themselves as an effective component in many applications; like in medicine (Zhang et al., 2007), construction (Lee et al., 2010), water treatment (Tiwari et al., 2008), cosmetic products (Pardeike et al., 2009), and so on. Therefore, the prime focus of the research would be in the direction of identifying effective nanomaterials and understanding the working mechanism of those NPs that have effective performance on mitigating gaseous emission. The selection of NPs was based on literature review and discussion with the experts considering their availability, economic and environmental benefits in real life use. In short, besides the determination of gaseous emission from a beef cattle feedlot, this research seek to identify the most suitable nano-materials used for mitigating of gaseous emission from livestock manure system; figuring out their appropriate application method and recovery; and environmental analysis.

Therefore, in this research both field and lab experiments had been undertaken. In the field condition, impacts of dietary manipulation on manure characteristics and gaseous emissions at feedlot pen surfaces had been measured. In addition, the performance evaluation of NPs and their application methods were investigated to reduce gaseous emissions in laboratory condition.

The overall objectives were as follows:

1. In the field study, the major objective was to evaluate the effect of dietary manipulation (protein and fat levels) on the gaseous emissions from the feedlot pen surface. Also, the effect of pen bedding on the feedlot pen surface had been investigated.
2. In the lab study, the major objective was to evaluate the performance of an innovative treatment technology such as nanotechnology to mitigate gaseous emissions from manure stored under anaerobic conditions. Additionally, the goal was to investigate different application methods to understand the treatment mechanisms. Specifically the objectives were:
    a. To evaluate the performance of Zinc oxide (nZnO) and Zirconium ($nZrO_2$) NPs to reduce H_2S and GHGs (CH_4 and CO_2) while treating with dairy and swine liquid manure.
    b. To evaluate the effectiveness of different application rates (100, 500 and 3000 mg L^{-1}) of nZnO, and to compare the effect of nano size ZnO with micro size ZnO in reducing gaseous emissions.
    c. To evaluate the effectiveness of different application methods of nZnO on gaseous emission and their recovery.

d. Lastly, to analyze the mechanism for the reduction of gases using different analysis and characterization techniques.

Dissertation organization

This dissertation is organized into five papers prefaced by the introduction and literature review and followed by the general conclusions, and suggestions for future works. The first and second papers cover the objective 1 while third, fourth and fifth papers cover the objective 2 discussed above.

LITERATURE REVIEW

Human civilization had adopted agriculture at around 10,000 BC, and since that period agriculture was referred as the domestication of some animals and plants (Manning, 2004; TimeMaps, 2015). Domestication of the animals was started mainly for food, fiber, and labor. It is found that people started the domestication of sheep and goats around 8000 BC; similarly at around 7,000 BC, pigs and swine were domesticated. Similarly, later at around 6,500 BC, domestication of cattle was started (OSU, 2015). Like this, people have started keeping different types of animals at home for the different purposes. Now dairy cattle, beef cattle, swine, goat, sheep, horse, donkey, mule, water buffalo, poultry, and turkey are some of the common domestic animals, and they are commonly called livestock. Currently, the human population is around seven billion; however, livestock population is about three times higher than human population (Livescience, 2011; WPF, 2010). The United States of America is one of the largest producers of livestock in the world and their number are listed in Table 1. In the farm, livestock, and human are the integrated components and the environment of livestock is closely related with health and environment of a human being. All kind of safety precautions should be adopted to maintain hygienic and healthy livestock products for the consumers, as well as the healthy indoor environment is needed for the safety of workers and sustainable livestock productivity. The concern should not be only on the products directly consumed by the consumer, but it also needs to address the byproducts which are the unavoidable part of the livestock industry. Manure management is a very important sector in livestock production which needs to manage for minimizing environmental concerns and social aspects.

Table 1. Livestock population according to the population census of 2012 (1,000 Head) (Source: USEPA, 2014).

Animals	Numbers
Dairy Cattle (*Bos* sp.)	13,816
Beef cattle (*Bos* sp.)	81,443
Pigs (*Sus* sp.)	66,516
Horse (*Equus* sp.)	4,413
Sheep (*Ovis* sp.)	30,158
Goat (*Capra* sp.)	3,141
Poultry and turkey (*Gallus* sp/ *Meleagris* sp.)	2,074,269
Mules and Asses (*Equus* sp.)	462
Bison (*Bison* sp.)	167

Animal manure consists of animal excreta dissolved in water or mixed with bedding materials, organic matter and may be used as an organic fertilizer. The manure management is one of the very important components in the livestock industry for sustainable growth of this industry and to minimize environmental degradation. Depending on livestock species and housing systems, livestock manure may be collected as solids, semi-solids and slurry (Hamilton, 2011). Based on the solid content of manure, they are addressed with a different name as in Figure 1.

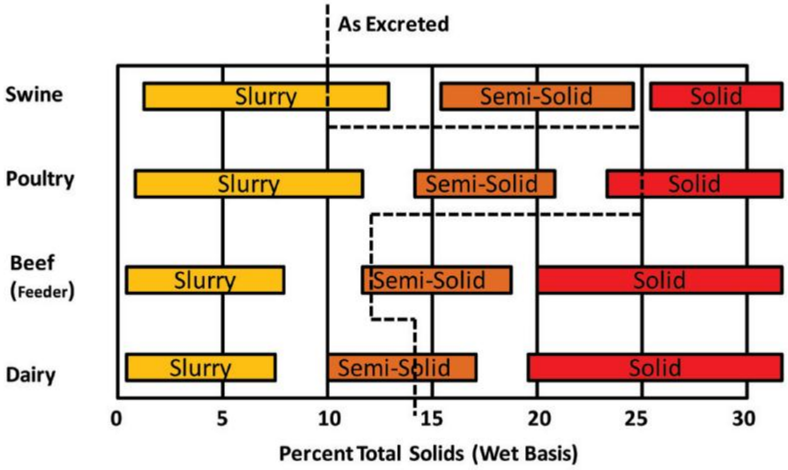


Figure 1. Consistency of various types of manure (Source: NRCS Agricultural waste management field handbook 1992 cited by Hamilton, 2011).

Based on the type and size of the animal, the amount of manure production may vary. Table 2 shows the daily and yearly production of manure and nutrients content like nitrogen, phosphorous, potash and sulfur from different types of livestock. Though manure is a good source of organic fertilizer for crops; however, if not managed properly, it could be a major source of air and water pollution. The emission of pollutant gases (i.e., ammonia-NH_3, hydrogen sulfide-H_2S, etc.), greenhouse gases (GHGs), Volatile organic compounds (VOCs), odor, the particulate material may cause health issue, global warming, and public nuisance, etc. Similarly, nutrients and pathogens in manure may cause eutrophication of surface water and may cause health risk from drinking contaminated water (Daniel et al., 1998).

Table 2. Approximate annual manure production per head and fertilizer content.

Animal		Daily production		Approximate annual production			
Type	Size (kg)	Manure (kg d^{-1})	Manure (kg yr^{-1})	Nitrogen (kg yr^{-1})	Phosphorous (kg yr^{-1})	Potash (kg yr^{-1})	Sulfur (kg yr^{-1})
Dairy cattle	227	20	7118	11	11	27	2
	635	54	19867	29	29	79	23
Beef cattle	340	20	7450	16	18	29	2
	567	34	12473	25	32	50	5
Finishing pig	68	4	1621	2	2	7	2
	91	6	2188	5	5	7	2
Sow and litter	170	10	3723	7	5	14	2
Layers	2	0.11	35	0.23	0.23	0.16	0.02
Broilers	1	0.05	23	0.16	0.16	0.11	0.02

Source. (http://learningstore.uwex.edu/assets/pdfs/a3601.pdf)

The odor from livestock production facility and manure management is mainly a public nuisance and an impediment for expanding existing facilities or establishing new facilities. There are more than 200 odorants and some of them are hazardous to the health of workers and environment also (Borhan et al., 2011; Zhu, 2000). Similarly, H_2S and NH_3 are the pollutant gases which directly effect on the health and environment aspect in livestock industry (Schiffman et al., 2001). Similarly, GHGs are the major contributor to global warming and livestock production facilities are generating GHGs and contributing to global warming. Therefore, many technologies have developed to control or reduce the emission from livestock production facilities and manure management activities, and still researchers are trying to develop new technologies.

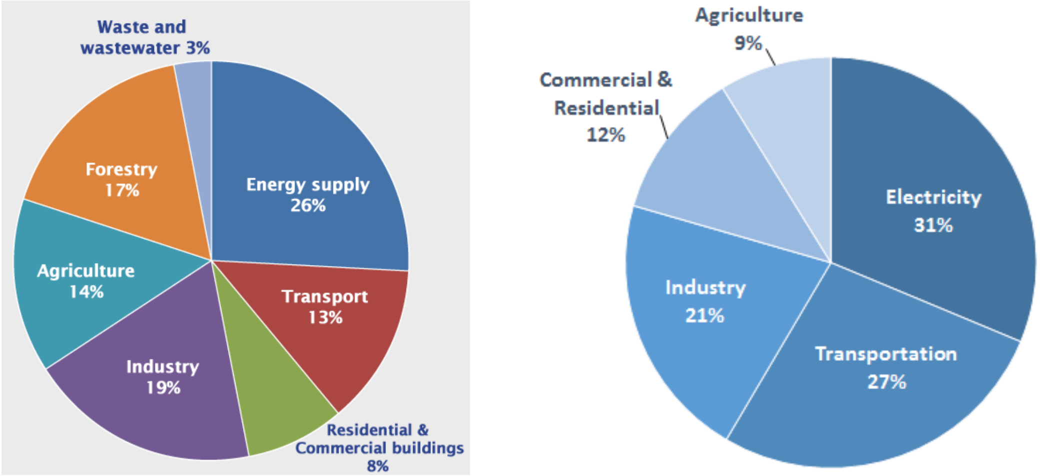
The earth surface temperature is increasing by 0.2°C per decade in the last 30 years (Hansen et al., 2006). GHGs have the potential to absorb and emit infrared radiation, which increases the earth's temperature (IPCC, 2001). The principal GHGs are water vapor, ozone (O_3), carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), chlorofluorocarbon, perfluorocarbon, and sulfur hexafluoride (Ehhalt et al., 2001); however, CO_2, CH_4, and N_2O are the most common GHGs responsible for global warming (Johnson et al., 2007). The global warming potential of CH_4 and N_2O are 25 and 298 times of CO_2, respectively (USEPA, 2015). Therefore, a small amount of N_2O or CH_4 could detrimentally affect the environment. Modernization in human developments and intensive agricultural system are emitting a huge amount of GHG (Dixon et al., 1996). From 1990 to 2013, in the United States, 82.5% of the total GHGs emission were contributed as CO_2 from the fossil fuel and industries; 9.5% as CH_4 and 5.3% as N_2O primarily emitted from agriculture (USEPA, 2015).

The agricultural sector is expected to contribute around 9% of US GHGs emission (USEPA, 2015).Within the agricultural sector, livestock production is a major emitter of GHGs, contributing approximately 3.4% of the total-GHGs-emissions in the USA (USEPA, 2009). Around 25.9% of total CH_4 is contributed from enteric fermentation and manure management system; and animals like beef and dairy cattle are the major sources of CH_4 emitted in the United State (USEPA, 2015). The rate of gaseous production depends on factors like animal species, diet composition, manure management, and environmental conditions like weather condition, types of housing system, and topographic features (Chadwick et al., 2000).

Besides the GHGs, the pollutant gases (NH_3 and H_2S) are the real life problem. The air quality is affected by odor, dust, and microorganism in the livestock production system (Copeland, 2010a). It is difficult to control the emission of these gases completely, but minimization up to a safe level would be a worthwhile option. Although all of the above-mentioned components affect air quality, this study focuses mainly on the mitigation of H_2S and GHGs from manure under anaerobic conditions.

Sources of gaseous emissions in livestock sector

Livestock production system is a major source of H_2S. The anaerobic decomposition of the organic compound resulted in H_2S production; however, its production amount solely depends on the sulfur content of that organic matter (Loneragan et al., 1998). Usually, even a small amount of H_2S from livestock production system can affect the safety of workers to a great extent. Occupational Safety and Health Administration (OSHA) of the United States has fixed the acceptable ceiling concentration of 20 ppm in the workplace while National Institute for Occupational Safety and Health (NIOSH) has recommended the value of 10 ppm for 10 minutes as maximum Recommended Exposure Limit (REL) for H_2S (ASTDR, 2006). Likewise, NH_3 is another pollutant gas produced during the anaerobic decomposition of nitrogenous compounds in manure. Ammonia is a major problem in poultry and swine facilities (Blunden and Aneja, 2008; Kristensen and Wathes, 2000). Similarly, agricultural land is the major source of NH_3 beside livestock. In general, the major sources of GHGs emission are from energy, industries, transportation, commercial and residual building, forestry; but a substantial amount of GHGs are also generating from agricultural activities and waste management (USEPA, 2015). The burning of fossil fuel for heat and electricity contribute the highest portion of GHGs emission followed by industry and transportation (Figure 2).



(a) (b)

Figure 2. a) Sources of GHG emission in global context and b) in USA (Source: IPCC, 2007; USEPA, 2015).

As mentioned above, agriculture contributes around 14% of the total GHGs emission globally (IPCC, 2007), including both crop and livestock production; and 26% of agricultural emission is from livestock production (USEPA, 2013). In the context of USA, beef cattle contribute the highest emission followed by dairy and swine. However, other livestock like horse, poultry, sheep and goat also contribute a minor fraction of GHGs. The CH_4 and CO_2 are produced by enteric fermentation and manure management activities including anaerobic storage of manure and composting. However, CO_2 and enteric CH_4 emission from CAFOs is not currently regulated by USEPA.

The United States is the number one country in the beef cattle production. In 2012, the total number of cattle raised were around 94 million, and among them around 81 million were beef cattle (USEPA, 2014). There are much research on the gaseous emission from dairy, swine, and poultry, however only few research have been carried out to measure GHGs emission from beef cattle feedlot surface (Beauchemin and McGinn, 2005; Borhan et al., 2011; Hao et al., 2001; Larney and Hao, 2007; Rahman et al., 2013). As different parameters are involved in the estimation of gaseous emission; therefore, the emission estimation or calculation for one place may not be directly applicable to another place mainly due to difference in animal diet, weather and management practices. Beef cattle production in North Dakota is one of the major livestock industries; however, here also the research on pollutant gases and GHGs emission are very limited. National Research Council (NRC) has emphasized that there is a need to measure emissions on farm scale to improve emission inventory.

Manure properties and their relation with gaseous emission

Livestock fecal matter, urine and any materials combined with waste feed, bedding material and waste water in the animal confinement is called manure (Spellman and Whiting, 2010). Livestock manure is not regarded as a hazardous waste according to the definition of Environmental Protection Agency (EPA) if it is handled in a proper way (Copeland, 2010b); moreover, it is one of the valuable products which have been intensively using as an organic nutrients sources for the plant growth. Manure is a source of the major nutrients such as nitrogen (N), phosphorous (P) and potassium (K), as well as minor source of micronutrients like copper (Cu), iron (Fe) cobalt (Cb), chromium (Cr), manganese (Mn), zinc (Zn), selenium (Se), iodine (I) and molybdenum (Md) as well (Franke et al., 2008). Manure also contains pathogens, hormones and antibiotics and if it is not managed properly may contaminate surface and ground water (Kumar et al., 2005; Sadeghi and Arnold, 2002).

Manure consists of organic and inorganic components such as cellulose, hemi-cellulose, fat, protein, volatile materials and some odorous compounds (Kerr et al., 2006). The manure properties depend on the composition of feed, digestibility, animal species, housing and environment of animals, weather, season and stage of production (Spellman and Whiting, 2010). The chemical interaction of these compounds in manure under specific environmental condition may result in the generation of different gases from them. Besides the normal metabolic process in the animal body, microbial decomposition of the organic material in the manure is the primary mechanism of gaseous emission. The gases like CH_4, CO_2, N_2O, H_2S, NH_3; particulate matter; volatile organic compounds and odors are the common forms of air pollutants emitted from manure. Types of animal species, confinement, feeding practices, manure management systems, and land application practices determine the substances to generate and their emission rate (Spellman and Whiting, 2010; USEPA, 2001).

Parameters affecting gaseous emissions in livestock production system

Factors like the weather, type of animal and their population size, diet composition, manure type, manure moisture content and manure storage conditions determine the quantity and rate of gaseous emission. Broadly these factors are:

Type of animal operation

Emission differs according to the types of animal and their operating practices. As shown in Table 3, the H_2S is a common form of emission from all kinds of livestock and from their operations; however, in case of dry solid manure from poultry and turkey housing system, H_2S emission is slightly less. Ammonia is a major problem in swine and poultry operations; however, it is generating in all types of livestock operations. Carbon dioxide is emitted from all types of operations. Nitrous oxide is mainly produced during the land application of manure. Methane is emitted from all types of manure under anaerobic storage. Particulate matters are emitted mainly in case of poultry, equine and others livestock operation where dry confinement environment is provided. The emission also depends on the age group of animals. Usually, the digestibility of animals are higher as they are getting bigger and older, thus the emission rate will be lower compared to baby livestock (Spellman and Whiting, 2010).

Table 3. Emission from different operation of livestock.

Animal Sector	Operations	H_2S	NH_3	CH_4	N_2O	CO_2	PM	VOC
Broilers, turkey, layers (dry)	Confinement					×	×	
	Manure storage					×	×	
	Land application				×	×	×	
Layers (liquid)	Confinement	×	×	×	×	×	×	×
	Manure storage	×	×	×	×	×		×
	Land application	×	×		×	×		×
Swine (flush)	Confinement	×	×			×	×	×
	Manure storage	×	×	×		×		×
	Land application	×	×		×	×		×
Swine (others)	Confinement	×	×		×	×		×
	Manure storage	×	×	×	×	×		×
	Land application	×	×		×	×		×
Dairy (flush, scrap)	Confinement	×	×			×	×	×
	Manure storage	×	×	×		×		×
	Land application	×	×		×	×		×
Dairy (drylot)	Confinement	×	×	×		×	×	×
	Manure storage	×	×	×		×	×	×
	Land application	×	×		×	×	×	×
Beef	Confinement	×	×	×		×	×	×
	Manure storage	×	×	×		×	×	×
	Land application	×	×		×	×	×	×

Source: Cited from USEPA, 2001 in (Spellman and Whiting, 2010).

Feeding practices

Depending on livestock species and ages, their feed compositions are different. Even within the same animal type, feeding composition may differ in different growth stages and also due to their availability. If the feed contains some sulfur compounds, higher emission of H_2S is expected; and if the feed contains more nitrogenous compound, then higher NH_3 is expected.

The composition of feed determines the digestibility; and if the feed is more digestible, there is less emission (Martin et al., 2008).

The enteric CH_4 production in the rumen is found be to be affected mainly by the feeding practice and feed composition. The type and amount of carbohydrate influence the production of individual volatile fatty acids (VFAs) formation which is directly related with CH_4 production. Feed containing more soluble sugars results in less CH_4 production than other carbohydrates. Feed containing more starch component favors propionate production resulting less CH_4 production. Similarly, feed containing more roughage favors acetate production increasing CH_4 production (Johnson and Johnson, 1995). High grain diet feed at high intake results higher rate of ruminal digestion and lower ruminal pH which inhibit the growth of methanogens and protozoa, and ultimately reduce the CH_4 formation (Hegarty, 1999). An increase in feeding level results decrease of CH_4 which is mainly due to the rapid passage of feed out of rumen i.e. decrease of retention time of feed in the rumen (Mathison et al., 1998; Waghorn and Hegarty, 2011). Methane production rate in ruminant differ according to forage species and the amount of production was found to be increased with a maturity of forage. Beef getting more grass forage showed more CH_4 yield than those getting legume forage (Moss et al., 2000; Waghorn et al., 2006). Feeding strategy like replacing grass silage by maize silage helped in reducing enteric CH_4 emission (Tamminga et al., 2007). Similarly, low feed frequency is likely to increase propionate production and lower acetate production i.e. propionate: acetate ratio tends to increase; resulting lower CH_4 production from beef cattle (Sutton et al., 2003). Besides these strategies, the techniques used for forage processing and preservation also exhibits some variation in CH_4 emission (Boadi et al., 2004).

Based on the feed intake and its nutrient composition, Kirchgessner; et al. (1995) have formulated an empirical model to determine the CH_4 production by enteric fermentation on cattle, which is described as:

$$\mathrm{E_{CH_4}} = \mathrm{a} + 79\mathrm{CF} + 10\mathrm{NFE} + 26\mathrm{CP} - 212\mathrm{F} \qquad (1)$$

where, *a* is the intercept (16 g CH_4 day^{-1} for beef cattle), CF is the crude fibers (kg day^{-1}), NFE is the intake of nitrogen-free extracts (kg day^{-1}), CP is the crude protein (kg day^{-1}) and F is the crude fat (kg day^{-1}). It shows that enteric CH_4 production has a direct relationship with the carbohydrate and protein composition of feed; however, it has an inverse relation with fat composition. In the other hand, the dietary manipulation influences on the carbon/nitrogen inputs which ultimately effect on the nutrient/mineral, odor and gaseous emissions from manure system (Bowman et al., 2000; Mirabelli et al., 2006).

Animal housing

With advanced production, handling, and management system; nowadays most of the livestock are raised in confined facilities, only limited animals are raised in an open environment. In confinement housing system, the structure such as a combination of the floor system, ventilation system, manure collection, and storage system collectively determine the level of gaseous emission including their concentration and amount (Borhan et al., 2012; Guidotti, 1994). Control of indoor climates like temperature, humidity, and air velocity has a great impact on gaseous emission (Milby and Baselt, 1999; Zschocke and Hoffmann, 2004). The ventilation system mainly influences the temperature, humidity and air movement inside the confinement (Zhang et al., 2005). In most of the cases, mechanical ventilation is provided in the animal housing, which help to control the indoor environment.

Similarly, the floor structure is adopted according to the manure handling and disposal system in the housing. Different livestock has their specific housing system, and emission varies according to the housing design, and manure production and collection system. In case of dairy cattle; free stall, tie stall and cubical are the common housing where the manure management is mainly based on slurry system (Bewley et al., 2001; Powell et al., 2008; Sommer et al., 2013). Similarly, beef cattle are usually raised in feedlots, a confined place in the open environment. In some case, they are provided with some shelter house to be safe from rain, snow and sun, and the manure is accumulated generally twice a year and taken for composting (Eghball and Power, 1994; Larney and Hao, 2007; Sommer et al., 2013). Likewise, in most of the case pigs are raised in the confinement house. The manure is collected mostly as a slurry (Ni et al., 1999); however, in some cases, pig houses could also have deep litter system (Groenestein and Van Faassen, 1996; Sommer et al., 2013) where the manure is collected in collection tank as semi-solid or keep as compost. Likewise, poultry has deep litter system in most of the case where the litter is collected and taken for composting. Few structures still have battery cage system, where the dropping is collected underneath in deep pit (Moore et al., 1995; Sommer et al., 2013). In general, higher CH_4 emission is observed from the slurry system. Housing where deep litter system is adopted, anaerobic environment got favored resulting higher CH_4 emission. Similarly, H_2S emission is also higher where the manure is storage inside the closed storage pit/ tank compared to open storage system. Likewise, higher N_2O emission is reported from the housing adopting deep litter system and compost system (Borhan et al., 2012; Jungbluth et al., 2001).

In the animal housing system, reduction of expose surface of manure, cooling of manure surface, decreasing pH of manure in storage system, and fast and complete removal of manure from floor to slurry storage system are some of the abatement principles that help to reduce gaseous emission from housing system (Borhan et al., 2012).

Manure storage system

Depending on the manure type and consistency (Figure 1), manure storage system differed. The storage of manure is one of the integrated parts in confinement facilities. According to the housing system, the manure storage system is designed. In case of dairy housing where tie stall barns adopted, the manure is collected in the storage tank. The manure from free stall barn is temporarily collected in a storage pit where the solid fraction is separated from the liquid portion and used as bedding material after drying or simply taken for composting. The liquid fraction is directly applied to the field (USEPA, 2012a; Spellman and Whiting, 2010). In case of swine, where deep litter system is adopted; in few cases, the solid fraction is collected and stored in the stack for composting. However, in most of the case the swine manure is handled as a liquid. The manure is usually stored in concrete storage pits just below the slotted floor, or pumped to a large outside storage. Similarly, liquid manure can be transfer to the lagoon from gutter or storage pits (USEPA, 2012b). Likewise, in case of poultry, manure is mostly handled as solid litter where the dropping is collected in the bedding material placed above the solid floor. In case of battery cages system, manure is collected in storage pit which can be transferred for composting or stored for the long term and applied directly to the field. In large scale farming, sometimes the manure is collected in the tank as liquid manure before applying to the field. They can also be stored in the lagoon for anaerobic digestion (USEPA, 2012c).

The emission of NH_3, H_2S, CH_4 and N_2O from solid manure is eventually determined by the temperature, moisture, compaction and aeration of the heap. Covering of heap reduce CH_4, NH_3 and H_2S emission (Heederik et al., 2007). The addition of straw to solid manure favors more aerobic condition, thus reduce CH_4 emission and increase N_2O emission (Radon et al., 2007). Slurry stores provide the anaerobic environment, so they are the major source of CH_4 emission; however N_2O emission is negligible (Donham et al., 2007). Similarly, slurry storage system enhances NH_3 and H_2S emission (Campagna et al., 2004).

Land application of manure

Depending on methods of land application of manure, the amount and rate of gaseous emission may vary. Broadcasting and surface application favor the volatilization of NH_3 compared to the injection system. Similarly, late incorporation of manure applied on the soil surface results in higher amount of emission as compared to injection system or immediate incorporation by plowing (Amon et al., 2006). The slurry or liquid manure application in the field provides a favorable environment for nutrient volatilization as compared to solid manure application in the field. Similarly, injection of manure may reduce volatilization of NH_3, but it increases N_2O emission (Wulf et al., 2002). Shallow injection provides anaerobic nature of slot environment, resulting higher CH_4 emission as compared to surface broadcast (Schiffman et al., 1995). The CH_4 emission is short-lived and occurred immediately after the manure application (Chadwick et al., 2000). During manure application, volatilization of NH_3 occurs within 48 h of manure application resulting a large quantity of nitrogen loss (Merchant et al., 2005). The manure application allows aerobic condition for nitrification, and N_2O is generated, but the process get delayed due to delay in mineralization and time required for manure carbon to become available (Bullers, 2005; Chadwick et al., 2011).

Environmental condition

The emission rates vary according to different climatic conditions. The regions with higher temperature bear higher emission rate. The emission rate increased along with increasing the wind velocity. The precipitation and humidity of the atmosphere also affect the emission rate. Besides the external environment, the physical and chemical characteristic of manure like moisture content, temperature and pH of manure also effect on the emission amount and emission rate (Garcia-Marco et al., 2014; Snyder et al., 2009).

Different gases from livestock production system

Different types of gases are emitted from different components of the livestock production system and from livestock themselves. Pollutant gases such as NH_3, H_2S; GHGs (CH_4, N_2O, and CO_2); particulate matter and volatile organic compounds (VOCs) are emitted from livestock production system. The manure management system contributes a major portion of the gaseous emission. In this research, the major focus will be on the emission from the manure management system, especially from manure storage system and research will be concentrated mainly on H_2S and GHGs emissions. Those gaseous components and the reaction mechanisms in their production from manure system are briefly described as follows:

Hydrogen sulfide

Hydrogen sulfide is a colorless, heavy gas that has a very unpleasant smell (rotten egg), and it is formed by the processes of sulfate reducing bacteria or the decomposition of sulfur-containing organic compounds in manure under anaerobic condition. Being a smelly and toxic gas, it is regarded as one of the major problems in the livestock production system from an environmental perspective and human health perspective (Thu, 2002). The sulfur amino acid contained in the feed, the inorganic sulfur compound used as feed and a trace sulfur mineral in drinking water are the major sources of sulfur in livestock manure. In liquid or slurry system, the anaerobic condition favors the production of H_2S. Hence, manure storage tank, lagoon, ponds and application sites are considered as primary source of H_2S. The amount of H_2S emission depends on bacterial populations coupled with temperature, pH and liquid content of manure (Spellman and Whiting, 2010; Washio et al., 2005).

Methane

Livestock, predominantly ruminants, are one of the major sources of CH_4 that contribute around 25% of total GHGs emissions from agriculture in the United States (USEPA, 2009). CH_4 is produced under anaerobic condition from the manure and other organic sources in the farmyard (Khan et al., 1997). *Methanobacterium, Methanobrevibacter, Methanococcus, Methanofollis, Methanogenium*, *Methanoseate,* and *Methanosarcina* are some of the common genera of methanogens responsible for CH_4 production (Hook et al., 2010; Liu and Whitman, 2008). During the process, the methanogen bacteria convert the organic matter in manure into CH_4 and CO_2. Manure in its liquid or slurry form provides anaerobic condition resulting CH_4 production; however, manure in the solid state would be usually in aerobic condition with less CH_4 production. Manure stored in the lagoon, pond or any kind of tanks would be in anaerobic condition; hence, CH_4 is produced mainly from that storage area during that period. Usually in the case of an anaerobic digester, CH_4 production is accompanied by CO_2 and the mixture of these two gases is called biogas. Commercially biogas is produced from organic materials in an anaerobic digester and usually it composed of 60 to 70% CH_4 and 30 to 40% CO_2 (Spellman and Whiting, 2010).

Methane generation mechanism during anaerobic digestion process

During the anaerobic digestion process, the decomposition of organic matter takes place in four different stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Figure 3). Hydrolysis is an extracellular step where hydrolytic bacteria hydrolyze the complex polymers into soluble oligomers and monomers in the presence of enzymes like cellulases, xylase, proteases, amylases and lipases (Daniel et al., 1998). In acidogenesis process, the simpler products obtained from hydrolysis process is converted into alcohols and volatile fatty acids (VFA). While in actenogenesis process; alcohol, VFA, and remaining products of hydrolysis are oxidized into CH_3COOH, CO_2, and H_2 by acetogens. Finally, the products of acidogenesis and acetogenesis such as CH_3COOH, H_2, CO_2 and other carbon compounds like carbon monoxide, methanol, methylamine are converted into CH_4 and CO_2 in the presence of obligate anaerobes, methanogens (Bruni, 2010; Gerardi, 2003; Raju, 2012). Two pathways are followed during CH_4 formation. One is acetoclastic methanogenesis (Equation 2) where CH_4 is produced from acetate, which accounts approximately 70% of the CH_4 formation. Similarly, another is hydrogenotrophic methanogenesis (Equation 3) where hydrogen and CO_2 combine to form CH_4 and water (Klass, 1984).

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad (2)$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \qquad (3)$$

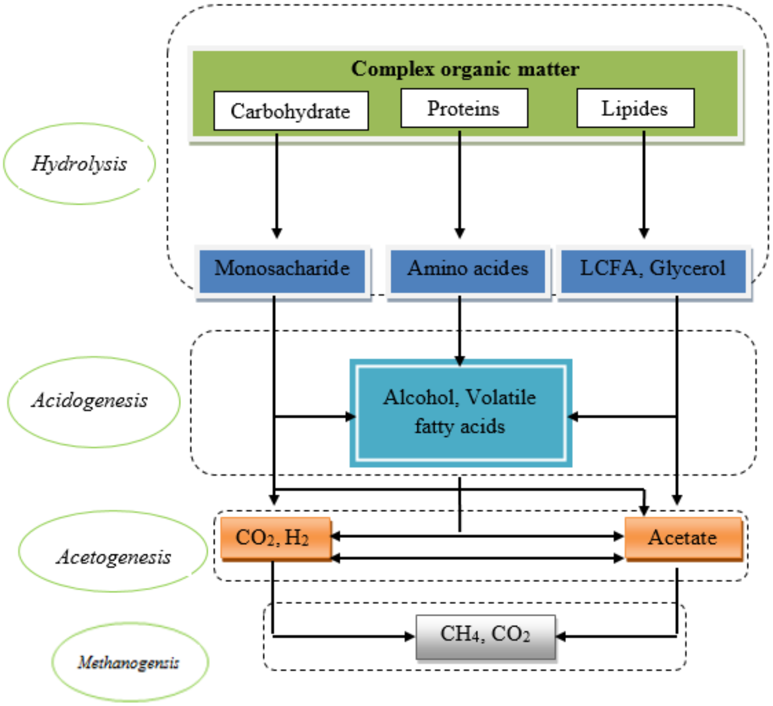


Figure 3. Anaerobic digestion process of organic matter (Source: Gautam, 2012).

Nitrous oxide

Manure application site is the major source of N_2O emission (Chadwick et al., 1999). The interaction of manure and soil emits N_2O during the process of oxidation or reduction of nitrogenous compounds. In short, N_2O is produced by the microbial decomposition of organic nitrogenous compounds in manure and it is emitted in the processes of nitrification followed by denitrification (Bremner and Blackmer, 1978; Chadwick, 2005; Spellman and Whiting, 2010). Actually when the manure is handled aerobically followed by anaerobically, N_2O emission occurs. In manure, most of the nitrogen is in NH_3 form, which upon aerobic nitrification process converts into nitrate form. Then under anaerobic de-nitrification, the nitrate change into nitrogen as a final product. N_2O is formed as an intermediate or side product during nitrification and de-nitrification process. In poorly drained soil where the anaerobic condition occurs resulting de-nitrification process, there is less conversion of organic nitrogen into nitrite and nitrate forms and ultimately N_2O emission will be high. Similarly, if manure applied at the site of the field where uptake by plants is less, there is more chance of N_2O formation and emission (Spellman and Whiting, 2010).

Nitrous oxide generation mechanism during nitrification and denitrification process

Nitrous oxide is formed during nitrification and de-nitrification processes (Equation 3-5). In nitrification process, the oxidation of NH_3 or ammonium takes place in the presence of *nitrosobacteria* (Equation 4) and converted into nitrite in the first step. Then nitrite gets oxidized to nitrate in the presence of *nitrobacteria* (Equation 5). Besides nitrosobacteria and nitrobacteria which are autotrophic, there are some heterotrophic nitrifiers which could be responsible for the generation of N_2O in nitrification process (Equation 7) (Borhan et al., 2012; Loh et al., 2008; McGinn et al., 2007). Heterotrophic nitrifiers are found to be active under aerobic condition.

(a) Nitrification:

$$2NH_4^+ + 2O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+ \tag{4}$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \tag{5}$$

In overall:

$$NH_3 \text{ or } NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^- \tag{6}$$

Nitrous oxide formation during nitrification process:

$$NH_3 \rightarrow NH_2OH \xrightarrow{\overset{N_2O}{\uparrow}} NO_2^- \xrightarrow{\overset{N_2O}{\uparrow}} NO_3^- \tag{7}$$

In de-nitrification process, the nitrate is reduced and converted into nitrite, nitrogen oxide, N_2O and finally into nitrogen gas (Equation 8-12). Though de-nitrification process takes place both in aerobic and anaerobic conditions; N_2O formation is favorable in anaerobic condition (McGinn et al., 2007). In de-nitrification process, the conditions like low organic carbon content, low oxygen pressure, high nitrogen content and low pH are believed to be favorable for N_2O formation (McGinn and Beauchemin, 2012).

(b) Denitrification:

$$NO_3^- + H_2O + 3e^- \rightarrow NO_2^- + 2OH^- \tag{8}$$

$$NO_2^- + e^- \rightarrow NO + O^{2-} \tag{9}$$

$$2NO + 2H^+ + 2e^- \rightarrow N_2O + H_2O \tag{10}$$

$$N_2O + 2H^+ + 2e^- \rightarrow N_2 + H_2O \tag{11}$$

In overall:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO + N_2O \rightarrow N_2 \tag{12}$$

Carbon-dioxide

In livestock production, CO_2 is produced during chemical and thermal decomposition, energy use, aerobic and anaerobic digestion of organic materials, and respiration. In aerobic condition, CO_2 and water are the end products; while in anaerobic condition CO_2 and CH_4 are the common end products. CO_2 emission from the agricultural sector does not contribute a long term increase in atmospheric CO_2 concentration (Spellman and Whiting, 2010). Hence, people are paying less attention to it as compared to others GHGs.

Ammonia

Ammonia is a light and colorless gas that has a sharp pungent odor in atmospheric condition. However, the odor of aqueous NH_3 is non-detectable by the human sense of smell (Marcus, 1991; Ruth, 1986). Ammonia is produced from the microbial decomposition of the organic nitrogen compounds in manure. The nitrogen in urea or uric acid hydrolyzes to form NH_3. Ammonia is water soluble, but highly volatile when manure gets dried. The volatilization of NH_3 depends on pH, temperature and storage period of manure. In acidic condition, ammonium is a predominant, while in the basic condition ammonium changes into NH_3 (Spellman and Whiting, 2010). Besides the direct impact on human and animal health, NH_3 can have a great impact on the environment. Ammonia can cause the eutrophication of surface water, acidification, and the promotion of bacterial growth that leads to the weathering and corrosive damage of the buildings. The problem of NH_3 is more on swine and poultry housing than other livestock operation (Drummond et al., 1980; Ndegwa et al., 2008).

Other gases

Livestock production system is a major source of volatile organic compounds (VOCs) emission which are responsible for generating an odorous environment in the farmyard. Volatile fatty acids (VFAs), p-cresol, phenol, 4-ethylphenol, indole, and skatole are some of the VOCs responsible for odor formation in livestock production system (Zicari, 2003). Phenol is a highly reactive volatile organic compound (HRVOC) and p-cresol is recognized as one of the major odor-causing compound emitted from livestock operation (Moore, 2006).

Particulate matter (PM) is a heterogeneous mixture of different types of suspended materials like spores, soil, dust and organic chemicals in the air having particle-like properties (Cambra-López et al., 2010). Though, PM is not included as a component under gaseous emission, it's one of a major component of air quality in livestock production system. Livestock production contributes around 8% and 4% of total PM10 and PM2.5 emission, respectively (Cambra-López et al., 2010). Particulate matter from livestock production is important regarding the health of farmers and people on the periphery, and health and welfare of livestock; as PM can cause a detrimental effect on the performance and production of livestock (Homidan et al., 2003; Andersen et al., 2004). PM has potential to cause respiratory problems like a chronic cough, phlegm, bronchitis, allergies and asthma in farmers and cause early death of children and ill (Donham et al. 2000, Radon et al., 2001). Moreover, it also effect on the animal health and reduce the productivity and efficiency. Poultry and pig houses are the main source of PM emission in livestock industry (Hartung and Saleh, 2007).

Measurement techniques of gaseous emission in the field

There are broadly two main methods: device independent and sampling device, commonly used to quantify gaseous emissions from ground level area sources in livestock production facilities (Borhan et al., 2012). In addition, IPCC tiers I and II algorithms, and Blaxter and Clapperton algorithms are mathematical models based on the assumptions of biochemical reactions driven by animal size, feed intake and feed quality in confined conditions (Loh et al., 2008; Rahman et al., 2012; Van Haarlem et al., 2008). The device-independent methods also referred as the micrometeorological techniques include atmospheric dispersion modelings such as inverse dispersion and backward Lagrangian stochastic models, flux gradient, boundary layer budgeting, eddy covariance and relaxed eddy accumulation. These techniques involve a combination of atmospheric turbulence theory and call for gas concentrations in the air along with the vertical and horizontal speed of the wind as well as meteorological parameters to estimate gas flux from a surface (McGinn and Beauchemin, 2012; McGinn et al., 2007; Rahman et al., 2012). Similarly, in the case of sampling device methods, a chamber or wind tunnel is deployed on an emitting surface under some recommended operating conditions. Those devices may be static (sealed or vented) or dynamic (flushed with zero grade air i.e. contaminant free air at a known flow rate). Both emissions measurement methods employ gas concentration measurements in the air. Gas chromatograph (GC), gas chromatography mass spectroscopy (GCMS), non-dispersive infrared detector (NDIR), open path Fourier transform infrared radiation (OP-FTIR), tunable diode laser absorption spectroscopy (TDLAS) and photoacoustic spectroscopy (PAS) are some common instruments widely used for measuring gas concentrations from sources (Borhan et al., 2012). For source-specific emissions measurement in livestock facilities, the use of wind tunnel, flux chamber, mass balance and tracer ratio method are quite common which falls under non-micrometeorological technique (Harper et al., 2011; Storm et al., 2012).

Technologies and management practices for reducing gaseous emission

Science and technology have developed different methods to control the environment of livestock production system. Regarding the case of gaseous emission, many technologies have been already developed to minimize the emission. Farmers are adopting many strategies for the mitigation of NH_3 emitted from the livestock production system. The reduction of nitrogen from excretion can be achieved through dietary modification (Satter et al., 2002). Ammonia volatilization from manure can be minimized by reducing pH (Jensen, 2002), segregating urine and feces (Von Bemuth et al., 2005), inhibiting hydrolysis (Varel, 1997), binding NH_3 (Portejoie et al., 2003), and applying biological treatment (Luostarinen et al., 2006). Acidification of manure is a popular practice where NH_3 emission gets reduced by converting the nitrogenous compounds in ammonium form in low pH (Kai et al., 2008). Similarly, livestock urine contains urea, which upon hydrolysis gives NH_3 in the presence of urease enzyme. Feces contain urease, and if urine can be separated from mixing with feces, then the NH_3 formation can be reduced (Von Bemuth et al., 2005). Likewise, Portejoie et al. (2003) used different types of covering or binding material like oil, plastic film, perforated polystyrene float, peat and zeolites in pig slurry to see the effect on NH_3 emission; and determined that oil could reduce NH_3 up to 40% and zeolites can reduce up to 71%. The use of filter and bio-filter are also found to be effective for reducing NH_3 emission from the manure, where the nitrogenous compounds are captured by the filter media and prevent from NH_3 volatilization process (Luostarinen et al., 2006). Furthermore, the improvement in building design and manure management can also reduce NH_3. In practice, the strategies to capture the emitted gas and to trap the fugitive gases by using different kinds of the medium is very common (Ndegwa et al., 2008).

Similarly, several management practices have been developed to reduce H_2S emission from livestock manure. Some of the management practices that are targeted for the reduction of NH_3 can also be effective for reducing H_2S. Hydrogen sulfide is produced from livestock housing, manure storage facilities and during land application of manure. In housing facilities, filter and bio-filters are commonly used to trap the emitted gas (Ruokojärvi et al., 2001). Use of vegetable oil also shows a significant reduction to H_2S emission (Powers, 1999). Selection of proper landscape for animal housing and use of windbreak around the buildings minimize the H_2S concentration in housing facilities. Manipulation of livestock diet also reduces the H_2S concentration in buildings by altering the composition of excreta (Nahm, 2002). H_2S emission from the storage facilities can be minimized by using covers, by adopting aeration, and by flowing composting process (Patterson, 2005). Furthermore, the strategies that minimize H_2S emission from the manure system include the injection or incorporation of manure, maintenance of dry manure system, and timely application of manure in the field (Work, 2010).

Many research have been conducted to mitigate enteric CH_4. Dietary manipulation and alteration in feeding practice are the major approaches adopted for controlling enteric CH_4. Feed containing more soluble sugars, starch components, and fewer roughages favor less enteric CH_4 production (Johnson and Johnson, 1995). Similarly, low feed frequency results lower CH_4 production (Sutton et al., 2003). The management practices used for forage processing and preservation also showed some variation in CH_4 emission. CH_4 production per unit intake found to be decreased by 30-40% in case of grinded or pelleted forage (Boadi et al., 2004). Similarly, CH_4 production was observed to be lower in case of ensiled forage than dried ones (Shingfield et al., 2005). Beside management strategies, manipulation on rumen diet also favored minimizing the enteric CH_4 production. The addition of fat to the cattle diet increases the energy density of the diet and decrease the CH_4 generation. Mathison et al. (1998) have reported 85% decrease in enteric CH_4 production while adding 4% of canola oil in the diet. Yang et al. (2009) have also reported that use of linseed as dietary supplements showed the effective result on the reduction of enteric CH_4 production. The addition of some ionophores such as monensin, lasalocid, tetronasin, lysocellin, narasin, salinomycin and laidomycin to ruminant diet have shown a positive effect on the reduction of enteric CH_4 (Newbold et al., 1988). Defaunation process (the removal of protozoa from rumen by dietary or chemical agent) could also reduce enteric CH_4 production by 20-50% (Finlay et al., 1994). Likewise, the use of some chemical inhibitors for the methanogenesis process could also be a possible option. Bromoethanesulphonate (BES) is a possible inhibitor which was able to show 71% reduction in enteric CH_4 production (Dong et al., 1999). The use of probiotics has shown 10-50 % CH_4 reduction during enteric fermentation (McGinn et al., 2004; Newbold et al., 2005).

In addition to mitigate enteric CH_4 from the rumen, different techniques or management practices have also been developed to mitigate CH_4 generated from manure. Dietary manipulation has regarded as a potential option for mitigating CH_4 from manure (Mirabelli et al., 2006). Use of bedding material favors the reduction of CH_4; however, it also favors the nitrification process resulting more N_2O emission (Chadwick et al., 2011). Frequent and regular removal of manure from the floor is found to be very effective for reducing CH_4 emission. The adoption of anaerobic digestion system for the collected manure helps to control CH_4 emission to the minimum level (Clemens et al., 2006). Composting of manure has shown a significant reduction in CH_4 emission (Pattey et al., 2005). Likewise, CH_4 emissions from lagoons can be controlled by covering the source and capturing the emitted gas (Clemens and Ahlgrimm, 2001). The optimum pH for methanogenic bacteria is 7-8 and CH_4 production is inhibited when acidification treatment (pH 5.5) is applied on whole manure (Sutaryo et al., 2012).

Nitrous oxide emission is found mainly in manure application site and the emission resulted from soil-manure-interaction. In practice, the adoption of the slurry system favors the anaerobic system and nitrification process get reduced, which ultimately reduces N_2O emission (Chadwick et al., 2011). Furthermore, the strategies of manure covering and composting also reduce N_2O emission along with other gases. Currently, researchers are exploring the best specific cropping system to reduce N_2O emission (Dalal et al., 2003). Biochar has also been used to reduce N_2O emission (Singh et al., 2010). Likewise; applying manure in the field also favors the minimization of CO_2 emission by promoting carbon sequestration (Reicosky et al., 2000). Spreading of biochar is a technique for the reduction of CO_2 along with N_2O emission (Woolf et al., 2010).

However, most of the technologies or management practices that have been described above are time-consuming, labor intensive, short time effective and targeted for specific gas only. Therefore, scientists are still looking for the innovative technologies or practices which could be effective for a long period and for multiple gases. As nanotechnology has shown promising results in many other applications, there is still a very limited application of this technology in the field of agriculture. Currently, some researchers are using nanoparticles to treat animal waste so as to reduce the gaseous emission (Predicala et al., 2012).

Applications of nanotechnology

A new and innovative way of mitigating gaseous emission is the application of nanotechnology. Nanotechnology is an emerging field of science. Nanotechnology deals with the manipulation of substance at its atomic and molecular level, and material form in this kind of small scale is called nanomaterials. Nano-materials are in different forms and one of the most common form of is nanoparticles (NPs). Their size is smaller than 100 nm at least in one dimension (Luoma, 2008). Any chemical on its nano-scale exhibits extremely high surface area which could be the very important feature for reacting to any substrate or media where it works.

The particle size is the major feature of NPs. Table 4 illustrates the effect of size reduction on surface area. On top of that, NPs could exhibit unique and distinct physical, chemical, and biological properties compared to their ordinary chemical form. Therefore, NPs are getting so popular nowadays. Nanoparticles have a wide range of applications in the fields of medicine, bio-material, electronics, energy production, civil construction, waste water treatment, and agriculture (Bolyard et al., 2013; Mueller and Nowack, 2008, Predicala et al., 2012, Sharma et al., 2003).

Table 4. Effect on surface area during the reduction of size to nano-scale for a cube.

Edge length	Number	Volume	Surface area
1 cm	1	1 cm^3	0.0006 m^2
1 mm	10^3	1 cm^3	0.006 m^2
1 µm	10^{12}	1 cm^3	6 m^2
1 nm	10^{21}	1 cm^3	6000 m^2

Though NPs are widely used in many sectors, they have very limited applications in the agricultural sector, especially livestock agriculture. It is assumed that material on its nano-scale could possess toxic nature, and the rumor about the toxicity of NPs might be a major factor for their limited applications in agricultural products. As the consumption of agricultural products is directly related to human health, so people seem to hesitate on using NPs on agricultural product. However, the application of NPs in manure (non-consumable product) could be an option in the agricultural sector. There are some research on NPs applications in the field of waste management and waste water treatment (Bolyard et al., 2013; Mueller and Nowack, 2008; Yang et al., 2012b). However, an intensive research on their application in livestock manure has not carried out yet. Some preliminary research has shown that NPs can effectively contribute for the reduction of harmful gaseous emission by the process of sorption of produced gas, chemical interaction with substrates, and amendment of microbial activity (Choi and Hu, 2009; Wang et al., 2008b).

As mentioned above, NPs possess the properties of sorption of gas, chemical conversion and amendment of microbial activity, a research hypothesis can be made that NPs can be used to control the pollutant gas emissions from livestock production system. The research will be primarily focused on the identification of some promising NPs, evaluating their performance and developing their application technique for mitigating target gases.

Nanoparticles and their environmental applications

Nanoparticles have wide applications even within the environmental sector also. They are basically used for wastewater treatment, air quality treatment, as a gaseous detector and so on. The commonly used NPs relevant to this specific research with their specific feature and application field are as follows:

Zinc oxide NPs (nZnO)

Zinc oxide NPs (nZnO) have wide applications; they are commonly used in nanogenerators (Gao et al., 2005), biosensors (Topoglidis et al., 2001), solar cells (Hames et al., 2010), photo-catalysts (Kamat et al., 2002), and photo-detector (Sharma et al., 2003) due to their significant electrical and optical properties. They have high surface activity and large surface area that are the key characteristics for a wide range of potential applications (Sayyadnejad et al., 2008). The specific chemical, structural and surface properties of nZnO made theme effective and popular in various applications. They are less expensive as compared to other NPs because of the involvement of simple techniques of their preparation (Talam et al., 2012).

Zinc oxide NPs has the potential to mitigate GHGs and other pollutant gases in the field of waste management. Many researchers (Abatzoglou and Boivin, 2009; Hernández et al., 2011; Sayyadnejad et al., 2008) have already used nZnO to remove H_2S during the purification of biogas, and drill-work of gas and oil. When nZnO are added to biomass, desulphurization occurs in which ZnO combines with H_2S, forming ZnS and water. In fact, nZnO are very reactive with sulfur compounds (Hernández et al., 2011). The reduction of H_2S could also be due to the chemical interaction with substrates biomass and amendment of microbial activity. The way nZnO reduce H_2S in the liquid phase (waste water, manure etc.) is not so clear, it could be either due to sorption or chemical conversion of emitted gas or the lethal effect of these NPs on the microbial community (Fang et al., 2006; Luna-delRisco et al., 2011). Zinc oxide NPs have an affinity to react with NH_3 gas as well. However, both H_2S and NH_3 produced from manure; there is a possibility that they will compete for the reaction with ZnO. Zinc oxide is most likely to reacts with H_2S instead of NH_3 because the reactivity of NH_3 is relatively lower than H_2S with ZnO (Chung et al., 2005).

Limited research has been carried out on the application of nZnO on manure. Predicala et al. (2012) have reported a reduction of H_2S caused by introducing nZnO on swine manure. The effect of nZnO on specific manure is generally understood, but a detailed study is still needed to understand the different application methods of NPs in different manures type, a dose of NPs, recover of NPs and the environmental analysis. Based on literature review and from previous experience, nZnO is found to be the most effective one for the specific purpose of this research. Therefore, it’s good to know about their synthesis process along with their physicochemical properties.

Synthesis of nano zinc oxide

There are many synthesizing processes of nZnO, but they are not limited to mechano-chemical, controlled precipitation, hydrothermal synthesis, vapor deposition, sol-gel process, precipitation from microemulsions and pyrolysis spray are some of the common methods used for the preparation of nZnO (Kołodziejczak-Radzimska and Jesionowski, 2014). However, the mechano-chemical process is a very common method of nanoparticles preparation on a large scale and nZnO is prepared using anhydrous $ZnCl_2$ and Na_2CO_3 as common starting materials, where following reaction occurred at a high-temperature condition (Equation 13-14) (Aghababazadeh et al., 2006; Ao et al., 2006).

$$\mathrm{ZnCl_2 + Na_2CO_3 \rightarrow ZnCO_3 + 2NaCl} \tag{13}$$

$$\mathrm{ZnCO_3 \xrightarrow{400-800^\circ C} ZnO + CO_2} \tag{14}$$

[1]Controlled precipitation is another widely used method for nZnO synthesis. The reduction of zinc salt solution in the presence of a reducing agent under specific temperature and time of precipitation give rise to nZnO (Hong et al., 2006; Jia et al., 2012). Wang et al. (2010) prepared nZnO by precipitating aqueous solutions of NH_4HCO_3 and $ZnSO_4$ as in reactions below (Equation 15-16). The two solution were mixed in room temperature (298 K) until the formation of crystal product of zinc hydroxyl carbonate. The byproduct $(NH_4)_2SO_4$ was removed by washing powder with distilled water. Then the washed power was taken for calcination at 100°C for overnight.

$$\mathrm{5ZnSO_4 + 10NH_4HCO_3 \rightarrow Zn_5(CO_3)_2(OH)_6 + 5(NH_4)_2SO_4 + 8CO_2 + 2H_2O} \tag{15}$$

$$\mathrm{Zn_5(C0_3)_2(OH)_6 \rightarrow 5ZnO + 2CO_2 + 3H_2O} \tag{16}$$

Similarly, a hydrothermal method is one of another simple and environmentally friendly technique where a mixture of substrates is heated to 100-300˚C in an autoclave and left for several days on specific pH (5-8) (Kołodziejczak-Radzimska and Jesionowski, 2014). Chen et al. (1999) synthesized nZnO using $ZnCl_2$ and NaOH (Equation 17-18), while Ismail et al. (2005)

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[1] According to the information provided by the manufacturer, the nZnO used for this research was also synthesized by using control precipitation followed by calcination process.

used $Zn(CH_3COO)_2$ and NaOH to synthesize nZnO as shown in the reaction below (Equation 19-20).

$$\mathrm{ZnCl_2 + 2NaOH \rightarrow Zn(OH)_2 + 2Na^+ + 2Cl^-} \tag{17}$$

$$\mathrm{Zn(OH)_2 \xrightarrow{100-300℃} ZnO + H_2O} \tag{18}$$

$$\mathrm{Zn(CH_3COO)_2 + 2NaOH \rightarrow Zn(OH)_2 + 2CH_3COONa} \tag{19}$$

$$\mathrm{Zn(OH)_2 \xrightarrow{100-300℃} ZnO + H_2O} \tag{20}$$

The sol-gel method is another simple, low-cost, more reliable and repeatable method for the synthesis of nZnO (Kołodziejczak-Radzimska and Jesionowski, 2014). In this process, films or tiny powder in the form of colloidal sol transformed into a gel in the presence of precursor (Mahato et al., 2009). Benhebal et al. (2013) obtained nZnO from zinc acetate dehydrate and oxalic acid using ethanol as a solvent while Ristic et al. (2005) used tetramethyl ammonium hydroxide (TMAH) and added to the solution of zinc ethyl hexanoate and propanol.

Physico-chemical properties of nZnO

As mentioned in the previous section, nZnO can be synthesized in many ways, but they represent almost very similar physical and chemical properties (Kołodziejczak-Radzimska and Jesionowski, 2014). Zinc oxide NPs are usually odorless powder, milky white in color, and spherical or usually irregular in shape (Gupta et al., 2009). The solubility and dissolution rate of nZnO are relatively higher than the bulk ZnO (Reed et al., 2012), but crystal structure resemble with bulk ZnO. Some of the physical properties of nZnO are listed in Table 5 (Vaseem et al., 2010).

The solubility of bulk ZnO is considered almost zero but Reed et al. (2012) measured the solubility of up to 7.4 mg L^{-1} in nano-pure water. Zinc oxide NPs exhibits wurtzite structure which is the most stable form, having a hexagonal unit cell (Vaseem et al., 2010). Though ZnO crystallizes in two main forms: hexagonal wurtzite and cubic zinc-blende; but wurtzite is the most stable in ambient condition and most common in occurrence.

Table 5. Physical properties of nZnO.

Properties	Values
Density (g cm^{-3})	5.606
Melting point (°C)	1975
Thermal conductivity (W cm^{-1} $°C^{-1}$)	0.6-1.2
Dielectric constant	8.656
Refractive index	2.008
Band gap (eV)	3.37
Linear expansion coefficient (^{o}C)	a_o: 5.6 $cm^3 \times 10^{-6}$ c_o: 3.0 $cm^3 \times 10^{-6}$

Zinc content in animal feed and animal manure

Regarding the scenario of nZnO application for manure treatment; it's important to understand about the usual concentration of zinc on manure, the permissible range of zinc concentration that can be applied in the land, and regulatory limit of manure quantity in the field. Livestock manure consists of different types of micro and macro nutrients, and the nutrient content of manure vary with the type of livestock, the digestibility of the ration, animal age, amount of feed and water consumed by animals, the type and amount of bedding used, and the amount of water used to remove manure from the buildings.

Chastain and Camberato (2004) presented the concentration of zinc in different types of dairy manure that is ready for the land application as in Table 6. Similarly, Nicholson et al. (1999) reported the average zinc concentration in the dairy cattle slurry, beef cattle farm yard manure, pig slurry and poultry manure as 209, 81, 575, 459 mg kg^{-1} dry matter of manure, respectively in their research, and reported the range of 5-727, 41-274, 5-2500, 350-632 mg kg^{-1} dry matter of manure, respectively based on the literature review.

Table 6. Concentrations of zinc in dairy manure (dry basis) that is land applied (source: Chastain and Camberato, 2004).

Manure type	liquid	slurry	Lagoon water	Lagoon sludge
Moisture %	96.2	93	99.4	93.9
Zn, mg kg^{-1}	372	353	1373	752

Usually, the zinc concentration in a normal soil is in the range of 10-300 mg kg^{-1} (Ahmad et al., 2013). The zinc concentration in the manure helps to change the zinc concentration in soil over a certain time period. As the nutrient composition of the manure is greatly affected by the feed composition and different type of feed ingredients. Therefore, the variation of zinc concentration according to the manure type is obvious. Nicholson et al. (1999) have reported the zinc concentration on various feed for livestock as in Table 7.

Table 7. Zn concentration in animal feeds (source: Nicholson et al., 1999).

1. Cattle feed type	Zn (mg kg^{-1} dry matter)
Dairy feed	
Dairy cake/nuts	39-289
Maize gluten	64-92
Sugar beet pulp	21-32
Grass silage	21-48
Maize silage	28-30
Minerals	1540-4530
Beef cattle feed	
Beef cake/nuts/pellets	56-777
Rolled oats and barley	22-59
Hay	17-41
Straw	43-252
Grass silage	26-53
2. Pig feed type	
Compound feed	150-2920
Home-mix feed	100-2580
3. Poultry feeds type	
Compound feed	102-311
Home-mix feed	24-4030

Silver NPs (nAg)

Silver NPs (nAg) also has wide applications such as in water purification, medical applications, antimicrobial uses, paints, coatings and food packaging (Li et al., 2008; Tolaymat et al., 2010). Nano silver is popular due to its wide applications and relatively low manufacturing cost. It can be found in different forms, such as nano-silver powder, colloidal silver, spun silver and polymeric silver (Perez, 2012). Among these, colloidal silver is one of the commonly used forms; as it has wide applications due to some distinct properties such as chemical stability, good conductivity, catalytic and antibacterial activity (Panáček et al., 2006). The antibacterial activity of nAg made it popular in the field of biological science (Sondi and Salopek-Sondi, 2004) because it hinders the growth of micro-organisms in the media as silver ion and silver based compounds are highly toxic to microorganisms (Kim et al., 2007).

Silver NPs are used in the field of waste management, like wastewater treatment and landfill decomposition, and they have demonstrated effective results on the control of pollutant gas emissions also (Choi and Hu, 2009; Yang et al., 2012a; Yang et al., 2012b). Choi and Hu (2009) have reported that silver NPs inhibit the nitrification process that leads to the reduction of NH_3 and N_2O emissions. Similarly, silver NPs also have a negative impact on methanogenesis process; however, the dose of NPs determines the level of inhibition. Yang et al. (2012b) have reported that when silver NPs of 10 mg kg^{-1} solids was introduced in the landfill site, then biogas production was reduced significantly; however, in a low concentration of 1 mg kg^{-1} solids, no significant difference in the cumulative biogas volume was observed. Likewise, Yang et al. (2012a) found that there is no inhibition of methanogenesis with silver NPs until 40 mg L^{-1} concentration in anaerobic condition. However, inhibition can be expected beyond that limit (high dose).

Zirconia NPs (nZrO$_2$)

Zirconia is one of the most studied ceramic materials that have poly-crystalline properties. Zirconia has many applications in the manufacture of electro-ceramic tools. Peterson et al. (2011) has reported the use of nZrO$_2$ for the removal of sulfur dioxide. Similarly, Jia et al. (2011) have reported the use of nZrO$_2$ for treating wastewater containing chromium. To date, very few researchers have investigated the effect of nZrO$_2$ in the environmental field but this NP is interesting for the research because it has been used as the anti-bacterial agent (Jangra et al., 2012, Pradhaban et al., 2014).

Other NPs

Titanium dioxide (nTiO$_2$) is one of the popular compound widely used for antibacterial application (Kong et al., 2010; Stoimenov et al., 2002). Likewise, nanoscale zero-valent iron (NZVI) is also widely used for the treatment of contaminated soil and ground water remediation. Now a day, carbon nanotubes (CNTs) have shown great potential in managing and improving soil, water and air quality (Masciangioli and Zhang, 2003). Absorption of dioxin (Liu et al., 2007) NO$_x$, SO$_2$ and CO$_2$ (Long and Yang, 2001) is possible using CNTs. Carbon nanotubes have also showed good results on the test of absorption of other gases like N$_2$, O$_2$, and CH$_4$ (Tan et al., 2012). The absorption of NH$_3$, H$_2$S, H$_2$ and acetone are feasible under high vacuum and pressure (Feng, 2005). Ren et al. (2009) used copper oxide NPs (nCuO) to test it as an antimicrobial agent and found that they can be effective for the activities of certain groups of pathogen like *Staphylococcus aureus* and *Escherichia coli.* They are mostly effective for Gram positive and Gram negative bacterial (Stoimenov et al., 2002). Activated carbon powder itself is a good absorbent of many chemical compounds including gas molecules due to its high surface area, micro-porous structure and high surface reactivity (Bansal and Goyal, 2010; Goyal and

Bansal, 2005). Activated carbons have shown good results in capturing the CO_2 gas and some odorous substances (Khan et al., 1981). Nanoparticles loaded on activated carbon have found to perform better than the nanoparticle or activated carbon itself (Ghaedi et al., 2012; Huwe and Fröba, 2007).

Recovery of NPs

Nano-particle recovery is important because NPs are expensive and releasing to the environment might have an environmental concern. A small amount of NPs could be effective for a bulk substrate, therefore the use of NPs is increasing. However, even that small amount NPs could be economically unaffordable and environmentally unsafe. Therefore, there has always been a high demand for a better technology for recovery, recycle, and reuse of NPs so s to reduce the cost and make them environmentally safe. Some of the conventional separation and recovery techniques are flocculation (Chen et al., 2002), filtration (Zhang et al., 2001), evaporation (Koetz et al., 2005), super-rate centrifugation (Zhang et al., 2001), field-flow fractionation (Dubascoux et al., 2008) and phase separation by the chemical reaction (Hu et al., 2005). However, some new techniques such as reverse micelles (Zhang et al., 2001) and microemulsion (Gan et al., 1996) have also shown prominent results.

Usually, NPs are very expensive. In the other hand, they could be harmful to the ecosystem as their fate and transportation in the environment is not so clear for most of the cases. The concept of direct application of manure might be effective only in the case if the NPs that are used would be really cheap, and non-toxic to the ecosystem and environment. If the application rate of those NPs is very low and effective for the specific purpose, and the concentration is within the maximum contamination level (MCL), then the direct method of application might be effective. However, in adverse condition, some indirect application methods need to be considered so as to recover and regenerate the applied NPs.

In the indirect method, the main goal would be stopping NPs from being dispersed in the slurry and to the environment. For that, one of the techniques is to entrap NPs into the polymer matrix or beads (Bezbaruah et al., 2009); so that they can be applied in the slurry and recovered after the treatment. When those polymer beads get contact with manure, then it can react with the manure without dispersing those nanoparticles from beads into the slurry. The permeability and porosity of the polymer could allow the manure to react with NPs remained inside the beads, not limiting the NPs on the surface only. When the treatment is complete, the polymer beads can be collected more easily by any means than collecting NPs because of the bigger size of beads.

The nAg is the most commonly used NPs found to be embedded in a different type of polymers and in different fashions (Balan et al., 2008; Kong and Jang, 2006; Porel et al., 2005). NPs dispersed in the polymer matrix are found to be performed as effectively as they are applied in bare forms. Bezbaruah et al. (2009) used a technique of entrapping of NZVI in calcium alginate beads for ground water remediation application *i.e.* for the removal of nitrate. Similarly, the removal of trichloroethylene (Bezbaruah et al., 2011; Kim et al., 2010) and arsenic (Escudero et al., 2009) were also examined by using embedded NZVI in sodium alginate beads. Liu et al. (2010) used NZVI entrapped chitosan beads for hexavalent chromium removal from the waste water. Chitosan beads are used for embedding Ag, Pd, Pt, Au and Cu NPs as well (He et al., 2008; Laudenslager et al., 2008; Wu et al., 2009). Polymers like Polyvinylidene fluoride (PVDF), Polyamide blends, Poly-ether ether ketone (PEEK) and Polyelectrolyte membrane are also popular for embedding NPs like Cu, Pd, Pt, Rh and Ag (Monticelli et al., 2005; Muraviev et al., 2007; Xu and Bhattacharyya, 2007). Zero-valent silver and Ni are found to be embedded in polyamide films i.e. resins with divinylbenzene matrix (Akamatsu et al., 2008); the hydrated ferric oxide is embedded in cation exchange resin with a sulfonic acid functional group (Cumbal and SenGupta, 2005).

Functionalized polymers or biopolymers supported metal or metal oxide nanoparticles are called polymer supported nanoparticles (PSNPs), exhibit unique properties which are not exhibited by the polymeric host or nanoparticle alone. As these metal or metal oxide PSNPs exhibit combines composite properties of biopolymers and nanoparticles, they have wide applications as sensors and biosensors, biomedical device, coating, paints, electro-conductive pastes ad glues and antifriction polymeric coating. These PSNPs are used for the sorption of different targeted species from water and wastewater.

As this metal or metal oxide polymer PSNPs exhibit almost similar properties as that of bare nanoparticles, it can be expected that they can show effective results as used in liquid or gas media (Sarkar et al., 2012). If the gas is allowed to pass through the NPs embedded dry beads or filters, consist of certain effective doped NPs in it, the pollutant gas could be absorbed into it (Figure 4).

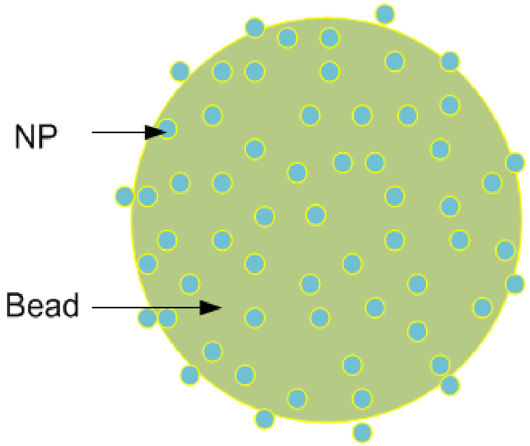

Figure 4. Absorption of pollutant gas by the NPs embedded in the beads when piled up in a column.

Characterization technologies for NPs and NPs entrapped media

Electron microscope

An electron microscope uses a beam of an electron instead of a ray of light as in ordinary microscope to make the image of a specimen. Electron microscope produces the image of very high resolution (up to 50 pm) and high magnification (up to 10,000,000X) (Erni et al., 2009). Transmission electron microscope (TEM) and scanning electron microscope (SEM) are two major electron microscope. In TEM, the beam of the electrons is transmitted through the sample specimen by interacting with it and form an image of high resolution and magnification. Besides that, TEM also gives the detail information about the internal composition of specimens and also provides the characteristics features such as morphology, crystallization, stress and even magnetic domains (Chescoe and Goodhew, 1984).

Similarly, SEMs give the image of the specimen by scanning the sample specimen with high energy electron beam. The electrons hitting the specimen generate various signals which provide the information about the morphology, topography, chemical composition, crystal orientation and structure of the sample (Williams and Carter, 1996). Unlike TEM, here the electron beams raster the surface of the sample and produced high-resolution image. Comparatively, the TEM provides much higher resolution image than SEM. The image on TEM is two dimension whereas it’s three dimensional in case of SEM.

Beside the production of a high regulation image of the sample, the secondary electron emitted by the atom is detected by different detectors attached to SEM to provide various information. Energy Dispersive Spectroscopy (EDS) in SEM determines the chemical composition of sample specimen (Angelidis and Sklavounos, 1995) while Electron Backscatter Diffraction (EBSD) determine its crystal structure and crystal orientation (Prior et al., 1999). As secondary electron emitted from the sample display compositional contract based on an atomic number of the elements. EDS provides the information about the particular element based on the relative atomic weight. EDS generates X-ray spectra from the entire scan area of the SEM and the spectral range of element depend on the energy level. So, in EDS analysis, Y-axis displays the number of X-rays spectra received by the detector (count) and X-axis displays the energy level of those counts (Williams and Carter, 1996).

X-ray photoelectron spectroscopy (XPS)

X-ray Photoelectron Spectroscopy (XPS), also called Electron Spectroscopy for Chemical Analysis (ESCA) is another widely used surface analysis technique. It provides the quantitative and chemical state information of the material’s surface. It gives the information of thin film composition i.e. surface layer up to 10 nm depth of sample specimen. In XPS, an electron energy analyzer measures the energy of the photoelectron emitted from the surface; and based on the binding energy and intensity of pick; chemical state and elemental identity can be determined (except hydrogen). Besides elemental identification, it provides the information about the chemical state of elements and relative composition of the constituents in the surface region (Watts, 1994). The XPS spectra are obtained while irradiating the surface of sample specimen with a beam of X-rays, and photoelectron spectrum is recorded counting the ejected electrons over the range of kinetic energy of electrons. So a plot of the intensity of photoelectron on Y-axis and binding energy or kinetic energy on X-axis can be observed in XPS spectrum. It is analogous to EDS of SEM; however it provides better compositional analysis of the sample, rastering in higher depth compared to EDS (Andrade, 1985).

Real-time polymeric chain reaction (RT-PCR)

Polymeric chain reaction (PCR) provides the accurate quantification of the targeted gene copies. The reliability of the technique is strongly dependent on the quality of the extracted genomic DNA (Dionisi et al., 2003). The main propose of PCR is to make numbers of copies of a gene which is necessary to have enough starting template for sequencing. As the name implies, polymeric chain reaction: One DNA molecule produces two, which becomes four, then eight, then sixteen and so forth. This doubling process is carried out with the help of a specific enzyme (protein) called polymerases which help to form a long molecular strand by string the individual DNA building block: adenine (A), thymine (T), cytosine (C) and guanine (G). The process also need a small fragment of DNA also called primer, where the building blocks get attached to form a template (Joshi and Deshpande, 2011). In PCR, there are major three steps: Denaturation, annealing, and polymerization or extension; which are temperature depended processes. In denaturation process, the double stranded DNA is opened and converted into single-stranded DNA through melting at elevated temperature (usually 94˚C). Whereas, in annealing process (usually from 55-60˚C), hybridization of two oligonucleotides used as primers to the target DNA takes place; i.e. ionic bonds are formed between single stranded primer and building blocks, and polymerization is just started copying the template. At an ideal temperature of 72˚C, the polymerization gets extended called the extension process (Bergmann, 2011; Joshi and Deshpande, 2011; Querci et al., 2006). Real-time polymerase chain reaction (RT-PCR), also called quantitative real time polymerase chain reaction (QPCR) is a very common laboratory technique based on the PCR. The principle of the RT-PCR is very similar to that of a conventional PCR where the target gene (DNA molecules) is amplified over a defined number of PCR cycles to quantify them. Conventional PCR allows only end-point detection where the end products of the reaction (amplified DNA fragments) are analyzed by electrophoresis. Whereby, RT-PCR permits the analysis of the products while the reaction is actually in progress. The concentration of the amplified target is monitored after each PCR cycle in RT-PCR applications using a fluorescent dye or probe, and intensity of fluorescence gives the quantitative value or concentration of amplified gene in real time (Bergmann, 2011; Zhang and Fang, 2006). RT-PCR is the fast and easy method to quantify the starting amounts cDNA since it is not necessary to perform electrophoresis or other procedure after the DNA amplification reaction (VanGuilder et al., 2008).

Economic and environmental analysis of the technology

There is a great debate on the need for proactive assessment of the potential health and environmental risk and benefits resulted from the unregulated nano-technologies (Sweet and Strohm, 2006). The market potential of technology, environment, health, safety, risk and uncertainties possessed by the nanotechnology need to be addressed in the early stage of the development before launching the product in the market. Environmental Protection Agency (EPA) has already brought a concept of the life-cycle impact of a new field before the technology is mature and set a benchmark for future (Shatkin, 2012). EPA has also emphasized on a point that the risk on use and disposal scenario has to be well defined before the technology implemented in practical life (Wardak et al., 2008).

As the concept of nanotechnology was developed just few years earlier, so still there are lots of things which are unknown and need more investigation regarding the environmental issue. For the implementation of this technology, the environmental law, and policy regarding the use of nanotechnology should also be well addressed. Though, EPA has set the maximum contamination level for the safe drinking water, surface water and in soil composition regarding the use of chemicals (Bove et al., 1995); however, the nano effect of the chemical on the biosphere is still uncertain (Renn and Roco, 2006).

Regarding the case of nanotechnology application on manure system, life cycle assessment is most essential. As there are different technologies and management practices which are already developed and implemented in real life. So, the new proposed technology should be economically viable, environmentally safe and legally acceptable regarding environmental law and policy of the state and the federal government.

Potential impacts of applied nanoparticles

Currently, NPs have been widely used in different fields but less attention has paid on their long effect. Those NPs may not show short time effect, but could have a hazardous effect on long term (Jarvie et al., 2009). The small size of nanoparticles has potential to interact with the biological entities such as cells, cellular components, and micro-organisms; and bear risk factor to affect soil, plant, human health, and the environment. It has been seen that smaller the NPs, the higher is the potential of taking up into the body and being toxic (Teow et al., 2011). The unsafe disposal of NPs use for any purpose can reach up to the food source of human being. The unsafe disposal of the NPs poses a larger threat to our water source. Once they reach to the water source, they can’t be easily separated even by the ultra-filtration process also and they have the potential of entering into the body following different pathways (Jarvie et al., 2009). Nanoparticles can enter the body following different pathways like inhalation, ingestion, dermal absorption and therapeutic application if the person exposed the environment containing NPs.

Many toxicological studies have been carried out specifically on nZnO to understand their potential risk human health and environment. Unfortunately, nZnO have shown some toxicological effect on some bacteria, Daphnia magna, freshwater microalgae, mice, and even human cells (Brayner et al., 2006; Franklin et al., 2007; Heinlaan et al., 2008; Sharma et al., 2009; Wang et al., 2008a). Zinc oxide NPs could have a negative dermatological effect as they have the potential to enter the skin. Sharma et al. (2009) had carried out the an experiment on the effect of nZnO on human skin and observed that nZnO possess the DNA damaging potential and even low concentration of nZnO possess genotoxic effect in the human epidermal cell.

In this research, NPs treated manure could be the potential source for the exposure of NPs in the environment. The treated manure could affect on the composition of soil and their properties. The plants may absorb those NPs which could have a negative impact on plant health and could pass to human and other livestock through the food chain (Franklin et al., 2007). Additionally, the person who is working in the field and manure management could have a direct impact of NPs on their health (Sharma et al., 2009). The land application of treated manure could have an environmental impact as they can be transported to a water body and can expose to the atmosphere and create water and air pollution. So, further research is required regarding the fate and transport of NPs on manure system including their impact on soil, plants, human and environment as a whole.

References

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

Aghababazadeh, R., Mazinani, B., Mirhabibi, A., and Tamizifar, M. 2006. ZnO nanoparticles synthesised by mechanochemical processing. *Journal of Physics*. 26(1): 312-314.

Ahmad, W., Watts, M., Imtiaz, M., Ahmed, I., and Zia, M. H. 2013. Zinc deficiency in soils, crops and humans: a review. *Agrochimica.* 56(1): 65-97.

Akamatsu, K., Adachi, S., Tsuruoka, T., Ikeda, S., Tomita, S., and Nawafune, H. 2008. Nanocomposite polymeric microspheres containing Ni nanoparticles with controlled microstructures. *Chemistry of Materials*. 20(9): 3042-3047.

Amon, B., Kryvoruchko, V., Amon, T., and 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.

Andersen, C. I., Von Essen, S. G., Smith, L. M., Spencer, J., Jolie, R., and Donham, K. J. 2004. Respiratory symptoms and airway obstruction in swine veterinarians: A persistent problem. *American journal of industrial medicine*. *46*(4): 386-392.

Andrade, J. D. 1985. X-ray photoelectron spectroscopy (XPS). In *Surface and interfacial aspects of biomedical polymers*, 105-195: Springer.

Angelidis, T., and Sklavounos, S. 1995. A SEM-EDS study of new and used automotive catalysts. *Applied Catalysis A: General*. 133(1): 121-132.

Ao, W., Li, J., Yang, H., Zeng, X., and Ma, X. 2006. Mechanochemical synthesis of zinc oxide nanocrystalline. *Powder Technology*. 168(3): 148-151.

ASTDR. 2006. Public Health Statement, Hydrogen sulfide, CAS# 7783-6-4. Reterieved on 9/10/2015 from http://www.atsdr.cdc.gov/ToxProfiles/tp114-c1-b.pdf.

Balan, L., Jin, M., Malval, J.-P., Chaumeil, H., Defoin, A., and Vidal, L. 2008. Fabrication of silver nanoparticle-embedded polymer promoted by combined photochemical properties of a 2, 7-diaminofluorene derivative dye. *Macromolecules*. 41(23): 9359-9365.

Bansal, R. C., and Goyal, M. 2010. *Activated carbon adsorption*: CRC press.

Basarab, J. A., Price, M. A., Aalhus, J. L., Okine, E. K., Snelling, W. M., and Lyle, K. L. 2003. Residual feed intake and body composition in young growing cattle. *Canadian Journal of Animal Science*. 83(2): 189-204.

Beauchemin, K. A., and McGinn, S. M. 2005. Methane emissions from feedlot cattle fed barley or corn diets. *Journal of animal science*. 83(3): 653-661.

Benhebal, H., Chaib, M., Salmon, T., Geens, J., Léonard, A., Lambert, S. D., Crine, M., and Heinrichs, B. 2013. Photocatalytic degradation of phenol and benzoic acid using zinc oxide powders prepared by the sol–gel process. *Alexandria Engineering Journal*. 52(3): 517-523.

Bergeson, L. L., and Dassa, I. 2007. TSCA and engineered nanoscale substances. *Nanotech. L. & Bus.* 4: 51.

Bergmann, I. 2011. Characterization of methanogenic Archaea communities in biogas reactors by quantitative PCR. Doctoral disseratation, Berlin: Universitätsbibliothek der Technischen Universität.

Bewley, J., Palmer, R., and Jackson-Smith, D. B. 2001. A comparison of free-stall barns used by modernized Wisconsin dairies. *Journal of Dairy Science*. 84(2): 528-541.

Bezbaruah, A. N., Krajangpan, S., Chisholm, B. J., Khan, E., and Elorza Bermudez, J. J. 2009. Entrapment of iron nanoparticles in calcium alginate beads for groundwater remediation applications. *Journal of hazardous materials*. 166(2): 1339-1343.

Bezbaruah, A. N., Shanbhogue, S. S., Simsek, S., and Khan, E. 2011. Encapsulation of iron nanoparticles in alginate biopolymer for trichloroethylene remediation. *Journal of Nanoparticle Research*. 13(12): 6673-6681.

Bhattarai, N., and Zhang, M. 2007. Controlled synthesis and structural stability of alginate-based nanofibers. *Nanotechnology*. 18(45): 455601.

Bhattarai, N., Li, Z., Edmondson, D., and Zhang, M. 2006. Alginate-based nanofibrous scaffolds: Structural, mechanical, and biological properties. *Advanced Materials*. 18(11): 1463-1467.

Blunden, J., and Aneja, V. P. 2008. Characterizing ammonia and hydrogen sulfide emissions from a swine waste treatment lagoon in North Carolina. *Atmospheric Environment*. 42(14): 3277-3290.

Boadi, D., Benchaar, C., Chiquette, J., and 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., and Santra, S. 2013. Behavior of engineered nanoparticles in landfill leachate. *Environmental science & technology*. 47(15): 8114-8122.

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

Borhan, S., Mukhtar, S., Capareda, S., and Rahman, S. 2012. Greenhouse gas emissions from housing and manure management systems at confined livestock operations.In *Waste management- An Intregrated Vision,* 260-296. INTECH Open Acess Publisher.

Bove, F. J., Fulcomer, M. C., Klotz, J. B., Esmart, J., Dufficy, E. M., and Savrin, J. E. 1995. Public drinking water contamination and birth outcomes. *American Journal of Epidemiology*. 141(9): 850-862.

Bowman, A., Mueller, K., and Smith, M. 2000. Increased animal waste protection from concentrated animal feeding operation (CAFOs): Potential implication for public and environmental health. Omaha, Nebraska: Nebraska Center For Rural Health Research.

Brayner, R., Ferrari-Iliou, R., Brivois, N., Djediat, S., Benedetti, M. F., and Fiévet, F. 2006. Toxicological impact studies based on Escherichia coli bacteria in ultrafine ZnO nanoparticles colloidal medium. *Nano Letters*. 6(4): 866-870.

Bremner, J. M., and Blackmer, A. M. 1978. Nitrous oxide: emission from soils during nitrification of fertilizer nitrogen. *Science*. 199(4326): 295-296.

Bruni, E. 2010. Improved anaerobic digestion of energy crops and agricultural residues. PhD thesis, Kongens Lyngby: Technical University of Denmark.

Buddle, B. M., Denis, M., Attwood, G. T., Altermann, E., Janssen, P. H., Ronimus, R. S., Pinares-Patiño, C. S., Muetzel, S., and Neil Wedlock, D. 2011. Strategies to reduce methane emissions from farmed ruminants grazing on pasture. *The Veterinary Journal*. 188(1): 11-17.

Bullers, S. 2005. Environmental stressors, perceived control, and health: the case of residents near large-scale hog farms in eastern North Carolina. *Human Ecology*. 33(1): 1-16.

Cambra-López, M., Aarnink, A. J., Zhao, Y., Calvet, S., and Torres, A. G. 2010. Airborne particulate matter from livestock production systems: A review of an air pollution problem. *Environmental Pollution*. 158(1): 1-17.

Campagna, D., Kathman, S. J., Pierson, R., Inserra, S. G., Phifer, B. L., Middleton, D. C., Zarus, G. M., and White, M. C. 2004. Ambient hydrogen sulfide, total reduced sulfur, and hospital visits for respiratory diseases in northeast Nebraska, 1998–2000. *Journal of Exposure Science and Environmental Epidemiology*. 14(2): 180-187.

Chadwick, D. R. 2005. Emissions of ammonia, nitrous oxide and from cattle manure heaps: effect of compaction and covering. *Atmospheric Environment*. 39(4): 787-799.

Chadwick, D. R., Pain, B. F., and Brookman, S. K. E. 2000. Nitrous oxide and methane emissions following application of animal manures to grassland. *Journal of environmental quality*. 29(1): 277-287.

Chadwick, D. R., Sneath, R. W., Phillips, V. R., and Pain, B. F. 1999. A UK inventory of nitrous oxide emissions from farmed livestock. *Atmospheric Environment*. 33(20): 3345-3354.

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

Chastain, J. P., and Camberato, J. J. 2004. Dairy manure production and nutrient content. *Confined Animal Manure Manager Certification Program Manual Dairy Version*: 1-16.

Chen, D., Jiao, X., and Cheng, G. 1999. Hydrothermal synthesis of zinc oxide powders with different morphologies. *Solid State Communications*. 113(6): 363-366.

Chen, Y., Müller, J. D., Ruan, Q., and Gratton, E. 2002. Molecular brightness characterization of EGFP in vivo by fluorescence fluctuation spectroscopy. *Biophysical journal*. 82(1): 133-144.

Chescoe, D., and Goodhew, P. J. 1984. *The operation of the transmission electron microscope*: Oxford Univ. London, New York, etc.: Press & Royal Microscopical Soc.

Choi, B. Y., Park, H. J., Hwang, S. J., and Park, J. B. 2002. Preparation of alginate beads for floating drug delivery system: effects of CO_2 gas-forming agents. *International journal of pharmaceutics*. 239(1): 81-91.

Choi, O., and Hu, Z. 2009. Nitrification inhibition by silver nanoparticles. *Water Sci Technol*. 59(9): 1699-1702.

Chung, Y. C., Lin, Y. Y., and Tseng, C. P. 2005. Removal of high concentration of NH_3 and coexistent H_2S by biological activated carbon (BAC) biotrickling filter. *Bioresource Technology*. 96(16): 1812-1820.

Clemens, J., and Ahlgrimm, H.-J. 2001. Greenhouse gases from animal husbandry: mitigation options. *Nutrient Cycling in Agroecosystems*. 60(1-3): 287-300.

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

Copeland, C. 2010a. Air quality issues and animal agriculture: a primer. *Animal Agriculture Research Progress*: 1.

Copeland, C. 2010b. Animal waste and hazardous substances: current laws and legislative issues. *Animal Agriculture Research Progress*: 75.

Cumbal, L., and SenGupta, A. K. 2005. Arsenic removal using polymer-supported hydrated iron (III) oxide nanoparticles: role of Donnan membrane effect. *Environmental science & technology*. 39(17): 6508-6515.

Dalal, R. C., Wang, W., Robertson, G. P., and Parton, W. J. 2003. Nitrous oxide emission from Australian agricultural lands and mitigation options: a review. *Soil Research*. 41(2): 165-195.

Daniel, T. C., Sharpley, A. N., and Lemunyon, J. L. 1998. Agricultural Phosphorus and Eutrophication: A Symposium Overview. *J. Environ. Qual.* 27(2): 251-257.

Dionisi, H. M., Harms, G., Layton, A. C., Gregory, I. R., Parker, J., Hawkins, S. A., Robinson, K. G., and Sayler, G. S. 2003. Power analysis for real-time PCR quantification of genes in activated sludge and analysis of the variability introduced by DNA extraction. *Applied and environmental microbiology*. 69(11): 6597-6604.

Dixon, R. K., Sathaye, J. A., Meyers, S. P., Masera, O. R., and Makarov, A. A. 1996. Greenhouse Gas Mitigation Strategies: Preliminary Results from the US. *Ambio*. 25(1): 26-32.

Dong, Y., Bae, H., McAllister, T., Mathison, G., and Cheng, K. J. 1999. Effects of exogenous fibrolytic enzymes, α-bromoethanesulfonate and monensin on fermentation in a rumen simulation (RUSITEC) system. *Canadian Journal of Animal Science*. 79(4): 491-498.

Donham, K. J., Blake, J. P., and Patterson, P. H. 2000. Occupational health hazards and recommended exposure limits for workers in poultry buildings. *2000 National Poultry Waste Management Symposium, Ocean City, MD, USA, 16-18 October 2000,* 92-109. National Poultry Waste Management Symposium Committee.

Donham, K. J., Wing, S., Osterberg, D., Flora, J. L., Hodne, C., Thu, K. M., and Thome, P. S. 2007. Community health and socioeconomic issues surrounding concentrated animal feeding operations. *Environmental health perspectives*: 317-320.

Drummond, J. G., Curtis, S. E., Simon, J., and Norton, H. W. 1980. Effects of aerial ammonia on growth and health of young pigs. *Journal of animal science*. 50(6): 1085-1091.

Dubascoux, S., Von Der Kammer, F., Le Hécho, I., Gautier, M. P., and Lespes, G. 2008. Optimisation of asymmetrical flow field flow fractionation for environmental nanoparticles separation. *Journal of Chromatography A*. 1206(2): 160-165.

Eghball, B., and Power, J. 1994. Beef cattle feedlot manure management. *Journal of Soil and Water Conservation*. 49(2): 113.

Ehhalt, D., Prather, M., Dentener, F., Derwent, R., Dlugokencky, E. J., Holland, E., Isaksen, I., Katima, J., Kirchhoff, V., and Matson, P. 2001. Atmospheric chemistry and greenhouse gases. Richland, WA: Pacific Northwest National Laboratory (PNNL).

Erni, R., Rossell, M. D., Kisielowski, C., and Dahmen, U. 2009. Atomic-resolution imaging with a sub-50-pm electron probe. *Physical Review Letters*. 102(9): 096101.

Escudero, C., Fiol, N., Villaescusa, I., and Bollinger, J.-C. 2009. Arsenic removal by a waste metal (hydr) oxide entrapped into calcium alginate beads. *Journal of hazardous materials*. 164(2): 533-541.

Fang, M., Chen, J.-H., Xu, X.-L., Yang, P.-H., and Hildebrand, H. F. 2006. Antibacterial activities of inorganic agents on six bacteria associated with oral infections by two susceptibility tests. *International journal of antimicrobial agents*. 27(6): 513-517.

Feng, X. 2005. Application of single walled carbon nanotubes in environmental engineering: adsorption and desorption of environmentally relevant species studied by infrared spectroscopy and temperature programmed desorption. PhD disseratation. Pittsburgh. Pennsylvania: University of Pittsburgh, Department of Civil and Environmental Engineering.

Finlay, B. J., Esteban, G., Clarke, K. J., Williams, A. G., Embley, T. M., and Hirt, R. P. 1994. Some rumen ciliates have endosymbiotic methanogens. *FEMS Microbiology Letters*. 117(2): 157-161.

Franke, A. C., Schulz, S., Oyewole, B. D., Diels, J., and Tobe, O. K. 2008. The role of cattle manure in enhancing on-farm productivity, macro-and micro-nutrient uptake, and profitability of maize in the Guinea savanna. *Experimental Agriculture*. 44(03): 313-328.

Franklin, N. M., Rogers, N. J., Apte, S. C., Batley, G. E., Gadd, G. E., and Casey, P. S. 2007. Comparative toxicity of nanoparticulate ZnO, bulk ZnO, and ZnCl2 to a freshwater microalga (Pseudokirchneriella subcapitata): the importance of particle solubility. *Environmental science & technology*. 41(24): 8484-8490.

Gan, L. M., Zhang, K., and Chew, C. H. 1996. Preparation of silica nanoparticles from sodium orthosilicate in inverse microemulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. 110(2): 199-206.

Gao, P. X., Ding, Y., Mai, W., Hughes, W. L., Lao, C., and Wang, Z. L. 2005. Conversion of zinc oxide nanobelts into superlattice-structured nanohelices. *Science*. 309(5741): 1700-1704.

Garcia-Marco, S., Ravella, S. R., Chadwick, D., Vallejo, A., Gregory, A. S., and Cardenas, L. M. 2014. Ranking factors affecting emissions of GHG from incubated agricultural soils. *European Journal of Soil Science*. 65(4): 573-583.

Gautam, D. P. 2012. Sourcing Novel Substrates for Anaerobic Digestion. Master thesis. Wageningen: Wageningen University, Ag & Bioresource Engineeirng.

Gerardi, M. H. 2003. The microbiology of anaerobic digesters. John Wiley & Sons, New Jersey.

Ghaedi, M., Biyareh, M. N., Kokhdan, S. N., Shamsaldini, S., Sahraei, R., Daneshfar, A., and Shahriyar, S. 2012. Comparison of the efficiency of palladium and silver nanoparticles loaded on activated carbon and zinc oxide nanorods loaded on activated carbon as new adsorbents for removal of Congo red from aqueous solution: Kinetic and isotherm study. *Materials Science and Engineering: C*. 32(4): 725-734.

Goyal, M., and Bansal, R. 2005. *Activated Carbon Adsorption*: CRC Press.

Greger, M., and Koneswaran, G. 2010. The public health impacts of concentrated animal feeding operations on local communities. *Family & community health*. 33(1): 11-20.

Groenestein, C., and Van Faassen, H. 1996. Volatilization of ammonia, nitrous oxide and nitric oxide in deep-litter systems for fattening pigs. *Journal of Agricultural Engineering Research*. 65(4): 269-274.

Guidotti, T. L. 1994. Occupational exposure to hydrogen sulfide in the sour gas industry: some unresolved issues. *International archives of occupational and environmental health*. 66(3): 153-160.

Gupta, R. K., Kennel, E., and Kim, K. J. 2009. *Polymer nanocomposites handbook*: CRC press.

Hames, Y., Alpaslan, Z., Kösemen, A., San, S. E., and Yerli, Y. 2010. Electrochemically grown ZnO nanorods for hybrid solar cell applications. *Solar Energy*. 84(3): 426-431.

Hamilton, D. 2011. Consistency of manure/water mixtures. *Stillwater, OK: Oklahoma Cooperative Extension Service. Retrieved on 07/10/2015 from http://pods.dasnr.okstate.edu/docushare/dsweb/Get/Document-7612/BAE-1751.pdf.*

Hansen, J., Sato, M., Ruedy, R., Lo, K., Lea, D. W., and Medina-Elizade, M. 2006. Global temperature change. *Proceedings of the National Academy of Sciences*. 103(39): 14288-14293.

Hao, X., Chang, C., Larney, F. J., and Travis, G. R. 2001. Greenhouse gas emissions during cattle feedlot manure composting. *Journal of environmental quality*. 30(2): 376-386.

Hao, X., Mir, P. S., Shah, M. A., and Travis, G. R. 2005. Influence of canola and sunflower diet amendments on cattle feedlot manure. *Journal of environmental quality*. 34(4): 1439-1445.

Hartung, J., and Saleh, M. 2007. Composition of dust and effects on animals. In *International interdisciplinary conference. Particulate matter in and from agriculture* (3-4).

Harper, L. A., Denmead, O. T., and Flesch, T. K. 2011. Micrometeorological techniques for measurement of enteric greenhouse gas emissions. *Animal Feed Science and Technology*. 166: 227-239.

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

He, X., Yuan, R., Chai, Y., and Shi, Y. 2008. A sensitive amperometric immunosensor for carcinoembryonic antigen detection with porous nanogold film and nano-Au/chitosan composite as immobilization matrix. *Journal of biochemical and biophysical methods*. 70(6): 823-829.

Heederik, D., Sigsgaard, T., Thorne, P. S., Kline, J. N., Avery, R., Bønløkke, J. H., Chrischilles, E. A., Dosman, J. A., Duchaine, C., and Kirkhorn, S. R. 2007. Health effects of airborne exposures from concentrated animal feeding operations. *Environmental health perspectives*: 298-302.

Hegarty, R. S. 1999. Reducing rumen methane emissions through elimination of rumen protozoa. *Australian Journal of Agricultural Research*. 50(8): 1321-1328.

Heinlaan, M., Ivask, A., Blinova, I., Dubourguier, H.-C., and Kahru, A. 2008. Toxicity of nanosized and bulk ZnO, CuO and TiO 2 to bacteria Vibrio fischeri and crustaceans Daphnia magna and Thamnocephalus platyurus. *Chemosphere*. 71(7): 1308-1316.

Hernández, S. P., Chiappero, M., Russo, N., and Fino, D. 2011. A novel ZnO-based adsorbent for biogas purification in H_2S production systems. *Chemical Engineering Journal*. 176: 272-279.

Homidan, A., Robertson, J. F., and Petchey, A. M. 2003. Review of the effect of ammonia and dust concentrations on broiler performance. *World's Poultry Science Journal*. 59(03): 340-349

Hong, R., Pan, T., Qian, J., and Li, H. 2006. Synthesis and surface modification of ZnO nanoparticles. *Chemical Engineering Journal*. 119(2): 71-81.

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

Hou, Y., Velthof, G. L., and Oenema, O. 2015. Mitigation of ammonia, nitrous oxide and methane emissions from manure management chains: a meta-analysis and integrated assessment. *Global Change Biology*. 21(3): 1293-1312.

Hribar, C., and Schultz, M. 2010. Understanding concentrated animal feeding operations and their impact on communities. East Gypsy Lane Road - Bowling Green, Ohio: National Association of Local Boards of Health.

Hu, J., Lo, I. M. C., and Chen, G. 2005. Fast removal and recovery of Cr (VI) using surface-modified jacobsite ($MnFe_2O_4$) nanoparticles. *Langmuir*. 21(24): 11173-11179.

Huwe, H., and Fröba, M. 2007. Synthesis and characterization of transition metal and metal oxide nanoparticles inside mesoporous carbon CMK-3. *Carbon*. 45(2): 304-314.

Illinois-EPA. 2012. Illinois Environmental Protection Agency, Summary ofMaximum Allowable Concentrations of Chemical Constituents In Uncontaminated Soil Used as Fill Material

At Regulated Fill Operations (35 Ill. Adm. Code 1100.Subpart F). Reterived on 7/2/2015 from http://www.epa.state.il.us/land/ccdd/new-max-allowable-concentrations-table.pdf.

IPCC. 2001. Second Assessment Report, Climate Change: The Scientific Basis Contribution of Working Group I to the Second Assessment Report of th IPCC.

IPCC. 2007. Third Assessment Report, Climate Change: The Scientific Basis Contribution of Working Group I to the Third Assessment Report of th IPCC.

Ismail, A. A., El-Midany, A., Abdel-Aal, E., and El-Shall, H. 2005. Application of statistical design to optimize the preparation of ZnO nanoparticles via hydrothermal technique. *Materials Letters*. 59(14): 1924-1928.

Jarvie, H. P., Al-Obaidi, H., King, S. M., Bowes, M. J., Lawrence, M. J., Drake, A. F., Green, M. A., and Dobson, P. J. 2009. Fate of silica nanoparticles in simulated primary wastewater treatment. *Environmental science & technology*. 43(22): 8622-8628.

Jensen, A. O. 2002. Changing the environment in swine buildings using sulfuric acid. *Transaction -American Society of Agriultural Engineering*. 45(1): 223-238.

Jia, W., Dang, S., Liu, H., Zhang, Z., Yu, C., Liu, X., and Xu, B. 2012. Evidence of the formation mechanism of ZnO in aqueous solution. *Materials Letters*. 82: 99-101.

Jia, Y., Liu, T. T., and Luo, C. 2011. Synthesis of Monoclinic Square ZrO_2 Nanoparticles and its Application of Treating Wastewater Containing Chromium (VI). *Nanoscience & Nanotechnology*. 2: 2002-2003.

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

Johnson, K. A., and Johnson, D. E. 1995. Methane emissions from cattle. *Journal of Animal Science*. 73(8): 2483-92.

Joshi, M., and Deshpande, J. 2011. Polymerase Chain Reaction: Methods, Principles and Application. *International Journal of Biomedical Research*. 2(1): 81-97.

Jungbluth, T., Hartung, E., and Brose, G. 2001. Greenhouse gas emissions from animal houses and manure stores. *Nutrient cycling in agroecosystems*. 60(1): 133-145.

Kai, P., Pedersen, P., Jensen, J. E., Hansen, M. N., and Sommer, S. G. 2008. A whole-farm assessment of the efficacy of slurry acidification in reducing ammonia emissions. *European Journal of Agronomy*. 28(2): 148-154.

Kamat, P. V., Huehn, R., and Nicolaescu, R. 2002. A “sense and shoot” approach for photocatalytic degradation of organic contaminants in water. *The Journal of Physical Chemistry B*. 106(4): 788-794.

Kerr, B. J., Ziemer, C. J., Trabue, S. L., Crouse, J. D., and Parkin, T. B. 2006. Manure composition of swine as affected by dietary protein and cellulose concentrations. *Journal of animal science*. 84(6): 1584-1592.

Khan, K. A., Suidan, M. T., and Cross, W. H. 1981. Anaerobic activated carbon filter for the treatment of phenol-bearing wastewater. *Journal (Water Pollution Control Federation)*: 1519-1532.

Khan, R. Z., Müller, C., and Sommer, S. G. 1997. Micrometeorological mass balance technique for measuring CH4 emission from stored cattle slurry. *Biology and fertility of soils*. 24(4): 442-444.

Kim, H., Hong, H.-J., Jung, J., Kim, S.-H., and Yang, J.-W. 2010. Degradation of trichloroethylene (TCE) by nanoscale zero-valent iron (nZVI) immobilized in alginate bead. *Journal of hazardous materials*. 176(1): 1038-1043.

Kim, J. S., Kuk, E., Yu, K. N., Kim, J.-H., Park, S. J., Lee, H. J., Kim, S. H., Park, Y. K., Park, Y. H., and Hwang, C.-Y. 2007. Antimicrobial effects of silver nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine*. 3(1): 95-101.

Kirchgessner;, M., Windisch;, W., Müller;, H. L., Engelhardt;, W. v., Leonhard-Marek;, S., Breves;, G., and D.Giesecke. 1995. In *Nutritional factors for the quantification of methane production. Proceedings 8th International Symposium on Ruminant Physiology, Germany,* 333-348.

Klass, D. 1984. Methane from anarobic fermentation. *Science*. 223: 1021-1028.

Koetz, J., Reichelt, S., Kosmella, S., and Tiersch, B. 2005. Recovery of nanoparticles produced in phosphatidylcholine-based template phases. *Journal of colloid and interface science*. 284(1): 190-198.

Kołodziejczak-Radzimska, A., and Jesionowski, T. 2014. Zinc oxide—from synthesis to application: a review. *Materials*. 7(4): 2833-2881.

Kong, H., and Jang, J. 2006. One-step fabrication of silver nanoparticle embedded polymer nanofibers by radical-mediated dispersion polymerization. *Chem. Commun.*(28): 3010-3012.

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

Kristensen, H. H., and Wathes, C. M. 2000. Ammonia and poultry welfare: a review. *World's Poultry Science Journal*. 56(03): 235-245.

Kumar, K., Gupta, S. C., Baidoo, S. K., Chander, Y., and Rosen, C. J. 2005. Antibiotic uptake by plants from soil fertilized with animal manure. *Journal of environmental quality*. 34(6): 2082-2085.

Larney, F. J., and Hao, X. 2007. A review of composting as a management alternative for beef cattle feedlot manure in southern Alberta, Canada. *Bioresource Technology*. 98(17): 3221-3227.

Laudenslager, M. J., Schiffman, J. D., and Schauer, C. L. 2008. Carboxymethyl chitosan as a matrix material for platinum, gold, and silver nanoparticles. *Biomacromolecules*. 9(10): 2682-2685.

Lee, J., Mahendra, S., and Alvarez, P. J. J. 2010. Nanomaterials in the construction industry: a review of their applications and environmental health and safety considerations. *Acs Nano*. 4(7): 3580-3590.

Lee, S. R., Han, J. K., Choi, Y. J., and Nam, K. 2007. Reduction of ammonia and hydrogen sulfide emission from swine manure using aqueous foams amended with microorganisms and chemical additives. *CLEAN–Soil, Air, Water*. 35(3): 230-234.

Li, Q., Mahendra, S., Lyon, D. Y., Brunet, L., Liga, M. V., Li, D., and Alvarez, P. J. 2008. Antimicrobial nanomaterials for water disinfection and microbial control: potential applications and implications. *Water Research*. 42(18): 4591-4602.

Liu, T., Zhao, L., Sun, D., and Tan, X. 2010. Entrapment of nanoscale zero-valent iron in chitosan beads for hexavalent chromium removal from wastewater. *Journal of hazardous materials*. 184(1): 724-730.

Liu, Y., and Whitman, W. B. 2008. Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. *Annals of the New York Academy of Sciences*. 1125(1): 171-189.

Liu, Y., Phenrat, T., and Lowry, G. V. 2007. Effect of TCE concentration and dissolved groundwater solutes on NZVI-promoted TCE dechlorination and H2 evolution. *Environmental science & technology*. 41(22): 7881-7887.

Livescience. 2011. How Many People Can Earth Support? Retrieved on 7/3/2015 from http://www.livescience.com/16493-people-planet-earth-support.html.

Loh, Z., Chen, D., Bai, M., Naylor, T., Griffith, D., Hill, J., Denmead, T., McGinn, S., and Edis, R. 2008. Measurement of greenhouse gas emissions from Australian feedlot beef production using open-path spectroscopy and atmospheric dispersion modelling. *Animal Production Science*. 48(2): 244-247.

Loneragan, G. H., Gould, D. H., Callan, R. J., Sigurdson, C. J., and Hamar, D. W. 1998. Association of excess sulfur intake and an increase in hydrogen sulfide concentrations in the ruminal gas cap of recently weaned beef calves with polioencephalomalacia. *Journal of the American Veterinary Medical Association*. 213(11): 1599-604.

Long, R. Q., and Yang, R. T. 2001. Carbon nanotubes as a superior sorbent for nitrogen oxides. *Industrial & engineering chemistry research*. 40(20): 4288-4291.

Luna-delRisco, M., Orupõld, K., and 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, J., Kulasegarampillai, M., Bolan, N., and Donnison, A. 2004. Control of gaseous emissions of ammonia and hydrogen sulphide from cow manure by use of natural materials. *New Zealand Journal of Agricultural Research*. 47(4): 545-556.

Luoma, S. N. 2008. Silver nanotechnologies and the environment. *Woodrow Wilson International Center for Scholars. Washington, DC, USA*: 72.

Luostarinen, S., Luste, S., and Rintala, J. 2006. Nitrogen removal from on-site treated anaerobic effluents using intermittently aerated moving bed biofilm reactors at low temperatures. *Water research*. 40(8): 1607-1615.

Mahato, T., Prasad, G., Singh, B., Acharya, J., Srivastava, A., and Vijayaraghavan, R. 2009. Nanocrystalline zinc oxide for the decontamination of sarin. *Journal of Hazardous Materials*. 165(1): 928-932.

Manning, R. 2004. *Against the grain: how agriculture has hijacked civilization*. North Point Press, New York.

Marcus, B. K. 1991. Process for sequestering ammonia and the odor associated therewith. US, Patent No. 5013335A.

Martin, C., Rouel, J., Jouany, J. P., Doreau, M., and 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., and Zhang, W.-X. 2003. Peer Reviewed: Environmental technologies at the nanoscale. *Environmental science & technology*. 37(5): 102A-108A.

Mathison, G. W., Okine, E. K., McAllister, T. A., Dong, Y., Galbraith, J., and Dmytruk, O. I. N. 1998. Reducing Methane Emissions from Ruminant Animals. *Journal of Applied Animal Research*. 14(1): 1-28.

McGinn, S. M., and Beauchemin, K. A. 2012. Dairy farm methane emissions using a dispersion model. *Journal of environmental quality*. 41(1): 73-79.

McGinn, S. M., Flesch, T. K., Crenna, B. P., Beauchemin, K. A., and Coates, T. 2007. Quantifying ammonia emissions from a cattle feedlot using a dispersion model. *Journal of environmental quality*. 36(6): 1585-1590.

McGinn, S., Beauchemin, K., Coates, T., and Colombatto, D. 2004. Methane emissions from beef cattle: Effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid. *Journal of Animal Science*. 82(11): 3346-3356.

Merchant, J. A., Naleway, A. L., Svendsen, E. R., Kelly, K. M., Burmeister, L. F., Stromquist, A. M., Taylor, C. D., Thorne, P. S., Reynolds, S. J., and Sanderson, W. T. 2005. Asthma and farm exposures in a cohort of rural Iowa children. *Environmental health perspectives*: 350-356.

Milby, T. H., and Baselt, R. C. 1999. Hydrogen sulfide poisoning: clarification of some controversial issues. *American journal of industrial medicine*. 35(2): 192-195.

Mirabelli, M. C., Wing, S., Marshall, S. W., and Wilcosky, T. C. 2006. Race, poverty, and potential exposure of middle-school students to air emissions from confined swine feeding operations. *Environmental health perspectives*: 591-596.

Mitloehner, F. M., and Schenker, M. B. 2007. Environmental exposure and health effects from concentrated animal feeding operations. *Epidemiology*. 18(3): 309-311.

Monticelli, O., Russo, S., Campagna, R., and Voit, B. 2005. Preparation and characterisation of blends based on polyamide 6 and hyperbranched aramids as palladium nanoparticle supports. *Polymer*. 46(11): 3597-3606.

Moore, M. N. 2006. Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? *Environment International*. 32(8): 967-976.

Moore, P., Daniel, T., Sharpley, A., and Wood, C. 1995. Poultry manure management: Environmentally sound options. *Journal of Soil and Water Conservation*. 50(3): 321-327.

Moss, A. R., Jouany, J.-P., and Newbold, J. 2000. Methane production by ruminants: its contribution to global warming. *Ann. Zootech.* 49(3): 231-253.

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

Muraviev, D. N., Macanás, J., Parrondo, J., Muñoz, M., Alonso, A., Alegret, S., Ortueta, M., and Mijangos, F. 2007. Cation-exchange membrane as nanoreactor: Intermatrix synthesis of platinum–copper core–shell nanoparticles. *Reactive and Functional Polymers*. 67(12): 1612-1621.

Nahm, K. H. 2002. Efficient feed nutrient utilization to reduce pollutants in poultry and swine manure. *Critical Reviews in Environmental Science and Technology*. 32(1): 1-16.

Ndegwa, P. M., Hristov, A. N., Arogo, J., and Sheffield, R. E. 2008. A review of ammonia emission mitigation techniques for concentrated animal feeding operations. *Biosystems Engineering*. 100(4): 453-469.

Newbold, C. J., López, S., Nelson, N., Ouda, J., Wallace, R. J., and Moss, A. 2005. Propionate precursors and other metabolic intermediates as possible alternative electron acceptors to methanogenesis in ruminal fermentation in vitro. *British Journal of Nutrition*. 94(01): 27-35.

Newbold, C. J., Wallace, R. J., Watt, N. D., and Richardson, A. J. 1988. Effect of the novel ionophore tetronasin (ICI 139603) on ruminal microorganisms. *Applied and Environmental Microbiology*. 54(2): 544-547.

Ni, J., Vinckier, C., Coenegrachts, J., and Hendriks, J. 1999. Effect of manure on ammonia emission from a fattening pig house with partly slatted floor. *Livestock Production Science*. 59(1): 25-31.

Nicholson, F., Chambers, B., Williams, J., and Unwin, R. 1999. Heavy metal contents of livestock feeds and animal manures in England and Wales. *Bioresource Technology*. 70(1): 23-31.

Novak, S. M., and Fiorelli, J.-L. 2010. Greenhouse gases and ammonia emissions from organic mixed crop-dairy systems: a critical review of mitigation options. *Agronomy for Sustainable Development*. 30(2): 215-236.

OSU. 2015. Oklahoma State University, Breeds of Livestock, Department of Animal Science. Retrieved on 7/1/2015 from http://www.ansi.okstate.edu/breeds.

Pain, B. F., Phillips, V. R., Clarkson, C. R., and Klarenbeek, J. V. 1989. Loss of nitrogen through ammonia volatilisation during and following the application of pig or cattle slurry to grassland. *Journal of the Science of Food and Agriculture*. 47(1): 1-12.

Panáček, A., Kvitek, L., Prucek, R., Kolar, M., Vecerova, R., Pizurova, N., Sharma, V. K., Nevečná, T. J., and Zboril, R. 2006. Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity. *The Journal of Physical Chemistry B*. 110(33): 16248-16253.

Pardeike, J., Hommoss, A., and Müller, R. H. 2009. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. *International journal of pharmaceutics*. 366(1): 170-184.

Park, J., Lee, S.-R., Han, J. K., and Nam, K. 2006. Mitigation of ammonia and hydrogen sulfide emissions by stable aqueous foam-microbial media. *Environmental science & technology*. 40(9): 3030-3035.

Patterson, P. H. 2005. Management strategies to reduce air emissions: Emphasis—Dust and ammonia. *The Journal of Applied Poultry Research*. 14(3): 638-650.

Pattey, E., Trzcinski, M. K., and Desjardins, R. L. 2005. Quantifying the reduction of greenhouse gas emissions as a resultof composting dairy and beef cattle manure. *Nutrient Cycling in Agroecosystems*. 72(2): 173-187.

Perez, M. A. 2012. The Effects of Silver Nanoparticles on Wastewater Treatment and *Escherichia coli* Growth. Florida State university, Department of Civil and Environmental Engineering.

Peterson, G. W., Rossin, J. A., Karwacki, C. J., and Glover, T. G. 2011. Surface chemistry and morphology of zirconia polymorphs and the influence on sulfur dioxide removal. *The Journal of Physical Chemistry C*. 115(19): 9644-9650.

Porel, S., Singh, S., Harsha, S. S., Rao, D. N., and Radhakrishnan, T. P. 2005. Nanoparticle-embedded polymer: in situ synthesis, free-standing films with highly monodisperse silver nanoparticles and optical limiting. *Chemistry of Materials*. 17(1): 9-12.

Portejoie, S., Martinez, J., Guiziou, F., and Coste, C. M. 2003. Effect of covering pig slurry stores on the ammonia emission processes. *Bioresource Technology*. 87(3): 199-207.

Powell, J., Broderick, G., and Misselbrook, T. 2008. Seasonal diet affects ammonia emissions from tie-stall dairy barns. *Journal of Dairy Science*. 91(2): 857-869.

Powers, W. J. 1999. Odor control for livestock systems. *Journal of animal science*. 77(2): 169-176.

Predicala, B., Alvarado, A., and Asis, D. 2012. Use of Zinc Oxide Nanoparticles to Control Hydrogen Sulphide, Ammonia and Odour Emissions from Pig Barns. In *9th International Livestock Environment Symposium American Society of Agricultural and Biological Engineers Valencia Conference Centre Valencia, Spain July 8 - 12, 2012.* Paper Number: ILES12-1507, ASABE. Retrieved on 8/20/2013 from http://elibrary.asabe.org/azdez.asp?JID=1&AID=41577&CID=iles2012&T=2.

Prior, D. J., Boyle, A. P., Brenker, F., Cheadle, M. C., Day, A., Lopez, G., Peruzzo, L., Potts, G. J., Reddy, S., and Spiess, R. 1999. The application of electron backscatter diffraction and orientation contrast imaging in the SEM to textural problems in rocks. *American Mineralogist*. 84: 1741-1759.

Querci, M., Jermini, M., and Eede, G. 2006. The analysis of food samples for the presence of genetically modified organisms. *World Health Organization-Joint Research Centre, European Commission Directorate General, Luxembourg*.

Radon, K., Weber, C., Iversen, M., Danuser, B., Pedersen, S., and Nowak, D. (2001). Exposure assessment and lung function in pig and poultry farmers. *Occupational and Environmental Medicine*. 58(6): 405-410.

Radon, K., Schulze, A., Ehrenstein, V., van Strien, R. T., Praml, G., and Nowak, D. 2007. Environmental exposure to confined animal feeding operations and respiratory health of neighboring residents. *Epidemiology*. 18(3): 300-308.

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

Rahman, S., Lin, D., and Zhu, J. 2012. Greenhous Gas (GHG) Emission from Mecanically Ventilated Deep Pit Swine Gestation Operation. *Civil and Environmental Engineering*. 2(1): 1-7.

Raju, C. S. 2012. Optimization of the anerobic digestion process by substrate pre-treatment and the application of NIRS. PhD thesis. Foulum: Aarhus University, Department of Biosystem Engineering.

Reed, R. B., Ladner, D. A., Higgins, C. P., Westerhoff, P., and Ranville, J. F. 2012. Solubility of nano-zinc oxide in environmentally and biologically important matrices. *Environmental Toxicology and Chemistry*. 31(1): 93-99.

Reicosky, D. D., Hatefield, J. L., and Sass, R. L. 2000. Agricultural contributions to greenhouse gas emissions. *Climate Change and Global Crop Productivity. eight ed. CABI Publishing, Wallingford, UK*: 37-50.

Ren, G., Hu, D., Cheng, E. W. C., Vargas-Reus, M. A., Reip, P., and Allaker, R. P. 2009. Characterisation of copper oxide nanoparticles for antimicrobial applications. *International journal of antimicrobial agents*. 33(6): 587-590.

Renn, O., and Roco, M. C. 2006. Nanotechnology and the need for risk governance. *Journal of Nanoparticle Research*. 8(2): 153-191.

Ristić, M., Music, S., Ivanda, M., and Popović, S. 2005. Sol–gel synthesis and characterization of nanocrystalline ZnO powders. *Journal of Alloys and Compounds*. 397(1): L1-L4.

Ruokojärvi, A., Ruuskanen, J., Martikainen, P. J., and Olkkonen, M. 2001. Oxidation of gas mixtures containing dimethyl sulfide, hydrogen sulfide, and methanethiol using a two-stage biotrickling filter. *Journal of the Air & Waste Management Association*. 51(1): 11-16.

Ruth, J. H. 1986. Odor thresholds and irritation levels of several chemical substances: a review. *American Industrial Hygiene Association Journal*. 47(3): A-142.

Sadeghi, A. M., and Arnold, J. G. 2002. A SWAT/microbial sub-model for predicting pathogen loadings in surface and groundwater at watershed and basin scales. *Total Maximum Daily Load (TMDL) Environmental Regulations*: 56-63.

Sarkar, S., Guibal, E., Quignard, F., and SenGupta, A. K. 2012. Polymer-supported metals and metal oxide nanoparticles: synthesis, characterization, and applications. *Journal of Nanoparticle Research*. 14(2): 1-24.

Satter, L. D., Klopfenstein, T. J., and Erickson, G. E. 2002. The role of nutrition in reducing nutrient output from ruminants. *Faculty Papers and Publications in Animal Science*: 472.

Sayyadnejad, M. A., Ghaffarian, H. R., and Saeidi, M. 2008. Removal of hydrogen sulfide by zinc oxide nanoparticles in drilling fluid. *International Journal of Environmental Science & Technology*. 5(4): 565-569.

Schiffman, S. S., Auvermann, B. W., and Bottcher, R. W. 2001. Health effects of aerial emissions from animal production waste management systems. In, 103-113.

Schiffman, S. S., Sattely Miller, E. A., Suggs, M. S., and 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.

Shalumon, K. T., Anulekha, K. H., Nair, S. V., Nair, S. V., Chennazhi, K. P., and Jayakumar, R. 2011. Sodium alginate/poly (vinyl alcohol)/nano ZnO composite nanofibers for antibacterial wound dressings. *International journal of biological macromolecules*. 49(3): 247-254.

Sharma, P., Sreenivas, K., and Rao, K. V. 2003. Analysis of ultraviolet photoconductivity in ZnO films prepared by unbalanced magnetron sputtering. *Journal of Applied Physics*. 93(7): 3963-3970.

Sharma, V., Shukla, R. K., Saxena, N., Parmar, D., Das, M., and Dhawan, A. 2009. DNA damaging potential of zinc oxide nanoparticles in human epidermal cells. *Toxicology letters*. 185(3): 211-218.

Shatkin, J. A. 2012. *Nanotechnology: health and environmental risks*: CRC Press.

Shingfield, K. J., Salo-Väänänen, P., Pahkala, E., Toivonen, V., Jaakkola, S., Piironen, V., and Huhtanen, P. 2005. Effect of forage conservation method, concentrate level and propylene glycol on the fatty acid composition and vitamin content of cows' milk. *Journal of Dairy Research*. 72(03): 349-361.

Singh, B. P., Hatton, B. J., Singh, B., Cowie, A. L., and Kathuria, A. 2010. Influence of biochars on nitrous oxide emission and nitrogen leaching from two contrasting soils. *Journal of environmental quality*. 39(4): 1224-1235.

Snyder, C., Bruulsema, T., Jensen, T., and Fixen, P. 2009. Review of greenhouse gas emissions from crop production systems and fertilizer management effects. *Agriculture, ecosystems & environment*. 133(3): 247-266.

Sommer, S. G., Christensen, M. L., Schmidt, T., and Jensen, L. S. 2013. *Animal manure recycling: treatment and management*: John Wiley & Sons.

Sondi, I., and Salopek-Sondi, B. 2004. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. *Journal of colloid and interface science*. 275(1): 177-182.

Spellman, F. R., and Whiting, N. E. 2010. *Environmental management of concentrated animal feeding operations (CAFOs)*: CRC Press.

Stoimenov, P. K., Klinger, R. L., Marchin, G. L., and Klabunde, K. J. 2002. Metal oxide nanoparticles as bactericidal agents. *Langmuir*. 18(17): 6679-6686.

Storm, I. M. L. D., Hellwing, A. L. F., Nielsen, N. I., and Madsen, J. 2012. Methods for measuring and estimating methane emission from ruminants. *Animals*. 2(2): 160-183.

Sutaryo, S., Ward, A. J., and Møller, H. B. 2012. Anaerobic digestion of the solid fractions of acidified manure. In *Energy, biomass and biological residues. International Conference of Agricultural Engineering-CIGR-AgEng 2012: Agriculture and Engineering for a Healthier Life, Valencia, Spain, 8-12 July 2012*, C-0680: CIGR-EurAgEng.

Sutton, J. D., Dhanoa, M. S., Morant, S. V., France, J., Napper, D. J., and Schuller, E. 2003. Rates of Production of Acetate, Propionate, and Butyrate in the Rumen of Lactating Dairy Cows Given Normal and Low-Roughage Diets. *Journal of Dairy Science*. 86(11): 3620-3633.

Sweet, L., and Strohm, B. 2006. Nanotechnology—life-cycle risk management. *Human and Ecological Risk Assessment*. 12(3): 528-551.

Talam, S., Karumuri, S. R., and Gunnam, N. 2012. Synthesis, characterization, and spectroscopic properties of ZnO nanoparticles. *ISRN Nanotechnology*. 2012.

Tamminga, S., Bannink, A., Dijkstra, J., and Zom, R. 2007. *Feeding strategies to reduce methane loss in cattle*: Animal Sciences Group.

Tan, C. W., Tan, K. H., Ong, Y. T., Mohamed, A. R., Zein, S. H. S., and Tan, S. H. 2012. Energy and environmental applications of carbon nanotubes. *Environmental Chemistry Letters*. 10(3): 265-273.

Teow, Y., Asharani, P. V., Hande, M. P., and Valiyaveettil, S. 2011. Health impact and safety of engineered nanomaterials. *Chemical communications*. 47(25): 7025-7038.

Thu, K. M. 2002. Public health concerns for neighbors of large-scale swine production operations. *Journal of agricultural safety and health*. 8(2): 175-184.

TimeMaps. 2015. The Coming of Farming. Retrieved on 7/1/2015 from http://www.timemaps.com/farming.

Tiwari, D. K., Behari, J., and Sen, P. 2008. Application of Nanoparticles in Waste Water Treatment. *World Applied Sciences Journal*. (3): 417-433.

Tolaymat, T. M., El Badawy, A. M., Genaidy, A., Scheckel, K. G., Luxton, T. P., and Suidan, M. 2010. An evidence-based environmental perspective of manufactured silver nanoparticle in syntheses and applications: A systematic review and critical appraisal of peer-reviewed scientific papers. *Science of the Total Environment*. 408(5): 999-1006.

Topoglidis, E., Cass, A. E. G., O'Regan, B., and Durrant, J. R. 2001. Immobilisation and bioelectrochemistry of proteins on nanoporous TiO_2 and ZnO films. *Journal of Electroanalytical Chemistry*. 517(1): 20-27.

USEPA. 2001. Emission from animal feeding operation, Research Triangle Park, NC: United States Environmental Protection Agency No. 68-D6-0011.

USEPA. 2009. Inventory of US Greenhouse Gas Emission and Sinks: 1990-2007. Washington DC.

USEPA. 2012a. United States Environmental Protection Agency; Dairy production: Common Manure Handling Systems. Retrieved on 7/1/2015. http://www.epa.gov/agriculture/ag101/dairymanure.html.

USEPA. 2012b. United States Environmental Protection Agency; Pork Production: Common Manure Handling Systems. Retrierved on 7/1/2015. http://www.epa.gov/agriculture/ag101/porkmanure.html.

USEPA. 2012c. United States Environmental Protection Agency; Poultry Production: Common Manure Handling Systems. Retrieved on 7/1/2015 from http://www.epa.gov/agriculture/ag101/poultrymanure.html.

USEPA. 2013. US Greenhouse Gas Inventory Report: Inventory of US Greenhouse Gas Emission and Sink:1990-2011. Washington DC, USA.

USEPA. 2014. Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990-2012, Washington DC, USA.

Van Haarlem, R. P., Desjardins, R. L., Gao, Z., Flesch, T. K., and Li, X. 2008. Methane and ammonia emissions from a beef feedlot in western Canada for a twelve-day period in the fall. *Canadian Journal of Animal Science*. 88(4): 641-649.

VanGuilder, H. D., Vrana, K. E., and Freeman, W. M. 2008. Twenty-five years of quantitative PCR for gene expression analysis. *Biotechniques*. 44(5): 619.

Varel, V. H. 1997. Use of urease inhibitors to control nitrogen loss from livestock waste. *Bioresource Technology*. 62(1): 11-17.

Vaseem, M., Umar, A., and Hahn, Y.-B. 2010. ZnO nanoparticles: growth, properties, and applications. American Scientific Publishers: New York, 1-36.

Von Bemuth, R. D., Hill, J. D., Henderson, E., Godbout, S., Hamel, D., and Pouliot, F. 2005. Efficacy of a liquid/solid isolation system for swine manure. *Transactions of the ASAE*. 48(4): 1537-1546.

Waghorn, G. C., and Hegarty, R. S. 2011. Lowering ruminant methane emissions through improved feed conversion efficiency. *Animal Feed Science and Technology*. 166–167(0): 291-301.

Waghorn, G. C., Woodward, S. L., Tavendale, M., and Clark, D. A. 2006. Inconsistencies in rumen methane production effects of forage composition and animal genotype. *International Congress Series*. 1293(0): 115-118.

Wang, B., Feng, W., Wang, M., Wang, T., Gu, Y., Zhu, M., Ouyang, H., Shi, J., Zhang, F., and Zhao, Y. 2008a. Acute toxicological impact of nano-and submicro-scaled zinc oxide powder on healthy adult mice. *Journal of nanoparticle research*. 10(2): 263-276.

Wang, B., Feng, W.-Y., Wang, T.-C., Jia, G., Wang, M., Shi, J.-W., Zhang, F., Zhao, Y.-L., and Chai, Z.-F. 2006. Acute toxicity of nano-and micro-scale zinc powder in healthy adult mice. *Toxicology letters*. 161(2): 115-123.

Wang, K. Y., Huang, D. D., Ying, H. C., and Luo, H. J. 2014. Effects of acidification during storage on emissions of methane, ammonia, and hydrogen sulfide from digested pig slurry. *Biosystems Engineering*. 122: 23-30.

Wang, X., Sun, T., Yang, J., Zhao, L., and Jia, J. 2008b. Low-temperature H_2S removal from gas streams with SBA-15 supported ZnO nanoparticles. *Chemical Engineering Journal*. 142(1): 48-55.

Wang, Y., Zhang, C., Bi, S., and Luo, G. 2010. Preparation of ZnO nanoparticles using the direct precipitation method in a membrane dispersion micro-structured reactor. *Powder Technology*. 202(1): 130-136.

Wardak, A., Gorman, M. E., Swami, N., and Deshpande, S. 2008. Identification of Risks in the Life Cycle of Nanotechnology-Based Products. *Journal of Industrial Ecology*. 12(3): 435-448.

Washio, J., Sato, T., Koseki, T., and Takahashi, N. 2005. Hydrogen sulfide-producing bacteria in tongue biofilm and their relationship with oral malodour. *Journal of medical microbiology*. 54(9): 889-895.

Watts, J. F. 1994. X-ray photoelectron spectroscopy. *Vacuum*. 45(6): 653-671.

Williams, D. B., and Carter, C. B. 1996. *The transmission electron microscope*: Springer.

Woolf, D., Amonette, J. E., Street-Perrott, F. A., Lehmann, J., and Joseph, S. 2010. Sustainable biochar to mitigate global climate change. *Nature communications*. 1: 56.

Work, H. B. 2010. Best Management Practices to Mitigate Air Quality Impacts from Animal Agriculture. Reterived on 9/20/2014 from http://ua.dnr.wi.gov/topic/AirQuality/documents/bmp/AppJ.pdf.

WPF. 2010. Human population: 6 billion Livestock population: at least 17 billion. Reterived on 7/3/2015 http://www.worldpreservationfoundation.org/blog/human-effects/human-population-6-billion-livestock-population-at-least-17-billion/#.UzxjYfldX_o.

Wu, S. J., Liou, T. H., and Mi, F. L. 2009. Synthesis of zero-valent copper-chitosan nanocomposites and their application for treatment of hexavalent chromium. *Bioresource Technology*. 100(19): 4348-4353.

Wulf, S., Maeting, M., and Clemens, J. 2002. Application technique and slurry co-fermentation effects on ammonia, nitrous oxide, and methane emissions after spreading. *Journal of environmental quality*. 31(6): 1795-1801.

Xiao, Y., Xie, G., and Lu, C. 2005. Economic values of nitrogen transformation in rice field ecosystems. *Yingyong Shengtai Xuebao*. 16(9): 1745-1750.

Xu, J., and Bhattacharyya, D. 2007. Fe/Pd nanoparticle immobilization in microfiltration membrane pores: Synthesis, characterization, and application in the dechlorination of polychlorinated biphenyls. *Industrial & engineering chemistry research*. 46(8): 2348-2359.

Yang, S. L., Bu, D. P., Wang, J. Q., Hu, Z. Y., Li, D., Wei, H. Y., Zhou, L. Y., and Loor, J. J. 2009. Soybean oil and linseed oil supplementation affect profiles of ruminal microorganisms in dairy cows. *Animal*. 3(11): 1562-1569.

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

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

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

Zhang, J., Han, B., Liu, J., Zhang, X., Liu, Z., and He, J. 2001. A new method to recover the nanoparticles from reverse micelles: recovery of ZnS nanoparticles synthesized in reverse micelles by compressed CO2. *Chem. Commun.*(24): 2724-2725.

Zhang, L., Gu, F. X., Chan, J. M., Wang, A. Z., Langer, R. S., and Farokhzad, O. C. 2007. Nanoparticles in medicine: therapeutic applications and developments. *Clinical Pharmacology & Therapeutics*. 83(5): 761-769.

Zhang, T., and Fang, H. H. 2006. Applications of real-time polymerase chain reaction for quantification of microorganisms in environmental samples. *Applied Microbiology and Biotechnology*. 70(3): 281-289.

Zhu, J. 2000. A review of microbiology in swine manure odor control. *Agriculture, ecosystems & environment*. 78(2): 93-106.

Zicari, S. M. 2003. Removal of hydrogen sulfide from biogas using cow-manure compost. Master thesis. Ithaca, New York: Cornell University, Department of Biological and Environmental Engineering.

Zschocke, J., and Hoffmann, G. F. 2004. *Vademecum metabolicum: manual of metabolic paediatrics*: Schattauer Verlag

EFFECTS OF PEN BEDDING AND FEEDING HIGH CRUDE PROTEIN DIETS ON MANURE COMPOSITION AND GREENHOUSE GAS EMISSIONS FROM A FEEDLOT PEN SURFACE [2]

Abstract

Greenhouse gas (GHG) emissions from concentrated animal feeding operations (CAFOs) vary by stage of production and management practices. The objective of this research was to study the effect of two dietary crude protein levels (12 and 16%) fed to beef steers in pens with or without corn stover bedding. Manure characteristics and GHG emissions were measured from feedlot pen surfaces. Sixteen equal sized feedlot pens (19×23 m) were used. Eight were bedded approximately twice a week with corn stover and the remaining eight feedlot pens were not bedded. Angus steers (n=138) were blocked by live-weights (lighter and heavier) with 7 to 10 animals per pen. The trial was a 2×2 factorial design with factors of two protein levels and two bedding types (bedding vs. non-bedding), with four replicates. The study was conducted from June through September and consisted of four ~28 day periods. Manure from each pen was scrapped once every 28 days and composite manure samples from each pen were collected. Air samples from pen surfaces were sampled in Tedlar bags using a vacuum pump coupled with a portable wind tunnel and analyzed with a greenhouse gas chromatograph (GC) within 24 hours of sampling. The manure samples were analyzed for crude protein (CP), total nitrogen (TN), ammonia (NH_3), total volatile fatty acid (TVFA), total carbon (TC), total phosphorus (TP), and potassium (K). The air samples were analyzed for methane (CH_4), carbon dioxide (CO_2), and

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[2] The material in this chapter was co-authored by Md Saidul Borhan, Dhan Prasad Gautam, Chandra Engel, Van L. Anderson, and Shafiqur Rahman. Dhan Prasad Gautam had primary responsibility for collecting and analyzing the samples. Dhan Prasad Gautam was the primary developer of the conclusions that are advanced here. The remaining co-authors served as proofreader and help Dhan in calculation and statistical analysis.

nitrous oxide (N_2O) concentrations. The concentration of TN was significantly higher ($P<0.05$) in manure from pens with cattle fed the high protein diets. The volatile fatty acids (VFAs) such as acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids concentrations were similar across both treatments. There were no significant differences in pen surface GHG emissions across manure management and dietary crude protein levels.

Introduction

The overall gaseous emissions from concentrated livestock operations vary by animal type and growth stage due to different diets, daily feed intake, quality of diet, and feed conversion mechanism. In the US, total GHG including CH_4 emissions from agricultural sources (livestock production, agricultural soils, and rice production) were estimated to be approximately 500 metric ton CO_2eq in 2010. The enteric CH_4 produced in the rumen of cattle represents one-third of the CH_4 emissions, and manure management accounts for about 15% of the total GHG from the Agriculture sector (USEPA, 2010). In 2010, GHG emissions from agriculture accounted for approximately 7% of total United States GHG; which is a 13% increase since 1990. The biggest driver for this increase has been the 51% growth in combined CH_4 and N_2O emissions from livestock manure management systems (USEPA, 2010). The agricultural sector is reported to be the greatest contributor of N_2O and the third greatest contributor of CH_4 in the United States (Luo and Saggar, 2008; Saggar et al., 2004). Thus, researchers continue to evaluate different options or technologies to mitigate GHG contribution from livestock production system and manure management.

One potential approach involves manipulating constituents of livestock diets with or without using feed additives to achieve desirable manure pH, concentrations and solubility of C and N, and other factors that are pertinent to CH_4 and N_2O emissions (Kebreab et al., 2010;

Sejian and Naqvi, 2012). Dietary manipulation limiting C/N inputs into the digestive systems of livestock appeared to be an effective tool in reducing nutrient/mineral pollution, and odorous and gaseous emissions (Sejian and Naqvi, 2012). Reduction in N excretion can be achieved by limiting both N content in the diet and changing the proportions of rumen-degradable protein supplements (synthetic Amino Acids) with low-CP (150 g/kg DM) diets (Castillo et al., 2001; Kebreab et al., 2010).

Corn-based DDGS is a readily available by-product from the ethanol industries and is used extensively in dairy and beef cattle diets. DDGS has traditionally been one of the lowest cost feed ingredients supplying both energy and protein (Garcia and Taylor, 2002; Schroeder, 2012). The National Research Council (NRC) recommends crude protein levels in beef feedlot diets between 12.5 and 13.5% crude protein (CP) depending on the animal growth stage and desired gains. Feedlot diets containing DDGS tend to be higher in protein than NRC recommendations often reaching levels of 15 to 18% CP. Several recent published articles evaluate the effects of protein concentrations in beef cattle diet on NH_3 emissions and manure quality (Archibeque et al., 2007; Cole et al., 2005; Spiehs et al., 2012; Todd et al., 2006), but the impact of dietary protein levels on the manure concentrations and GHGs emissions are deficient. Especially, in the Northern Plains with abundant DDGS, no research has been done comparing the effect of high protein diet and NRC recommended protein diet fed to cattle on manure composition and GHG emissions from the feedlot surfaces.

In a production scaled feedlot situation**,** Hao et al. (2009) studied the effects of wheat dry distiller grains with soluble (DDGS) in feedlot cattle diets (backgrounding and finishing) on feces and manure composition. They found that as the ratios of wheat DDGS (e.g., 0, 20, 40, and 60%) in animal diets increased, the manure TP and TN also increased. They also reported significant increases of isobutyric, valeric and isovaleric acids in the manure from cattle fed 40 and 60% wheat DDGS diets, although there was no difference in the total VFA content due to diet (Hao et al., 2009). However, the backgrounding diets' CP concentration of the 20, 40, and 60 % of the wheat DDGS were 13, 17, 24 %, respectively, whereas CP values for 0, 20, 40, and 60 % DDGS for the finishing diets were13, 18, 24, and 29 %, respectively. Spiehs et al. (2012) evaluated the effect of wet distillers grains with solubles (WDGS) and control diet (no WDGS) to beef cattle on manure composition and air quality and reported an increase in TP in manure cattle fed diets containing WDGS compared to pens fed the control. They also observed that NH_3 concentrations in the air samples collected from pens fed the high WDGS diets was higher than in the air samples collected from the pens fed control diet. However, concentrations of the VOC were similar between the two treatments.

Recently, Borhan et al. (2011) conducted GHG emissions at ground level area source from dairy and cattle feedlot operation in Texas. They found that pen surfaces have significantly higher CO_2 and N_2O emissions than the compost pile and runoff pond. The feedlot cattle were fed corn silage, steam flaked corn, protein (wet distillers corn and condensed corn distillers), fat, beef finisher (rumensin and tylan), and trace elements (vitamin A and D) at 19, 57.2, 20.1, 0.8, 2.1, 0.8% on an as fed basis, respectively. The median emission rates for CH_4, CO_2, and N_2O were 3.8, 1399, 0.68 g hd^{-1} d^{-1} (1.7 kg CO_2e hd^{-1} d^{-1}), respectively, from the beef cattle feedlot pen surface measured with dynamic flux chamber (Borhan et al., 2011). In the relatively cooler climate of North Dakota, Rahman et al. (2012) reported emission rates for CH_4, CO_2, and N_2O were 38 g hd^{-1} d^{-1}, 17 kg hd^{-1} d^{-1}, and 26 g hd^{-1} d^{-1}, respectively, from a research feedlot measured with a custom-made wind tunnel (Rahman, 2012a). The diet was formulated to achieve low (13% crude protein) and high protein (17 % crude protein) diets but beef cattle were contained in the same feedlot, Thus, feeding effect from that study could not be separated.

The chemical composition of the diet is a critical factor, affecting rumen chemistry and CH_4 emissions by ruminant animals. The dietary carbohydrate source alters the microbial fermentation process in the rumen and the VFA products that are produced. Feeding cattle with a high starch and low fiber diet reduced ruminal acetate production, thus lowering enteric CH_4 production (Beauchemin et al., 2009; Kebreab et al., 2010; Osada et al., 2011), and has been shown to reduce CH_4 production during manure storage (Beauchemin et al., 2008; Eckard et al., 2010; Kebreab et al., 2001).

In a controlled laboratory environment, the effects of dietary protein concentration levels on the manure nutrient composition and gaseous emissions from the incubated fresh dairy manure and manure-amended soil were investigated (Lee et al., 2012). Fresh manure from lactating Holstein cows on a high crude protein (HCP; 16.7%, dry basis) diet, showed higher total nitrogen content than manure from cows fed a low protein (LCP; 14.8%, dry basis) diet. In contrast, the emitting potential and cumulative GHG emissions (CH_4, CO_2, N_2O) between HCP and LCP were not statistically different. Similarly, with manure–amended soil, cumulative CH_4 and N_2O emissions were not significantly different between HCP and LCP. However, CO_2 emission was increased with LCP diets (Lee et al., 2012).

Another GHG mitigation option might be manure management with and without bedding on the pen, which could also affect manure pH and soluble C and N levels and thus, the emissions during manure storage and treatment. Providing animals with bedding is a good animal husbandry practice which means less environmental stress and more healthy and productive animals (Anderson and Bird, 1993; Birkelo and Lounsbery, 1992; Stanton and

Schultz, 1996).While winter in the Northern Great Plain can be severe, wind protection and bedding can make significant differences in cattle performance and profit, in addition to sequestering more nutrients in the manure that will increase fertilizer value. Amon et al. (2001) used an environmental dynamic chamber that covered 27 m^2 of emitting surface, in a tie stall dairy, to measure and compare the effect of slurry and straw bedded pen to a slurry based manure management systems as to CH_4, N_2O, and NH_3 emissions. They observed no significant differences in average CH_4 (194.4 and 194.4 g LU^{-1} d^{-1}) and N_2O (610 to 619 mg LU^{-1} d^{-1}) emissions for slurry and straw system, respectively (Amon et al., 2001). Similarly, under northern climatic conditions bedding is used on the feedlot pen surface for animal comfort, to absorb and hold water (Spiehs et al., 2012). Additionally, bedding may reduce the “heat load” of heavyweight cattle, and lower the volatilization of GHG, nitrogen, and odor while sequestering more nutrients in the bedding pack. It is critical to understand optimum bedding management practices on beef cattle productivity and GHG emissions in the Northern Plains.

Little attention has been given to the environmental impact of manure, including GHG emissions, and the loss of nitrogen due to volatilization from feedlot pen surfaces from these higher protein diets as well as bedding effects. Additionally, data pertaining to the effects of dietary protein levels on nutrients composition and GHG emissions from the feedlot pen surfaces in climates similar to North Dakota is not available. Therefore, it is important to determine the effects of dietary protein levels fed to the cattle on the nutrient composition and gaseous emissions from the manure accumulated on the feedlot pen surface in a research feedlot (production scaled) situation. The main goal of this research was to assess whether diet and bedding provide a simple, inexpensive and practical means of reducing GHG emissions in a North Dakota feedlot. The specific objective was to investigate the effect of two dietary protein levels fed to the beef cattle in pens with and without bedding on the manure characteristics and subsequent GHG emissions from a feedlot pen surface.

Materials and methods

Experimental unit and design

This study was conducted in a feedlot with 16 pens located at North Dakota State University Carrington Research Extension Center (CREC) beef research feedlot (Figure 5). Each pen had an area of 437 m^2 (dimension 19 m × 23 m), with an overall aggregate 3% slope. The trial was a 2×2 factorial design with factors of dietary protein level and bedding. One hundred thirty-eight Angus and Angus crossbred steer calves were blocked by weight and allocated across 16 pens with eight pens per treatment (protein level) and 7 to 10 animals per pen. Thus, there were 4 replicates per treatment (two protein levels × two bedding levels. Average initial live weights for light, medium light, medium-heavy, and heavy animal ranged from 264 (219-298), 330 (300-370), 401 (373-419), 447 (422-501) kg, respectively. The average stocking densities per unit in each pen varied from 61.8 to 43.3 m^2. Table 8 shows animal weight and growth stage, diets and bedding information experimental information in each pen in the experimental layout.

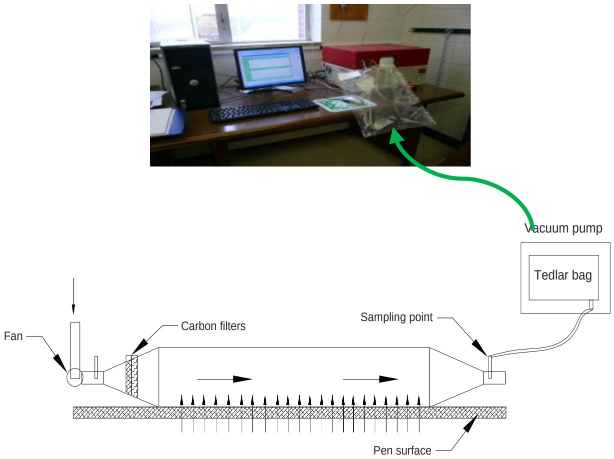

Figure 5. Schematic diagram of a wind tunnel and greenhouse gas chromatography (GC) used in this study (drawing not to scale).

Table 8. Summary of animal weight and growth stage, diets, and bedding information in each pen.

Pen ID	Animal weight	Growth stages	Diets	Bedding
Pen 1	Light	Growing/Finish	High Protein	No-bedding
Pen 2	Light	Growing/Finish	Low Protein	Bedding
Pen 3	Light	Growing/Finish	Low protein	No-bedding
Pen 4	Light	Growing/Finish	High Protein	Bedding
Pen 5	Medium light	Growing/Finish	Low protein	No-bedding
Pen 6	Medium light	Growing/Finish	Low protein	Bedding
Pen 7	Medium light	Growing/Finish	High Protein	No-bedding
Pen 8	Medium light	Growing/Finish	High Protein	Bedding
Pen 9	Medium heavy	Finish	High Protein	Bedding
Pen 10	Medium heavy	Finish	High Protein	No-bedding
Pen 11	Medium heavy	Finish	Low protein	No-bedding
Pen 12	Medium heavy	Finish	Low protein	Bedding
Pen 13	Heavy	Finish	High Protein	Bedding
Pen 14	Heavy	Finish	Low protein	No-bedding
Pen 15	Heavy	Finish	High Protein	No-bedding
Pen 16	Heavy	Finish	Low protein	Bedding

Light weight range (219 to 370 kg); Heaver weight range (373 to 501 kg); High protein (16%); Low protein (12%).

The study was divided into four ~28 day periods: period 1 (June 14 to July 12), period 2 (July 13 to August 9), period 3 (August 10 to September 5), and period 4 (September 6 to October 2). Live weights were recorded on the last day of each period. Cattle with lighter initial weights (n = 8 pens) were fed a growing diet containing 54 Mcal NEg and either 12.36 (control: NRC recommended) or 16.58 % CP for 56 days. The eight pens of heavier weight cattle were fed a finishing diet containing 62 Mcal NEg and either 12.00 (control; NRC recommended) or 16.00% CP through all four feeding periods. Similarly, eight of the 16 pens were bedded with corn stover and the remaining pens were not bedded as listed in Table 8. Two CP diets were prepared monthly and thoroughly mixed samples were collected analyzed monthly to verify CP levels and nutrient composition of diets during the growing and finishing periods as shown in Table 9.

Table 9. Diets composition for growing and finishing beef cattle used in this study.

Item	High protein finish diet	Control finishing diet	High protein growing diet	Control growing diet
Corn, dry rolled, %	32.52	50.76	25.90	45.29
Barley, dry rolled, %	10.27	10.07	---	---
Wheat midds, pelleted, %	0.30	0.54	1.08	0.78
Modified Distillers Grains, %	32.70	14.32	39.16	19.65
Corn Silage, %	8.11	8.14	16.21	16.62
Hay[1], %	14.18	14.25	15.18	15.32
Supplement[2], %	1.93	1.89	2.45	2.34
Crude Protein, %	15.84	12.26	16.08	12.24
NEg, Mcal kg^{-1}	0.25	0.25	0.22	0.22

[1] This was an alfalfa grass hay for periods 1 and 2 and pea-wheat straw forage for the remaining periods;
[2] Includes ionophore, Mineral mix, and Calcium Carbonate.

Manure sampling and analysis

From each pen surface, manure samples were collected randomly from 5 to 7 spots, kept in a zip-locked plastic bag to prepare a composite sample, and stored in a cooler during sampling. Following collection, samples were brought back to the lab and stored at 4°C until lab analysis. Thus, in each sampling period, a total of 16 composite samples were collected and each composite sample weighs roughly 500-1000 g. Before analysis, samples were mixed thoroughly again, divided and sent to labs for analysis using standard laboratory method presented in Table 10. Manure and air samplings were collected monthly, and pen surface temperature were measured using an infrared thermometer (Model: MINITEMP-MT6, Instrumart, Carlsbad, CA) during each sampling period for four months. Manure samples were analyzed for ash content, total nitrogen (TN), crude protein (CP), total phosphorus (TP), total carbon (TC), potassium (K), fecal ammonia (NH_3), and total volatile fatty acid contents (TVFA). A total of 64 manure samples were collected during the study period.

Table 10. Method/protocol used in this study to analyze manure samples.

Parameters	Method/protocol used/Measurement range
TN	Recommended Methods of Manure Analysis, A3769[1] Macro-Kjeldahl method (adapted from Kane, 1998)
TP	Recommended Methods of Manure Analysis, A3769[1]
K	Recommended Methods of Manure Analysis, A3769[1]
TC	US EPA method 415.1. Catalytic combustion and Non-dispersive Infrared Detection (NDIR) method.
Ash	Official Method 942.05, AOAC International (2005) 18th ED., AOAC International Gaithersburg, MD, USA.
CP	Official Method 2001.11, AOAC International (2005) 18th ED., AOAC International Gaithersburg, MD, USA.
NH_3	Sigma Technical Bulletin #640. Sigma Diagnostics, St. Louis, MO 63178
VFA	Method of Goetsch and Galyean, 1983. Agilent 6890N Gas Chromatograph with an FID (flame ionization detector) and the 7683 Series auto-injector and autosampler. Column used was the Supelco brand, NUKOL Fused Silica Column, 15 m x 0.53 mm x 0.5 um

AOAC = Association of Official Agricultural Chemists,

[1] http://learningstore.uwex.edu/assets/pdfs/A3769.pdf

Air sampling and analysis

Air samples were collected from each pen surface in 5L Tedlar® bags using a portable wind tunnel (0.80 m × 0.40 m) and Vac-u-chamber (SKC Inc., Eighty Four, PA) (Figure 5). A uniform air flow rate (1.75 $m^3 s^{-1}$) was maintained inside the tunnel throughout the sampling period using a DC motor regulator (to calculate emission rate). In this way duplicated air samples were collected from each pen. Within 24 hours of sampling, air samples were analyzed for CH_4, CO_2, and N_2O using a greenhouse gas GC (Model No. 8610C, SRI Instruments, 20720 Earl St., Torrance, CA 90502) (Figure 5) equipped with a flame ionization detector (FID) and an electron captured detector (ECD). A total of 128 air samples were collected during the study period. A detailed description of the GHG measurement procedure using GHG GC can be found in Rahman et al. 2012. Ambient temperature during the monitoring period was downloaded from the nearest weather station maintained by North Dakota Agricultural Weather Network. (NDAWN).

Emission calculation

To compare the estimated emission factors (EFs) among months, the measured volumetric concentrations were standardized at standard pressure (1 atm) and temperature (25°C). Equations 21 to 24 were used to calculate the mass concentration of particular gas, flux rates (FR) *and emission factors (EF).*

$$C_{mass} = \frac{C_{ppm} \times MW_{GHG}}{24.45} \tag{21}$$

$$\mathrm{FR_{GHG}} = \frac{\mathrm{C_{mass}} \times \mathrm{V_{WT}} \times 3600 \times 24}{\mathrm{A_{wT}} \times 1000} \tag{22}$$

$$\mathrm{AU} = \frac{\mathrm{TAN} \times \text{Indicidual live weight}}{500} \tag{23}$$

$$\mathrm{EF_{GHG}} = \frac{\mathrm{FR_{GHG}} \times \mathrm{A_{SC}}}{\mathrm{AU}} \tag{24}$$

where, C_{ppm} = Volumetric concentration of a target compound (ppm)

C_{MASS} = Mass concentration of a target compound (mg m^{-3})

MW_{GHG} = Molecular weight of a target compound (g $gmol^{-1}$)

24.45 = Volume per mole of an ideal gas at standard temperature and pressure (L $gmol^{-1}$)

FR_{GHG} = GHG emission flux rate from pen surface (g m^{-2} d^{-1})

EF_{GHG} = GHG emission rate from pen surface (g hd^{-1} d^{-1})

V_{WT} = Airflow rate through wind tunnel (m^3 s^{-1})

A_{WT} = Surface area covered by the wind tunnel (0.4 × 0.8 m^2)

A_{sc} = Surface area of the source (m^2)

AU = Animal unit is the total live weight of animal in kg divided by 500 kg.

Statistical analysis

The effect of diets and bedding on the manure nutrient composition and greenhouse gas emissions at each measurement event and among the events were statistically analyzed and means were compared using the GLM procedure in SAS software (SAS 1999). Initially, a univariate Randomized Complete Block Design (RCBD) was fitted to the data for each time event (month) with animal weights (lighter and heavier) as blocks and two factors, diets and beddings, in a 2×2 factorial arrangement. All significant tests were evaluated at P =0.05. As there were only two levels of the diet and bedding factors, a significant F test for a factor indicates that the treatment levels were statistically different so post hoc tests such as LSD were required. Subsequently, we fit a multivariate repeated measures model to assess the effect of time (month), the two factors (diets and bedding), and their interactions on nutrient composition and GHG emissions. The null hypothesis tested was that mean nutrient concentrations and GHG emissions (gas concentrations, FRs, and ERs) within and among the various months in treatment were equal.

Results and discussion

Background weather information

Daily average ambient air temperatures, bare soil temperatures, and solar radiations during June, July, and August were very similar and ranged from 20.5 to 22.5 °C, 25.6 to 28.2 °C, 23 to 27 MJ m^{-2}, respectively. During September, daily average air and soil temperature, and solar radiation were much lower (9.4 °C, 13.7 °C, 10.5 MJ m^{-2}, respectively) than those observed in the previous sampling months (Table 11). Interestingly, pens bedded with corn stover showed lower temperature (20.5 to 43.5 °C) than those pen surfaces with no bedding (22.4 to 47.3 °C) during the study period (Table 12). Lower pen surface temperature might reduce heat stress and provide comfort to cattle.

Table 11. Weather data during each sampling period gathered from a nearby weather station of NDAWN (http://ndawn.ndsu.nodak.edu/wind-directions.html).

Sampling date	Air temperature (°C)			Bare soil temperature (°C)	Solar radiation (MJ m^{-2})
	Average	Minimum	Maximum		
June 27, 2012	20.52	13.33	27.45	26.10	27.05
July 18, 2012	22.46	14.44	28.87	28.24	25.92
August 23, 2012	21.07	11.67	31.56	25.63	23.00
September 20, 2012	9.40	6.11	16.87	13.75	10.54

Effect of pen bedding and dietary protein levels on the manure composition

The concentrations of TS, VS, Ash, CP, TN, fecal NH_3, TVFA, TC, TP, and K measured at different treatment combinations during four measurement periods are presented in Table 12. The measured nutrients content in manure especially fecal NH_3, TP, and K concentrations were widely varied between the treatment, within, and among the sampling months as indicated by the standard deviation. This average nutrient concentrations difference between treatments was most likely due to CP levels in diets. Differences in nutrient concentration among months were likely due to feed digestibility and rumen chemistry.

Manure ash content in manure with high protein diets was generally higher compared to low protein diet manure. Average ash contents in manure measured during June and September were significantly higher with high protein diet than those of low protein diet manure. Similarly, during July and August, the ash content of high protein diet manure were higher but not significantly different than those of low protein diet manure ($P>0.05$) (Table 12). Similar to ash content, CP content in the manure was significantly higher with high protein diets than those with low protein diets except for the beginning month (June). It is likely that high protein diets fed to the animals should produce higher CP content in the excreted manure.

Table 12. Effect of pen bedding with corn stover and dietary protein levels fed to the animals on the nutrients composition of excreted manure (dry matter basis).

Sample Date	Pen surface temp. and nutrient composition	Pen Bedding		Protein Level	
		No-bedding	Bedding	High (16%)	Low (12%)
27-Jun-12	Temperature (℃)	42.88 a ± 1.6	38.92 a ± 2.4	40.36 x ± 3.1	41.44 x ± 2.6
	Ash (%)	14.69 a ± 2.8	14.38 a ± 5.1	16.71 x ± 4.3	12.37 y ± 2.2
	CP (%)	15.04 a ± 1.7	14.71 a ± 1.9	15.58 x ± 2.2	14.17 x ± 1.0
	TN (%)	2.41 a ± 0.3	2.35 a ± 0.3	2.49 x ± 0.4	2.27 x ±0.2
	Rumen NH_3 (mM)	20.26 a ± 9.9	14.04 a ± 8.3	20.34 x ± 11.4	13.95 x ± 5.9
	TVFA (mM)	76.21 a ± 3.9	77.09 a ± 4.8	77.46 x ± 3.5	75.84 x ± 5.0
	TC (%)	159.32 a ± 34.5	167.36 a ± 26.8	163.98 x ± 29.5	162.70 x ± 32.7
	TP (%)	0.93 a ± 0.52	0.82 a ± 0.16	1.04 x ± 0.4	0.70 x ± 0.25
	K (%)	0.50 a ± 0.17	0.53 a ± 0.25	0.63 x ± 0.19	0.40 y ± 0.16
18-Jul-12	Temperature (℃)	47.34 a ± 7.9	43.49 a ± 6.6	45.73 x ± 7.3	45.10 x ± 7.8
	Ash (%)	12.38 a ± 2.1	12.16 a ± 2.7	13.00 x ± 2.4	11.54 x ± 2.2
	CP (%)	14.97 a ± 1.5	15.40 a ± 1.6	16.09 x ± 1.6	14.28 y ± 0.6
	TN (%)	2.40 a ± 0.24	2.46 a ± 0.26	2.58 x ± 0.25	2.28 y ± 0.10
	Rumen NH_3 (mM)	15.68 a ± 6.6	13.78 a ± 6.6	18.54 x ± 6.8	10.92 y ± 3.2
	TVFA (mM)	81.23 a ± 3.2	82.56 a ± 3.4	83.87 x ± 2.9	79.92 y ± 2.3
	TC (%)	182.79 a ± 24.7	171.92 a ± 34.3	187.92 x ± 10.8	166.79 x ± 38.5
	TP (%)	1.26 a ± 0.67	0.89 a ± 0.24	1.35 x ± 0.59	0.80 y ± 0.25
	K (%)	0.59 a ± 0.28	0.46 a ± 0.20	0.59 x ± 0.28	0.45 x ± 0.19
23-Aug-12	Temperature (℃)	46.88 a ± 6.88	44.04 a ± 4.4	45.85 x ± 6.3	45.07 x ± 5.6
	Ash (%)	16.04 a ± 7.0	13.07 a ± 2.1	16.62 x ± 6.6	12.50 x ± 2.5
	CP (%)	14.96 a ± 1.3	16.65 b ± 2.1	16.76 x ± 1.8	14.85 y ± 1.5
	TN (%)	2.39 a ± 0.20	2.66 b ± 0.34	2.68 x ± 0.29	2.38 y ± 0.24
	Rumen NH_3 (mM)	24.59 a ± 13.6	21.51 a ± 12.2	30.74 x ± 12.1	15.36 y ± 7.6
	TVFA (mM)	82.54 a ± 3.2	82.38 a ± 4.1	83.90 x ± 2.9	81.02 x ± 3.8
	TC (%)	184.41 a ± 15.7	189.09 a ± 12.6	190.42 x ± 17.0	183.08 x ± 9.9
	TP (%)	1.12 a ± 0.29	1.16 a ± 0.54	1.34 x ± 0.44	0.94 x ± 0.30
	K (%)	0.94 a ± 1.82	1.05 a ± 1.9	1.01 x ± 1.7	0.98 x ± 1.9
20-Sep-12	Temperature (℃)	22.38 a ± 2.0	20.52 a ± 4.2	21.51 x ± 2.5	21.39 x ± 4.21
	Ash (%)	12.65 a ± 2.2	13.76 a ± 2.7	14.63 x ± 1.9	11.78 y ± 2.2
	CP (%)	15.30 a ± 1.9	16.81 a ± 2.0	17.31 x ± 1.8	14.80 y ± 1.4
	TN (%)	2.45 a ± 0.31	2.69 a ± 0.32	2.77 x ± 0.29	2.37 y ± 0.22
	Rumen NH_3 (mM)	13.28 a ± 6.2	13.10 a ± 3.8	16.97 x ± 3.0	9.42 y ± 3.4
	TVFA (mM)	29.29 a ± 5.66	37.13 a ± 14.2	35.14 x ± 13.8	31.28 x ± 8.4
	TC (%)	186.78 a ± 13.0	198.10 a ± 27.3	199.56 x ± 26.6	185.32 x ± 12.9
	TP (%)	1.02 a ± 0.23	1.06 a ± 0.27	1.11 x ± 0.20	0.96 x ± 0.27
	K (%)	0.73 a ± 0.15	0.78 a ± 0.28	0.68 x ± 0.17	0.83 x ± 0.24

Values followed by the same letter in a row for a particular parameter are not significantly different at $P \leq 0.05$.
Temperature indicates the pen surface temperature.

The concentrations of TN and fecal NH_3 in the manure from high protein diets were significantly higher ($P<0.05$) during sampling periods when compared with those of low protein diets except for June (Table 12). In contrast, TN and fecal NH_3 concentration in manure during June from the high protein diets were not significantly higher than that of low protein diets. Total nitrogen concentrations in the pen surface manure with high protein diets during July, August, and September were13, 23, and 17 %, respectively, higher than low protein diets. Similarly, fecal NH_3 concentrations in the manure from the high protein diets were 72, 100, and 80% higher than those measured from low protein diets during those dates, respectively.

The concentration of TC was similar for pens fed the high and low protein diets at all sampling events. Concentrations of TVFA were similar for pens of cattle fed both diets on all sampling events except July when TVFA was higher in manure from cattle fed the high protein diet compared to manure from cattle fed the low protein diet. Relatively higher ambient (air) and pen surface temperatures in July compared to other months might have contributed in significantly higher TVFA from high protein diet manure. Potassium concentration in manure was similar among pens of cattle fed the high and low protein diets on all sampling dates except June, when it was higher for manure from cattle fed high protein diet. This was likely due to the introduction of a different level of protein diets that might increase K level in June, but no increase in K content on later sampling dates, since cattle were on the same diet after June. In a similar study with increasing DDGS (0, 12, 24, and 36%) in a barley based diets, Anderson et al (2011) observed no significant differences in manure K contents when manure analyzed 56 d after the trial was initiated. Although TP concentrations from manure with the high protein diets during June, July, August, and September were 49, 72, 42, and 16 %, respectively, higher than the manure from low protein diets, but they were not statistically significant. It was also reported by other researchers that higher TP excretion in manure by feedlot cattle fed diets with distillers co-products compared to cattle fed non-distillers co-products diets (Spiehs et al. 2012; Spiehs and Varel 2009).

The manure nutrient concentrations between bedding and no bedding were similar ($p>0.05$) during all months except August. In August, CP and TN concentrations from bedded pens were significantly higher (Table 12). This was likely that bedded pens had greater water absorbance capacity and bedding materials also provide blending of manure and bedding that reduce N loss (Rahman et al., 2012). The fecal NH_3 concentrations in manure samples from the bedded pen showed slightly lower than those manure samples collected from the non-bedded pen and the differences were not significant. In contrast, TN concentrations from the bedded pen were slightly higher than those from the non-bedded pen, except in June. Overall, this research showed that bedding had little effect on the nutrients composition of the manure on the feedlot surface. Further analysis for investigating the effect of feeding duration (time or month) coupled with the interactions of diets and bedding on manure compositions revealed that with the exception of potassium, there was no significant month × bedding, month × diet, or month × bedding × diet interactions.

Effect on the manure volatile fatty acids (VFAs) compositions

Manure samples were analyzed for VFAs such as acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids are reported in Table 13. The measured VFAs concentrations across two treatments, within and among sampling months varied widely as revealed by the standard deviation. Except valeric acid, measured VFAs in manure were not statistically significant between bedding. Perhaps CP ranges 12-16% in the diets produced a similar amount of ruminal VFAs and ultimately excreted along with manure. Manure VFAs were dominated by acetic, propionic, and butyric acid and all together they account for about 95% of total VFA. This showed an agreement with previous findings (Hao et al., 2009; Hao et al., 2005; McGinn et al., 2002). Valeric acid concentrations were significantly lower with bedded than not bedded pens in June and July ($P \leq 0.05$). This was likely due to the low surface temperature that limited volatilization of VFAs from bedded pen surface.

VFA concentrations in manure varied between manure collected from pens cattle fed high-protein diets and low protein diets among months. In June, isovaleric and valeric; in July, isovaleric; in August, propionic were significantly different for cattle manure fed with low protein diets. Hao et al (2009) reported a significant and positive correlation between the sum of fecal isobutyric, valeric, and isovaleric acid contents and feed CP content ($r = 0.615$). In this research, the significant variations in VFAs over the sampling months were likely due to the changes in dry matter intake, protein utilization efficiency, the amount of N excreted by cattle, VFA volatility, and manure temperature (Hao et al., 2009).

Acetic acid had the greatest concentration among the VFAs measured and its concentrations in the manure showed no significance irrespective of treatments and sampling time (Table 13). Propionic acid and butyric acid had similar concentrations in all manure samples, except in September manure samples. Propionic acid and butyric acid were significantly different between pens fed high and low protein diets in August and they were similar in the other three months. A previous study with wheat DDGS reported a significant increase in isobutyric, valeric and isovaleric acids in the manure with 40 and 60% DDGS diets, although there was no change in the total VFA content with diet (Hao et al., 2009). However, the CP contents evaluated in that research were 18 and 29 % which were much higher than the highest CP in the current study. Acetic and propionic acids are recognized to trigger GHG (CH_4)

production from anaerobic waste (Hill et al., 1987; Marchaim and Krause, 1993). Acetic acid is the desired intermediate among VFAs since it is a direct substrate for methanogenesis and accounts for approximately 70% of CH_4 production from acetic acid in an anaerobic setting (Hill et al., 1987). It was previously reported that the ratio of propionic acid to acetic acid (1:4) was used as an indicator of efficient digester performance (Marchaim and Krause, 1993). The above-mentioned scenario is true for strictly anaerobic condition. The aerobic condition of the feedlot pen surface did not show the contribution of VFAs on GHG emissions.

Table 13. Effect of pen bedding and dietary protein levels fed to the animals on the VFA constituents of excreted manure in the feedlot pen surfaces (dry matter basis).

Month	VFAs	Bedding conditions		Crude Protein levels	
		Not-bedded	Bedded	High (16 %)	Low (12 %)
June	Acetic	45.25 a ± 5.4	42.43 a ± 4.2	41.51 x ± 4.2	46.17 x ± 4.6
	Propionic	22.65 a ± 5.1	22.61 a ± 4.8	20.85 x ± 4.2	24.41 x ± 4.9
	Isobutyric	1.48 a ± 0.8	1.28 a ± 0.5	1.77 x ± 0.7	0.99 y ± 0.3
	Butyric	26.99 a ± 5.1	31.16 a ± 6.9	31.84 x ± 7.0	26.31 x ± 0.3
	Isovaleric	1.66 a ± 1.1	1.29 a ± 0.5	2.00 x ± 0.9	0.95 y ± 0.3
	Valeric	1.96 a ± 0.9	1.23 b ± 0.5	2.03 x ± 0.8	1.16 y ± 0.5
July	Acetic	40.67 a ± 3.6	43.39 a ± 3.4	42.34 x ± 3.4	41.72 x ± 4.1
	Propionic	28.39 a ± 2.0	27.73 a ± 2.4	28.00 x ± 2.5	28.12 x ± 2.0
	Isobutyric	1.16 a ± 0.5	1.06 a ± 0.4	1.29 x ± 0.4	0.93 x ± 0.4
	Butyric	26.14 a ± 5.7	25.28 a ± 2.6	24.91 x ± 4.5	26.51 x ± 4.3
	Isovaleric	1.16 a ± 0.4	1.07 a ± 0.5	1.37 x ± 0.4	0.86 y ± 0.4
	Valeric	2.48 a ± 0.4	1.46 b ± 0.6	2.09 x ± 0.79	1.85 x ± 0.7
August	Acetic	40.18 a ± 2.6	42.20 a ± 3.4	42.81 x ± 2.6	39.57 x ± 2.8
	Propionic	27.36 a ± 3.2	27.77 a ± 3.3	26.08 x ± 2.0	29.04 y ± 3.5
	Isobutyric	1.01 a ± 0.5	1.05 a ± 0.5	1.26 x ± 0.3	0.79 y ± 0.4
	Butyric	27.61 a ± 2.5	25.20 a ± 3.9	24.62 x ± 3.1	28.19 y ± 3.0
	Isovaleric	1.15 a ± 0.6	1.11 a ± 0.6	1.48 x ± 0.5	0.79 y ± 0.5
	Valeric	2.68 a ± 1.2	2.68 a ± 2.1	3.75 x ± 1.6	1.62 y ± 0.7
September	Acetic	75.01 a ± 7.3	68.10 a ± 8.2	72.34 x ± 9.5	70.76 x ± 7.5
	Propionic	24.17 a ± 6.9	27.02 a ± 5.4	23.15 x ± 6.1	28.05 x ± 5.5
	Isobutyric	0.21 a ± 0.1	0.94 a ± 0.9	0.84 x ± 1.3	0.31 x ± 0.3
	Butyric	0.16 a ± 0.1	1.89 a ± 1.7	1.65 x ± 1.4	0.40 x ± 0.3
	Isovaleric	0.45 a ± 0.2	2.05 a ± 1.9	2.02 x ± 1.9	0.48 x ± 0.4
	Valeric	0.00 a ± 0.0	0.00 a ± 0.0	0.00 x ± 0.0	0.00 x ± 0.0

Values followed by the same letter in a row for a particular parameter are not significantly different at $P \leq 0.05$.

Table 14. Effect of bedding and dietary protein levels on GHG concentration, emission flux (EFlux), and emission factors (EF) estimated based on the animal unit (AU = 500 body weight).

Sampling Date	Manure composition	Bedding condition		Protein level (%)	
		No-bedding	Bedding	High (16 %)	Low (12 %)
June 27, 2012	CH_4 Concentration (ppm)	1.84 a ± 0.11	1.79 a ± 0.05	1.78 x ± 0.05	1.84 x ± 0.11
	CO_2 Concentration (ppm)	448.55 a ±13.5	447.82 a ± 14.9	451.79 x ± 11.9	444.58 x ± 15.3
	N_2O Concentration (ppm)	1.50 a ± 0.81	1.03 a ± 0.27	1.20 x ± 0.33	1.34 x ± 0.85
	CH_4 Eflux ($g\ m^{-2}\ d^{-1}$)	0.89 a ± 0.06	0.87 a ±0.02	0.87 x ± 0.03	0.90 x ± 0.05
	CO_2 Eflux ($g\ m^{-2}\ d^{-1}$)	598.94 a ± 18.1	597.96 a ± 19.9	603.27 x ± 15.9	593.64 x ± 20.4
	N_2O Eflux ($g\ m^{-2}\ d^{-1}$)	1.68 a ± 0.40	1.38 a ± 0.36	1.60 x ± 0.45	1.46 x ± 0.35
	CH_4 EF ($g\ d^{-1}AU^{-1}$)	60.21 a ± 16.0	60.39 a ± 18.7	59.67 x ± 18.1	60.94 x ± 16.6
	CO_2 EF ($kg\ d^{-1}AU^{-1}$)	40.75 a ± 12.6	41.80 a ± 14.1	41.94 x ± 14.4	40.61 x ± 12.4
	N_2O EF ($g\ d^{-1}AU^{-1}$)	116.59 a ± 50.3	97.29 a ± 47.6	114.79 x ± 59.2	99.09 x ± 37.0
July 18, 2012	CH_4 Concentration (ppm)	2.03 a ± 0.14	2.16 a ± 0.31	2.07 x ± 0.24	2.12 x ± 0.26
	CO_2 Concentration (ppm)	510.54 a ± 25.3	522.38 a ± 43.1	523.44 x ± 43.4	509.48 x ± 24.1
	N_2O Concentration (ppm)	1.60 a ± 0.99	1.88 a ± 1.2	1.89 x ± 1.2	1.60 x ± 0.99
	CH_4 Eflux ($g\ m^{-2}\ d^{-1}$)	0.99 a ± 0.07	1.05 a ± 0.15	1.01 x ± 0.11	1.03 x ± 0.13
	CO_2 Eflux ($g\ m^{-2}\ d^{-1}$)	681.71 a ± 33.7	697.53 a ± 57.5	698.94 x ± 57.9	680.31 x ± 32.2
	N_2O Eflux ($g\ m^{-2}\ d^{-1}$)	1.81 a ± 0.41	1.84 a ± 0.92	1.85 x ± 0.91	1.80 x ± 0.43
	CH_4 EF ($g\ d^{-1}AU^{-1}$)	59.00 a ± 17.3	64.24 a ± 18.1	60.86 x ± 19.3	62.38 x ± 16.4
	CO_2 EF ($kg\ d^{-1}AU^{-1}$)	40.79 a ± 12.3	42.94 a ± 13.1	42.27 x ± 13.5	41.46 x ± 11.9
	N_2O EF ($g\ d^{-1}AU^{-1}$)	110.12 a ± 52.1	112.37 a ± 52.6	110.66 x ± 51.8	111.83 x ± 52.8
August 23, 2012	CH_4 Concentration (ppm)	1.85 a ± 0.16	2.13 a ± 0.39	1.95 x ± 0.28	2.03 x ± 0.38
	CO_2 Concentration (ppm)	521.43 a ± 33.3	538.16 a ± 32.3	548.00 x ± 27.1	511.58 x ± 28.7
	N_2O Concentration (ppm)	1.32 a ± 1.0	1.11 a ± 0.36	1.14 x ± 0.34	1.29 x ±1.0
	CH_4 Eflux ($g\ m^{-2}\ d^{-1}$)	0.90 a ± 0.08	1.09 a ± 0.30	0.95 x ± 0.14	1.04 x ±0.31
	CO_2 Eflux ($g\ m^{-2}\ d^{-1}$)	696.26 a ± 44.5	718.59 a ± 110.0	731.74 x ± 36.3	683.11 y ± 119.7
	N_2O Eflux ($g\ m^{-2}\ d^{-1}$)	1.43 a ± 0.53	1.49 a ± 0.47	1.52 x ± 0.45	1.39 x ± 0.55
	CH_4 EF ($g\ d^{-1}AU^{-1}$)	46.95 a ± 15.6	54.66 a ± 10.9	49.67 x ± 12.86	51.94 x ± 15.3
	CO_2 EF ($kg\ d^{-1}AU^{-1}$)	35.98 a ± 10.63	38.75 a ± 9.9	38.88 x ± 11.9	35.85 x ± 8.3
	N_2O EF ($g\ d^{-1}AU^{-1}$)	78.64 a ± 48.8	86.42 a ± 53.32	85.97 x ± 50.8	79.09 x ± 51.5
Sept. 20, 2012	CH_4 Concentration (ppm)	1.79 a ± 0.11	2.22 b ± 0.32	2.08 x ± 0.37	1.93 x ± 0.26
	CO_2 Concentration (ppm)	508.28 a ± 12.8	521.53 a ± 20.3	518.09 x ± 15.2	511.72 x ± 20.5
	N_2O Concentration (ppm)	1.22 a ± 1.1	0.75 a ± 0.20	0.83 x ± 0.26	1.14 x ± 0.13
	CH_4 Eflux ($g\ m^{-2}\ d^{-1}$)	0.87 a ± 0.05	1.08 a ± 0.15	1.01 x ± 0.18	0.94 x ± 0.13
	CO_2 Eflux ($g\ m^{-2}\ d^{-1}$)	678.70 a ± 17.1	696.40 a ± 27.0	691.80 x ± 20.3	683.29 x ± 27.4
	N_2O Eflux ($g\ m^{-2}\ d^{-1}$)	1.30 a ± 0.59	1.00 a ± 0.27	1.11 x ± 0.35	1.19 x ± 0.58
	CH_4 EF ($g\ d^{-1}AU^{-1}$)	39.66 a ± 7.9	52.25 a ± 16.4	47.65 x ± 16.2	44.26 x ± 12.4
	CO_2 EF ($kg\ d^{-1}AU^{-1}$)	31.04 a ± 7.4	33.51 a ± 8.1	32.43 x ± 8.2	32.12 x ± 7.6
	N_2O EF ($g\ d^{-1}AU^{-1}$)	62.38 a ± 40.8	49.86 a ± 25.3	54.46 x ± 29.3	57.78 x ± 39.1

Values followed by the same letter in a row for a particular parameter are not significantly different at $P \leq 0.05$.

Effect of pen bedding and dietary protein levels on GHG emissions

Table 14 shows the measured GHG (CH_4, CO_2, and N_2O) concentrations, and estimated flux rates (FRs) and emissions factors (EFs) at different treatment combinations during four measurement periods. The measured CH_4, CO_2, and N_2O concentrations between the treatments, within, and among the months varied widely as depicted by the standard deviation, but differences were not significant except for two instances (Table 14). Higher CO_2 concentration in manure with high protein level in August was probably due to the higher VFA content coupled with high surface temperature (25 to 31℃) during sampling time. During most of the sampling time, pen surface was relatively dry, which might facilitate partial aerobic condition. Under aerobic condition in the feedlot pen surface, most soil microorganism use O_2 as an electron acceptor, releasing CO_2 into the atmosphere (Li, 2007).

It is well established that CH_4 production in the anaerobic processes (manure storage and treatment structures such as lagoons and settling basins, slurry, and solid manure) are strongly dependent on manure temperature, physic-chemical characteristics of manure, and storage duration (Husted, 1994; Massé et al., 2003; Weiske et al., 2006). In this study, CH_4 concentration was significantly different in September between bedded and non-bedded pens, which was likely due to manure temperature, pen surface condition, and manure storage duration. During September sampling, manure was accumulated on the pen surface and fermentation was likely to be started by the anaerobic environment of the feedlot pen manure (fresh and accumulated manure) and bedding materials (Saggar et al., 2004) as compared with non-bedded manure. Methane emissions generally vary with manure loading rates, manure microbial activity, and fluctuations in surface temperature (Borhan et al., 2011; Mukhtar et al., 2008). Bedded pen surface temperatures were always lower than those measured from non-bedded pen surfaces although the differences were not statistically significant (*P>0.05*). However, during the summer time, lower pen surface temperature in the bedded pens would reduce heat stress and thus provide comfort to cattle.

Further analysis including the effect of feeding duration (month) coupled with the interactions of diets and pen bedding on GHG emissions revealed that CH_4, CO_2, and N_2O emissions (concentrations, FRs, and EFs) varied significantly ($P \leq 0.05$) over the sampling period (Table 15).

Table 15. Table cells show probability values based on the repeated measure multivariate analysis along with time and treatments interaction.

Parameters	Time effect	Interactions		
		Month*Bedding	Month*Diets	Month*Bedding *Diets
Pen Surface Temperature (℃)	< 0.01	0.75	0.60	0.60
CH_4 Concentration (ppm)	< 0.01	< 0.01	0.10	0.45
CO_2 Concentration (ppm)	< 0.01	0.24	0.28	0.82
N_2O Concentration (ppm)	< 0.01	0.14	0.41	0.37
CH_4 Eflux (g m^{-2} d^{-1})	< 0.01	< 0.01	0.10	0.45
CO_2 Eflux (g m^{-2} d^{-1})	< 0.01	0.24	0.28	0.82
N_2O Eflux (g m^{-2} d^{-1})	< 0.01	0.14	0.41	0.37
CH_4 EF (g $d^{-1}AU^{-1}$)	< 0.01	< 0.01	0.10	0.45
CO_2 EF (kg $d^{-1}AU^{-1}$)	< 0.01	0.24	0.28	0.82
N_2O EF (g $d^{-1}AU^{-1}$)	< 0.01	0.14	0.41	0.37

A previous study in a controlled laboratory environment reported no significant difference in the emitting potential and cumulative GHG emissions (CH_4, CO_2, and N_2O) between high crude protein (14.8%) and low crude protein (16.7 %) diets (Lee et al. 2012). The CH_4, CO_2, and N_2O concentrations ranged over sampling events (June to September) were 1.8 to 2.1, 448 to 530, and 0.9 to 1.3, ppm, respectively. Similar CH_4, CO_2, and N_2O concentrations were also reported previously in a study in Texas (Borhan et al. 2011) and North Dakota (Rahman et al. 2012) feedlots with high protein diets. The minimum and maximum measured

EFs for CH_4, CO_2, and N_2O were 46 to 60 g $d^{-1}AU^{-1}$, 32 to 42 kg $d^{-1}AU^{-1}$, and 56 to 107 g $d^{-1}AU^{-1}$), respectively, during the sampling period.

The results obtained in this research using wind tunnel were lower than those reported using micrometeorological mass difference, dispersion, and other modeling techniques (McGinn et al. 2008; van Haarlem et al. 2008; Zoe et al. 2008) based on source integrated or whole farm approach. Ideally, measured concentration and emission factors with source integrated method would be higher using open-path sensors (entire open air area sources including feedlot, retention pond, compost piles, a portion of enteric GHGs, etc.) than a source specific method using flux chamber and wind tunnel.

Similar results of bedding effect on the CH_4 and N_2O emissions were previously reported. Amon et al. (2001) evaluated slurry and straw bedded-pen manure management systems for a tie stall dairy. When identical diets were fed to the cows, no significant differences were observed in average EFs for CH_4 (194.4 and 194.4 g LU^{-1} d^{-1} for slurry and straw system, respectively) and N_2O (610 to 619 mg LU^{-1} d^{-1} for slurry and straw system, respectively) (Amon et al., 2001). There was no interaction effect of diets and bedding on GHG emissions observed from the feedlot pens. While previous results have indicated that a high protein diet namely WDGS may decrease CH_4 emissions from excreted manures, possibly due to efficient digestion of WDGS in the rumen and due to the decrease in the starch concentration in the feces (Hales et al. 2013). Hales et al. (2013) used WDGS that has a low starch inherently. Thus, with 30% WDGS and 17 % CP decreased the CH_4 concentration by decreasing starch concentration in the excreted manure. In this study, DDGS used had higher starch content than WDGS and it is likely to have high starch content in the manure. However, the starch of the DDGS was not quantified in the study. However, this research did not find enough evidence to conclude that either pen bedding or high protein diets had a significant effect on GHG emissions from excreted manure from the feedlot pen surface.

Conclusions

An experiment was conducted on research feedlot pen surfaces with and without bedding to investigate the effect of two dietary crude protein concentrations (12 and 16 %) on the manure nutrients content and GHG (CH_4, CO_2, and N_2O) emissions. This study was conducted over a 4-month period, during North Dakota summer climatic conditions and consisted of four ~28 day feeding periods. The analyzes revealed that the two CP levels fed to the beef cattle in the bedded and non-bedded pens had shown a little effect on nutrient composition (Ash, TN, NH_3, TVFA, TP, and K) and GHG emissions from excreted manure obtained from the feedlot surface. Total N and fecal NH_3 concentrations in the pen surface manure with high protein diets during July, August, and September were significantly higher than low protein diets. In general, TC and TP concentrations in the pen surface manure between high and low protein were similar. Overall, this research showed that bedding had little effect on the nutrients composition of the manure on the feedlot surface.

References

Amon, B., Amon, T., Boxberger, J., and Alt, C. 2001. Emissions of NH_3, N_2O and CH_4 from dairy cows housed in a farmyard manure tying stall (housing, manure storage, manure spreading). *Nutrient cycling in agroecosystems*. 60(1-3): 103-113.

Anderson, V., and Bird, J. 1993. Effect of shelterbelt protection on the performance of feedlot steers during a North Dakota winter. *Beef Production Field Day Proceedings, Carrington Research Extension Center, North Dakota State University*. 16: 19-21.

Archibeque, S., Freetly, H., Cole, N., and Ferrell, C. 2007. The influence of oscillating dietary protein concentrations on finishing cattle. II. Nutrient retention and ammonia emissions. *Journal of animal science*. 85(6): 1496-1503.

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

Beauchemin, K., McGinn, S., Benchaar, C., and Holtshausen, L. 2009. Crushed sunflower, flax, or canola seeds in lactating dairy cow diets: effects on methane production, rumen fermentation, and milk production. *Journal of Dairy Science*. 92(5): 2118-2127.

Birkelo, C., and Lounsbery, J. 1992. Effect of straw and newspaper bedding on cold season feedlot performance in two housing systems. *South Dakota beef report*. Reterived on 9/12/2012 from https://www.sdstate.edu/ars/species/beef/beef-reports/upload/Cattle_92-11_Birkelo.pdf.

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

Castillo, A., Kebreab, E., Beever, D., Barbi, J., Sutton, J., Kirby, H., and France, J. 2001. The effect of protein supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets. *Journal of animal science*. 79(1): 247-253.

Cole, N., Clark, R., Todd, R., Richardson, C., Gueye, A., Greene, L., and McBride, K. 2005. Influence of dietary crude protein concentration and source on potential ammonia emissions from beef cattle manure. *Journal of animal science*. 83(3): 722-731.

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

Garcia, A., and Taylor, G. 2002. Economics of feeding distillers grains to dairy cows. In Extension publication Ex 4025: South Dakota State University.

Hao, X., Benke, M. B., Gibb, D. J., Stronks, A., Travis, G., and McAllister, T. A. 2009. Effects of dried distillers' grains with solubles (wheat-based) in feedlot cattle diets on feces and manure composition. *Journal of environmental quality*. 38(4): 1709-1718.

Hao, X., Mir, P. S., Shah, M. A., and Travis, G. R. 2005. Influence of canola and sunflower diet amendments on cattle feedlot manure. *Journal of environmental quality*. 34(4): 1439-1445..

Hill, D., Cobb, S., and Bolte, J. 1987. Using volatile fatty acid relationships to predict anaerobic digester failure. *Trans. ASAE; (United States)*. 30(2).

Husted, S. 1994. Seasonal variation in methane emission from stored slurry and solid manures. *Journal of environmental quality*. 23(3): 585-592.

Kebreab, E., France, J., Beever, D., and 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., and France, J. 2010. Impact of dietary manipulation on nutrient flows and greenhouse gas emissions in cattle. *Revista Brasileira de Zootecnia*. 39: 458-464.

Lee, C., Hristov, A., Dell, C., Feyereisen, G., Kaye, J., and Beegle, D. 2012. Effect of dietary protein concentration on ammonia and greenhouse gas emitting potential of dairy manure. *Journal of Dairy Science*. 95(4): 1930-1941.

Li, C. 2007. Quantifying greenhouse gas emissions from soils: Scientific basis and modeling approach. *Soil Science and Plant Nutrition*. 53(4): 344-352.

Luo, J., and Saggar, S. 2008. Nitrous oxide and methane emissions from a dairy farm stand-off pad. *Animal Production Science*. 48(2): 179-182.

Marchaim, U., and Krause, C. 1993. Propionic to acetic acid ratios in overloaded anaerobic digestion. *Bioresource Technology*. 43(3): 195-203.

Massé, D., Croteau, F., Patni, N., and Masse, L. 2003. Methane Emissions from Dairy Cow and Swine Manure Slurres stored at 10°C and 15°C. *Canadian Biosystems Engineering*. 45: 6.1-6.1.

McGinn, S., Koenig, K., and Coates, T. 2002. Effect of diet on odorant emissions from cattle manure. *Canadian Journal of Animal Science*. 82(3): 435-444.

Mukhtar, S., Mutlu, A., Capareda, S. C., and Parnell, C. B. 2008. Seasonal and spatial variations of ammonia emissions from an open-lot dairy operation. *Journal of the Air & Waste Management Association*. 58(3): 369-376.

Osada, T., Takada, R., and 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.

Rahman, S. 2012. Suitability of sunflower-hulls-based turkey litter for on-farm turkey carcass composting. *Canadian Biosystems Engineering*. 54: 6.1-6.8.

Rahman, S., Lin, D., and Zhu, J. 2012. Greenhous Gas (GHG) Emission from Mecanically Ventilated Deep Pit Swine Gestation Operation. *Civil and Environmental Engineering*. 2(104): doi 10.4172/2165-784X.1000104.

Saggar, S., Bolan, N. S., Bhandral, R., Hedley, C., and Luo, J. 2004. A review of emissions of CH_4, NH_3, and N_2O from animal excreta deposition and farm effluent application in grazed pastures. *New Zealand Journal of Agricultural Research*. 47(4): 513-544.

Schroeder, J. W. 2012. Distillers grains as a protein and energy supplement for dairy cattle. In Extension publication. AS 1241, Department of Animal Sciences: North Dakota State University.

Sejian, V., and Naqvi, S. 2012. *Livestock and climate change: mitigation strategies to reduce methane production*: INTECH Open Access Publisher.

Spiehs, M., Miller, D., Woodbury, B., Eigenberg, R., Varel, V., and Parker, D. 2012. Effect of feeding wet distillers grains with solubles to beef cattle on air and manure quality. *Applied engineering in agriculture*.

Stanton, T., and Schultz, D. 1996. Effect of bedding on finishing cattle performance and carcass characteristics. *Colorado State Beef Program Rep., CSU, Fort Collins, CO*: 37-41.

Todd, R. W., Cole, N. A., and Clark, R. N. 2006. Reducing crude protein in beef cattle diet reduces ammonia emissions from artificial feedyard surfaces. *Journal of environmental quality*. 35(2): 404-411.

USEPA. 2010. Methane and Nitrous Oxide Emissions From Natural Sources. Washington, DC 20460.

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

THE EFFECT OF FEEDING A HIGH-FAT DIET ON MANURE COMPOSITION AND GASEOUS EMISSION FROM THE MANURE OF BEEF CATTLE FEEDLOT

Abstract

Dietary manipulation is a common practice to mitigate gaseous emission from livestock production facilities, and the variation of fat level in the diet has shown great influence on ruminal volatile fatty acids (VFA) and enteric methane generation. The changes in dietary fat levels influence rumen chemistry that could modify a few crucial variables such as pH, VFAs, and carbon-nitrogen ratio of manure, which determines the overall manure nutrient composition along with odor and gaseous emissions from manure management facilities. A field experiment was carried out on beef cattle feedlots to investigate the effect of four level dietary fat concentrations (3 to 5.5%) on the manure composition and gaseous emissions (methane-CH_4, nitrous oxide-N_2O, carbon dioxide-CO_2 and hydrogen sulfide-H_2S) from the pen surface. The experiment was carried out over a 5-month period from May to October during North Dakota's summer-fall climatic condition. Air and manure sampling were conducted five times at an interval of 20-30 days. Overall, this research showed that fat levels in diet have no or little effect on the nutrient composition of manure and gaseous emission from pens with cattle feed with different diet. Though significant variation of gaseous emission and manure composition were observed between different sampling periods, no effect of high fat diet was observed on manure composition and gaseous emission on any of the months.

Introduction

The United States of America is one of the largest producers of livestock and number one producer of beef cattle in the world (Spellman and Whiting, 2010). According to the USDA, as of July 2015, there are 89.9 million beef cattle in the United states (USDA, 2015) and approximately 1.5 billion kg of manure (according to ASABE Standard D384.2, manure production from a beef cattle is 20-34 kg of manure per day) is generated daily only from beef cattle. Livestock manure is a good nutrient source for crops. At the same time, it is also a major source of pollutant gases (ammonia-NH_3, hydrogen sulfide-H_2S, etc.), greenhouse gases (GHGs), volatile organic compounds (VOCs), odor, and particulate material (PM). Gaseous emission of pollutant gases and GHGs are becoming an important issue for human and animal health, and environment (Portejoie et al., 2002; Viguria et al., 2015). In a livestock production system, the rate and amount of gaseous emissions depend on animal species, diet composition, manure management, weather, types of housing system, and topographic features (Chadwick et al., 2000).

In a confined livestock operation, the gaseous emission of pollutant gases has impact on workers' health, livestock welfare and productivity. The exposure of pollutant gas like H_2S can cause dizziness, headache, respiratory problem, bronchitis, pulmonary paralysis, unconsciousness and even fetal effect (Bowman et al., 2000; Guidotti, 1994; Milby and Baselt, 1999). Similarly, the higher concentration of NH_3 can cause respiratory irritation, chemical burns to the respiratory track, skin and eyes, severe cough, and chronic lung diseases (Hribar and Schultz, 2010). Besides the impacts on human and animal health; those pollutant gases have impact on environment. For example, NH_3 is one of the responsible factors for the nutrient build up and eutrophication of surface water, acidification, and the promotion of bacterial growth that leads to the weathering and corrosive damage of the buildings (Dang et al., 2010; Hartung and Phillips, 1994; Menz and Seip, 2004). Livestock production system is generating GHGs and they are likely to contribute to the global warming (Owen and Silver, 2015; Philippe and Nicks, 2015).

The GHGs have the potential to absorb and emit infrared radiation that increases the earth's temperature and cause global warming (IPCC, 2001). The principal GHGs are water vapor, ozone (O_3), carbon dioxide (CO_2), methane (CH_4) nitrous oxide (N_2O), chlorofluorocarbon, per-fluorocarbon and sulfur hexafluoride; however CH_4, N_2O and CO_2 are the major GHGs emitted from livestock production system (Owen and Silver, 2015; Philippe and Nicks, 2015). It is estimated that 3.4% of the total GHGs emissions in the USA is emitted from livestock (USEPA, 2009). Methane is emitted mostly from livestock production system as a result of enteric fermentation in rumen and decomposition of manure in the manure treatment and management facilities. Similarly, N_2O is produced during alternate aerobic and anaerobic decomposition of livestock manure (Spellman and Whiting, 2010). Though the reported contribution of CH_4 and N_2O are only around 9.5% and 5.3%, respectively, to the total GHG emissions (USEPA, 2015); the global warming potential of these gases are 25 and 298 times of CO_2, respectively (USEPA, 2015). On the other hand, CH_4 and N_2O emission from manure management has increased by 68% and 25%, respectively, since 1990 (USEPA, 2014). Researchers around the world are seeking technologies and management practices to mitigate gaseous emission of these gases from livestock production facilities (Hristov et al., 2013; Meale et al., 2012; Schader et al., 2014). Among treatment options, diet manipulation is one of the prominent options for minimizing the total gaseous emission (enteric and from manure management) (Chuntrakort et al., 2014; Hulshof et al., 2012; Osada et al., 2011).

The manure management is one of the major source for both CH_4 and N_2O emission; however, a larger portion of CH_4 (25.9% of total CH_4 emission) is also emitted from enteric fermentation in rumens (USEPA, 2015). Basically, the enteric CH_4 production in rumen is affected by the feeding practice and feed composition of cattle. The chemical composition of feed influence the rumen chemistry, which ultimately determine the CH_4 production (Dutreuil et al., 2014). Specifically, the diet composition can affect rumen pH, carbon nitrogen ratio, nutrient composition of manure, odor, and gaseous emissions from manure system (Bowman et al., 2000; Mirabelli et al., 2006). In animal diet, carbohydrate and amount of intake influence the production of individual volatile fatty acids (VFAs), which is directly related with CH_4 production. Feed containing more sugar and starch component favors propionic acid production resulting in less CH_4 production (Johnson and Johnson, 1995). Carbohydrate has the greatest impact on pH, microbial population, and VFA concentration which influences on CH_4 production. Similarly, an increase of fat level on cattle diet increases the energy density of the diet, and also help to decrease enteric CH_4 generation (Mathison et al., 1998).

The addition of supplemental fat in the diet is common for beef cattle. The fat content of commercial beef cattle feed usually consists of 2-5% of fat in composite sample (Eastridge, 2002). If the fat content in feed exceed 6%, it can cause digestive disturbance, diarrhea, and reduce feed intake (Ziegler, 2007). Many researchers have conducted the experiments using fat and oil in beef cattle diets and observed its impact on body performance, weight gain, cold tolerance, and gaseous emission from body and manure. Engstrom et al. (1994) conduct a feeding trial on feedlot performance and carcass quality with beef cattle in Canada using 0%, 2% and 4% fat from canola oil in diet. They found an increase of 9.8% in daily weight gain with an addition of 4% fat in diet during the first 56 days.

The increase of fat level in the diet may affect metabolic changes in the ruminant. It may favor the production of propionic acid, which can reduce CH_4 generation. In addition, the supplementary fat can also lower the digestibility of fermentable substrate in rumen, bio-hydrogenate the unsaturated fat, and decrease methanogens population in rumen by producing hydrogen acceptor; then ultimately reduce CH_4 emission (Johnson and Johnson, 1995). Mathison (1997) reported 33% reduction in enteric CH_4 production is achievable by adding 4% of canola oil in a steer diet containing 85% concentrate. Beauchemin and McGinn (2006) carried out an experiment using fumeric acid, essential oil, and canola oil on the beef cattle diet to see their effect on enteric CH_4 emission. Their results showed a reduction on CH_4 emission using canola oil; though essential oil and fumeric acid did not influence ruminal fermentation or CH_4 emission. Similarly, Beauchemin et al. (2009) used the fat sources from different oil seeds like sunflower, canola and flaxseed to feed the cattle, and observed significant CH_4 reduction in all cases.

Corn based distiller's dried grain with solubles (DDGS) is a by-product from the ethanol industries and widely used in livestock diets. Usually, DDGS contains 12 to 15% oil on dry basis; however, partial removal of corn oil is common in the ethanol industry. In general, presence of 3 to 9% corn oil has been reported in the processed DDGS (Anderson and Engel, 2014). In beef cattle diet, DDGS is playing a major ingredient comprising of 42% of the total diet (Lardy and Anderson, 2014). Besides DDGS; corn silage, hay, sunflower meal, and concentrated separator by-product (CSB) are some other common ingredients added in beef cattle diet. The variation on fat level on the overall diet can be achieved by mixing the right amount of DDGS. However, no studies have been reported on the effect of various fat levels from DDGS on gaseous emission and manure composition from the feedlot pen surfaces. Therefore, the objective of this study was to investigate the effect of different fat levels in beef cattle diet on manure nutrient composition and GHG emission from feedlot pen surfaces.

Materials and methods

Feedlot description and experimental design

The research was carried out in a research feedlot at Carrington Research Extension Center (CREC) of North Dakota State University. This feedlot had 16 pens and each pen had an area of 433 m^2 (≈19 m × 23 m). The overall slope of the feedlot was around 3%. Total 182 Angus-steer calves were used in this study. Fall-born (n=92) and spring-born (90) Angus-steer calves were blocked by weight (four groups viz. light, medium light, medium heavy and heavy). Four types of diets having different concentration of fat level (high, medium, low and control) were provided to the steers. The steers were allocated to one of four treatment groups. After blocking, the group of steers were allocated to one of 16 pens so that each pen had 11 to 12 steers. In brief, the placement of the steers was such that each group was provided with four different diet levels as treatment with four replication per treatments in a randomized complete block design. Initially, the finishing ration was provided to heavy and medium heavy animals while the growing ration was provided to light and medium light animals. So, the blocking of animals were basically on heavy and light weight basis of animals. However, after June same ration (finishing) was provided to both group. This study was conducted from June to October of 2013. The information about animal number, blocking groups, feeding strategies, treatment category and weight of animals on each pen on different time period has been provided in Table 16.

Weather condition

During each sampling, the pen surface temperatures were measured using an infrared thermometer (MiniTemp-MT6 Instrument, Carlsbad, CA). Ambient temperature, wind speed, solar radiation, and rainfall were collected from the North Dakota Agricultural Weather Network

- NDAWN site, NDSU Carrington Research and Extension center, which was 2 km from the study site.

Dietary composition

In this study, the effects of four different fat levels of diet (high, medium, low and control) on beef cattle performance, manure composition and gaseous emissions from feedlot pen surfaces were studied. Three different DDGS product sourced from different ethanol plants were used to obtain different oil level. High fat treatment group consisted of DDGS purchased from High-water Ethanol, Lamberton, MN; and consisted of 12.96% corn oil (no corn removal). Medium fat treatment group consisted of DDGS purchased from Blue Flient Ethanol, Washburn, ND; which consisted of 8.05% corn oil (partial removal). Similarly, low fat treatment group consisted of DDGS purchased from POET, Groton, SD; consisted of 5.47% corn oil (higher removal). The control diet included sunflower meal consisted of 2.44% oil, which represent the general condition of North Dakota. Besides DDGS, other ration ingredients were chopped grass hay, dry-rolled corn grain, corn silage, condensed separator by produce and supplements like vitamins and minerals. The diets were formulated to meet the nutrient requirement recommended by NRC (1996). Overall, the fat content of high, medium, low, and control diet (composite diet) were 5.07, 4.12, 3.6, and 3.19%, respectively in the growing ration and they were 5.48, 4.52, 4.02, and 3.58%, respectively in the finishing ration. The diet ingredients and the nutrient composition of composite diet is listed in Tables 17, and the nutrient composition of each ingredient is listed in Table 18**.**

Table 16. Summary of animal weight, feeding stage, treatment diet, and animal weight at a different period.

Pen	Animal weight	Feeding stage	Treatment diet	Animal number	Weight of animals (kg)				
					7 June	17 July	14 August	11 Sept.	2 Oct.
Pen 1	Heavy	Finish	Medium fat	11	448	528	588	642	683
Pen 2	Heavy	Finish	Low fat	12	451	533	595	654	697
Pen 3	Heavy	Finish	High fat	11	448	535	608	671	715
Pen 4	Heavy	Finish	Control	11	446	529	586	651	695
Pen 5	Medium- heavy	Finish	Medium fat	11	411	504	569	632	677
Pen 6	Medium- heavy	Finish	Control	11	413	494	559	619	661
Pen7	Medium- heavy	Finish	High fat	11	412	490	556	622	661
Pen 8	Medium- heavy	Finish	Low fat	12	413	489	548	613	655
Pen 9	Medium-light	Growing/Finish	Medium fat	11	358	426	491	552	628
Pem10	Medium-light	Growing/Finish	Low fat	11	358	426	487	545	629
Pen 11	Medium-light	Growing/Finish	High fat	12	360	433	508	572	649
Pen 12	Medium-light	Growing/Finish	Control	12	360	429	500	554	638
Pen 13	Light	Growing/Finish	Medium fat	11	307	380	441	505	585
Pen 14	Light	Growing/Finish	High fat	12	307	384	455	516	596
Pen 15	Light	Growing/Finish	Control	11	306	383	448	505	594
Pen 16	Light	Growing/Finish	Low fat	12	309	386	443	506	588

Table 17. Diet ingredient and nutrient composition of growing and finishing ration.

Diet ingredients	Growing Rations				Finishing Rations			
	Control	High Fat	Med. Fat	Low Fat	Control	High Fat	Med. Fat	Low Fat
Corn (%)	48.76	43.25	42.76	43.09	66.68	61.02	60.95	60.96
DDGS (%)	--	18.89	18.76	18.7	--	19.4	19.34	19.4
Sunflower meal (%)	13.24	--	--	--	13.3	--	--	--
Hay (%)	16.96	16.95	17.06	16.96	11.13	11.27	11.29	11.29
Corn silage (%)	12.85	12.9	13.18	13.04	--	--	--	--
CSB (%)	6.32	6.3	6.27	6.29	6.76	6.73	6.73	6.73
Supplement (%)	1.87	1.71	1.96	1.92	1.56	1.58	1.69	1.62
Nutrient Composition								
CP (%)	12.09	11.85	12.39	12.53	12.42	12.12	12.7	12.88
NEm (Mcal kg^{-1})	0.37	0.37	0.37	0.37	0.37	0.41	0.41	0.41
NEg (Mcal kg^{-1})	0.23	0.23	0.23	0.23	0.24	0.27	0.27	0.27
Fat (%)	3.19	5.07	4.12	3.64	3.58	5.48	4.52	4.02

Note: DDGS = Distiller's dried grains with solubles; CSB = Concentrated separator by-product; CP= Crude protein; NEm= Net energy of maintenance; NEg = net energy of gain.

Table 18. Nutrient composition in each diet ingredient.

Ingredient	DM %	CP %	ADF %	TDN %	NEm Mcal kg^{-1}	NEg Mcal kg^{-1}	Fat %
Corn	87.66	8.33	3.58	87	0.45	0.31	4.37
Corn silage	32.90	7.53	28.69	68	0.33	0.20	2.59
Mixed hay	85.74	7.42	44.70	52	0.22	0.10	1.88
Sunflower meal	90.63	39.44	22.77	70	0.34	0.21	2.44
DDGS medium fat	89.26	31.90	16.48	87	0.44	0.29	8.05
DDGS high fat	88.74	28.76	15.74	88	0.44	0.29	12.96
DDGS low Fat	88.83	32.69	11.93	92	0.43	0.29	5.47
CSB	71.56	10.07	0.19	86	0.42	0.28	1.28

Note: DDGS = Distiller's dried grains with solubles; CSB = Concentrated separator by-product; DM = Dry matter; CP = Crude protein, ADF= Acid detergent fiber; TDN = Total digestible nutrients; NEm = Net energy for maintenance and NEg = Net energy for gain.

Gaseous sampling and analysis

Air samples from the pen surface were collected for five times during June to October 2013 with a sampling interval of 30±10 days. Air samples were collected using a custom built portable wind tunnel (0.8 m × 0.4 m), Tedlar bag, and Vac-U-Chamber (SKC Inc., Eighty Four, PA) (Figure 6). In each sampling location, a 5 L Tedlar bag was placed inside the air sample box and a uniform air flow rate (1.75 m^3 sec^{-1}) was maintained inside the tunnel throughout the sampling period using a DC motor. Additional sampling protocol can be found at Rahman et al. (2013). In each pen, two samples were collected; one from the front end of the pen next to feeding area, and another one from the backside of the pen. So, a total of 160 air samples (16 pens × 2 samples per pen × 5 times) were collected and they were brought back to the laboratory for hydrogen sulfide (H_2S) and GHGs (i.e. methane-CH_4, carbon dioxide-CO_2, and nitrous oxide-N_2O) analysis. Within 24 hours of sampling, they were analyzed for GHGs using a greenhouse gas monitoring gas-chromatography (Model No. 8610C, SRI Instruments, and 20720 Earl St., Torrance, CA 90502), and H_2S using a Jerome meter (Jerome® 631-X, Arizona instrument, Arizona, USA). The GC was equipped with a flame ionization detector (FID) to measure CO_2 and CH_4 and an electron captured detector (ECD) to analyze N_2O. GHG was analyzed following the procedure described in Rahman et al. (2013).

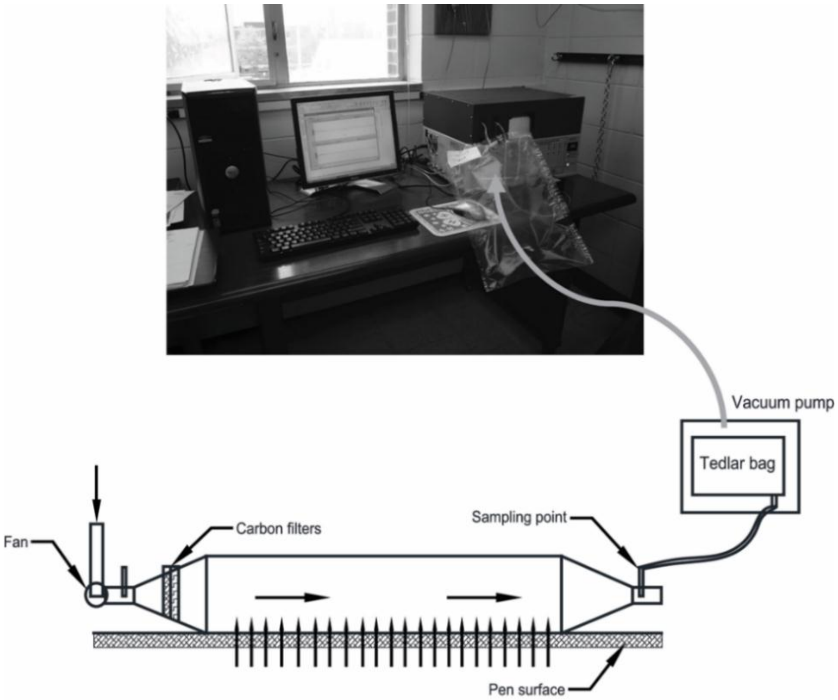

Figure 6. Schematic diagram of field gas collection system and GHG analysis using a gas chromatograph (drawing not to scale).

Manure sampling and analysis

During each sampling event, composite manure samples were collected from each pen in a zip-locked bag, brought back to the lab, and stored in a refrigerator at around 4°C until analysis. Then, samples were subdivided and analyzed for pH, moisture and ash content. Likewise, the sub-samples were also analyzed for volatile fatty acids (VFAs), total carbon (TC), crude protein (CP), total phosphorous (TP), potassium (K), total nitrogen (TN), and fecal ammonia (NH_3) using the methods listed in Table 19.

Table 19. Method/protocol used to analyze manure samples.

Parameters	Methods/protocol used
TN	Recommended methods of manure analysis, A3769 Macro-Kjeldahl method (adapted from Kane, 1998)
K	Recommended method of manure analysis, A3769
TP	Recommended method of manure analysis, A3769
TC	U.S. EPA method 415.1: Catalytic combustion and non-dispersive infrared detection (NDIR) method
CP	Official Method 2001.11, AOAC International (2005) 18th ED., AOAC International Gaithersburg, MD, USA
Fecal NH_3	Sigma Technical Bulletin #640. Sigma Diagnostics, St. Louis, MO 63178
VFA	Method of Goetsch and Galyean, 1983. Agilent 6890N Gas Chromatograph with an FID (flame ionization detector) and the 7683 Series auto-injector and autosampler. Column used was the Supelco brand, NUKOL Fused Silica Column, 15 m x 0.53 mm x 0.5 um

AOAC = Association of Official Agricultural Chemists

Emission calculation

In order to estimate the emission rate; the volumetric gas concentration was standardized at standard pressure and temperature (1 atmosphere and 25°C). Mass concentration of the compound was calculated from normalized volumetric concentration (Equation 25). Flux rates (g m^{-2} d^{-1}) was calculated using the average airflow through the wind tunnel, mass concentration of the target gas and the surface area covered by the wind tunnel as shown in Equation 26. Finally, emission rate was estimated using the surface area of the pen, flux rate, and animal unit (AU) in the pen (Equation 27).

$$C_{mass} = \frac{C_{ppm} \times MW}{24.45} \tag{25}$$

$$\mathrm{FR} = \frac{\mathrm{C_{mass}} \times \mathrm{V_{WT}} \times 3600 \times 24}{\mathrm{A_{wT}} \times 1000} \tag{26}$$

$$\mathrm{ER} = \frac{\mathrm{FR} \times \mathrm{A_{SC}}}{\mathrm{AU}} \tag{27}$$

where, C_{ppm}= Volumetric concentration of the target gas (ppm)

C_{mass}= Mass concentration of the target gas (mg m^{-3})

MW= Molecular weight of the target gas (g mol^{-1})

FR= Emission flux rate from pen surface (g m^{-2} d^{-1})

24.25= Volume per mole of an ideal gas at standard temperature and pressure (L mol^{-1})

V_{wt}= Airflow rate through wind tunnel (m^3 s^{-1})

A_{wt}= Surface area covered by the wind tunnel (0.4 × 0.8 m^2)

ER= Emission rate from pen surface (g hd^{-1} d^{-1})

A_{sc}= Surface area of the source (m^2)

AU= Animal unit (total weight of animals in pen divided by 500 kg live weight)

Statistical analysis

The effect of fat levels in the diet on GHG emission and manure composition were compared using the Generalized Liner Model (GLM) procedure in SAS software (SAS 9.3, 2002-2010). Randomized complete block design was chosen for each sampling event (months) with animal weight as a block (light and heavy) for four treatments (control, low, medium and high). However, during analysis no significant difference of treatments were observed separating the animals on weight basis. Therefore, a comparative study of different treatments were carried considering the animal types as a single block. All significance tests were evaluated at P=0.05. The null hypothesis of the analysis was that the means value of GHGs concentrations, emission flux, emission rates, and manure's nutrient and VFAs concentrations were equal within and among treatments and sampling time.

Results and discussion

Ambient weather and feedlot pen surface temperature

The daily mean air temperature, wind speed, solar irradiation, and rainfall at the sampling locations during each sampling period are listed in Table 20. August sampling time had the highest ambient temperature, while October had the lowest ambient temperature. Likewise, the highest pen surface temperature was noted in August, which equate to the ambient temperature. Similarly, the lowest pen surface temperature was observed in September (Figure 7). Overall, average pen surface temperatures were very consistent among pens in each sampling time. Besides temperature, solar radiation was also the highest in August, and the lowest in September. During the sampling time, no noticeable rainfall was observed, which might have some effects on gaseous emission from the manure pen surface.

Table 20. Ambient weather condition at the study site.

Sampling date	Air temperature (^{o}C)			Average wind speed (mph)	Solar radiation (MJ m^{-2})	Rainfall (mm)
	Average	Minimum	Maximum			
20-Jun-13	20.56	17.78	23.33	11.40	7.57	0.00
30-July-13	16.67	10.56	22.78	4.80	16.99	0.80
20-Aug-13	26.11	16.11	36.11	6.20	23.01	0.00
18-Sep-13	18.33	13.89	22.78	5.70	5.19	0.00
9-Oct-13	11.11	2.78	19.44	3.40	10.08	0.00

It is known that temperature variation on the pen surface effects the gaseous emission. Usually, higher temperature enhances CH_4 production (van Winden et al., 2012). The temperature range of 25 - 30˚C is considered as optimum temperature for CH_4 production

(Boeckx and Van Cleemput, 1996). Surface temperature also influences on N_2O emission. Luo et al. (2013) reported the highest N_2O emission in case of the moist and warm soil, and the soil exposed to freezing and thawing condition. Lang et al. (2012) had also experienced the promotion of nitrification and N_2O emission under higher soil temperature. In this study, higher emission of CH_4 and N_2O is expected on July and August months due to an environmental condition.

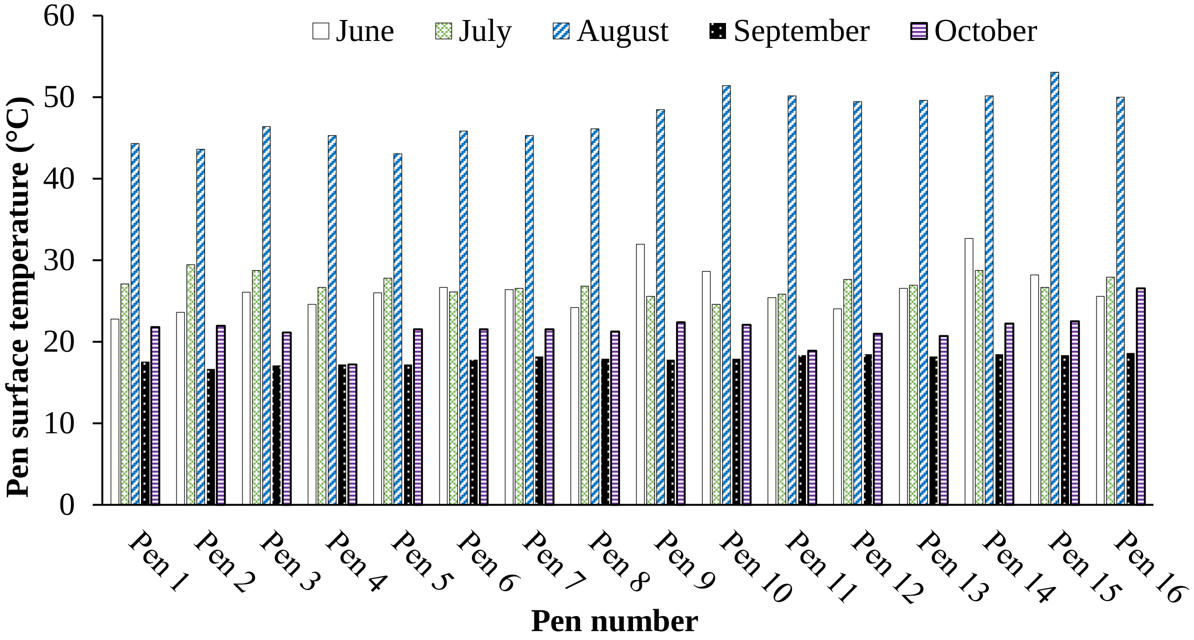

Figure 7. Variation in feedlot pen surface temperature during the experimental period.

Effect of dietary fat levels on manure composition

Overall, no significant differences in manure composition were observed among the treatments in most of the months, but significant differences on some manure composition were observed over the sampling period. In August and September, the total volatile fatty acid (TVFA) content were significantly lower in the high fat group than the others (Table 21), which may contribute to lower CH_4 emission. Likewise in August, the moisture content, crude protein (CP), and TN were also significantly lower in the manure from pens with cattle fed with high fat diet than the other treatment groups.

Table 21. Manure composition for each month based on treatment.

Sampling date	Manure composition	Treatments			
		control	Low fat	Medium fat	High fat
20-jun-13	pH	5.4 a±.0.2	5.6 a ± 0.4	5.5 a ± 0.4	5.4 a ± 0.2
	Moisture %	76.7 a ±1.9	76.9 a ± 1.0	74.2 a ± 5.5	77.4 a ± 1.6
	Ash %	11.1 a ± 1.4	13.9 a ± 4.2	10.0 a ± 0.8	10.4 a ± 1.6
	CP %	14.9 a[1] ± 0.8	14.9 a ± 1.4	13.7 a ± 2.8	15.1 a ± 0.6
	TN %	2.4 a ± 0.1	2.4 a ± 0.2	2.2 a ± 0.5	2.4 a ± 0.1
	NH$_3$ (mM)	5.6 a ± 1.2	4.9 a ± 1.7	5.5 a ± 1.6	4.7 a ± 1.4
	TC (%)	43.7 a ± 1.0	43.2 a ± 1.5	44.8 a ± 0.5	43.7 a ± 1.0
	TP %	0.1 a ±0.1	0.2 a ± 0.1	0.2 a ± 0.1	0.1 a ± 0.0
	K %	0.1 a ± 0.0	0.1 a ± 0.0	0.1 a ± 0.0	0.1 a ± 0.0
	TVFA (mM)	120.7 a ± 27.6	101.7 a ± 37.4	105.1 a ± 8.6	127.1 a ± 24.2
30-Jul-13	pH	5.5 a ± 0.1	5.4 a ± .2	5.5 a ± .4	5.5 a ± 0.1
	Moisture %	77.8 a ± 0.6	77.2 a ± 1.5	76.8 a ± 1.5	75.5 a ± 2.6
	Ash %	9.1 a ± 0.8	8.2 a ± 0.3	10.2 a ± 1.3	8.5 a ± 1.6
	CP %	15.6 a ± 1.5	15.4 a ± 1.1	16.3 a ± 0.8	14.9 a ± 1.0
	TN %	2.5 a ± 0.3	2.5 a ± 0.2	2.6 a ± 0.1	2.4 a ± 0.1
	NH$_3$ (mM)	5.2 a ± 1.7	5.5 a ± 1.5	9.4 a ± 5.7	8.9 a ± 2.1
	TC (%)	44.4 a ± 0.4	43.6 a ± 1.8	44.3 a ± 0.4	45.4 a ± 0.8
	TP %	0.1 a ± 0.0	0.1 a ± 0.0	0.1 a ± 0.0	0.1 a ± 0.0
	K %	0.1 a ± 0.0	0.1 a ± 0.0	0.1 a ± 0.0	0.1 a ± 0.0
	TVFA (mM)	150.2 a ± 18.9	149.0 a ± 32.7	176.4 a ± 42.2	148.7 a ±10.9
20-Aug-13	pH	5.2 a ± 0.1	5.1 a ± 0.3	5.1 a ± 0.1	5.0 a ± 0.2
	Moisture %	73.7 ab ± 1.2	73.3 ab ± 2.2	75.6 a ± 0.7	72.2 b ± 1.9
	Ash %	8.1 a ± 0.5	8.9 a ± 1.1	7.7 a ± 0.6	7.4 a ± 1.0
	CP %	15.2 ab ± 1.1	17.6 a ± 1.4	17.4 ab ± 1.8	14.9 b ± 0.6
	TN %	2.4 ab ± 0.2	2.8 a ± 0.2	2.8 ab ± 0.3	2.4 b ± 0.1
	NH$_3$ (mM)	12.8 a ± 1.2	17.1 a ± 9.0	16.8 a ± 6.8	11.3 a ± 3.7
	TC (%)	44.2 a ± 1.3	45.0 a ± 2.2	44.3 a ± 0.5	42.9 a ± 1.8
	TP %	0.1 a ± 0.0	0.1 a ± 0.1	0.1 a ± 0.0	0.2 a ± 0.0
	K %	0.1 a ± 0.0	0.1 a ± 0.0	0.1 a ± 0.0	0.1 a ± 0.0
	TVFA (mM)	147.6 ab ± 34.8	178.2 a ± 19.9	142.0 ab ± 42.5	128.8 b ± 47.5
18-Sep-13	pH	5.6 a ± 0.2	5.4 a ± 0.2	5.4 a ± 0.3	5.5 a ± 0.1
	Moisture %	75.4 a ± 1.4	74.9 a ± 2.0	75.1 a ± 2.8	75.7 a ± 1.3
	Ash %	9.5 a ± 2.1	8.0 a ± 1.3	8.7 a ± 1.0	7.9 a ± 1.0
	CP %	15.0 a ± 1.5	15.0 a ± 2.6	15.6 a ± 0.8	15.0 a ± 1.1
	TNm %	2.4 a ± 0.2	2.4 a ± 0.4	2.5 a ± 0.1	2.4 a ± 0.2
	NH$_3$ (mM)	10.0 ab ± 2.9	7.6 b ± 3.3	8.0 ab ± 2.1	11.5 a ± 2.3
	TC (%)	44.4 a ± 0.8	44.1 a ± 0.7	43.5 a ± 1.7	44.9 a ± 0.4
	TP %	0.2 a ±0.1	0.1 b ± 0.0	0.1 b ± 0.0	0.1 b ± 0.0
	K %	0.1 a ± 0.0	0.1 a ± 0.0	0.1 a ± 0.0	0.1 a ± 0.1
	TVFA (mM)	129.2 a ± 15.0	109.0 ab ± 14.4	114.5 ab ± 17.9	105.3 b ± 11.1
9-Oct-13	pH	5.5 a ± .01	5.6 a ± 0.1	5.9 a ± .02	5.3 a± 0.0
	Moisture %	72.6 a ± 0.5	72.9 a ± 0.0	72.4 a ± 3.6	72.4 a ± 3.0
	DM %	95.6 a ± 0.2	96.7 a ± 0.0	96.6 a ± 0.5	96.1 a ± 0.3
	Ash %	8.7 a ± 1.3	8.5 a ± 1.2	19.9 a ± 10.9	6.9 a ± 0.9
	CP %	16.4 a ± 1.2	14.9 a ± 1.2	13.9 a ± 1.1	15.0 a ± 0.6
	TN %	2.6 a ± 0.2	2.4 a ± 0.2	2.2 a ± 0.2	2.4 a ± 0.1
	NH$_3$ (mM)	6.9 a ± 0.9	14.9 a ± 2.7	19.0 a ± 5.7	8.0 a ± 1.1
	TC (%)	44.0 a ± 0.9	39.9 a ± 5.0	37.2 a ± 7.2	44.8 a ± 0.2
	TP %	0.1 a ± 0.0	0.2 a ± 0.1	0.1 a ± 0.0	0.1 a ± 0.0
	K %	0.1 a ± 0.0	0.2 a ± 0.0	0.2 a ± 0.0	0.1 a ± 0.0
	TVFA (mM)	144.5 a ± 18.4	108.8 a ± 0.5	113.5 a ± 16.2	123.1 a ±8.0

Values followed by the same letter in row are not significantly different at $P \leq 0.05$.

However, when the analysis was simply carried out on a time basis (comparison among months), a significant difference on most of the parameters of manure composition were observed (Table 22). Manure pH was significantly lower in August as compared to other months. Similarly, the moisture content of manure was significantly lower in October as compared to June, July, and September as shown in Table 22. Ash content of manure was the highest in June and the lowest in August. Crude protein, TP, and fecal NH_3 content in manure were the lowest in June and the highest in August. Total carbon (TC) in manure was significantly lower in August as compared to other months. Likewise, the TVFA content of manure was significantly higher in July and August compared to other months (Table 22), which is likely due to temperature effect on VFA production. Due to higher TVFA, comparatively higher CH_4 emission can be expected during July and August. In 2012 summer, Borhan et al. (2013) had also measured the nutrient composition of the manure in the same feedlot under similar condition and the values of different nutrient parameters were almost comparable with this study.

Table 22. Manure composition on monthly basis.

Parameters	June	July	August	September	October
pH	5.5 a[*] ± 0.1	5.5 a ± 0.0	5.1 b ± 0.1	5.5 a ± 0.1	5.6 a ± 0.2
Moisture %	76.32 a ± 1.2	76.8 a ± 0.8	73.7 bc ± 1.2	75.3 ab ± 0.3	72.6 c ± 0.2
Ash %	11.3 a ± 1.5	9.0 abc ± 0.8	8.0 c ± 0.6	8.5 bc ± 0.5	11.0 ab ± 5.2
CP %	14.6 a ± 0.6	15.5 ab ± 0.5	16.2 b ± 1.2	15.1 ab ± 0.3	15.0 ab ± 0.9
TN %	2.3 a ± 0.1	2.5 ab ± 0.1	2.6 b ± 0.2	2.4 ab ± 0.0	2.4 ab ± 0.1
NH_3 (mM)	5.2 d ± 0.4	7.3 cd ± 1.9	14.5 a ± 2.5	9.3 bc ± 1.6	12.2 ab ± 5.0
TC (%)	43.8 a ± 0.6	44.4 a ± 0.6	44.1 a ± 0.8	44.2 a ± 0.5	41.5 b ± 3.1
TP %	0.1 a ± 0.0	0.1 a ± 0.0	0.1 a ± 0.0	0.2 a ± 0.0	0.1 a ± 0.0
K %	0.1 a ± 0.0	0.1 a ± 0.0	0.1 a ± 0.0	0.1 a ± 0.0	0.1 a ± 0.0
TVFA (mM)	113.7 b ± 10.6	156.1 a ± 11.8	149.2 a ± 18.1	114.5 b ± 9.1	122.5 b ± 13.7

[*] Values followed by the same letter in row are not significantly different at $P \leq 0.05$.

Effect of dietary fat level on volatile fatty acid (VFAs) composition of manure

No or little significant differences in any of VFAs concentration were observed among treatments during the study period. However, in July, isovaleric acid was significantly higher in manure from pens with cattle fed the low-fat diet than the control. Likewise, in September the butyric acid was significantly higher in the manure from pens with cattle fed the medium fat diets compared to the control (Table 23). However, when the analysis carried out on a timely basis, the lowest acetic acid concentration and the highest propionic acid concentrations were observed in August (Table 24).

During anaerobic decomposition of manure; acetic, propionic, butyric and valeric acids are the common VFAs produced by the micro-organisms. Acetic acid is the major VFA responsible for CH_4 production from the anaerobic biomass which accounts more than two third of CH_4 production (Zinder, 1990). Propionic and butyric acids are considered as the inhibitory agents in the anaerobic process (Ward et al., 2008). Higher concentration of propionic usually inhibits the CH_4 production in case of an anaerobic digester (Boone and Xun, 1987); however, some researchers have mentioned that it's the effect rather than cause for the inhibition of CH_4 production (Gourdon and Vermande, 1987; Pullammanappallil et al., 2001). The ratio of acetic acid and propionic acid is another important factor for determining the CH_4 production rate. Higher acetic acid (>800 mg L^{-1}), following propionic acid and acetic acid ratio more than 1.4 is taken as the indicator for failure of the anaerobic process (Marchaim and Krause, 1993). However, in this study, the ratio of propionic acid acetic acid was <1:4 (Table 23), which was an indicator of anaerobic process on the pen surface.

Environmental temperature has also an impact on ruminal VFA content. Kelley et al. (1967) have reported that higher the ambient temperature, higher is the acetic acid and lower the propionic acid in ruminal fluid of cattle. Lippke (1975) observed that temperature variation has slight shifts in ruminal VFA concentrations, which might have effects on manure VFAs. However, in this study the ruminal VFA was not measured.

Table 23. Volatile fatty acids (VFAs) content in manure measured based on the treatment.

Sampling date	VFAs (mM)	Treatments			
		control	Low fat	Medium fat	High fat
20-Jun-13	Acetic	49.5 a* ± 3.2	51.3 a ± 3.5	46.0 a ± 1.5	46.5 a ± 4.8
	Propionic	17.0 a ±2.1	17.3 a ± 2.5	20.2 a ± 4.1	19.4 a ± 4.0
	Isobutyric	2.1 a ± 1.3	2.0 a ± 0.6	2.0 a ± 1.4	2.1 a ± 0.6
	Butyric	24.0 a ± 2.6	21.7 a ± 3.0	25.7 a ± 3.7	23.0 a ± 1.5
	Isovaleric	4.6 a ± 1.4	3.2 a ± 1.9	4.6 a ± 1.7	3.9 a ± 1.3
	Valeric	2.8 a ± 3.0	4.5 a ± 1.3	1.5 a ± 1.5	5.1 a ± 1.8
30-Jul-13	Acetic	51.4 a ± 2.2	49.5 a ± 2.4	51.1 a ± 2.9	50.9 a ± 2.8
	Propionic	23.5 a ± 1.4	19.8 a ± 3.4	21.3 a ± 3.3	19.5 a ±2.3
	Isobutyric	1.4 a ± 0.3	1.5 a ± 0.4	1.7 a ± 0.6	1.7 a ± 0.2
	Butyric	21.0 a ± 1.2	24.4 a ± 2.3	22.1 a ± 1.8	22.8 a ± 1.7
	Isovaleric	1.3 b ± 0.3	3.5 a ± 0.5	2.2 ab ± 0.8	3.4 ab ± 0.3
	Valeric	1.4 a ± 1.3	1.4 a ± 1.0	1.6 a ± 1.7	1.6 a ± 0.9
20-Aug-13	Acetic	46.0 a ± 3.7	44.7 a ± 2.8	45.6 a ± 2.4	49.0 a ± 5.6
	Propionic	25.4 a ± 1.3	25.6 a ± 3.9	24.7 a ± 2.1	22.0 a ± 3.5
	Isobutyric	1.5 a ± 0.2	1.4 a ± 0.3	1.2 ab ± 0.4	0.7 b ± 3.6
	Butyric	22.4 a ± 2.4	22.4 a ± 2.4	23.4 a ± 1.4	24.6 a ± 3.6
	Isovaleric	1.3 a ± 0.1	1.5 a ± 0.6	1.3 a ± 0.4	0.9 a ± 0.3
	Valeric	3.5 a ± 0.5	4.4 a ± 1.7	1.3 a ± 1.2	2.9 a ± 1.1
18-Sep-13	Acetic	48.9 b ± 0.6	50.8 ab ± 0.6	53.5 a ± 2.1	51.2 ab ± 2.5
	Propionic	22.7 a ± 1.7	22.4 a ± 1.1	20.9 a ± 2.3	21.9 a ± 1.0
	Isobutyric	1.1 a ± 0.3	0.8 a ± 0.1	1.0 a ± 0.5	1.2 a ± 0.3
	Butyric	23.7 a ± 1.0	22.4 ab ± 1.8	20.6 b ± 1.6	21.3 ab ± 1.0
	Isovaleric	1.0 a ± 0.3	0.8 a ± 0.2	1.0 a ± 0.5	1.2 a ± 0.2
	Valeric	2.5 a ± 0.6	2.8 a ± 1.0	3.0 a ± 1.3	3.6 a ± 1.2
9-Oct-13	Acetic	53.5 a ± 0.9	49.8 b± 0.0	51.9 ab ± 0.3	50.2 b ±0.8
	Propionic	20.9 a ±0.1	20.6 a ± 0.6	20.2 a ± 1.7	22.8 a ± 0.7
	Isobutyric	0.9 a ± 0.0	1.2 a ± 0.0	1.4 a ± 0.4	0.4 a ± 0.5
	Butyric	21.8 a ± 0.2	23.7 a ± 0.9	21.8 a ± 0.5	24.4 a ± 1.3
	Isovaleric	0.9 a ± 0.3	1.2 a ± 0.1	1.4 a ± 0.3	0.6 a ± 0.2
	Valeric	1.9 a ± 0.2	3.5 a ± 1.5	3.3 a ± 1.5	1.5 a ± 1.5

*Values followed by the same letter in row are not significantly different at P ≤ 0.05.

Table 24. Monthly volatile fatty acid (VFA) analysis of manure.

Parameters	June	July	August	September	October
Acetic	48.3 bc* ± 2.2	50.7 ab ± 0.7	46.3 c ± 1.6	51.1 ab ± 1.6	51.4 a ± 1.5
Propionic	18.5 c ± 1.4	21.0 b ± 1.6	24.4 a ± 1.4	22.0 b ± 0.7	21.1 b ± 1.0
Isobutyric	2.0 a ± 0.1	1.6 ab ± 0.1	1.2 bc ± 0.3	1.0 c ± 0.2	1.0 c ± 0.4
Butyric	23.6 a ± 1.5	22.6 a ± 1.2	23.2 a ± 0.9	22.0 a ± 1.2	22.9 a ±1.1
Isovaleric	4.1 a ± 0.6	1.5 b ± 0.1	1.2 b ± 0.2	1.0 b ± 0.1	1.0 b ± 0.3
Valeric	3.5 a ± 1.4	2.6 a ± 0.9	3.7 a ± 0.6	2.9 a ± 0.2	2.6 a ± 0.8

*Values followed by the same letter in row are not significantly different at P ≤ 0.05.

Effect of dietary fat level on GHG emission

Overall, no significant difference in GHGs emissions was observed from the feedlot pen surfaces with beef cattle fed four levels of fat (control, low, medium, high) in the diets (Table 25). However, some variations on GHG emission were observed when the measurement was compared between months. In July and September, the highest CO_2 efflux was observed from pen surface with cattle fed medium fat content diet. The increased of fat source in the diet is most likely to increase dietary energy, suppress methanogens decreasing CH_4 emission (both enteric and from manure) as well as reduce nitrogen emission from manure (Johnson and Johnson, 1995; Machmüller et al., 2006).

The effect of fat on gaseous emission depends on many factors; such as the type of fat, the amount of fat in feed, and environmental condition. As Beauchemin and McGinn (2006) had also used three different types of fat sources (canola oil, essential oil, and fumaric acid) to feed beef cattle; and found only the effect of canola oil on enteric CH_4 production. Similarly, Beauchemin et al. (2009) added 3.1 to 4. 2% of fat from 3 different sources like sunflower, canola, and flaxseed individually to the feed of dairy cow; and observed 10, 16 and 18% reduction in enteric CH_4 production, respectively. Though, the addition of fat might have an effect on enteric CH_4 production, but it may not greatly influence the CH_4 production from the pen surface area. In pen surface area, the emission is most likely to influence from the environmental factors. The environmental condition were almost similar in all the pen surfaces; therefore, very less variation in gaseous emission might have observed under different treatments conditions. In addition, the reduction of CH_4 concentration using supplementary fat may not be applicable for corn oil; or the application rate of corn oil used in this research may not be sufficient for a significant reduction on gaseous emission from pen surface.

When the gaseous emission were compared between different months, a significant difference in the gaseous parameter was observed. The CH_4 emission was significantly higher during September and October from the pen surfaces as compared to June, July, and August. Higher emission of CH_4 was expected due to a higher temperature in July and August (van Winden et al., 2012). Though the CH_4 concentration was observed higher in August and July compared to June, the concentration in September and October were even higher than July and August. This could be due to the accumulation of manure on the pen surface that provide an anaerobic condition for CH_4 emission. Nitrous oxide emission was significantly lower during September and October and higher during June, July, and August (Table 26). The higher temperature during June, July and August could be a reason for higher N_2O emission (Lang et al., 2012). Similarly, the dry and wet condition of the pen surface may provide an aerobic and anaerobic condition on the pen surface, thus the variation of N_2O emission was observed. The significantly lowest N_2O and CO_2 fluxes during October is most likely due to prevailing dry surface and ambient condition (Table 25).

Comparing the results with the previous study; in 2011, Rahman and Swanson (2013) measured GHG emission from the same feedlot pen surface and they found that CH_4, CO_2, and N_2O emission are 38, 26, and 17 g $hd^{-1}d^{-1}$, respectively, during the summer period. Similarly, in 2012, Borhan et al. (2013) studied the effects of two dietary crude proteins (12% and 16%) on GHG emission on the similar condition. They found that CH_4, CO_2, and N_2O emission ranged 40-61, 31-43, and 50-116 $gAU^{-1}d^{-1}$ (0.8-1.1, 593-431, and 1-1.9 g $m^{-2}d^{-1}$), respectively, during the summer months. They noticed no significant differences on gaseous emission due to different protein level.

Table 25. Analysis of greenhouse gas emissions based on treatment.

Sampling date	Emission parameters	Treatments			
		control	Low fat	Medium fat	High fat
20-Jun-13	CH_4 concentration (ppm)	2.2 ab* ± 0.0	2.2 a ± 0.2	2.3 b ± 0.2	2.1 a ± 0.0
	CO_2 concentration (ppm)	378.0 a ± 24.0	378.3 a ± 17.9	390.0 a ± 40.4	374.3 a ± 15.8
	N_2O concentration (ppm)	0.9 a ± 0.1	1.2 a ± 0.5	0.8 a ± 0.4	1.4 a ± 0.4
	CH_4 FR ($g\ m^{-2}\ d^{-1}$)	1.1 a ± 0.0	1.1 a ± 0.1	1.1 a ± 0.1	1.0 a ± 0.0
	CO_2 FR ($g\ m^{-2}\ d^{-1}$)	504.8 a ± 32.0	505.2 a ± 23.9	520.8 a ± 54.0	499.8 a ± 21.1
	N_2O FR ($g\ m^{-2}\ d^{-1}$)	1.2 a ± 0.2	1.6 a ± 0.6	1.1 a ± 0.5	1.8 a ± 0.6
	CH_4 EF ($g\ AU^{-1}\ d^{-1}$)	54.3 a ± 8.5	53.4 a ± 9.4	55.7 a ± 6.3	51.4 a ± 6.0
	CO_2 EF ($kg\ AU^{-1}\ d^{-1}$)	25.8 a ± 2.8	24.7 a ± 2.9	27.1 a ± 2.2	25.9 a ± 1.7
	N_2O EF ($g\ AU^{-1}\ d^{-1}$)	62.2 a ± 8 .9	78.2 a ± 26.5	54.5 a ± 20.4	93.8 a ± 35.4
30-Jul-13	CH_4 concentration (ppm)	2.8 ab ± 0.3	2.8 b ± 0.1	3.1 a ± 0.2	2.6 b ± 0.1
	CO_2 concentration (ppm)	467.9 b ± 70.3	485.4 b ± 67.0	518.0 a ± 75.0	473.5 b ± 58.8
	N_2O concentration (ppm)	1.0 a ± 0.4	0.8 a ± 0.2	1.3 a ± 0.1	1.0 a ± 0.3
	CH_4 FR ($g\ m^{-2}\ d^{-1}$)	1.4 ab ± 0.2	1.3 b ± 0.0	1.5 a ± 0.1	1.3 b ± 0.1
	CO_2 FR ($g\ m^{-2}\ d^{-1}$)	624.7 b ± 93.9	648.2 b ± 89.5	691.7 a ± 100.2	632.2 b ± 78.5
	N_2O FR ($g\ m^{-2}\ d^{-1}$)	1.4 a ± 0.5	1.1 a ± 0.3	1.7 a ± 0.1	1.4 a ± 0.4
	CH_4 EF ($g\ AU^{-1}\ d^{-1}$)	58.4 ab ± 5.6	54.8 a ± 6.3	64.3 b ± 4.2	52.8 a ± 3.6
	CO_2 EF ($g\ AU^{-1}\ d^{-1}$)	26.2 a ± 1.6	26.1 a ± 2.9	29.6 b ± 2.2	25.9 a ± 1.7
	N_2O EF ($kg\ AU^{-1}\ d^{-1}$)	58.6 ab ± 23.5	42.5 a ± 7.0	74.1 b ± 9.5	55.5 ab ± 13.2
20-Aug-13	CH_4 concentration (ppm)	3.2 a ±1.0	2.8 a ± 0.4	2.7 a ± 0.5	2.8 a ± 0.7
	CO_2 concentration (ppm)	431.5 a ± 48.3	471.1 a ± 93.1	487.4 a ± 131.1	447.0 a ± 64.2
	N_2O concentration (ppm)	0.9 a ± 0.4	1.4 a ± 0.6	0.9 a ± 0.4	1.2 a ± 0.3
	CH_4 FR ($g\ m^{-2}\ d^{-1}$)	1.6 a ± 0.5	1.4 a ± 0.2	1.3 a ± 0.2	1.4 a ± 0.4
	CO_2 FR ($g\ m^{-2}\ d^{-1}$)	576.2 a ± 64.4	629.0 a ± 124.3	650.9 a ± 175.1	596.9 a ± 85.8
	N_2O FR ($g\ m^{-2}\ d^{-1}$)	1.3 a ± 0.6	1.9 a ± 0.8	1.2 a ± 0.4	1.7 a ± 0.8
	CH_4 EF ($g\ AU^{-1}\ d^{-1}$)	57.9 a ± 15.3	49.0 a ± 2.6	49.7 a ± 6.0	48.2 a ± 9.0
	CO_2 EF ($kg\ AU^{-1}\ d^{-1}$)	21.2 a ± 1.6	22.1 a ± 1.6	24.3 a ± 4.3	21.1 a ± 1.8
	N_2O EF ($g\ AU^{-1}\ d^{-1}$)	48.5 a ± 25.6	67.3 a ± 31.6	48.5 a ± 18.7	59.1 a ± 28.6
18-Sep-13	CH_4 concentration (ppm)	3.3 a ±0.3	3.4 a ± 0.7	3.1 a ± 0.5	3.6 a ± 0.7
	CO_2 concentration (ppm)	389.3 a ± 28.1	381.1 a ± 60.6	423.3 a ± 70.3	422.2 a ± 44.7
	N_2O concentration (ppm)	0.6 a ± 0.1	0.6 a ± 0.1	0.6 a ± 0.1	0.6 a ± 0.1
	CH_4 FR ($g\ m^{-2}\ d^{-1}$)	1.6 a ± 0.1	1.6 a ± 0.4	1.5 a ± 0.3	1.7 a ± 0.4
	CO_2 FR ($g\ m^{-2}\ d^{-1}$)	519.9 a ± 37.5	508.9 a ± 80.9	565.2 a ± 93.9	563.8 a ± 59.7
	N_2O FR ($g\ m^{-2}\ d^{-1}$)	0.8 a ± 0.3	0.7 a ± 0.1	0.7 a ± 0.3	0.8 a ± 0.4
	CH_4 EF ($g\ AU^{-1}\ d^{-1}$)	54.2 a ± 6.0	51.6 a ± 5.7	50.8 a ± 4.0	54.7 a ± 7.7
	CO_2 EF ($kg\ AU^{-1}\ d^{-1}$)	17.3 ab ± 0.6	16.1 b ± 1.1	19.1 a ± 2.1	17.9 ab ± 1.6
	N_2O EF ($g\ AU^{-1}\ d^{-1}$)	26.9 a ± 7.8	24.3 a ± 4.5	24.4 a ± 9.2	23.9 a ± 9.4

Table 25. Analysis of greenhouse gas emissions based on treatment (continued).

Sampling date	Emission parameters	Treatments			
		control	Low fat	Medium fat	High fat
9-Oct-13	CH_4 concentration (ppm)	4.3 a ± 1.4	3.9 a ± 1.1	2.5 a ± 0.2	3.4 a ± 0.2
	CO_2 concentration (ppm)	367.2 a ± 35.1	381.6 a ± 23.9	345.7 a ± 11.3	379.8 ± 2.9
	N_2O concentration (ppm)	0.4 a ± 0.0	0.3 a ± 0.1	0.4 a ± 0.1	0.4 a ± 0.0
	CH_4 FR (g m^{-2} d^{-1})	2.1 a ± 0.7	1.9 a ± 0.5	1.2 a ± 0.1	1.6 a ± 0.1
	CO_2 FR (g m^{-2} d^{-1})	490.3 a ± 46.9	509.5 a ± 31.9	461.6 a ± 15.0	507.2 a ± 3.9
	N_2O FR (g m^{-2} d^{-1})	0.5 a ±0.0	0.5 a ± 0.1	0.5 a ± 0.1	0.5 a ± 0.0
	CH_4 EF (g AU^{-1} d^{-1})	62.2 a ± 16.3	59.1 a ± 15.5	38.8 b ± 1.5	47.8 a ± 5.2
	CO_2 EF (kg AU^{-1} d^{-1})	14.9 a ± 0.3	15.8 a ± 1.2	15.0 a ± 0.0	14.7 a ± 0.7
	N_2O EF (g AU^{-1} d^{-1})	14.6 a ± 0.7	14.0 a ± 2.1	15.7 a ± 2.8	13.6 a ± .09

[*] Values followed by the same letter in row are not significantly different at $P \leq 0.05$

Table 26. Greenhouse gas emissions on monthly basis.

Parameters	June	July	August	September	October
CH_4 concentration (ppm)	2.2 d[*] ± 0.0	2.8 c ± 0.2	2.9 bc ± 0.2	3.3 ab ± 0.2	3.5 a ± 0.7
CO_2 concentration (ppm)	380.2 b ± 5.9	486.2 a ± 19.4	459.3 a ± 21.5	404.0 b ± 19.0	368.6 b ± 14.3
N_2O concentration (ppm)	1.1 a ± 0.2	1.0 a ± 0.2	1.1 a ± 0.2	0.6 b ± 0.0	0.4 b ± 0.0
CH_4 FR (g m^{-2}d^{-1})	1.1 d ± 0.0	1.4 c ± 0.1	1.4 bc± 0.1	1.6 ab ± 0.1	1.7 a ± 0.3
CO_2 FR (g m^{-2}d^{-1})	507.6 b ± 7.9	649.2 a ± 26.0	613.3 a ± 21.5	539.4 b ± 19.0	492.1 b± 14.3
N_2O FR (g m^{-2}d^{-1})	1.4 a ± 0.3	1.4 a ± 0.2	1.5 a ± 0.3	0.8 b ± 0.0	0.5 b ± 0.0
CH_4 EF (g d^{-1} hd^{-1})	40.5 b ± 1.2	52.7 ab ± 3.8	54.2 b ± 3.8	62.4 a ± 2.3	63.7 a ± 11.6
CO_2 EF(g d^{-1} hd^{-1})	19487 b± 624	24958 a± 326	23584 a ± 326	20693 b ± 143	18541 b ± 431
N_2O EF (g d^{-1} hd^{-1})	55.2 a ± 11.0	53.3a ± 9.3	58.2 a ± 9.6	29.6b ± 1.4	17.7 b ± 0.9
CH_4 EF (g d^{-1} AU^{-1})	53.2 ab ± 7.9	57.6 a ± 6.7	51.2 b ± 10.2	52.8 ab ± 6.2	52.0 ab ± 14.9
CO_2 EF(kg d^{-1} AU^{-1})	25.5 a ± 2.6	26.9 a ± 2.0	22.2 b ± 2.9	17.5 c± 1.8	15.1 c ± 0.8
N_2O EF(g d^{-1} AU^{-1})	67.0 a ± 29.0	57.7 a ± 18.5	55.8 a ± 27.7	24.9 b ± 8.1	14.5 c ± 2.0

[*] Values followed by the same letter in row are not significantly different at $P \leq 0.05$

Further analysis was carried out to see the interaction of diet and time on GHG emissions. The results reveal that all CH_4, CO_2 and N_2O emissions (concentration and emission rate) varied significantly ($P \leq 0.05$) over the sampling period; however diet does not have any interaction with time to show the effect on GHG emission (Table 27).

Table 27. Probability values based on the repeated measure multivariate analysis along with time and treatment interactions.

Parameters	Interaction	
	Time	Diet*Time
CH_4 ppm	<0.01	0.68
CO_2 ppm	<0.01	0.43
N_2O ppm	<0.01	0.37
CH_4 g m^{-2} d^{-1}	<0.01	0.68
CO_2 g m^{-2} d^{-1}	<0.01	0.43
N_2O g m^{-2} d^{-1}	<0.01	0.37
CH_4 g AU^{-1} d^{-1}	0.03	0.41
CO_2 kg AU^{-1} d^{-1}	<0.01	0.97
N_2O g AU^{-1} d^{-1}	<0.01	0.48

Hydrogen sulfide emission

Hydrogen sulfide concentration was very low (<80 ppb) at the pen surfaces throughout the measurement period. Other researchers have also reported the concentration around 50 ppb in the feedlot (Sullivan, 1999). There was no significant difference in H_2S emission rate as well among the treatments. However, variations in H_2S emission rates were observed during different among sampling periods (Figure 8). The H_2S emission rate was fairly low (< 0.18 g m^{-2} d^{-1}) in the first month since pen surfaces had thin layer of manure on the surface. The H_2S concentration gradually increased over time and reached up to 0.7 g m^{-2} d^{-1} in August (Figure 8). However, as the temperature started decreasing (Figure 7), the H_2S emission rate also declined gradually (Figure 12). This study shows that H_2S emission rate measured on the feedlot pen surfaces were correlated with temperature change and manure accumulation (Figure 7 and 8). Other researchers have also observed very low emission rate of H_2S from the feedlot. Wood et al. (2001) have also reported the emission rate 103 μg m^{-2} min^{-1}. Similarly, Baek et al. (2003) and Koziel et al. (Koziel et al., 2005) reported the H_2S emission rate as 1.88 μg m^{-2} min^{-1}, and 1.39 μg m^{-2} min^{-1}, respectively.

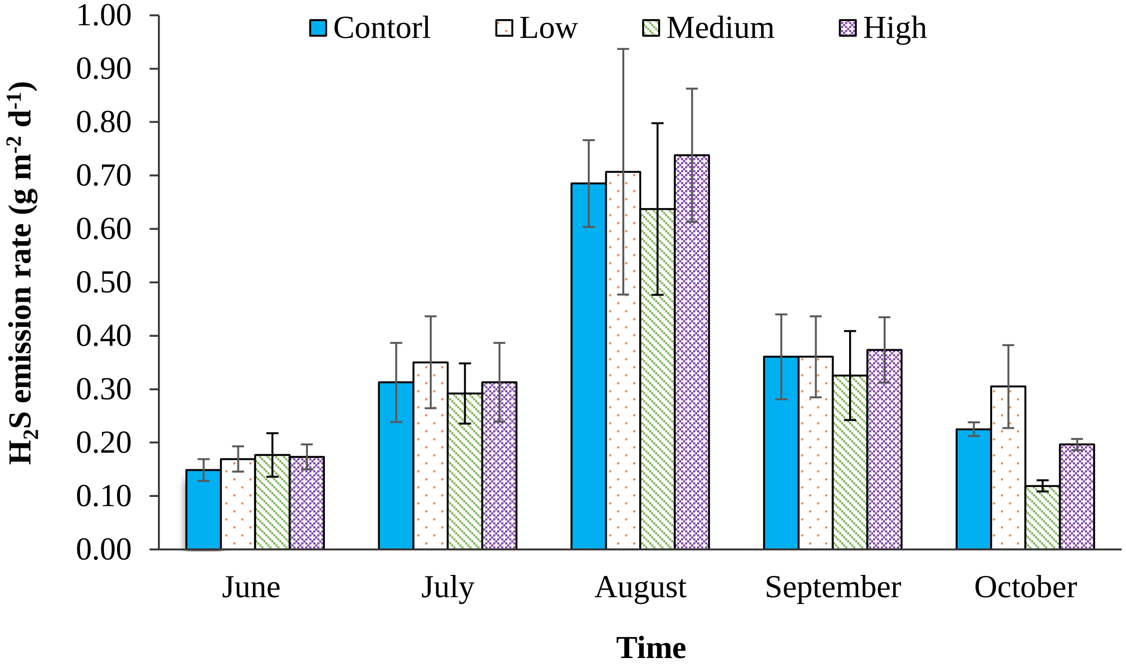

Figure 8. Hydrogen sulfide emission from feedlot pen surfaces in different time period.

Conclusions

In this study, the effect of four dietary fat concentrations (3 to 5.5% in the composite sample) feed to beef cattle was evaluated in term of manure nutrient composition, VFA concentration, H_2S and GHG (CH_4, CO_2, and N_2O) emissions. The study was conducted over a 5-month period from June to October for a five ~28-day feeding periods. Overall, the fat levels in the diet showed no or little effect on the manure compositions, VFA, and H_2S and GHGs emissions. However, some variation in the above mentioned parameters were observed among different measurement periods. Though, other researchers have reported the effect of supplementary fat on ruminal VFA and enteric CH_4 emission; this research did not reflect any effect of fat level variation of diet on GHGs and H_2S emission, as well as on manure composition.

References

Anderson, V., and Engel, C. 2014. Effects of fat level in distillers grain on feedlot finishing performance and carcass traits. North Dakota Beef Report-AS1736. Fargo: North Dakota State University.

Baek, B.-H., Koziel, J. A., Spinhirne, J. P., Parker, D. B., and Cole, N. A. 2003. Estimation of ammonia and hydrogen sulfide emissions from cattle feedlots in Texas. In *Proceeding, Conference of Air Pollution from Agricultural Operations III, 12-15 October 2003,* 123-130. Research Triangle Park, North Carolina: ASAE.

Beauchemin, K. A., and McGinn, S. M. 2006. Methane emissions from beef cattle: Effects of fumaric acid, essential oil, and canola oil. *Journal of animal science*. 84(6): 1489-1496.

Beauchemin, K. A., McGinn, S. M., Benchaar, C., and Holtshausen, L. 2009. Crushed sunflower, flax, or canola seeds in lactating dairy cow diets: effects on methane production, rumen fermentation, and milk production. *Journal of Dairy Science*. 92(5): 2118-2127.

Boeckx, P., and Van Cleemput, O. 1996. Methane oxidation in a neutral landfill cover soil: influence of moisture content, temperature, and nitrogen-turnover. *Journal of environmental quality*. 25(1): 178-183.

Boone, D. R., and Xun, L. 1987. Effects of pH, temperature, and nutrients on propionate degradation by a methanogenic enrichment culture. *Applied and environmental microbiology*. 53(7): 1589-1592.

Borhan, M. S., Gautam, D. P., Engel, C., Anderson, V. L., and 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.

Bowman, A., Mueller, K., and Smith, M. 2000. Increased animal waste protection from concentrated animal feeding operation (CAFOs): Potential implication for public and environmental health. Omaha, Nebraska: Nebraska Center For Rural Health Research.

Chadwick, D. R., Pain, B. F., and Brookman, S. K. E. 2000. Nitrous oxide and methane emissions following application of animal manures to grassland. *Journal of environmental quality*. 29(1): 277-287.

Chuntrakort, P., Otsuka, M., Hayashi, K., Takenaka, A., Udchachon, S., and Sommart, K. 2014. The effect of dietary coconut kernels, whole cottonseeds and sunflower seeds on the intake, digestibility and enteric methane emissions of Zebu beef cattle fed rice straw based diets. *Livestock Science*. 161: 80-89.

Dang, H., Li, J., Chen, R., Wang, L., Guo, L., Zhang, Z., and Klotz, M. G. 2010. Diversity, abundance, and spatial distribution of sediment ammonia-oxidizing betaproteobacteria in response to environmental gradients and coastal eutrophication in Jiaozhou Bay, China. *Applied and environmental microbiology*. 76(14): 4691-4702.

Dutreuil, M., Wattiaux, M., Hardie, C. A., and Cabrera, V. E. 2014. Feeding strategies and manure management for cost-effective mitigation of greenhouse gas emissions from dairy farms in Wisconsin. *Journal of Dairy Science*. 97(9): 5904-5917.

Eastridge, M. 2002. Effects of feeding fats on rumen fermentation and milk composition. In *Proc. 37th Annual Conf. Pacific Northwest Animal Nutrition, Vancouver, Canada*, 47-57.

Engstrom, D. F., Goonewardene, L. A., Volek, R., Grimson, R. E., Stilborn, R. P., and McKinnon, P. J. 1994. Effects of feeding added protein and fat on feedlot performance and carcass quality in large frame steers. *Canadian Journal of Animal Science*. 74(3): 547-549.

Gourdon, R., and Vermande, P. 1987. Effects of propionic acid concentration on anaerobic digestion of pig manure. *Biomass*. 13(1): 1-12.

Guidotti, T. L. 1994. Occupational exposure to hydrogen sulfide in the sour gas industry: some unresolved issues. *International archives of occupational and environmental health*. 66(3): 153-160.

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

Hribar, C., and Schultz, M. 2010. Understanding concentrated animal feeding operations and their impact on communities. East Gypsy Lane Road Bowling Green, Ohio: National Association of Local Boards of Health. Reterived on 9/10/2015 from http://www.cdc.gov/nceh/ehs/docs/understanding_cafos_nalboh.pdf.

Hristov, A. N., Ott, T., Tricarico, J., Rotz, A., Waghorn, G., Adesogan, A., Dijkstra, J., Montes, F., Oh, J., Kebreab, E., Oosting, S. J., Gerber, P. J., Henderson, B., Makkar, H. P. S., and Firkins, J. L. 2013. SPECIAL TOPICS-Mitigation of methane and nitrous oxide emissions from animal operations: III. A review of animal management mitigation options. *Journal of animal science*. 91(11): 5095-5113.

Hulshof, R. B. A., Berndt, A., Gerrits, W. J. J., Dijkstra, J., van Zijderveld, S. M., Newbold, J. R., and Perdok, H. B. 2012. Dietary nitrate supplementation reduces methane emission in beef cattle fed sugarcane-based diets. *Journal of animal science*. 90(7): 2317-2323.

IPCC. 2001. Second Assessment Report, Climate Change: The Scientific Basis Contribution of Working Group I to the Second Assessment Report of th IPCC.

Johnson, K. A., and Johnson, D. E. 1995. Methane emissions from cattle. *Journal of animal science*. 73(8): 2483-2492.

Kelley, R., Martz, F., and Johnson, H. 1967. Effect of environmental temperature on ruminal volatile fatty acid levels with controlled feed intake. *Journal of Dairy Science*. 50(4): 531-533.

Koziel, J. A., Parker, D. B., Baek, B.-H., Bush, K. J., Rhoades, M., and Perschbacher-Buser, Z. 2005. Ammonia and hydrogen sulfide flux from beef cattle pens: implications for air quality measurement methodologies and evaluation of emission controls.

Lang, M., Li, P., and Zhang, X. 2012. Effects of land use type and incubation temperature on soil nitrogen transformation and greenhouse gas emission. *The Journal of Applied Ecology*. 23(10): 2670-2676.

Lardy, G., and Anderson, V. 2014. Feeding Coproducts of the Ethanol Industry to Beef Cattle, AS1242. NDSU extension service. North Dakota State University, Fargo, ND.

Lippke, H. 1975. Digestibility and volatile fatty acids in steers and wethers at 21 and 32C ambient temperature. *Journal of Dairy Science*. 58(12): 1860-1864.

Luo, G., Kiese, R., Wolf, B., and Butterbach-Bahl, K. 2013. Effects of soil temperature and moisture on methane uptake and nitrous oxide emissions across three different ecosystem types. *Biogeosciences*. 10(5): 3205-3219.

Machmüller, A., Ossowski, D. A., and Kreuzer, M. 2006. Effect of fat supplementation on nitrogen utilisation of lambs and nitrogen emission from their manure. *Livestock Science*. 101(1–3): 159-168.

Marchaim, U., and Krause, C. 1993. Propionic to acetic acid ratios in overloaded anaerobic digestion. *Bioresource Technology*. 43(3): 195-203.

Mathison, G. 1997. Effect of canola oil on methane production in steers. *Can. J. Anim. Sci*. 77: 545.

Mathison, G., Okine, E., McAllister, T., Dong, Y., Galbraith, J., and Dmytruk, O. 1998. Reducing methane emissions from ruminant animals. *Journal of Applied Animal Research*. 14(1): 1-28.

Meale, S. J., McAllister, T. A., Beauchemin, K. A., Harstad, O. M., and Chaves, A. V. 2012. Strategies to reduce greenhouse gases from ruminant livestock. *Acta Agriculturae Scandinavica Section a-Animal Science*. 62(4): 199-211.

Menz, F. C., and Seip, H. M. 2004. Acid rain in Europe and the United States: an update. *Environmental Science & Policy*. 7(4): 253-265.

Milby, T. H., and Baselt, R. C. 1999. Hydrogen sulfide poisoning: clarification of some controversial issues. *American journal of industrial medicine*. 35(2): 192-195.

Mirabelli, M. C., Wing, S., Marshall, S. W., and Wilcosky, T. C. 2006. Race, poverty, and potential exposure of middle-school students to air emissions from confined swine feeding operations. *Environmental health perspectives*: 591-596.

NRC. 1996. Nutrient Requirements of Beef Cattle. 7th Revised Edition. National Academy of Science, Washington, D.C.

Osada, T., Takada, R., and 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*. 166-67: 562-574.

Owen, J. J., and Silver, W. L. 2015. Greenhouse gas emissions from dairy manure management: a review of field-based studies. *Global Change Biology*. 21(2): 550-565.

Philippe, F. X., and Nicks, B. 2015. Review on greenhouse gas emissions from pig houses: Production of carbon dioxide, methane and nitrous oxide by animals and manure. *Agriculture Ecosystems & Environment*. 199: 10-25.

Portejoie, S., Martinez, J., and Landmann, G. 2002. Ammonia of farm origin: impact on human and animal health and on the natural habitat. *Productions Animales*. 15(3): 151-160.

Pullammanappallil, P. C., Chynoweth, D. P., Lyberatos, G., and Svoronos, S. A. 2001. Stable performance of anaerobic digestion in the presence of a high concentration of propionic acid. *Bioresource Technology*. 78(2): 165-169.

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

Schader, C., Jud, K., Meier, M. S., Kuhn, T., Oehen, B., and Gattinger, A. 2014. Quantification of the effectiveness of greenhouse gas mitigation measures in Swiss organic milk production using a life cycle assessment approach. *Journal of Cleaner Production*. 73: 227-235.

Spellman, F. R., and Whiting, N. E. 2010. *Environmental management of concentrated animal feeding operations (CAFOs)*: CRC Press.

Sullivan, J. 1999. *Feedlot Air Quality Summary: Data Collection, Enforcement and Program Development*: Minnesota Pollution Control Agency.

USDA. *Cattle inventory – United States: July 1.* National agricultural statistics service (NASS), agricultural statistics board, united states department of agriculture (USDA). Reterieved on 9/10/2015 from http://www.Usda.Gov/nass/pubs/todayrpt/catl0715.Pdf.

USEPA. 2009. Inventory of US Greenhouse Gas Emission and Sinks: 1990-2007. Washington DC.

USEPA. 2014. US Greenhouse Gas Inventory Report: Inventory of US Greenhouse Gas Emission and Sink:1990-2012. Washington DC.

USEPA. 2015. Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990-2013, Washington DC, USA.

van Winden, J. F., Reichart, G.-J., McNamara, N. P., Benthien, A., and Damsté, J. S. S. 2012. Temperature-induced increase in methane release from peat bogs: a mesocosm experiment. *PLoS one*. 7(6): 1-5.

Viguria, M., Sanz-Cobena, A., Lopez, D. M., Arriaga, H., and Merino, P. 2015. Ammonia and greenhouse gases emission from impermeable covered storage and land application of cattle slurry to bare soil. *Agriculture Ecosystems & Environment*. 199: 261-271.

Ward, A. J., Hobbs, P. J., Holliman, P. J., and Jones, D. L. 2008. Optimisation of the anaerobic digestion of agricultural resources. *Bioresource Technology*. 99(17): 7928-7940.

Wood, S., Schmidt, D., Janni, K., Jacobson, L., Clanton, C., and Weisberg, S. 2001. Odor and air emissions from animal production systems. In *ASAE Annual International Meeting Sponsored by ASAE*.

Ziegler, K. 2007. Nutrition and Management: Feeding Fats and Oils in Feedlot Diets. Retrieved on 9/05/2015 from http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/beef11670.

Zinder, S. H. 1990. Conversion of acetic acid to methane by thermophiles. *FEMS Microbiol. Rev*. 75: 125-138.

APPLICATION OF NANOPARTICLES (NPs) IN LIVESTOCK MANURE AND THEIR EFFECTS ON AIR EMISSION[3]

Abstract

Emission of pollutant gases (ammonia and hydrogen sulfide) and greenhouse gases (GHGs) from the livestock production facility is a major environmental concern. Scientists are continuously striving for innovative and appropriate technologies for mitigating pollutant gases and GHGs emissions from livestock production facility. In recent years nanoparticles (NPs) have been shown to be promising additive to control the pollutant gas and GHG emissions. The objective of this research was to compare the effectiveness of zinc oxide NPs (nZnO) and zirconia NPs ($nZrO_2$) in minimizing hydrogen sulfide (hydrogen sulfide-H_2S) and GHG (methane-CH_4, and carbon dioxide-CO_2) emissions from swine and dairy manures. Three replications of each NP treatment including three controls were performed in 2-L Erlenmeyer flasks with a working volume of 1.5 L for 25-30 days under anaerobic conditions. For each treatment, NPs were added to manure at a rate of 3 g L^{-1}, mixed thoroughly and flasks were sealed with rubber stoppers with a hole to which a 1-L Tedlar bag was connected to collect the head-space gas. Headspace gas was analyzed for H_2S and GHGs every 2-3 days during the experimental period. Zinc oxide NP_S showed promising results in reducing H_2S and GHG production under that experimental conditions. Zinc oxide NPs reduced the total volume of gas production by 64 and 82% in cases of dairy and swine manure, respectively. Likewise, nZnO

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[3] This is a slightly alter version of the proceeding paper presented in International Symposium of Animal Environment and Welfare, October 19-22, 2013; Rongchang, Chongqing, China. The material in this chapter was co-authored by Dhan Prasad Gautam, Shafiqur Rahman, Md Saidul Borhan and Achintya N. Bezbaruah. Dhan Prasad Gautam had primary responsibility for the collection and analyses of samples, and was the primary developer of the conclusions that are advanced here. Other authors served as proofreader and checked the calculation conducted by Dhan Prasad Gautam.

reduced H_2S and CH_4 concentrations by more than 99 and 67%, from both dairy and swine manure, respectively, when compared with control. Similarly, nZnO also showed a significant reduction in CO_2 concentration. However, zirconia NPs ($nZrO_2$) did not show any promising results for any gaseous production and gaseous concentration. Additionally, the effectiveness of different application rates of nZnO (100 and 500 mg L^{-1}) was tested on both the manures and it was observed that these lower application rates were not effective in reducing GHGs concentration but effective for slight reduction of H_2S concentration and total gas production. Zinc oxide NPs showed the minimal effect on pH, crude protein, nitrogen, ammonia, and volatile fatty acids content in the manures.

Introduction

The major pollutant gases emitted from livestock production facilities include ammonia (NH_3) and hydrogen sulfide (H_2S). Greenhouse gases (GHGs) such as water vapor (H_2O), ozone (O_3), carbon-dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) are drawing attention due to their contribution to global warming. Methane, CO_2, and N_2O are the major GHGs emitted from manure (Johnson et al., 2007). Methane is produced during the anaerobic decomposition of manure and other organic matter (Khan et al., 1997), N_2O is produced during nitrification or denitrification in manure (Bremner and Blackmer, 1978; Chadwick, 2005), and CO_2 is produced during aerobic and anaerobic decomposition. Similarly, H_2S is formed by the process of bacterial sulfate reduction and decomposition of sulfur-containing organic compounds in manure under anaerobic condition (Thu, 2002). Ammonia is a major problem in swine and poultry housing (Drummond et al., 1980; Ndegwa et al., 2008) and is produced during ammonification and volatilization of nitrogenous compounds in manure. Though it is difficult to stop generation of these pollutant gases and GHGs completely, the production can be minimized by applying a different treatment of additives at different stages of livestock production and manure management process.

Scientists are consciously striving for innovative and appropriate technologies for mitigating pollutant gas and GHG emission from livestock production facilities. A lot of research has been conducted for mitigating pollutant gas resulting from animal production facilities (Basarab et al., 2003; Buddle et al., 2011; Waghorn et al., 2006), feedstock (Hao et al., 2005; Novak and Fiorelli, 2010; Waghorn et al., 2006), and lagoon (Ndegwa et al., 2008; Petersen and Miller, 2006; Rahman et al., 2011). Ammonia mitigation can be achieved by modifying dietary composition (Satter et al., 2002), reducing pH (Jensen, 2002), segregating urine from feces (Von Bemuth et al., 2005), inhibiting hydrolysis (Varel, 1997), binding NH_3 (Portejoie et al., 2003), applying biological treatment (Luostarinen et al., 2006), and using filters or bio-filters (Ndegwa et al., 2008). Similarly, H_2S can be mitigated using filters and biofilters (Ruokojärvi et al., 2001), using vegetable oil (Powers, 1999), manipulating livestock diet (Nahm, 2002), using lagoon covers, and adopting aeration and composting processes (Patterson, 2005). Likewise, many techniques have been developed to mitigate GHGs from manure. Methane emission can be reduced significantly by removing manure frequently from the floor or pen surface, adopting the anaerobic digestion system for manure (Clemens et al., 2006), composting the manure (Pattey et al., 2005), covering and capturing the emitted gas (Clemens and Ahlgrimm, 2001). Similarly, adoption of a manure storage system (slurry vs. deep litter system) may favor the anaerobic system, which ultimately reduces N_2O emission (Chadwick et al., 2011). Likewise, manure application in the field favors the minimization of CO_2 emission by promoting carbon sequestration (Reicosky et al., 2000) but may increase N_2O emissions (Li et al., 2005; Velthof et al., 2003). Though different techniques have been developed for the mitigation of pollutant gases, there is still a demand of a new technology which can control the emission of multiple gases at the source such as during storage. Use of nanoparticles (NPs) could be a potential technology for reducing pollutant gas generation and emissions.

Nanoparticles have found numerous applications in many sectors (Mueller and Nowack, 2008; Roco, 1999) but they have not been extensively in agriculture. Applications of NPs in the field of waste management and wastewater treatment (Mueller and Nowack, 2008), in adsorbing toxins (Chen et al., 2010), odor (MacDonald et al., 2011), removal of pathogens (Mishra et al., 2011), and mitigation gases like H_2S (Miao et al., 2007) have been carried out but limited researches have been carried out to control H_2S, NH_3 and GHG emissions from manure.

Research has shown that the NPs such as zinc oxide (nZnO), silver (nAg), titanium dioxide ($nTiO_2$), and copper oxide (nCuO) are effective in limiting microbial growth (Brar et al., 2010; Brayner et al., 2006; Choi et al., 2008). The antibacterial property of the silver NPs has made them popular in the field of biological science (Sondi and Salopek-Sondi, 2004). They hinder the growth of micro-organisms in the media as silver ion and silver-based compounds are highly toxic to microorganisms (Kim et al., 2007). Choi and Hu (2009) reported that nAg inhibits the nitrification process which leads to the reduction of NH_3 and N_2O emission. Similarly, nAg also has an impact on methanogenesis process; however, the applied dose of NPs determines the level of inhibition. Yang et al. (2012) have reported that even very low concentration of nAg (10 mg per kg solids) is able to reduce GHG emissions significantly from the municipal waste landfill site.

Similarly, nZnO has wide applications due to its high surface activity, large surface area, low cost and ease of manufacturing (Sayyadnejad et al., 2008). Researchers (Abatzoglou and Boivin, 2008; Hernández et al., 2011; Sayyadnejad et al., 2008) have used nZnO for removing

H_2S during the purification of biogas, and drilling of gas and oil work. Desulphurization reaction occurs when nZnO combines with H_2S, forming zinc sulfide (ZnS) and water. In fact, nZnO have high affinity towards the absorption of sulfur compounds (Hernández et al., 2011). The reduction of H_2S can also be due to the chemical interaction with substrates and amendment of microbial activity. In the case of anaerobic digestion of manure, Luna-delRisco et al. (2011) have reported a 74% reduction in total gas production using 240 mg L^{-1} of nZnO in manure. Predicala et al. (2012) have reported more than 95% reduction of H_2S from swine manure using nZnO at a dose of 3g L^{-1}. However, most of the previous research focused on a single component, and none of them focused on GHG mitigation from anaerobic storage of manure using NPs.

Zirconia NPs ($nZrO_2$) is one of the most studied nanomaterials for applications in ceramics. It has poly-crystalline properties and is mostly used in electroceramic applications. To date, no research has been done to investigate the effect of $nZrO_2$ in mitigating pollutant gases or GHG emission. However, the researchers have used $nZrO_2$ a*s* an antimicrobial agent (Jangra et al., 2012, Pradhaban et al., 2014). Therefore, in this study the effectiveness of nZnO and $nZrO_2$ were studied in mitigating these gases from swine and dairy manures stored under anaerobic conditions. In addition, the evaluation of the effectiveness of different application rates (100, 500 and 3000 mg L^{-1}) of nZnO was performed; and the a comparative study on the effect of nano and micro size of zinc oxide on reducing gaseous emissions was also carried out.

Materials and methods

In this study zinc oxide (ZnO, US3580, US Research nano-materials, Inc., Texas, USA) and zirconium oxide (ZrO_2-3Y, US3610, US Research nano-materials, Inc., Texas, USA) NPs were used to evaluate the effectiveness of these NPs to reduce gas production and pollutant gas concentration under anaerobic storage conditions (Table 28). Dairy and swine manures were collected from North Dakota State University (NDSU) dairy and swine farms, respectively, and the same manure was used during the study period. Some of the manure characteristics are listed in Table 29. All experiments in this study were performed under room temperature (around 25^{o}C) and atmospheric pressure (760 mm Hg).

Table 28. Characteristics of the nanoparticles under investigation.

Nanoparticles	CAS Number	Size, nm	Purity, %	SSA (m^2 g^{-1})	Form	Color
ZnO	1314-13-2	35-45	99.95	40-70	Powder	Milky white
ZrO_2	1314-23-4	40	99.95	30-60	Powder	White

SSA = Specific surface area; CAS = Chemical Abstracts Service

Table 29. Characteristics of dairy liquid manure and swine slurry.

Characteristics	Swine slurry				Dairy liquid manure			
	Initial	After 27 d incubation			Initial	After 25 d incubation		
		Control	nZnO	$nZrO_2$		Control	nZnO	$nZrO_2$
pH	7.2	7.2	7.5	7.2	7.3	6.7	7.1	7.00
Total solids (%)	8	9	10	10	13	14	15	15
Volatile solids (%)	74	67	69	68	82	71	73	73

Note: Initial means the manure collected from source before starting the experiment, and Control means the manure kept in a flask for 25/27 days without treating with NPs.

Experimental setup

The experiment was conducted in 2-L Erlenmeyer flasks with a working volume of 1.5 L, fitted with a rubber stopper. A glass tube of around 5 cm long was inserted at the center of the stopper, and connected to a 1-L Tedlar bag (SKC Gulf coast Inc., Texas, USA) using a Teflon tube (Figure 9). All connections were checked to avoid leakage.

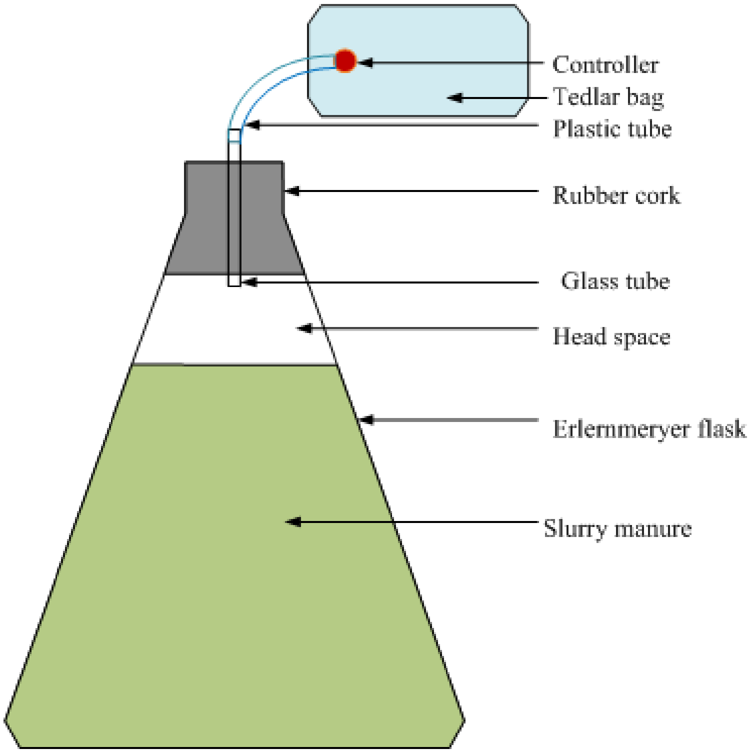


Figure 9. Schematic diagram of the experimental setup.

After collecting both manure samples, they were kept in room temperature for a day before setting up the experiment. Each manure sample was mixed thoroughly to have homogenous manure samples before pouring into flasks. A total of nine 2-L Erlenmeyer flasks with a working volume of 1.5 L was prepared. First three flasks were control, and second three flasks were treated with nZnO (3 g L^{-1}), and last there were treated with $nZrO_2$ (3 g L^{-1}).

After adding treatments in respective flasks, NPs and manure were well mixed by glass rod followed by handshaking. Following mixing, flasks were purged with nitrogen for 2 min and sealed with rubber stoppers and connected with Tedlar bags to accumulate the head space gas. All preparation was done under a fume- hood and personal protections were taken during mixing and preparation phase.

Additionally, an experiment was conducted to find an appropriate dose of nZnO to be used for further experiments. A dose (3 g L^{-1}) was chosen based on a previous study (Predicala et al., 2012) that was used to reduce H_2S production from swine manure. However, it was hypothesized that lower dose should reduce GHGs and H_2S production. Therefore, an experiment was carried out with four treatments: First with 100 mg L^{-1}, second with 500 mg L^{-1}, third with 3000 mg L^{-1} and fourth as a control (without adding any NPs). The experiment was carried out taking both swine and liquid dairy manure.

Similarly, another experiment was carried out to investigate the effect of particles size of ZnO on gaseous production. In this study, the effectiveness of regular nZnO (35-50 nm, used in all other experiment mentioned above) was compared with ZnO chemical power (Zinc oxide-Analytical Reagent, US 8832, Mallingckroad Chemical Work, Saint Louis, USA) having approximately 1µm particle size. The experiment was conducted similar to the other experiments as mentioned above, taking 1 L working volume of dairy manure and treating manure with 3g L^{-1} for both nZnO and micro ZnO.

Gas analysis

Headspace gas accumulated in a Tedlar bag was analyzed for hydrogen sulfide (H_2S) and GHGs (CH_4 and CO_2) every 2-6 days depending on the volume of gas accumulated in the bag. Total gas volume in each bag was determined using a graduated gas tight syringe (SGE Analytical Syringe, 500 mL, Australia). A fixed amount of gas sample (2.5 or 5 mL) was withdrawn from the Tedlar bag using a small graduated syringe (309604 - 10 mL BD Luer Lok^{TM} Tip Syringe, New Jersey, USA) and transferred to another clean Tedlar bag. Then, the sample was diluted with nitrogen gas to bring the concentration to the detection limit of a gas chromatograph (GC) (8610C, SRI Instrument, California, USA). The GC was equipped with a flame ionized detector (FID) for detecting CH_4 and CO_2.

Before each measurement, GC was calibrated using the calibration quality standard gases (20, 100, 1000 ppm for CH_4; 100, 1000, 2500 ppm for CO_2). Then, the standard curves were developed by regressing the peak areas (GC responses) and different concentration levels of each component through the origin. The performance of the GC was established by determining Method Detection Limit (MDL). To determine MLD, 2 ppm of CH_4 and 500 ppm of CO_2 were introduced seven times and MDLs were calculated following the USEPA guidelines, as the product of the standard deviation of replicates and the Student's t-value at the 99% confidence level as described in (Borhan et al., 2011).

Similarly, H_2S gas concentration was measured using a hydrogen sulfide analyzer (Jerome® 631-X, Arizona Instrument, Arizona, USA). For the measurement of H_2S, samples were also diluted with nitrogen gas to bring the gas concentration to the instrument's maximum detection limit of 50 ppm. After measuring the concentration of the diluted sample, the actual concentration of samples was calculated by multiplying with the dilution factor. The quality control of the data was ensured measuring the standard and blanks concentrations in every ten samples. The data was accepted with a variation within 2% compared to the known concentration tested.

A preliminary experiment was carried out at the beginning to find the appropriate dose of nZnO to be used for further experiments. The previous study by Predicala et al. (2012) used 3 g L^{-1} to reduce H_2S production from swine manure; however, even the lower dose is expected to work for reducing GHGs and H_2S production. Therefore, an experiment was carried out with the similar experimental set up as mentioned above, taking a lower dose of nZnO (100 mg L^{-1} and 500 mg L^{-1}) and 3000 mg L^{-1} (Predicala et al., 2012) doses of nZnO to treat the swine manure.

Similarly, one more experiment was carried out to investigate the effect of particles size of ZnO on gaseous production while adding to the manure. In this study, the effective of regular nZnO (35-50 nm, used in all other experiment mentioned above) was compared with ZnO chemical power (Zinc oxide-Analytical Reagent, US 8832, Mallingckroad Chemical Work, Saint Louis, USA) having approximately 1µm particle size. The experiment was conducted similar to the other experiments as mentioned above, taking 1 L working volume of dairy manure and treating manure with 3g L^{-1} for both nZnO and micro ZnO.

Manure sample analysis

Additionally, manure samples were also taken from each flasks before and after the experiment and analyzed for pH, total solid (TS), volatile solid (VS), total nitrogen (TN), ammonia (NH_3), crude protein (CP), and volatile fatty acids (VFAs) using the standard methods as described in Table 30.

Table 30. Method or protocol used to analyze manure samples.

Parameters	Methods/ protocol used
pH	EPA SW-846, Method 9040
TS	Recommended Method of Manure Analysis, A3769
VS	Official Method 942.05, AOAC International (2005) 18th ed., AOAC International Gaithersburg, MD
TN	Recommended Methods of Manure Analysis, A3769 Macro-Kjeldahl method (adapted from Kane, 1998)
CP	Official Method 2001.11, AOAC International (2005) 18th ed., AOAC International Gaithersburg, MD
NH_3	Sigma Technical Bulletin #640. Sigma Diagnostics, St. Louis, MO 63178
VFA	Method of Goetsch and Galyean, 1983. Agilent 6890N Gas Chromatograph with an FID (flame ionization detector) and the 7683 Series auto-injector and autosampler. Column used was the Supercool brand, NUKOL Fused Silica Column, 15 m x 0.53 mm x 0.5 um

AOAC = Association of Official Agricultural Chemists

Statistical analysis

Data were analyzed in SAS 9.4(2010) utilizing PROC ANOVA procedure. The null hypothesis tested was that mean GHG concentrations and quantity, nutrient compositions and volatile fatty acids concentrations across control and NPs treatments were equal. The significance level tested was 95% ($P \leq 0.05$).

Results and discussion

Effect of NPs on manure properties

Nitrogen (N), ammonia (NH_3), pH, and volatile fatty acids (VFAs) were measured at the beginning and at the end of an experiment for both swine and dairy manures. Some of the manure properties are listed in Tables 29, 31 and 32. Measured pH of swine manure before and after the experimental period was in the range of 7.2 - 7.5 while it was in the range of 6.9 - 7.3 for dairy manure (Table 29). The initial pH values of swine and dairy manure were close; however, the pH of dairy manure reduced slightly while pH of swine manure remained steady after the experiment. This is likely due to differences in TS and VFA concentration in the two manures. Dairy manure had higher TS, which might have decomposed over time and might change VFA concentration and finally altered the pH. The TS increased and VS decreased in control and NPs treated manure after the experiment compared to the manure before experiment for both dairy and swine (Table 29). The decrease of VS could be due to volatilization of the solid fraction into gaseous during decomposition of manure throughout the experimental period. Similarly, there were no significant differences in crude protein, total nitrogen (TN), and NH_3 concentrations between initial, control and NPs treated manure for both dairy and swine (Tables 31 and 32).

Table 31. Comparison of nutrient composition of liquid dairy manure before and after the completion of experiment.

Nutrient Concentrations	Initial		After digesting anaerobically for 25 d.					
			Control		$nZrO_2$		nZnO	
	Mean	Std.	Mean	Std.	Mean	Std.	Mean	Std.
Crude protein (%)	15.8a	0.8	15.4a	0.3	15.5a	1.4	16.3a	1.0
Nitrogen (%)	2.5a	0.1	2.5a	0.0	2.5a	0.2	2.6a	0.2
NH_3 (%)	143.2a	26.8	182.8a	23.5	194.9a	25.1	154.4a	37.8

Note: Values followed by the same letter in a row are not significantly different at $P \leq 0.05$; Std. = standard deviation.

Table 32. Comparison of nutrient composition of liquid swine manure before and after the completion of experiment

Nutrient Concentrations	Initial		After digesting anaerobically for 27 d.					
			Control		$nZrO_2$		nZnO	
	Mean	Std.	Mean	Std.	Mean	Std.	Mean	Std.
Crude protein (%)	21.5a	0.1	26.7a	3.6	22.9a	3.1	25.2a	6.2
Nitrogen (%)	3.4a	0.0	4.3a	0.6	3.7a	0.5	4.0a	1.0
NH_3 (%)	346.4a	40.2	342.2a	60.7	355.6a	54.7	301.6a	41.9

Note: Values followed by the same letter in a row are not significantly different at $P \leq 0.05$; Std. = standard deviation.

The total volatile fatty acid (TVFA) content of the dairy manure at the beginning of the experiment (initial) was 198 mM. At the end of the experiment total VFA in control, $nZrO_2$, and nZnO treated dairy manure were 425, 495, and 331 mM, respectively (Table 33). The TVFA increased significantly in control and $nZrO_2$ treated manure as compared to initial manure. However, the nZnO treated manure showed no significant difference compared to control and initial manure. Among the various VFAs, acetic acid was the dominant VFA. Acetic acid is considered as a prime VFA component for CH_4 production (Hill et al., 1987) and contribute to GHG. The average acetic acid concentration of the initial dairy manure was 143 mM, whereas, at the end of the experiment, the acetic acid concentrations of control, $nZrO_2$, and nZnO treated manure were 175, 226, and 166 mM, respectively (Table 33). Most of VFA components were significantly higher in control and $nZrO_2$ treated manure compared to initial manure; however, there was no significant difference in the VFA components in nZnO treated manure compared to both control and initial manure.

Table 33. Comparison of volatile fatty acid concentrations (VFA) of liquid dairy manure before and after the completion of the experiment.

Volatile fatty acid (mM)	Initial		After 25 days					
			Control		$nZrO_2$		nZnO	
	Mean	Std.	Mean	Std.	Mean	Std.	Mean	Std.
Acetic	142.9 b	10.9	175.2 ab	49.7	225.8 a	31.9	166.5ab	30.1
Propionic	25.5 b	2.0	57.7 a	21.3	65.8 a	20.5	42.8 ab	4.2
Isobutyric	4.7 b	0.9	24.4 a	3.1	25.5 a	10.3	15.0 ab	7.4
Butyric	16.2 b	1.6	98.0 a	18.0	107.3 a	47.4	66.7 ab	27.5
Isovaleric	5.8 b	0.7	38.7 a	5.0	37.3 a	18.3	22.5 ab	14.2
Valeric	2.7 b	0.1	31.4 a	4.0	33.0 a	18.3	17.7 ab	11.5
Total VFA	197.7 b	14.3	425.4 a	100.3	494.6 a	137.8	331.2ab	58.1

Note: Values followed by the same letter in a row are not significantly different at $P \leq 0.05$; Std. = standard deviation.

Similarly in case of swine manure, the TVFA including other VFA components like acetic acid, propionic acid, isobutyric acid and isovaleric acids were found to be significantly higher in the control as compared to initial manure. Similar to dairy manure, there was also no significant difference in TVFA of swine manure treated with nZnO compared to initial manure (Table 34).

Table 34. Comparison of volatile fatty acid concentrations (VFA) of swine manure before and after the completion of the experiment.

Volatile fatty acids (mM)	Initial		After 27 days					
			Control		$nZrO_2$		nZnO	
	Mean	Std.	Mean	Std.	Mean	Std.	Mean	Std.
Acetic	194.7 b	20.5	267.8 a	13.4	240.7 a	24.0	200.4 b	6.0
Propionic	53.6 c	4.9	78.4 a	3.2	68.8 b	3.4	63.4 b	1.4
Isobutyric	19.6 c	1.2	37.0 a	2.3	29.4 b	3.4	28.5 b	4.5
Butyric acid	71.6 a	6.7	74.1 a	5.1	70.3 a	10.4	71.3 a	13.4
Isovaleric	26.8 c	1.7	43.1 a	3.9	34.4 b	2.8	37.2 ab	6.7
Valeric acid	16.9 a	1.0	16.6 a	1.1	14.6 b	1.3	15.2 ab	1.2
Total VFA	383.2 c	32.8	517.0 a	19.3	458.2 b	24.4	416.0 bc	1.3

Note: Values followed by the same letter in a row are not significantly different at $P \leq 0.05$; Std. = standard deviation.

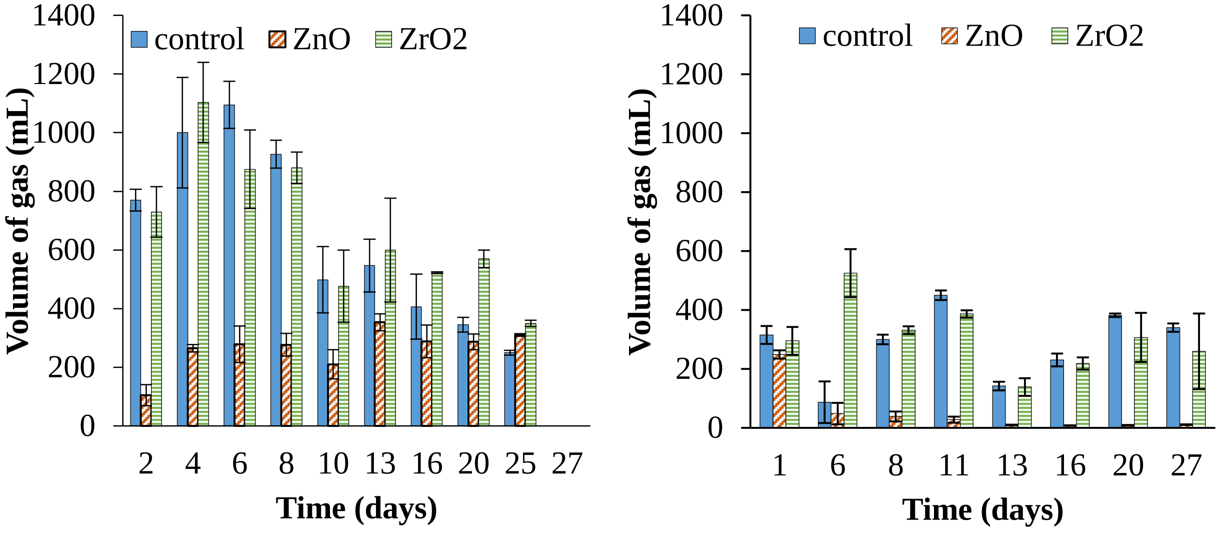
This research has showed that NPs do not affect the acidification step involved during anaerobic storage of both swine and dairy manure. A recent report (Mu et al., 2011) has pointed out that acidogenic (acid forming bacteria) are more resistant to metal toxicity than methanogens. In nZnO treated manure, methanogenic processes are more likely to be affected by the toxicity of nZnO than acidogenic process.

Effect of NPs on gas production from dairy manure

Total gas production from the control and nZnO treated dairy manure are presented in Figure 10a. The cumulative gas production from 1.5 L dairy manure treated with $nZrO_2$, nZnO, and control were 5758, 2063 and 5909 mL, respectively, for a period of 25 days. The average gas production per day per liter dairy manure were 153, 55 and 157 (mL d^{-1}) for $nZrO_2$, nZnO, and control treatment, respectively. The nZnO treatment reduced the gas production under anaerobic condition by 64% as compared with control (Figure 10a). In contrast, $nZrO_2$ treated manure showed no significant difference ($P=0.64$) in total gas production compared to control. Therefore, nZnO can be a suitable additive to reduce gas production under anaerobic storage condition.

Effect of NPs on gas production from swine manure

Like dairy manure, a similar trend was also observed with swine manure treated with NPs. The cumulative gas production from 1.5 L of swine manure were 2472, 399, 2245 mL for $nZrO_2$, nZnO, and control, respectively, over a period of 27 days (Figure 10b). The average gas production per day per liter swine manure were 61, 9, 55 (mL d^{-1} L^{-1}) for $nZrO_2$, nZnO, and control treatments, respectively. Gas production from the manure treated with nZnO was significantly reduced by 82% as compared to control. However, there was no significant difference (P=0.74) in the total gas production from the swine manure when treated with $nZrO_2$ compared to control.

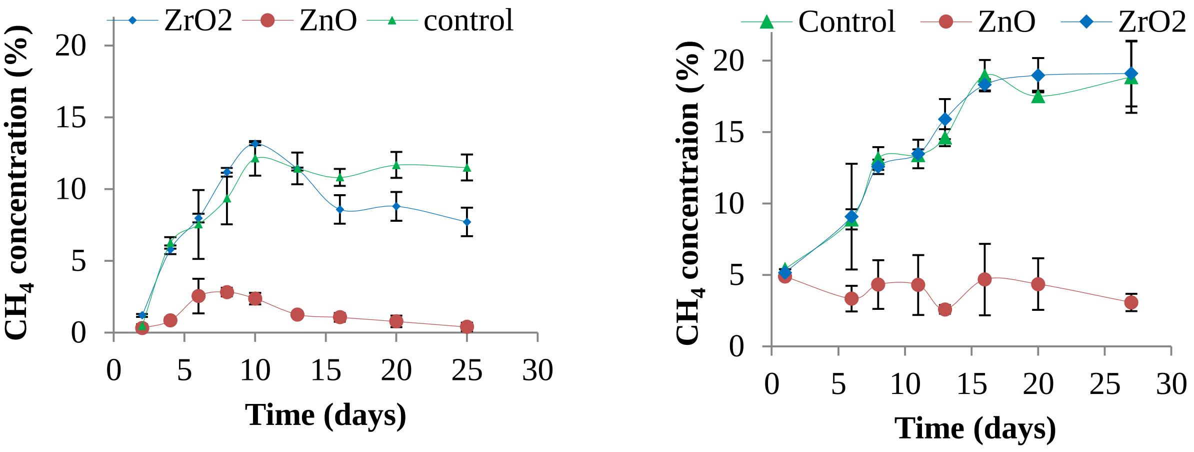


a) b)

Figure 10. Comparison of gas production from a) liquid dairy manure and b) swine slurry treated with NPs. The error bars are the standard deviations calculated from three replications of each treatment.

Effect of NPs on CH_4 production from dairy and swine manure

The trend of CH_4 concentrations for control and treated dairy samples are presented in Figure 11. The average CH_4 concentration with $nZrO_2$ gradually increased from 1.2 to 13 % up to day 10, and then gradually decreased to 7.7% at day 25 (Figure 11). Similarly, average CH_4 concentration with nZnO treated manure varied from 0.3 to 2.8% steadily increased to 2.8% up to day 8, and then gradually decreased to 0.4% up to day 25 (Figure 11). Similarly, in case of control, the average CH_4 concentration gradually increased from 0.5 to 12% in 10 days and remained almost constant (~11.5 %) up to day 25. Methane concentrations in the manure treated with nZnO were consistently lower those in control and manure treated with $nZrO_2$. Average CH_4 concentration with nZnO treated manure from day 10 to day 25 was approximately 10 times lower than the control treatment. The total CH_4 production from the dairy manure treated with nZnO, ZrO_2, and control were approximately 471, 515 and 33 mL, respectively. Like this, approximately 93% reduction in the total CH_4 production was observed from the manure treated with nZnO compared to control.



a)

b)

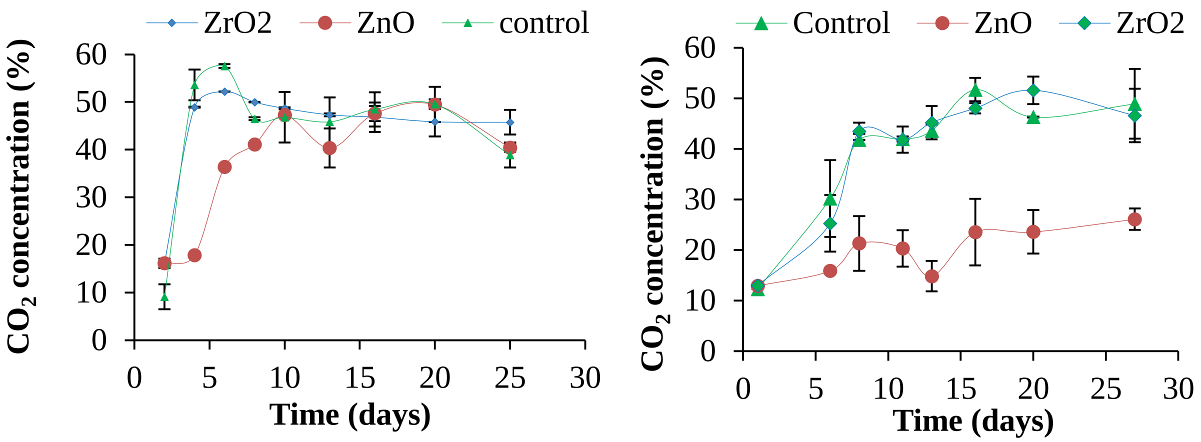
Figure 11. Trends of CH_4 concentrations from a) liquid dairy manure and b) swine manure treated with NPs. The error bars are the standard deviations calculated from three replications of each treatment.

Similarly, the trends of CH_4 concentration in control and treated swine manure were similar to dairy manure. The average CH_4 concentration varied from 5.2 to 19.1%, 2.6 to 4.9%, and 5.2 to 18.8%, respectively, for $nZrO_2$, nZnO, and control treatment for swine manure (Figure 11). In case of swine manure, the average CH_4 concentration with nZnO treatment was approximately four times lower than the control. Similarly, the total CH_4 production from the swine manure treated with nZnO, ZrO_2, and control were approximately 18, 320 and 326 mL; and there was around 94% reduction in the total CH_4 production from the manure treated with nZnO compared to control.

Comparing the CH_4 production between swine and dairy manure; though swine manure produced the gas with higher CH_4 concentrations for all treatments, the amount of total CH_4 production were lower as compared to dairy manure.

Effect of NPs on CO_2 production from liquid dairy and swine manure

Measured CO_2 concentrations varied from 16 to 52%, 16 to 50%, and 9 to 58% with dairy manure samples treated with $nZrO_2$ and nZnO, and control, respectively. The dairy manure treated with nZnO produced consistently lower CO_2 concentration as compared to the control and manure treated with $nZrO_2$ up to day 10. However, the concentrations of CO_2 remained almost constant (~48%) from day 13 onward for all of three treatments (Figure 12a). In case of swine manure, measured CO_2 concentrations varied from 13 to 52%, 15 to 26%, and 12 to 52% manure samples treated with $nZrO_2$ and nZnO, and control, respectively. Similar to CH_4, overall CO_2 concentration measured from nZnO treated swine manure was lower than those of control (12 to 52%) and manure treated with ZrO_2 (13 to 52%). Concentrations of CO_2 were almost steady for the control treatment (~49%), followed by $nZrO_2$ (~47%), and nZnO (~23%) from day 16 to 27 (Figure 12b). The study showed that nZnO reduces the CO_2 concentration from dairy manure only for first few days (10-12 days), but the reduction was throughout the experiment period in case of swine manure. However, there the overall CO_2 production reduced significantly ($P \leq 0.05$ for both type of manure) from the manure treated with nZnO compared to control in both in cases. During 25 days of the experiment, the total volume of CO_2 production from dairy manure treated with nZnO, ZrO_2, and control were 2617, 2726 and 926 mL, respectively. Similarly, the CO_2 production from swine manure treated with nZnO, ZrO_2, and control were 61, 903 and 923 mL, respectively; considering 27 days experiment period. Like this, nZnO was able to reduce 65 and 93% total CO_2 production from dairy and swine manure, respectively, compared to control. Therefore, nZnO may be used to reduce CO_2 production and emission from manure stored under anaerobic conditions.

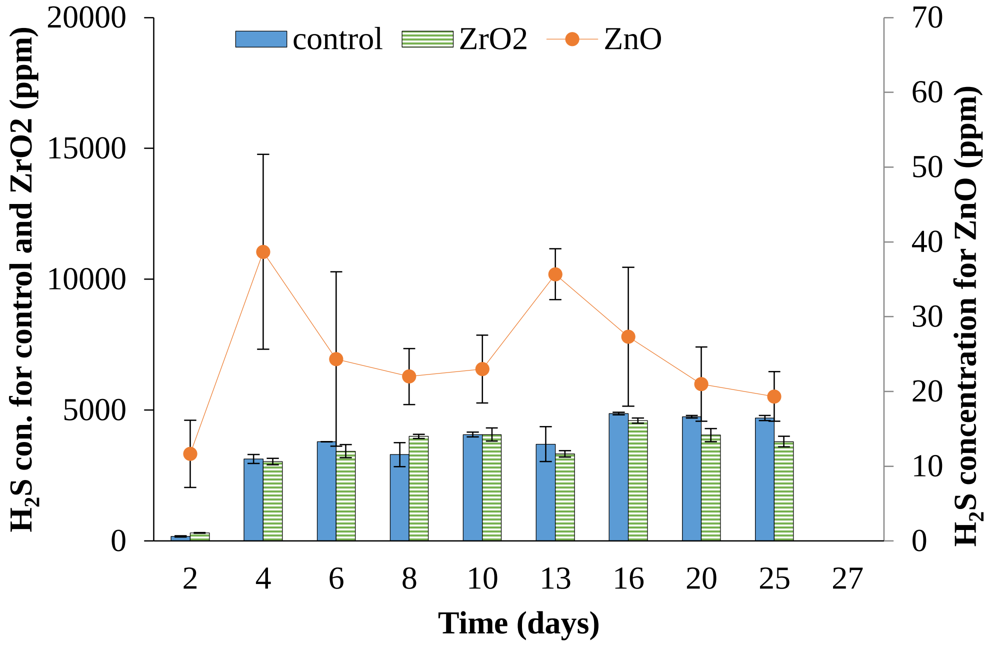


a) b)

Figure 12. Trends of CO_2 concentrations from a) liquid dairy manure and b) swine manure. The error bars are the standard deviations calculated from three replications of each treatment.

Effect of NPs on H_2S production from liquid dairy and swine manure

Similar to CH_4 and CO_2, H_2S concentrations in both swine and dairy manure treated with nZnO were consistently lower than those with $nZrO_2$ and control treatments over the study period. In both types of manure, there was more than 99% reduction in H_2S concentration as well as total H_2S volume from the manure treated with nZnO compared to control. In contrast, the measured average H_2S concentrations between control and $nZrO_2$ treatments were close throughout the experimental period for both swine as well as dairy manure (Figure 13). Comparing the gas production from both types of manure, H_2S concentration measured from control swine manure and treated with $nZrO_2$ were much higher than those with dairy manure as shown in Figure 13. Overall, the study showed that application of nZnO might be an option for mitigating the pollutant gas (H_2S) from the manure stored under the anaerobic system. However, disposal and recovery of nZnO need to be addressed.



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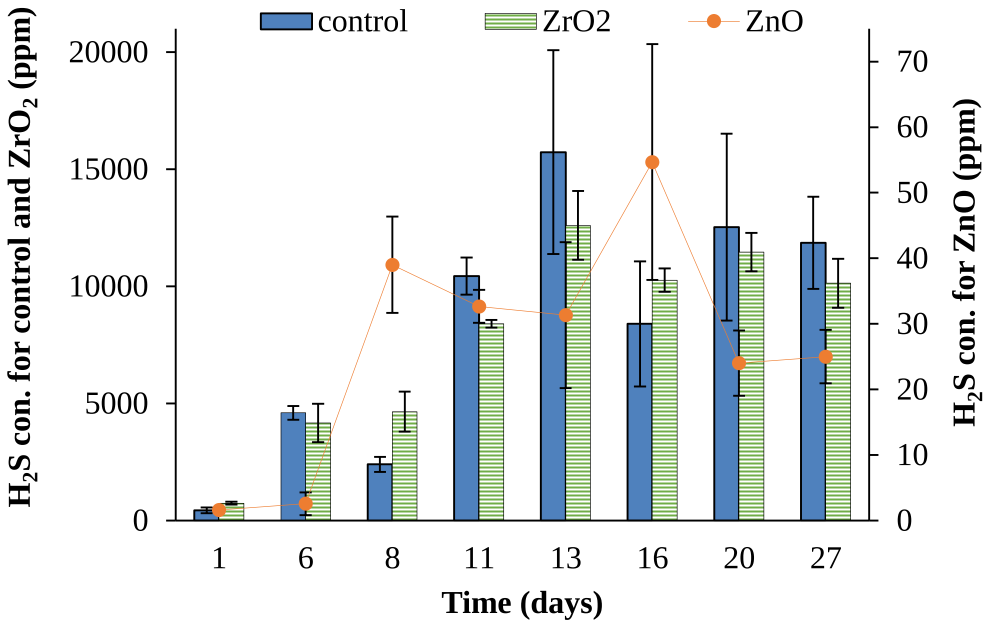
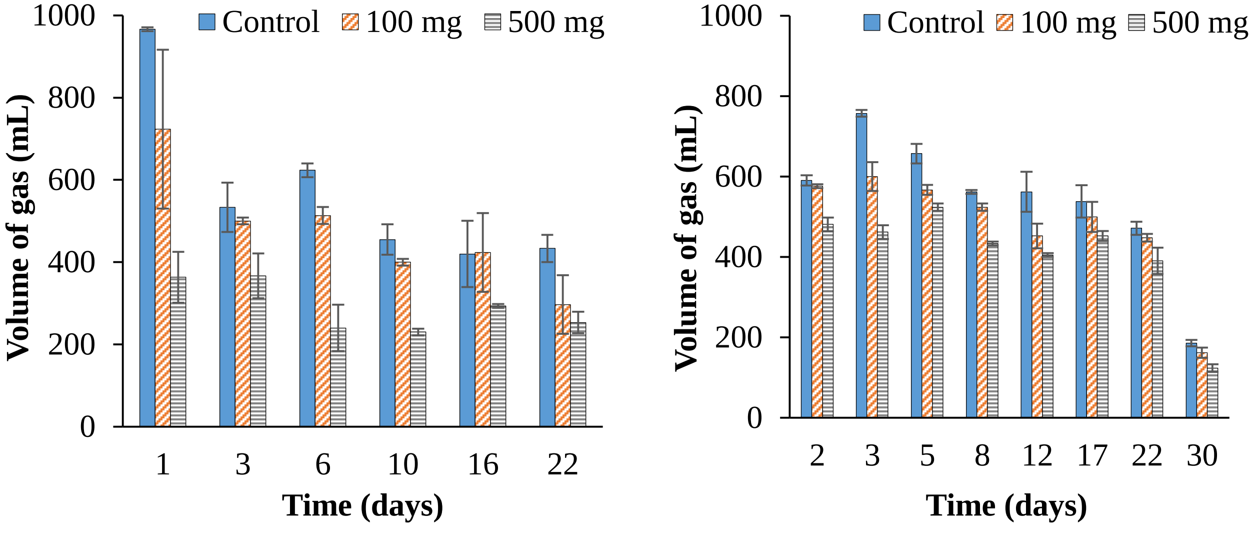


b)

Figure 13. Trends of H_2S concentrations from a) dairy and b) swine manure treated with NPs. The error bars are the standard deviations calculated from three replications of each treatment.

Effect of doses on gases production and on their concentration

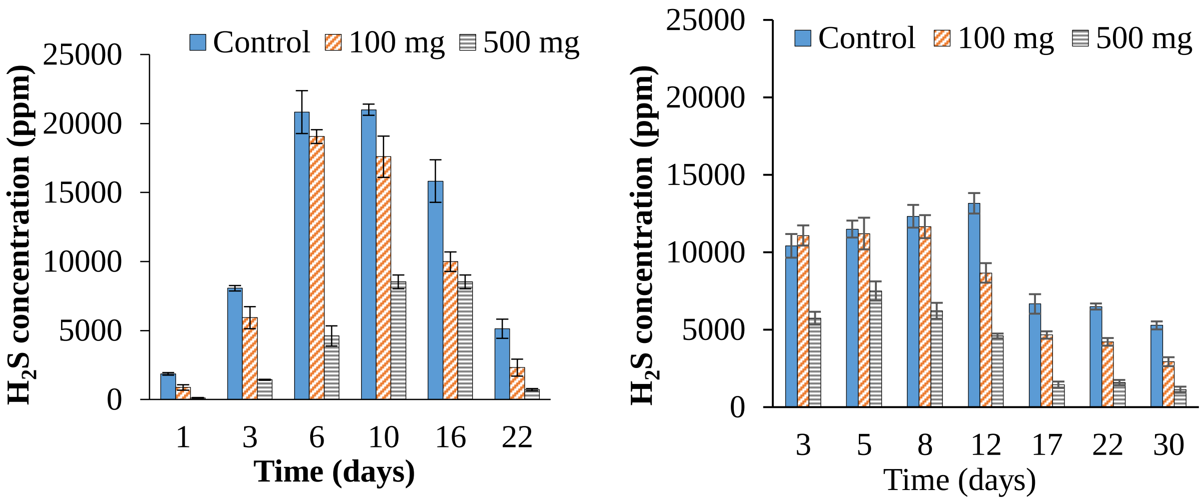
Initially, this experiment was conducted at an application rate of 3 g L^{-1} based on published literature (Predicala et al., 2012). This application rate seems high, so another set of experiment was conducted with a lower application rates of nZnO at doses of 100 and 500 mg L^{-1} in dairy manure and swine manure. In case of dairy manure, gas production was reduced by 17 and 49% by using 100 mg L^{-1} and 500 mg L^{-1} nZnO, respectively, in 24 days of the experimental period. Similarly, the gas production was found to be decreased by 11 and 24% while using 100 and 500 mg L^{-1} nZnO, respectively (Figure 14) in swine manure within the experimental period of 30 days. Definitely, the higher application rate is more effective in controlling pollutant gases, and an application rate of 500 mg L^{-1} would reduce application cost.



a) b)

Figure 14. Gas production from a) dairy manure while using 100 mg L^{-1} and 500 mg L^{-1} nZnO and b) using 100 mg L^{-1}and 500 mg L^{-1} nZnO from swine manure. The error bars are the standard deviations calculated from three replications of each treatment

Similarly, there was a significant reduction in H_2S gas production using lower application rate of nZnO. In dairy manure, the H_2S concentration was found to be significantly lower from manure treated with both 100 mg L^{-1} and 500 mg L^{-1} nZnO. In overall, there the average reduction of H_2S concentrations from the manure treated with 100 mg L^{-1} of nZnO and 500 mg L^{-1} were 33 and 74 %, respectively (Figure 15). However, in case of swine manure, though there was no significant different in the H_2S concentration from the manure treated with 100 mg L^{-1} nZnO and control on the early days (up to 8th day), the overall H_2S production rate showed significant different in all the treatment. The production reduced by 30 and 67% from the manure treated with 100 mg L^{-1} nZnO and 500 mg L^{-1} nZnO compared to control. Though the performance of lower dose of (100 mg L^{-1} and 500 mg L^{-1}) of nZnO was not as comparable to 3 g L^{-1} nZnO application, the lower dose can also be used to reduce gas volume and H_2S gas concentration from both dairy and swine manure.



a) b)

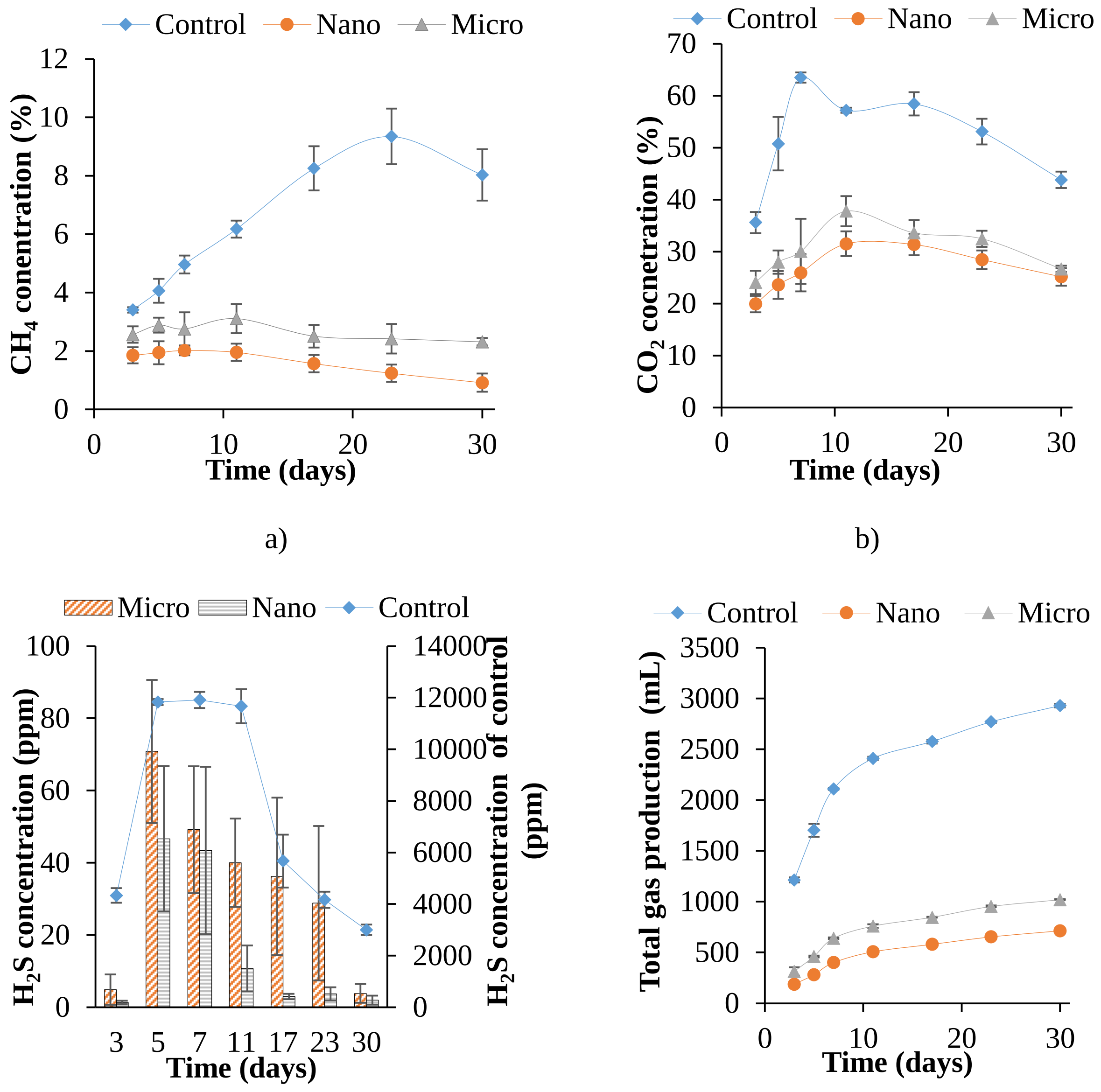
Figure 15. H_2s concentration from a) dairy manure while using 100 mg L^{-1} and 500 mg L^{-1} nZnO and b) using 100 mg L^{-1}, 250 mg L^{-1} and 500 mg L^{-1} nZnO from swine manure The error bars are the standard deviations calculated from three replications of each treatment.

No significant difference in the CH_4 and CO_2 concentration were observed between control and manure treated with 100 mg L^{-1} and 500 mg L^{-1} nZnO in both dairy and swine.

Effect of particle size of ZnO on gases production and on their concentration

To investigate the effect of particles size of ZnO, one more set of experiment was carried out taking 3 g L^{-1} of regular nZnO (35-50 nm) and ZnO (Zinc oxide-Analytical Reagent, US 8832, Mallingckroad Chemical Work, Saint Louis, USA) having the size of approximately 1µm in liquid dairy manure. During 1 month experimental period, the total gas production was found to be 2930, 713, 1020 mL L^{-1} from control, the manure treated with nZnO and micro ZnO, respectively. The reduction in total gas production was found to be 76 and 65% from manure treated with nZnO and micro ZnO compared to control (Figure 16d). Like this 9% difference in the reduction of the gaseous volume was observed in between nano and micro size ZnO. Similarly, in all the measurements, the CH_4 concentration was found to be significantly lower ($P \leq 0.05$) from the manure treated with micro ZnO compared to manure treated with nZnO the average CH_4 concentration was found to be approximately 52% lower in manure treated with micro ZnO, and 69% lower in manure treated with nZnO compared to control (Figure 16a). The study showed that comparatively a huge reduction (~17% difference) in CH_4 concentration can be achieved by using nZnO instead of micro ZnO. Total CH_4 production during 30 days of experimental period was found to be 139, 12 and 26 mL L^{-1} from control, manure treated with nZnO and micro ZnO, respectively. In the other hand, though no significant difference in the CO_2 concentration was found in most of the measurement in manure treated with micro ZnO and nZnO, but the average CO_2 concentration was found to be 48 and 41% lower in the manure treated with nZnO and micro ZnO, respectively, compared to control (Figure 16b). The CO_2 production rate was 1364, 180 and 280 mL L^{-1} in control and manure treated with nZnO and micro ZnO; and there was 87 and 79% percent reduction in the CO_2 production from the manure treated with nZnO and micro ZnO compared to control.

Both nZnO and micro ZnO were found to be very effective in reducing H_2S concentration. The average H_2S concentration was reduced by more than 99% in both manure treated with nZnO and micro ZnO compared to control (Figure 16c).



a)

b)

c)

d)

Figure 16. a) Methane concentration b) CO_2 concentration c) H_2S concentration and d) Cumulative gas production from a liter of dairy manure treated with micro ZnO, nZnO, and control.

Conclusions

This study was conducted to assess the impact of nZnO and $nZrO_2$ on the gas production from swine slurry and liquid dairy manure stored under anaerobic condition. The experiment was conducted by using 3 g L^{-1} NPs on the manure. The 3 g L^{-1} dose of nZnO showed good results for the reduction total gas production, and H_2S, CH_4 and CO_2 concentration from both swine and dairy manure. Compared to control, nZnO reduced the total gas production by 64 and 82% in dairy and swine manure, respectively. The H_2S concentration was reduced by more than 99% in both manure compared to control. Similarly, the CH_4 concentration also reduced by 67 and 78% in dairy and swine manure, respectively compared to control. The nZnO also reduced CO2 concentration from both dairy and swine manure. However, nZnO showed the minimal effect on the nutrient composition and volatile fatty acids content in the manures compared to control. On contrast, $nZrO_2$ treated manure samples did not show any effect on reducing pollutant gas production and on their concentrations. Even the lower nanoparticle application rate (100 and 500 mg L^{-1}) of nZnO may reduce H_2S gas concentration and total gas production but not GHG concentration. The comparative study of nZnO with micro ZnO showed better performance with nano-sized nZnO in term of total gas volume and gases concentration. Though nZnO performed well on mitigating GHGs and H_2S gas from livestock manure under anaerobic storage condition, further research is needed to implement this technology for real life applications.

References

Abatzoglou, N., and Boivin, S. 2008. A review of biogas purification processes. *Biofuels, Bioproducts and Biorefining*. 3(1): 42-71.

Basarab, J., Price, M., Aalhus, J., Okine, E., Snelling, W., and Lyle, K. 2003. Residual feed intake and body composition in young growing cattle. *Canadian Journal of Animal Science*. 83(2): 189-204.

Brar, S. K., Verma, M., Tyagi, R., and Surampalli, R. 2010. Engineered nanoparticles in wastewater and wastewater sludge–Evidence and impacts. *Waste Management*. 30(3): 504-520.

Brayner, R., Ferrari-Iliou, R., Brivois, N., Djediat, S., Benedetti, M. F., and Fiévet, F. 2006. Toxicological impact studies based on Escherichia coli bacteria in ultrafine ZnO nanoparticles colloidal medium. *Nano Letters*. 6(4): 866-870.

Bremner, J. M., and Blackmer, A. M. 1978. Nitrous Oxide: Emission from Soils During Nitrification of Fertilizer Nitrogen. *Science*. 199(4326): 295-296.

Buddle, B. M., Denis, M., Attwood, G. T., Altermann, E., Janssen, P. H., Ronimus, R. S., Pinares-Patiño, C. S., Muetzel, S., and Neil Wedlock, D. 2011. Strategies to reduce methane emissions from farmed ruminants grazing on pasture. *The Veterinary Journal*. 188(1): 11-17.

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

Chadwick, D. R. 2005. Emissions of ammonia, nitrous oxide and methane from cattle manure heaps: effect of compaction and covering. *Atmospheric Environment*. 39(4): 787-799.

Chen, Y., Chen, Z., He, Y., Lin, H., Sheng, P., Liu, C., Luo, S., and Cai, Q. 2010. L-cysteine-capped CdTe QD-based sensor for simple and selective detection of trinitrotoluene. *Nanotechnology*. 21(12): 125502.

Choi, O., Deng, K. K., Kim, N.-J., Ross, L., Surampalli, R. Y., and ZHIQIANG, H. 2008. The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth. *Water Research*. 42(12): 3066-3074.

Choi, O., and Hu, Z. 2009. Nitrification inhibition by silver nanoparticles. *Water science and technology: a Journal of the International Association on Water Pollution Research*. 59(9): 1699.

Clemens, J., and Ahlgrimm, H.-J. 2001. Greenhouse gases from animal husbandry: mitigation options. *Nutrient Cycling in Agroecosystems*. 60(1): 287-300.

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

Drummond, J. G., Curtis, S. E., Simon, J., and Norton, H. W. 1980. Effects of aerial ammonia on growth and health of young pigs. *Journal of Animal Science*. 50(6): 1085-1091.

Hao, X., Mir, P. S., Shah, M. A., and Travis, G. R. 2005. Influence of canola and sunflower diet amendments on cattle feedlot manure. *Journal of Environmental Quality*. 34(4): 1439-1445.

Hernández, S. P., Chiappero, M., Russo, N., and Fino, D. 2011. A novel ZnO-based adsorbent for biogas purification in H2 production systems. *Chemical Engineering Journal*. 176: 272-279.

Hill, D., Cobb, S., and Bolte, J. 1987. Using volatile fatty acid relationships to predict anaerobic digester failure. *Transaction of American Society of Agricultural Engineers.* 30(2): 496-501.

Jangra, S. L., Stalin, K., Dilbaghi, N., Kumar, S., Tawale, J., Singh, S. P., & Pasricha, R. 2012. Antimicrobial activity of zirconia (ZrO2) nanoparticles and zirconium complexes. *Journal of nano-science and nanotechnology*. 12(9): 7105-7112.

Jensen, A. 2002. Changing the environment in swine buildings using sulfuric acid. *Transactions-American Society of Agricultural Engineering*. 45(1): 223-238.

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

Khan, R. Z., Müller, C., and Sommer, S. G. 1997. Micrometeorological mass balance technique for measuring CH_4 emission from stored cattle slurry. *Biology and Fertility of Soils*. 24(4): 442-444.

Kim, J. S., Kuk, E., Yu, K. N., Kim, J.-H., Park, S. J., Lee, H. J., Kim, S. H., Park, Y. K., Park, Y. H., and Hwang, C.-Y. 2007. Antimicrobial effects of silver nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine*. 3(1): 95-101.

Li, C., Frolking, S., and Butterbach-Bahl, K. 2005. Carbon sequestration in arable soils is likely to increase nitrous oxide emissions, offsetting reductions in climate radiative forcing. *Climatic Change*. 72(3): 321-338.

Luna-delRisco, M., Orupõld, K., and 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.

Luostarinen, S., Luste, S., and Rintala, J. 2006. Nitrogen removal from on-site treated anaerobic effluents using intermittently aerated moving bed biofilm reactors at low temperatures. *Water Research*. 40(8): 1607-1615.

MacDonald, J. G., McGrath, K. P., Kuznetsov, I., Kim, J., and Huang, L. 2011. Method for reducing odor using colloidal nanoparticles. Google Patents No. 7,879,350.

Miao, Z., Wu, Y., Zhang, X., Liu, Z., Han, B., Ding, K., and An, G. 2007. Large-scale production of self-assembled SnO_2 nanospheres and their application in high-performance chemiluminescence sensors for hydrogen sulfide gas. *Journal of Materials Chemistry*. 17(18): 1791-1796.

Mishra, A., Tripathy, S. K., and Yun, S.-I. 2011. Biosynthesis of gold and silver nanoparticles from Candida guilliermondii and their antimicrobial effect against pathogenic bacteria. *Journal of Nanoscience and Nanotechnology*. 11(1): 243-248.

Mu, H., Chen, Y., and 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.

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

Nahm, K. 2002. Efficient feed nutrient utilization to reduce pollutants in poultry and swine manure. *Critical Reviews in Environmental Science and Technology*. 32(1): 1-16.

Ndegwa, P., Hristov, A., Arogo, J., and Sheffield, R. 2008. A review of ammonia emission mitigation techniques for concentrated animal feeding operations. *Biosystems Engineering*. 100(4): 453-469.

Novak, S., and Fiorelli, J. 2010. Greenhouse gases and ammonia emissions from organic mixed crop-dairy systems: a critical review of mitigation options. *Agronomy for Sustainable Development*. 30(2): 215-236.

Patterson, P. 2005. Management strategies to reduce air emissions: Emphasis—Dust and ammonia. *The Journal of Applied Poultry Research*. 14(3): 638-650.

Pattey, E., Trzcinski, M., and Desjardins, R. 2005. Quantifying the Reduction of Greenhouse Gas Emissions as a Result of Composting Dairy and Beef Cattle Manure. *Nutrient Cycling in Agroecosystems*. 72(2): 173-187.

Petersen, S. O., and Miller, D. N. 2006. Greenhouse gas mitigation by covers on livestock slurry tanks and lagoons? *Journal of the Science of Food and Agriculture*. 86(10): 1407-1411.

Portejoie, S., Martinez, J., Guiziou, F., and Coste, C. 2003. Effect of covering pig slurry stores on the ammonia emission processes. *Bioresource Technology*. 87(3): 199-207.

Powers, W. 1999. Odor control for livestock systems. *Journal of Animal Science*. 77(2): 169-176.

Pradhaban, G., Kaliaraj, G. S., & Vishwakarma, V. 2014. Antibacterial effects of silver–zirconia composite coatings using pulsed laser deposition onto 316L SS for bio implants. *Progress in Biomaterials*. *3*(2-4). 123-130.

Predicala, B., Alvarado, A., and Asis, D. 2012. Use of Zinc Oxide Nanoparticles to Control Hydrogen Sulphide, Ammonia and Odour Emissions from Pig Barns. In *9th International Livestock Environment Symposium American Society of Agricultural and Biological Engineers Valencia Conference Centre Valencia, Spain July 8 - 12, 2012.* Paper Number: ILES12-1507, ASABE. Retrieved on 8/20/2013 from http://elibrary.asabe.org/azdez.asp?JID=1&AID=41577&CID=iles2012&T=2.

Rahman, S., DeSutter, T., and 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): 1-9.

Reicosky, D. C., Hatfield, J. L., and Sass, R. L. 2000. Agricultural contributions to greenhouse gas emissions. *Climate Change and Global Crop Productivity*: 37-50.

Roco, M. C. 1999. Nanoparticles and nanotechnology research. *Journal of Nanoparticle Research*. 1(1): 1-6.

Ruokojärvi, A., Ruuskanen, J., Martikainen, P. J., and Olkkonen, M. 2001. Oxidation of gas mixtures containing dimethyl sulfide, hydrogen sulfide, and methanethiol using a two-stage biotrickling filter. *Journal of the Air & Waste Management Association*. 51(1): 11-16.

Satter, L., Klopfenstein, T. J., and Erickson, G. E. 2002. The role of nutrition in reducing nutrient output from ruminants. *Faculty Papers and Publications in Animal Science*: 472(1): 143-155.

Sayyadnejad, M., Ghaffarian, H., and Saeidi, M. 2008. Removal of hydrogen sulfide by zinc oxide nanoparticles in drilling fluid. *Int. J. Environ. Sci. Tech*. 5(4): 565-569.

Sondi, I., and Salopek-Sondi, B. 2004. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. *Journal of Colloid and Interface Science*. 275(1): 177-182.

Thu, K. M. 2002. Public health concerns for neighbors of large-scale swine production operations. *Journal of Agricultural Safety and Health*. 8(2): 175-184.

Varel, V. H. 1997. Use of urease inhibitors to control nitrogen loss from livestock waste. *Bioresource Technology*. 62(1): 11-17.

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

Von Bemuth, R., Hill, J., Henderson, E., Godbout, S., Hamel, D., and Pouliot, F. 2005. Efficacy of a liquid/solid isolation system for swine manure. *Transactions of the ASAE*. 48(4): 1537-1546.

Waghorn, G., Woodward, S., Tavendale, M., and Clark, D. 2006. Inconsistencies in rumen methane production effects of forage composition and animal genotype. *International Congress Series*. 1294 (0): 115-118.

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

EVALUATION OF CALCIUM ALGINATE ENTRAPPED NANO ZINC OXIDE TO REDUCE GASEOUS EMISSIONS FROM LIQUID DAIRY MANURE[4]

Abstract

Direct application of nanoscale zinc oxide (nZnO) particles has shown the promising result in controlling gaseous emissions (hydrogen sulfide-H_2S, methane-CH_4, and carbon dioxide-CO_2) from livestock liquid manure under anaerobic storage conditions. However, application of bare (unmodified) nanoparticles (NPs) could raise environmental concerns as their fate and transport are not well documented. Keeping this in mind, an innovative method has been adopted where NPs were entrapped in biopolymer beads that ensured that NPs were not released to the environment and can be recovered. The objective of this research was to evaluate the performance of calcium (Ca)-alginate entrapped nZnO (alginate-nZnO beads) and associated mechanisms involved in controlling gaseous emission from liquid dairy manure. Experiments were conducted in 1-L Erlenmeyer flasks with a working volume of 500 mL, where alginate-nZnO beads containing 3 g L^{-1} of NPs were applied freely as well as loosely contained in mosquito net bags. Headspace gas was collected every 2-5 days during the 33-day experiment and analyzed for H_2S, CH_4, and CO_2 concentration. Bacterial growth analysis and sorption test were also carried out to see the effect of NPs on microbial survivability and gaseous sorption capability, respectively. The concentration of H_2S, CH_4, CO_2, and total gas production was significantly reduced by 99, 51, 27

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[4] This is an accepted paper in press of Applied Engineering in Agriculture Journal. The material in this chapter was co-authored by Dhan Prasad Gautam, Shafiqur Rahman, Achintya N. Bezbaruah and Md Saidul Borhan. Dhan Prasad Gautam had primary responsibility for the collection and analyses of samples, and was the primary developer of the conclusions that are advanced here. Other authors served as proofreader and checked the math in the statistical analysis conducted by Dhan Prasad Gautam

and 43%, respectively, when beads were applied freely; while these reductions were 96, 18, 14 and 20%, respectively, when beads were applied loosely in bags ($P \leq 0.05$). Though both methods performed well, free beads application method outperformed the other application method.

Introduction

Usually Nanoparticles (< 100 nm) exhibit unique and distinct physical, chemical and biological properties compared to their bulk counterparts. Nanoparticles (NPs) have wide applications in the fields of medicine (Rejinold et al., 2015), bio-material (Ahamed et al., 2015), electronics (Vidor et al., 2014), energy production (Wang et al., 2014), civil construction (Rashad, 2013) and environmental remediation (Wu et al., 2015). Though NPs have found wide applications in different sectors, they have limited applications in the field of agriculture. Recently research publications, however, indicate a shift and reported the applications of NPs such as zinc oxide (nZnO), copper oxide (nCuO) and silver (nAg) in livestock manure to control gaseous emission (Gautam et al., 2013; Hu et al., 2005; Luna-delRisco et al., 2011; Predicala et al., 2012). Among these tested NPs, zinc oxide (nZnO) is found to be promising to control gaseous emissions of hydrogen sulfide (H_2S), methane (CH_4), and carbon dioxide (CO_2) from livestock liquid manure (Gautam et al., 2013; Luna-delRisco et al., 2011; Predicala et al., 2012). Gautam et al. (2013) reported a reduction of H_2S and CH_4 by 99% and 67%, respectively, while using 3 g L^{-1} nZnO in liquid dairy manure. Predicala et al. (2012) reported more than 95% reduction in H_2S concentration from swine manure with 3 g L^{-1} application rate of nZnO. Similarly Luna-delRisco et al. (2011) also reported 74% reduction of biogas yield at an application rate of 240 mg L^{-1} nZnO. However, the researchers (Gautam et al., 2013; Luna-delRisco et al., 2011) applied NPs directly to manure which may have other environmental consequences such as endemic bacterial death and residual toxicity; therefore, this is recognized as an ambiguous area needing further research (Bour et al., 2015; Lopez-Serrano et al., 2014). The concept of direct application of NPs in manure could be effective only if NPs are either non-toxic, effective at low doses, or transforms into benign end products. However, even the low dose applications of NPs could also be an issue, as the long-term effect of most of the NPs and their environmental fates are still not clear (Bragaru et al., 2013; Lopez-Serrano et al., 2014). The toxicity of NPs on endemic bacteria should be evaluated before adopting NPs for applications in the agricultural sector (Buffet et al., 2014; Mishra and Singh, 2015). In the lack of a clear picture about toxicity, the fate and transport of NPs, the disposal or land application of manure treated with NPs is a concern. Therefore, it is important to devise a method for effective recovery of NPs after the application on manure for gas emission reduction.

Many technologies have been developed for separation and recovery in order to reuse the valuable nanomaterials. Some of the separation and recovery techniques are light-induced flocculation (Vesperinas et al., 2007), filtration (Geukens and De Vos, 2013; Pesch et al., 2014), solvent evaporation (Koetz et al., 2005), micro-flotation (Mishchuk et al., 2012), temperature control (Abécassis et al., 2009), addition of anti-solvent CO_2 after reverse micelles process (Zhang et al., 2002), and micro-emulsion (Myakonkaya et al., 2011). The use of entrapped NPs on polymer matrix is one of the techniques which was tried for different applications to prevent the exposure of NPs in the environment (Balan et al., 2008; Bezbaruah et al., 2009; Wu et al., 2009). However, these applications were mostly confined to water and wastewater and have not been tested in livestock manure.

Nanoparticles dispersed in the polymer matrix are found to be as effective as in the bare form with a little change in reactivity (Bezbaruah et al., 2009). Silver NPs are the most commonly used NPs found to be entrapped in different types of polymers and in different fashions (Balan et al., 2008; Kong and Jang, 2006; Porel et al., 2005). Nanoscale zero-valent iron (nZVI) entrapped in Ca-alginate beads were used to remove nitrate (Bezbaruah et al., 2009), trichloroethylene-TCE (Bezbaruah et al., 2011; Kim et al., 2010) and arsenic (Escudero et al., 2009) from contaminated groundwater. Liu et al. (2010) used nZVI entrapped in chitosan beads for hexavalent chromium removal from wastewater. Chitosan beads were also used for entrapping silver (Ag), palladium (Pd), platinum (Pt), gold (Au) and copper (Cu) NPs (He et al., 2008; Laudenslager et al., 2008; Wu et al., 2009). Polymers such as Polyvinylidene fluoride (PVDF), Polyamide blends, Poly-ether ether ketone (PEEK) and Polyelectrolyte membrane are also popular for entrapping NPs like copper (Cu), palladium (Pd), platinum (Pt), rhodium (Rh) and silver (Ag) (Monticelli et al., 2005; Muraviev et al., 2007; Xu and Bhattacharyya, 2007). Similarly, zero-valent silver (Ag) and nickel (Ni) have been entrapped in polyamide films, i.e., resins with divinylbenzene matrix (Akamatsu et al., 2008) Hydrated ferric oxide has been entrapped in cation exchange resin with a sulfonic acid functional group (Cumbal and SenGupta, 2005).

Previous research (Gautam et al., 2013) has demonstrated that bare nZnO can effectively reduce H_2S and CH_4 generated from livestock manure. However, the NPs couldn’t be recovered back from the manure, thus, posing an environmental concern. One of the safer application methods could be entrapping the NPs into the biodegradable polymer matrix as beads (Bezbaruah et al., 2009) for application in liquid manure to reduce gas generation and recover the beads after use. Alginate is a biodegradable polymer derived from brown seaweed; and it is non-toxic, hydrophilic, biocompatible and cost-effective (Shalumon et al., 2011). It is a negatively charged polysaccharide and this negative charge is gained from the carboxyl group (Bhattarai and Zhang, 2007). Shalumon et al. (2011) used sodium alginate-nZnO beads as an antibacterial agent. In the alginate beads production procedure, calcium chloride solution is required, as calcium ion cross-linked in the polymer and harden the alginate drop, forming a solid spherical structure (Bezbaruah et al., 2009; Bezbaruah et al., 2011; Choi et al., 2002) (Equation 28).

$$2\mathrm{NaAlginate} + \mathrm{CaCl_2} \rightarrow 2\mathrm{NaCl} + \mathrm{CaAlginate} \qquad (28)$$

This work explored the possibility of the use of calcium alginate-nZnO beads for the adsorption of gases generated in livestock manure. It was expected that the NPs entrapped in the beads will be easy to apply into liquid manure and recoverable at the end of their useful life. The permeability of the polymer beads would allow gas transfer into the beads and would be adsorbed or reacted upon.

Though the performance evaluation of the direct application of NPs on the manure system for mitigating GHGs and pollutant gas has been carried out (Gautam et al., 2013; Predicala et al., 2012), the mechanism behind the reduction of gaseous emission is not well studied. Therefore, the specific objectives of this research were: 1) to evaluate the performance of alginate-nZnO beads in reducing gaseous emission from manure, and 2) to assess the indicators (*e.g.,* microbial study, sorption test, and SEM analysis) which help to explain the reduction of gaseous emission from manure by nZnO.

Materials and methods

From the preliminary experiment with bare nZnO, the reduction in the concentration of gases from manure was observed. Therefore, a hypothesis was made that the reduction of gases is either due to sorption or the chemical conversion of producing gas. As a proof of concept, the sorption test of gases generated from manure (H_2S and CH_4) was carried out. To avoid the exposure of NPs in the environment, they were entrapped in alginate beads which facilitate the easy recovery after the treatment. Subsequently a simple experiment was carried out to evaluate the performance of alginate-nZnO beads on the gaseous emission from livestock manure. Similarly, the bacterial study of manure was also carried out to test the hypothesis that the reduction of gaseous emission is likely due to the toxic effect of NPs on microorganisms.

In this experiment, dairy manure was collected from the dairy farm at North Dakota State University (NDSU) and treated with alginate beads. All the experiments were performed at room temperature (~ 22±2°C) and room atmospheric pressure (~ 760 mm Hg). To understand the mechanism of NPs effect on gaseous emission, the population density analysis of total coliform bacteria in manure was performed using the plate counting method. Also, the sorption test of hydrogen sulfide (H_2S) gas was performed using bare nZnO, and alginate-nZnO beads. The alginate bead preparation method, the experimental set-up, sampling, and analysis processes of experiments has been discussed in the following sections.

Preparation of alginate beads

Calcium alginate beads were prepared by entrapping nZnO (ZnO, US3580, US Research Nano-materials, Inc., Texas, USA) in sodium alginate solution ($(C_6H_7O_6Na)_n$, S1118, Spectrum, Gardena, CA90248, New Brunswick, NJ 08901), followed by hardening in calcium chloride solution ($CaCl_2.2H_2O$, BDHO224, VWR international LLC 1310 Parkway, West Chester, PA 19380). Firstly, 6 g of nZnO was placed in an Erlenmeyer flask containing 1L of deionized (DI) water and stirred using a magnetic stirrer for around 10 min at 350 rpm. Sodium alginate powder (15 g) was slowly added with stirring and continued stirring for around 48 h at ~50°C. Once a complete dissolution was obtained, the flask was transferred into a sonicator (Bransonic® CPXH Ultrasonic Cleaning Bath, Brason, USA) and kept there for ~1 h to disperse the NPs uniformly in the solution. Then the mixture solution was transferred with a 60 mL syringe and poured dropwise into the 3.5% calcium chloride solution. As soon as the drops of NP-alginate mixture came in contact with calcium chloride solution, they formed beads. The synthesized alginate beads were kept in a 3.5% calcium chloride solution for an additional period of 6-8 h for complete hardening of the beads and to ensure porosity for solute transport (Bezbaruah et al., 2009). The hardened beads were washed with DI water and kept in DI water until they were used. Following the same procedure, alginate beads without nanoparticles (blank beads) were prepared and stored in DI water for later use.

Experimental set up

Sorption of gas: proof of concept studies

This experiment was conducted to test the hypothesis that the reduction of gas concentration is likely due to both chemical conversion and sorption of gas by the NPs. Bare nZnO (2 mg in each flask) was transferred to four 500 mL Erlenmeyer flasks (flushed with ultra-pure nitrogen gas) and closed tight immediately with rubber stoppers. A similarly treated flask was kept as control (no nZnO added). Headspace gas (180 mL) was removed from each of the five flasks using a gas tight syringe without disturbing NPs at the bottom. The same amount (180 mL) of standard H_2S calibration gas (~25 ppm) was injected into each flask. Immediately after injection, all the flasks were shaken consistently by hand for approximately 15 s to ensure the maximum possible interaction of the NPs with the injected H_2S gas. The flasks were allowed to stand undisturbed before sample collection. Air samples from the headspace were taken at definite time intervals (2.5, 5, 10 and 20 min) in Tedlar bags (inert bag to collect gaseous sample, SKC Gulf Coast Inc., Texas, USA). Initially, the air samples from the control were measured in different time intervals, but no difference in the values were observed. Thus, air sample from the control flask was taken only at around 5 min in each experiment to compare the values. All air samples were analyzed immediately for H_2S using the Jerome meter (Jerome® 631-X, Arizona Instrument, Arizona, USA). The same experiment was repeated with alginate-nZnO and blank alginate beads. Each batch of beads contained the same amount of nZnO (2 mg). Similar experiments were carried out for CH_4 (~22.2 ppm gas) taking 2 mg, 10 mg, 100 mg and 3 g nZnO.

Entrapped nano zinc experiments

Preliminary studies were carried out using bare nZnO at an application rate of 100 mg L^{-1}, 500 mg L^{-1}, and 3 g L^{-1}. Best results (in terms total gas production and gas concentration reduction) were obtained from the 3 g L^{-1} dose for H_2S and CH_4 removal from manure (Gautam et al., 2013). In this study, when entrapped NPs alginate beads were prepared, nZnO concentration 3 g L^{-1} was maintained. This was done assuming that each bead contains an equal amount of nZnO.

The experiments were carried out in 1 L Erlenmeyer flasks having 500 mL working volume. Three treatments were: controls (manure with no NPs or beads) (Figure 17a), manure treated with loose alginate-nZnO beads (Figure 17b), and alginate-nZnO beads placed in porous bags (0.8 mm polyester mosquito netting (American Home and Habitat Inc., Sealy, TX, USA) (Figure 17c). Controls were manure with no NPs or beads. While beads were introduced in the first treatment as loose beads, it would be difficult to recover them after treatment and as the bags were used for a possible easy recovery of the used beads. Each flask was shaken thoroughly to ensure proper mixing and the head space was purged with nitrogen gas for ~3 min and closed immediately with a rubber stopper attached to 1 L Tedlar bag (SKC Gulf Coast Inc., Texas, USA). This experiment was continued approximately for 33 days.

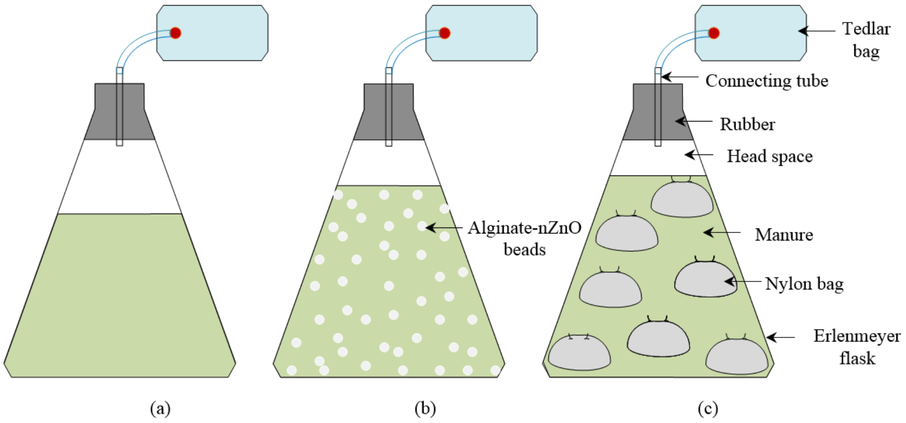


Figure 17. Schematic diagram of the experimental setup, Erlenmeyer flasks tightly fitted with 1 L Tedlar bag, containing a) manure only as control, b) manure treated with loose alginate-nZnO beads, and c) manure treated with alginate-ZnO beads inside nylon bags.

Sampling and analysis of headspace gas

Headspace gas collected in a Tedlar bag was analyzed for hydrogen sulfide (H_2S) and GHGs (CH_4 and CO_2) every 2-5 days depending on the volume of gas accumulated in the bag. Total gas volume in each bag was determined using a graduated gas tight syringe (SGE Analytical Syringe, 500 mL, Australia). A fixed amount of gas sample (2.5 or 5 mL) was withdrawn from the Tedlar bag using a small graduated syringe (309604 - 10 mL BD Luer – Lok^{TM} Tip Syringe, New Jersey, USA) and transferred to another clean Tedlar bag. Then, the sample was diluted with nitrogen gas to bring the concentration to the detection limit of a gas chromatograph (GC) (8610C, SRI Instrument, California, USA). The GC was equipped with a flame ionized detector (FID) for detecting CH_4 and CO_2.

Before each measurement, GC was calibrated using the calibration quality standard gases (20, 100, 1000 ppm for CH_4; 100, 1000, 2500 ppm for CO_2). For each concentration, five to seven replicated measurements were made. Minimum detection limit (MDL) was calculated following the USEPA guidelines, as the product of the standard deviation of replicates and the Student’s t-value at the 99% confidence level as described in Rahman et al. (2013).

Similarly, H_2S gas concentration was measured using a hydrogen sulfide analyzer (Jerome® 631-X, Arizona Instrument, Arizona, USA). For the measurement of H_2S, samples were also diluted with nitrogen gas to bring the gas concentration to the instrument's maximum detection limit of 50 ppm as previously described by Rahman and Newman (2012). After measuring the concentration of the diluted sample, the actual concentration of samples was calculated by multiplying with the dilution factor. The quality control of the data was ensured measuring the standard and blanks concentrations in every ten samples. The data was accepted with a variation within 2% compared to the known concentration tested.

Manure characterization

Manure samples were collected from each flask before and after completing an experiment. Samples were analyzed for pH, moisture content (MC), ash content, total nitrogen (TN), crude protein (CP), fecal ammonia (NH_3), and volatile fatty acids (VFAs) (Table 35).

Table 35. Method/ protocol used to analyze manure samples.

Parameters	Methods/ protocol used
pH	EPA SW-846, Method 9040
MC	Recommended Method of Manure Analysis, A3769
Ash	Official Method 942.05, AOAC International (2005) 18th ed., AOAC International Gaithersburg, MD
TN	Recommended Methods of Manure Analysis, A3769 Macro-Kjeldahl method (adapted from Kane, 1998)
CP	Official Method 2001.11, AOAC International (2005) 18th ed., AOAC International Gaithersburg, MD
NH_3	Sigma Technical Bulletin #640. Sigma Diagnostics, St. Louis, MO 63178
VFA	Method of Goetsch and Galyean, 1983. Agilent 6890N Gas Chromatograph with an FID (flame ionization detector) and the 7683 Series auto-injector and autosampler. Column used was the Supercool brand, NUKOL Fused Silica Column, 15 m x 0.53 mm x 0.5 um

AOAC = The Association of Official Agricultural Chemists

Bacterial study

To evaluate the effects of NPs on microbial community in manure, the population density analysis of bacteria (total coliform bacteria) was done using the plate count method (Total and Fecal coliform test). Batch experiments with 100 mg L^{-1} bare nZnO, 3 g L^{-1} bare nZnO, with 3 g L^{-1} alginate-nZnO beads were run in 50 mL plastic vials at a room temperature (22 ± 1^{o}C). The headspace of each vial was flushed with nitrogen gas and the vial was tightly closed with a plastic (Polypropylene) stoppers. Samples (1 mL) were collected from the batch reactors after 72 h and diluted with sterile water (dilution factor of 10^3). The first sampling was done at 72 h time to ensure that anaerobic microbial activities are in the growth phase (Ameur et al., 2011). A sterile membrane filter with absorbent pad (47 mm diameter, 0.45 µm pore size, WCN type, Whatman Limited, Japan) was placed in a sterile petri-dish (Anaerobic, Sterile petri dishes, 60 mm diameter and 15 mm height, VWR, USA) and an ampule of M-Endo broth (23735-50, HACH LANCH GmbH, Willstatterstrasse 11, Dusseldorf, Germany) was poured evenly over the entire surface of the absorbent pad. The diluted manure sample was poured onto the absorbent pad, and lids were closed. The petri-dishes were incubated at 35°C for 24 h. the petri-dishes were removed from the incubator after 24 h and colonies of bacteria were counted in a counting chamber using a 10-20X magnifying glass. All bacterial plate counting preparations were done inside a sterile environmental hood.

A parallel experiment was conducted with matured alginate-nZnO beads (beads recovered from manure treatment experiments after 33 days) to test the toxicity of entrapped nZnO on the microbial community over time. The matured alginate beads were collected manually separating them from manure, washed with copious amount of water before using them in batch studies with fresh manure. Samples (1 mL) were collected from the batch reactors after

72 h and bacterial plate count was done as described earlier. Control experiments (without any NPs or beads) were conducted and bacterial counting was done after 72 h.

Quality control and statistical analysis

All experiments were carried out in triplicate and the average values are reported with standard deviations. The effects of NPs on H_2S and GHG emissions among treatments were statistically compared and analyzed using PROC ANOVA procedure in SAS 9.4 (2010) software. The hypothesis was tested at 95% ($P \leq 0.05$) significance level.

Results and discussion

Manure properties

Moisture content (MC), pH, crude protein (CP), total nitrogen (TN), ammonia (NH_3), and volatile fatty acids (VFAs) were measured at the beginning and at the end of an experiment (manure treated with alginate-nZnO beads and control) (Table 36 and 37).

There was no significant difference in pH among treated samples at the end the experiment (pH = 7.65-7.67) but pH values dropped significantly (from pH 7.74 to 7.65) during the experimental period. A pH range of 6.5 - 7.5 is favorable for the CH_4 formation during anaerobic digestion (Babel et al., 2004). The CH_4 formation is inhibited due to NH_3 production above this pH range (Jiang et al., 2013). As the pH decreased from 7.74 towards the pH range favorable for CH_4 formation, more gas formation could be expected.

The average initial ash content in the manure was 19.10% and a significant increase (23.25%) was observed when loose alginate beads were used freely. However, the ash content in the controls and manure treated with alginate-nZnO beads in bags did not change considerably (20.50 to 20.90%). The increase in ash content could be due to an additional carbonaceous substance present in alginates–nZnO beads.

Table 36. Mean nutrient concentration of liquid dairy manure before and after the completion of the experiment.

Treatment	pH	Moisture %	Ash %	CP %	TN %	Ammonia mM
[1] Initial	7.74 a	90.64 a	19.10 a	15.63 a	2.50 a	149.65 a
[2]Control	7.67 b	91.58 b	20.90 b	14.72 b	2.36 b	174.96 b
Beads in bag	7.65 b	91.52 b	20.50 b	14.47 b	2.32 b	139.39 c
Free beads	7.65 b	91.49 b	23.25 c	15.85 a	2.54 a	118.01 d

*Values followed by the same letter in row are not significantly different at $P \leq 0.05$

[1]Initial means the fresh manure collected from source before starting the experiment, and [2]control means the manure kept in a flask for 33 days without treating with NPs or any beads.

Table 37. Comparison of mean volatile fatty acid (VFA) concentrations of liquid dairy manure before (initial) and after the completion of the experiment (control, beads in bags and loose beads).

Treatment	Acetic mM	Propionic mM	Isobutyric mM	Butyric mM	Isovaleric mM	Valeric mM	Total mM
[1]Initial	174.86 a	65.29 a	12.45 a	51.39 a	18.57 a	15.82 a	338.39 a
[2]Control	268.74 b	91.98 b	21.55 b	54.29 a	35.43 b	32.53 b	504.53 b
Beads in bags	224.68 c	66.46 a	13.58 a	52.52 a	19.93 a	19.32 c	396.50 c
Loose beads	154.52 d	30.56 c	5.60 c	35.70 b	8.43 c	8.04 d	242.85 d

*Values followed by the same letter in a row are not significantly different at $P \leq 0.05$.

Crude protein and TN were found to be lower in the control and samples treated with beads in bags as compared to manure treated with free beads and initial manure (Table 36). The higher TN would be helpful for the growth of bacteria. The fecal NH_3 was significantly higher in controls (174.96 mM) compared to treated samples (118.01 mM-139.39 mM). Manure treated with free beads resulted in the lowest NH_3 concentration (149.65 mM). The higher NH_3 content can result in higher nitrous oxide gas generation (Muñoz et al., 2010). Further, excess NH_3 may inhibit the CH_4 formation (Jiang et al., 2013).

The total VFA ranged between 242.85 mM to 504.53 mM among the manure samples. Total VFA was found highest in control (504.53 mM) and lowest in the manure treated with loose alginate-nZnO beads (242.85 mM). Most of the VFA components were found to be higher in treated samples as compared to initial samples. The acetic acid was found to be significantly higher in control (268.74 mM) as compared to others treatments (154.52 mM to 224.68 mM). All VFA components were found to be the lowest in manure treated with loose alginate-nZnO beads among all treatments.

Acetic and propionic acids are known to trigger CH_4 production (Hill et al., 1987; Lahav and Loewenthal, 2000). Acetic acid is the desired intermediate VFA since it is a substrate for methanogenic bacteria and accounts for approximately 70% of CH_4 production in an anaerobic setting (Hill et al., 1987). The lower value of acetic acid and TVFAs in alginate-nZnO treated samples compared to control indicate that the application of alginate-nZnO beads either promoted VFA conversion to CH_4 or inhibited VFAs production. Gautam et al. (2013) reported no significant effects on VFAs while treating the manure with bare nZnO, but observed a 67% reduction in average CH_4 concentration. The application of nZnO in manure could have inhibited either hydrolysis, acidogenesis or acetogenesis process in the anaerobic conversion pathway, and retarded the formation of VFAs, thus likely to affect the CH_4 formation.

Characterization of alginate beads

The beads were spherical in shape with an average diameter of 3 mm (n = 12, SD=0.68 mm). The alginate beads without NPs were colorless or translucent while fresh alginate-nZnO beads appeared white and the color changed into brown once treated with manure (Figure 18).

Figure18. Alginate-nZnO bead; freshly synthesized (left) and recovered (right) from treated liquid manure.

Scanning electron microscopy (SEM) micrographs (Figure 19a-d) were used to analyze the morphology of alginate beads. The porous structure of the alginate beads was visible (Figure 23b) which might have ensured mobility and surface contact of manure with NPs. The problem of agglomeration of NPs has well illustrated from micrographs (Figure 19c-d). Both fresh and treated alginate-nZnO beads showed the agglomerated nZnO (Figure 19c-d) inside beads. Bezbaruah et al. (2009) also reported such heterogeneous distribution of nZVI in calcium alginate beads. Therefore, still some modification on the process of NPs incorporation can be suggested. Analysis of the morphology of fresh (Figure 19c) and used (Figure 19d) alginate-nZnO bead indicated that nZnO retained in the beads (not released to the environment) even after treating the manure for a period of 33 d.

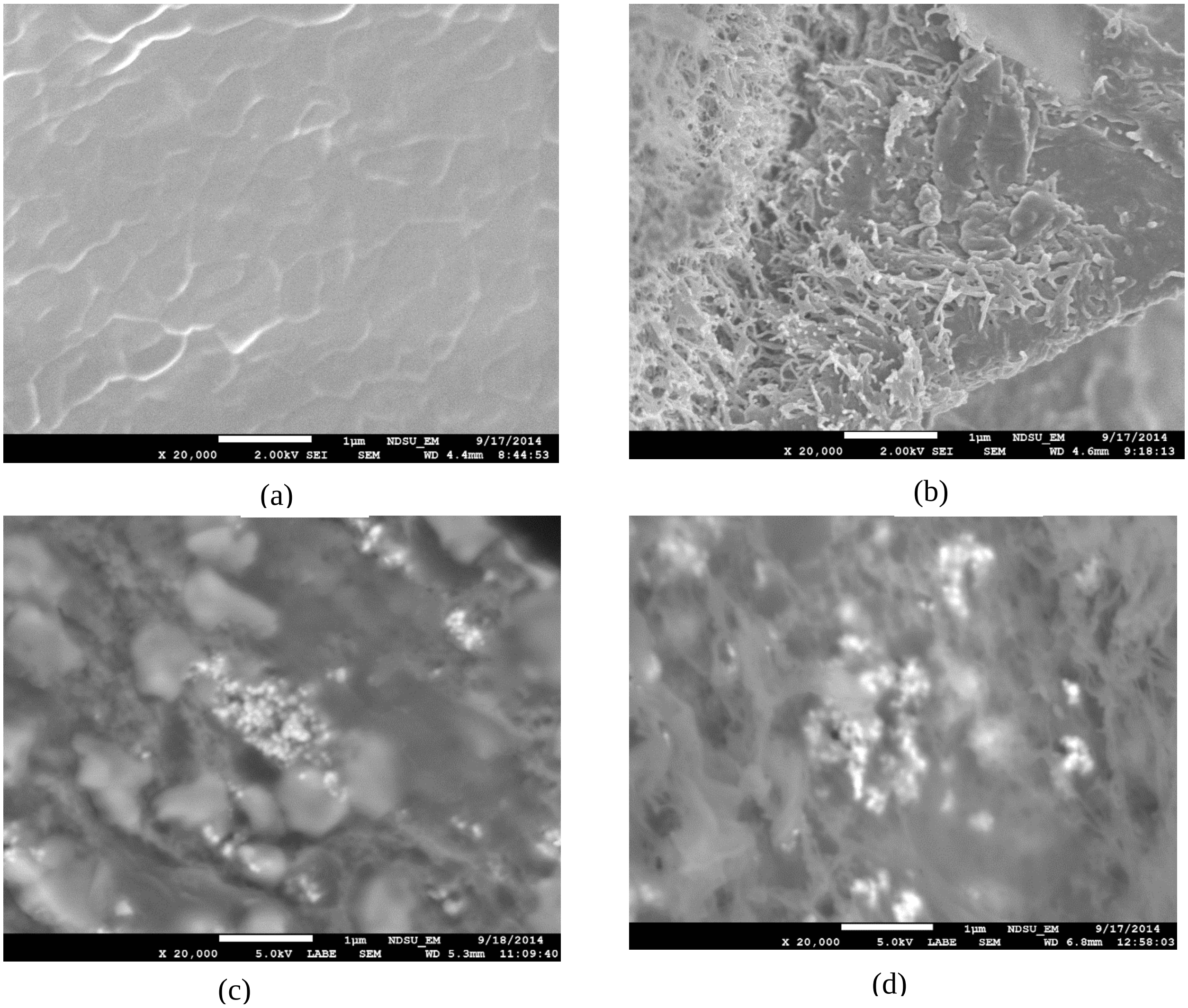


Figure 19. Scanning electron microscopy (SEM) micrographs: a) Surface of untreated alginate bead without NP b) Cross-section of treated alginate bead without NP c) Surface of untreated alginate-nZnO bead showing nZnO (tiny white particles like substances in the middle) d) Surface of treated alginate-nZnO bead showing nZnO (tiny white particles).

Sorption test of gases

Application of the minimal amount (2 mg) of bare nZnO in 500 mL flask was able to reduce the gas concentration significantly (Figure 20). Approximately, 96% of H_2S concentration reduction was achieved in 2.5 min, and more than 99% reduction in 20 min was achieved with bare nZnO (Figure 20). Similarly, application of alginate-nZnO beads (AB-NP) at the same amount of nZnO showed a similar pattern of H_2S gas reduction. The reduction was ~ 83% in the first 2.5 min and the H_2S concentration decreased gradually thereafter (98% at 20 min) (Figure 20).

The most plausible reason for the H_2S gas reduction resulting by bare nZnO is due to the conversion of H_2S to zinc sulfide (ZnS) (Equation 29, Mortezaali and Moradi, 2014).

$$\mathrm{ZnO} + \mathrm{H_2S} \rightarrow \mathrm{H_2O} + \mathrm{ZnS} \tag{29}$$

Application of the same amount of alginate bead without NPs (AB-WNP) also showed ~64% reduction of H_2S concentration in the first 2.5 min. This result showed that alginate beads itself (without NPs) can also show a significant H_2S concentration reduction. Though the solubility of H_2S in water is not fairly high (only 4 g kg^{-1} at 20 °C; source: http://www.engineeringtoolbox.com/gases-solubility-water-d_1148.html), wet AB-WNP showed comparatively higher reduction of H_2S concentration which may be due to the sorption process exhibited by the porous wet bed or the chemical conversion of H_2S reacting with the ions of components (calcium ion, chloride ion, sodium ion etc.) presents in AB-WNP. On the other hand, relatively higher reduction of gas concentration while using AB-NP may be due to both the chemical reaction of H_2S with the ions of components present in the beads in aqueous form (zinc ion along with other ions like in AB-WNP) and the sorption of porous beads.

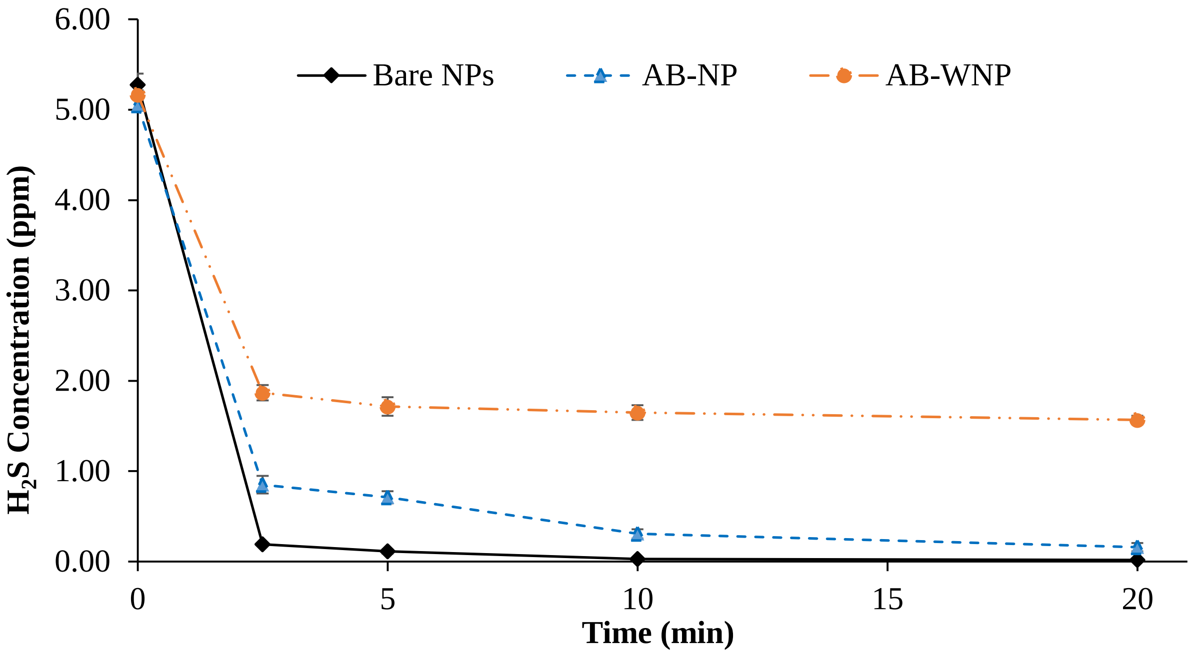


Figure 20. Sorption test of H_2S for bare NPs, alginate-nZnO beads (AB-NP) and alginate beads without NPs (AB-WNP). The error bars are the standard deviations calculated from three replications of each treatment.

A similar experiment was carried out with CH_4 taking 2 mg, 10 mg, 100 mg and even 3g bare nZnO in a flask, but the concentration of CH_4 remained almost constant over the time period. This showed that nZnO do not absorb or react with CH_4 under normal temperature and pressure. However under high temperature and pressure, formations of some complexes and syngas are reported (Ebrahim and Jamshidi, 2001; Su et al., 2006).

Bacterial study

Average number of colony forming units (CFU) were 5.8×10^4, 2.8×10^4, 0.3×10^4, 3.8×10^4 and 4.0×10^4 colony mL^{-1} in control, manure treated with bare nZnO at an application rate of 100 mg L^{-1}, manure treated with bare nZnO at an application rate of 3 g L^{-1}, manure treated with fresh alginate-nZnO beads (3 g L^{-1} NPs), and 33 days matured alginate-nZnO beads (3 g L^{-1} NPs), respectively (Figure 21). The results show that the application of bare nZnO (3 g L^{-1}) resulted in the lowest bacterial counts (reduced by 95%) followed by bare nZnO at100 mg L^{-1}

(60% reduction). Manure treated with fresh and 33 days manure alginate-nZnO beads (used) resulted in higher microbial survival compared to bare nZnO treatment. Thus, NPs would potentially reduce the activity and/or numbers of microorganisms producing GHGs and H_2S emissions. This is likely to affect overall gas production and concentration of gases under different treatment. The manure treated with bare NPs is likely to produce lesser gas followed by the manure treated with alginate-NPs beads.

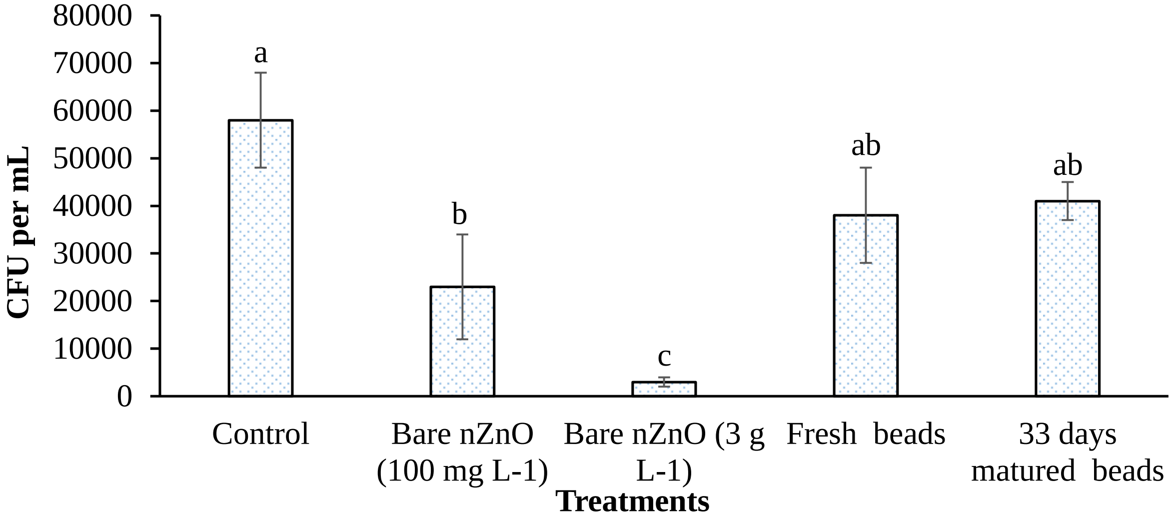


Figure 21. Number of colonies of total bacteria in the manure under different treatment conditions (control, the low application rate of 100 mg L^{-1} bare NPs, the high application rate of 3g L^{-1} bare NPs, fresh alginate-nZnO beads and matured alginate-nZnO beads). The error bars are the standard deviations calculated from three replications of each treatment and total number of observation was 15 (n=15). The treatments with the same letter above error bar are not significantly different at $P \leq 0.05$.

Effects of NPs application methods

Gas production

The cumulative gas production per liter of manure was calculated based on the gas measurement in between the experiments of 33 days (Figure 22a). The total gas production per unit volume of manure treated with loose alginate beads, beads in bags and control were 2770, 3913 and 4873 mL L^{-1}, respectively. Therefore, the corresponding gas production rates per unit time per unit volume of these treatments were 84, 119 and148 (mL d^{-1} L^{-1}), respectively. In all of the treatments, the gas production rate was quite high up to 12-15 days as indicated by the steeper slope (Figure 22a). However, the production rate gradually declined towards the end as indicated by the relatively flat slope. The application of loose beads and beads in bags into the manure reduced the total gas production by 43 and 20%, respectively, when compared with the gas production from the control treatment (Figure 22a). Similarly, cumulative CH_4 production was reduced by 68% and 29% when using loose beads vs bagged beads, respectively, as compared to the control (Figure 22b). Thus, this study demonstrated that total gas production may be reduced by applying NPs in both ways, but entrapped NPs in alginate beads were relatively less effective than the application of bare nZnO. Gautam et al. (2013) observed the reduction of total gas production by 64% in dairy liquid manure using the bare NPs at the same application rate of 3 g L^{-1}. While the application of loose beads and loosely bagged beads resulted in reduced total gas production by 43 and 20%, respectively. This reduction of total gas production among bare and alginate beads application methods are likely due to microbial survivability. As discussed previously, application of bare nZnO resulted in the lowest bacterial counts (Figure 21), thus likely to affect the total gas production. Besides, interactions of Ca-Alginate with nZnO during polymerizations; especially speciation, agglomeration, contact surface area and porosity of the alginate beads might restrain the fluid exchange, and thus reduced the effectiveness of NPs. Additionally, the application methods (loose beads and beads in bags) may also affect the effectiveness of entrapped NPs to a great extent. The use of alginate beads in bags might provide less opportunity to react with manure while loose alginate beads in manure provided more surface area and likely reacts with more manure; therefore better reduction opportunity. However, the application of beads in bags provides an easy access to recover the beads after use, thus likely to reduce the environmental concern, if there is any. In conclusion, there are opportunities for the improvements of NPs entrapment into polymer(s) as well as application method in manure for greater fluid exchange, thus potentially reducing gaseous emission while maintaining reasonable numbers of microorganisms in manure.

In this study, the total gas and CH_4 production rate and its concentration were very low (total gas 73.2 L kg VS^{-1} and CH_4 6.8 L kg VS^{-1}) compared to anaerobic digestion (total gas >213 L kg $VS^{-1}$and CH_4 >125 L kg VS^{-1}) (Amon et al., 2007). This is likely due to differences in the experimental set up between this study and anaerobic digestion studies. This study was conducted under room temperature, whereas anaerobic digestion is conducted under a target temperature (35°C) and optimum pH. However, at low temperature (15°C), Masse (2003) has reported the total CH_4 production rate in the range of 0.28-0.39 L L^{-1} or 3.64-11.53 L kg Vs^{-1} from dairy manure stored for180 days; which is comparable to this research.

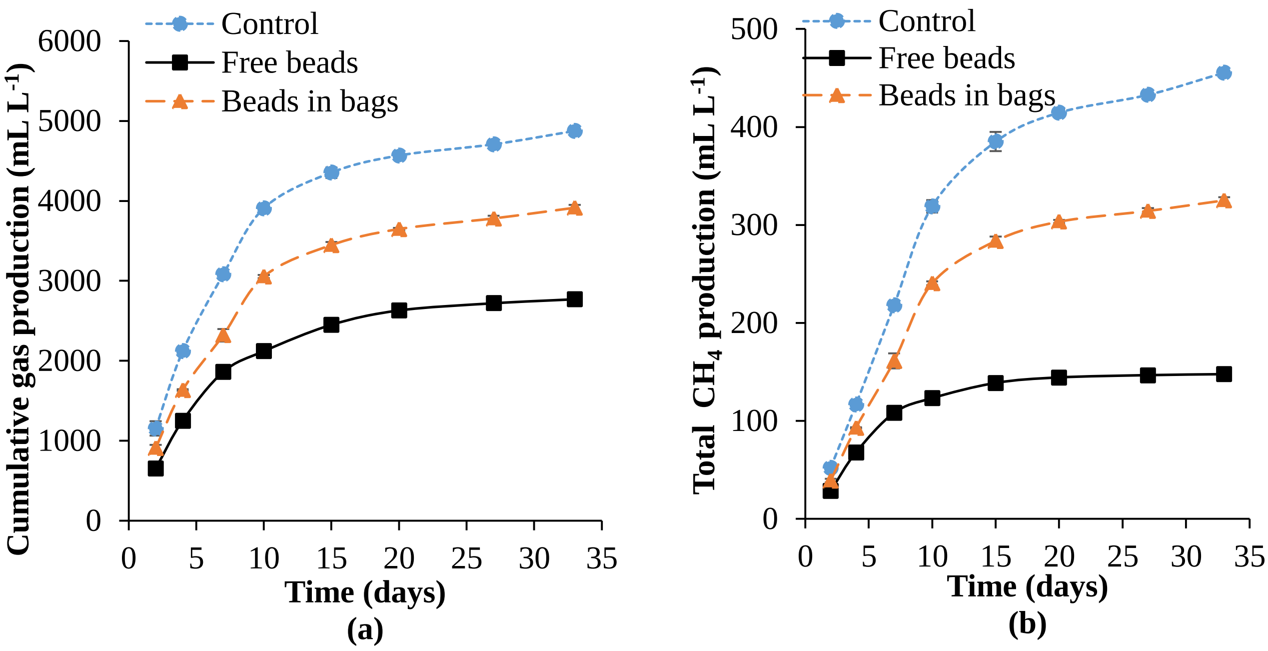


Figure 22. Cumulative volume of a) total gas production and b) CH_4 production per unit volume of liquid dairy manure treated with nZnO entrapped loose alginate beads, alginate beads in nylon bags, and control. The error bars are the standard deviations calculated from three replications of each treatment.

Hydrogen sulfide production

The alginate-nZnO beads (AB-NP) were effective in reducing H_2S in gaseous form. When they were applied in liquid manure, they were able to show effective results in reducing H_2S even in the liquid phase (liquid manure). In the control experiment, the concentration of H_2S increased gradually and reached around 2000 ppm on day 7 of the experiment, then declined gradually. However, in the alginate-nZnO beads treated manure, the H_2S concentrations were below 50 ppm in all the measurements, except the first measurement of alginate beads in bags (~127 ppm). Considering 33 days of the experiment period, the total H_2S gas production rates were 5.51, 0.01, and 0.20 mL L^{-1} of manure from control, manure treated with loose alginate-nZnO beads, and manure treated with alginate-ZnO beads in bags, respectively. Similarly, when the alginate-nZnO beads were used freely in dairy manure, the reduction in H_2S production was around 99%. Similarly, with alginate-nZnO beads kept in bags, the reduction was slightly lower (96%) than that of beads applied freely (Figure 23). A previous study reported that bare NPs reduce the H2S concentration from dairy manure about 99% compared to control (Gautam et al., 2013). Although, in gaseous form, bare NPs outperformed the alginate bead entrapped NPs in reducing H_2S concentration, but in liquid form their effectiveness is comparable. This is likely that beads are porous and during the experimental time they had enough time to come in contact and react with NPs, thus resulting in better performance. Therefore, both application methods would be able to reduce H_2S gas production significantly, however, application in bags might be a better option due to recovery option.

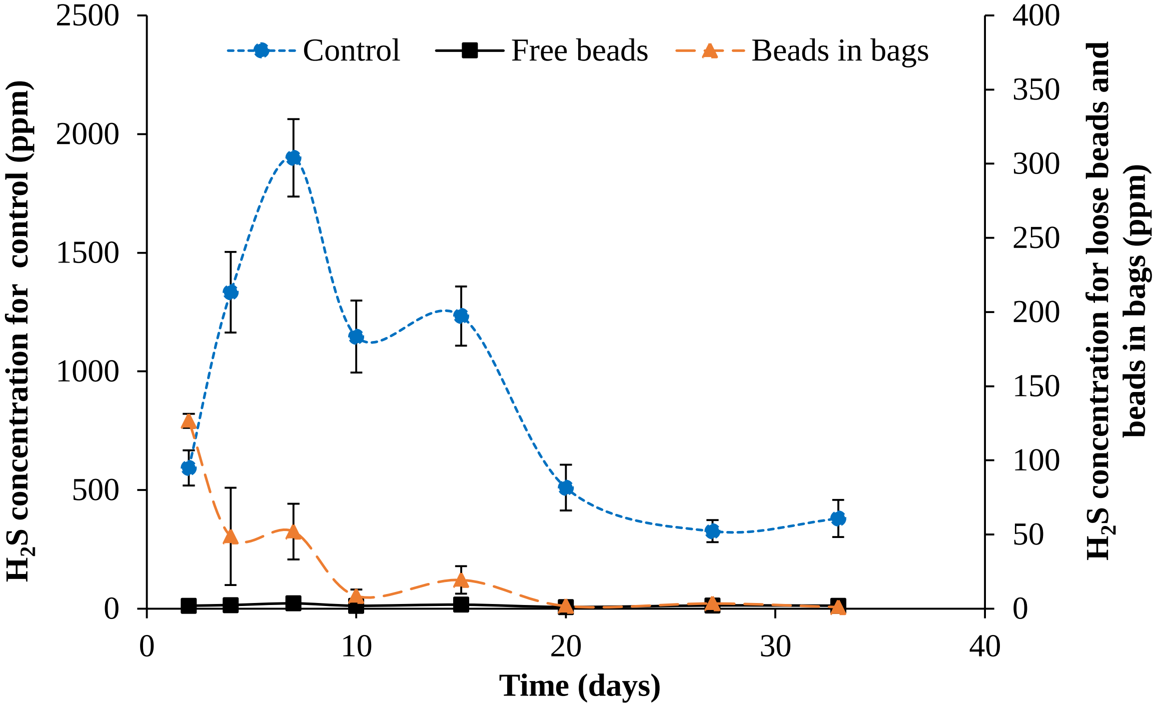


Figure 23. Hydrogen sulfide (H_2S) concentrations from the liquid dairy manure treated with alginate-nZnO beads in freely and in nylon bags. The error bars are the standard deviations calculated from three replications of each treatment.

Methane production

Methane production during anaerobic storage of manure under laboratory conditions varied widely during the experimental period for different treatments (Figure 24). Initial CH_4 concentration (2%) was similar for all treatments. As the treatment time progressed, CH_4 concentration increased to 15% in 15 days and remained at 14% until the experiment was terminated.

In case of loose alginate-nZnO beads treated experiments, the maximum CH_4 concentration (7%) reached at day 4 and thereafter decreased gradually and reached even lower than the initial concentration. In case of manure treated with alginate-nZnO beads in bags, CH_4 concentration gradually increased till day 15 and reached around 11%, then gradually decreased and reached to 8% at the end of the study period (Figure 24). It seems that loose alginate-nZnO beads were very effective in reducing CH_4 concentration (89% maximum and 51% on average) compared to alginate-nZnO beads in bags (41% maximum and 18% in average). The CH_4 formation is most likely from the hydrogenotrophic methanogenesis process rather than acetoclastic methanogenesis process (Horn et al., 2003). Although, in this study different processes were not studied, but most of the CH_4 production might be from CO_2 and hydrogen reaction compared to VFAs conversion (Horn et al., 2003; Kotsyurbenko et al., 2004). However, it is difficult to recover freely applied alginate beads that may end up in the environment. Hence, NP entrapped beads is likely a better option, although effectiveness is reduced significantly. Therefore, new polymer development or modification of existing polymer and improved application option are likely to overcome some of this issue.

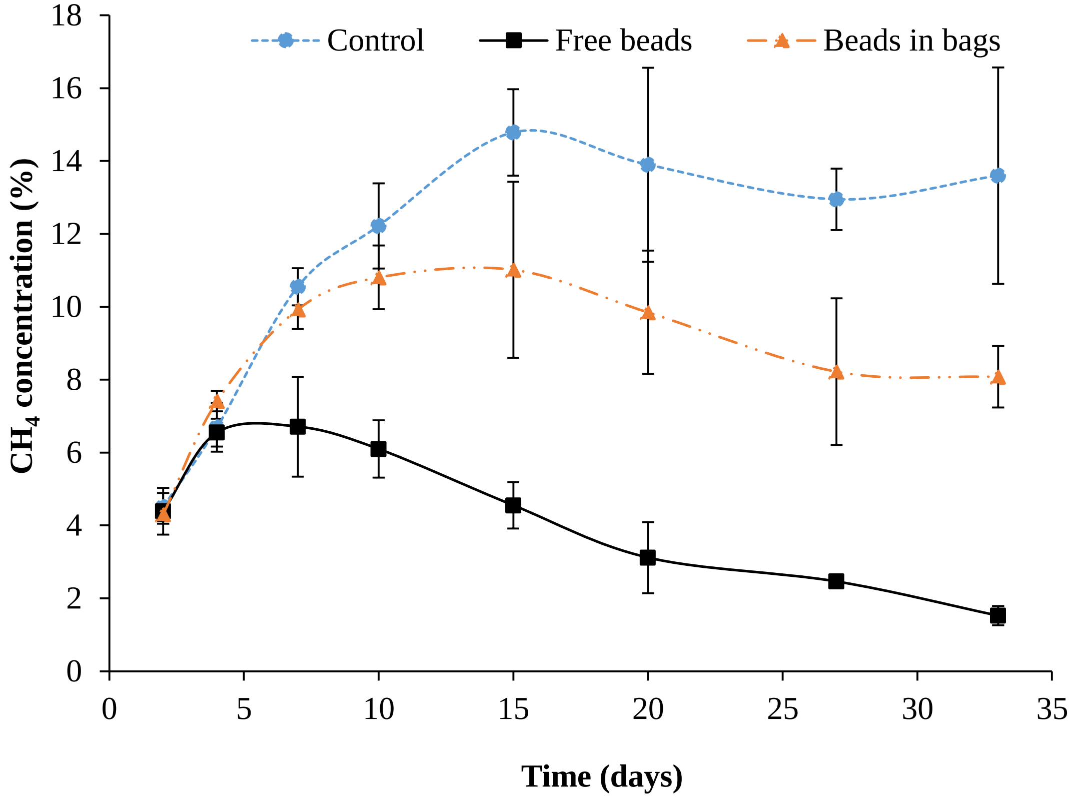


Figure 24. Methane concentration from the manure treated with alginate-nZnO beads applying freely and keeping in bags. The error bars are the standard deviations calculated from three replications of each treatment.

Carbon dioxide production

In all treatments, the CO_2 concentration shows a similar trend to CH_4 (Figure 25). Initial CO_2 concentration was similar and their concentrations increased up to five days, thereafter decreased gradually and dropped close to the initial concentration. Control treatment resulted in the highest CO_2 concentration followed by the manure treated with alginate beads in bags and manure treated with free or loose beads, in that order (Figure 25). Use of alginate-nZnO beads inside the nylon bags reduced CO_2 concentration by 14%, whereas addition of loose alginate beads reduced CO_2 concentration by 27% on average as compared to control. Thus, application of alginate-nZnO beads by keeping in bags were not as effective as freely applied beads, but they were also able to reduce CO_2 concentration noticeably. Overall, irrespective of application methods, alginate-nZnO beads were not very effective in reducing CO_2 as compared to H_2S and CH_4.

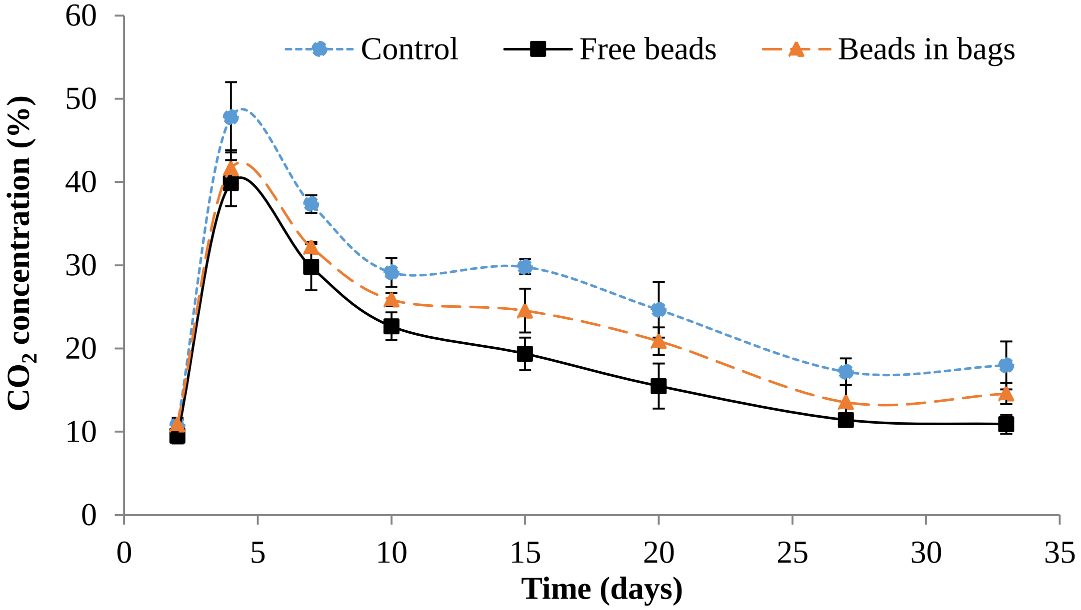


Figure 25. Carbon dioxide concentration from the manure treated with alginate-nZnO beads applying freely and keeping in nylon bags. The error bars are the standard deviations calculated from three replications of each treatment.

Effect of alginate beads without NPs on gaseous production

As in the gas absorption study, a separate study was conducted with manure and alginate beads to examine the effects of alginate bead only on liquid manure. No significant reduction of CH_4 and H_2S (Figure 26) reduction was observed as compared to the control. A similar trend was observed with CO_2 concentration and total gas production. Thus, it can be concluded that based on this lab study, the reduction of total gas production or concentration reduction of a particular gas is likely due to the effects of NPs, not from the polymer.

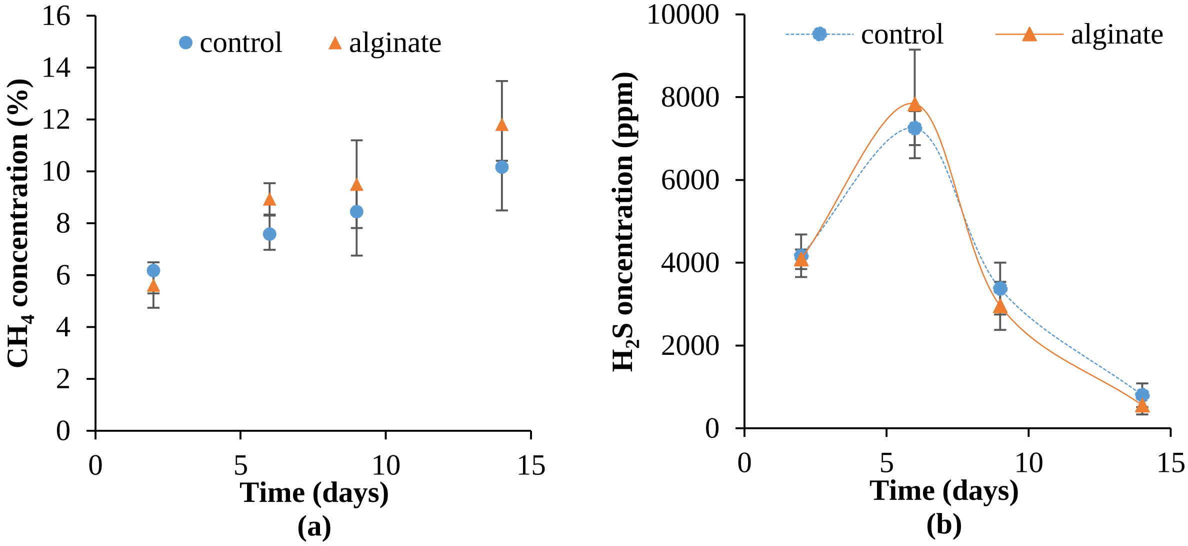


Figure 26. Concentration of a) methane (CH_4) and b) hydrogen sulfide (H_2S) when only alginate beads without nZnO were used in manure. The error bars are the standard deviations calculated from three replications of each treatment.

Conclusions

In this study, the performance of alginate-nZnO beads on gaseous emission and manure characteristic were laboratory tested under anaerobic conditions. It was found that when the entrapped NPs beads were applied freely in dairy manure, the average H_2S and CH_4 concentration reduction were 99 and 51%, respectively, and the reduction in total gas production was 43% as compared to control ($P \leq 0.05$). Similarly, with alginate beads in bags, the average

H_2S, CH_4 and total gas production were significantly reduced by 96, 18, and 20%, respectively, as compared to control ($P \leq 0.05$). This reduction was likely due to reduced activity and/or numbers of microbial microorganisms responsible for gaseous production, sorption, or chemical conversion of gases. Results show that both application methods (loose beads and beads in bags) were effective, but loose beads performed better than the beads kept in bags. However, bagged beads would provide a better recovery and reuse option compared to the free application method. Therefore, both application methods are very effective in mitigating H_2S, CH_4, and CO_2 concentration under the anaerobic storage of manure; however, a better placement method of alginate beads in bags may be developed to improve the effectiveness.

References

Abécassis, B., Testard, F., and Zemb, T. 2009. Gold nanoparticle synthesis in worm-like catanionic micelles: microstructure conservation and temperature induced recovery. *Soft Matter*. 5(5): 974-978.

Ahamed, M. I. N., Sankar, S., Kashif, P. M., Basha, S. K. H., and Sastry, T. P. 2015. Evaluation of biomaterial containing regenerated cellulose and chitosan incorporated with silver nanoparticles. *International journal of biological macromolecules*. 72: 680-686.

Akamatsu, K., Adachi, S., Tsuruoka, T., Ikeda, S., Tomita, S., and Nawafune, H. 2008. Nanocomposite polymeric microspheres containing Ni nanoparticles with controlled microstructures. *Chemistry of Materials*. 20(9): 3042-3047.

Ameur, H., Ghoul, M., and Selvin, J. 2011. The osmoprotective effect of some organic solutes on Streptomyces sp. mado2 and nocardiopsis sp. mado3 growth. *Brazilian Journal of Microbiology*. 42(2): 543-553.

Amon, T., Amon, B., Kryvoruchko, V., Zollitsch, W., Mayer, K., and Gruber, L. 2007. Biogas production from maize and dairy cattle manure influence of biomass composition on the methane yield. *Agriculture, ecosystems & environment*. 118(1): 173-182.

Babel, S., Fukushi, K., and Sitanrassamee, B. 2004. Effect of acid speciation on solid waste liquefaction in an anaerobic acid digester. *Water Research*. 38(9): 2417-2423.

Balan, L., Jin, M., Malval, J.-P., Chaumeil, H., Defoin, A., and Vidal, L. 2008. Fabrication of silver nanoparticle-embedded polymer promoted by combined photochemical properties of a 2, 7-diaminofluorene derivative dye. *Macromolecules*. 41(23): 9359-9365.

Bezbaruah, A. N., Krajangpan, S., Chisholm, B. J., Khan, E., and Elorza Bermudez, J. J. 2009. Entrapment of iron nanoparticles in calcium alginate beads for groundwater remediation applications. *Journal of hazardous materials*. 166(2): 1339-1343.

Bezbaruah, A. N., Shanbhogue, S. S., Simsek, S., and Khan, E. 2011. Encapsulation of iron nanoparticles in alginate biopolymer for trichloroethylene remediation. *Journal of Nanoparticle Research*. 13(12): 6673-6681.

Bhattarai, N., and Zhang, M. 2007. Controlled synthesis and structural stability of alginate-based nanofibers. *Nanotechnology*. 18(45): 455601.

Bour, A., Mouchet, F., Silvestre, J., Gauthier, L., and Pinelli, E. 2015. Environmentally relevant approaches to assess nanoparticles ecotoxicity: A review. *Journal of Hazardous Materials*. 283: 764-777.

Bragaru, A., Kusko, M., Vasile, E., Simion, M., Danila, M., Ignat, T., Mihalache, I., Pascu, R., and Craciunoiu, F. 2013. Analytical characterization of engineered ZnO nanoparticles relevant for hazard assessment. *Journal of nanoparticle research*. 15(1): 1-17.

Buffet, P. E., Zalouk-Vergnoux, A., Chatel, A., Berthet, B., Metais, I., Perrein-Ettajani, H., Poirier, L., Luna-Acosta, A., Thomas-Guyon, H., Risso-de Faverney, C., Guibbolini, M., Gilliland, D., Valsami-Jones, E., and Mouneyrac, C. 2014. A marine mesocosm study on the environmental fate of silver nanoparticles and toxicity effects on two endobenthic species: The ragworm Hediste diversicolor and the bivalve mollusc Scrobicularia plana. *Science of the Total Environment*. 470: 1151-1159.

Choi, B. Y., Park, H. J., Hwang, S. J., and Park, J. B. 2002. Preparation of alginate beads for floating drug delivery system: effects of CO_2 gas-forming agents. *International journal of pharmaceutics*. 239(1): 81-91.

Cumbal, L., and SenGupta, A. K. 2005. Arsenic removal using polymer-supported hydrated iron (III) oxide nanoparticles: role of Donnan membrane effect. *Environmental science & technology*. 39(17): 6508-6515.

Ebrahim, H. A., and Jamshidi, E. 2001. Kinetic study of zinc oxide reduction by methane. *Chemical Engineering Research and Design*. 79(1): 62-70.

Escudero, C., Fiol, N., Villaescusa, I., and Bollinger, J.-C. 2009. Arsenic removal by a waste metal (hydr) oxide entrapped into calcium alginate beads. *Journal of hazardous materials*. 164(2): 533-541.

Gautam, D. P., Rahman, S., Borhan, M. S., and Bezbaruah, A. N. 2013. Applications of nanoparticles (NPs) in livestock manure and their effects on air emissions. In *International Symposium on Animal Environment and Welfare, October 19-22, 2013* Rongchang, Chongqing, China

Geukens, I., and De Vos, D. E. 2013. Recovery of Metallic Nanoparticles. In *Nanomaterials in Catalysis*, 311-330: Wiley-VCH Verlag GmbH & Co. KGaA.

He, X., Yuan, R., Chai, Y., and Shi, Y. 2008. A sensitive amperometric immunosensor for carcinoembryonic antigen detection with porous nanogold film and nano-Au/chitosan composite as immobilization matrix. *Journal of biochemical and biophysical methods*. 70(6): 823-829.

Hill, D., Cobb, S., and Bolte, J. 1987. Using volatile fatty acid relationships to predict anaerobic digester failure. *Transactions of the ASAE American Society of Agricultural Engineers*. 30(2): 496-501.

Horn, M. A., Matthies, C., Küsel, K., Schramm, A., and Drake, H. L. 2003. Hydrogenotrophic methanogenesis by moderately acid-tolerant methanogens of a methane-emitting acidic peat. *Applied and environmental microbiology*. 69(1): 74-83.

Hu, J., Lo, I. M. C., and Chen, G. 2005. Fast removal and recovery of Cr (VI) using surface-modified jacobsite (MnFe2O4) nanoparticles. *Langmuir*. 21(24): 11173-11179.

Jiang, X., Hayashi, J., Sun, Z. Y., Yang, L., Tang, Y. Q., Oshibe, H., Osaka, N., and Kida, K. 2013. Improving biogas production from protein-rich distillery wastewater by decreasing ammonia inhibition. *Process Biochemistry*. 48(11): 1778-1784.

Kim, H., Hong, H.-J., Jung, J., Kim, S.-H., and Yang, J.-W. 2010. Degradation of trichloroethylene (TCE) by nanoscale zero-valent iron (nZVI) immobilized in alginate bead. *Journal of hazardous materials*. 176(1): 1038-1043.

Koetz, J., Reichelt, S., Kosmella, S., and Tiersch, B. 2005. Recovery of nanoparticles produced in phosphatidylcholine-based template phases. *Journal of colloid and interface science*. 284(1): 190-198.

Kong, H., and Jang, J. 2006. One-step fabrication of silver nanoparticle embedded polymer nanofibers by radical-mediated dispersion polymerization. *Chem. Commun.*(28): 3010-3012.

Kotsyurbenko, O. R., Chin, K. J., Glagolev, M. V., Stubner, S., Simankova, M. V., Nozhevnikova, A. N., and Conrad, R. 2004. Acetoclastic and hydrogenotrophic methane production and methanogenic populations in an acidic West-Siberian peat bog. *Environmental microbiology*. 6(11): 1159-1173.

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

Laudenslager, M. J., Schiffman, J. D., and Schauer, C. L. 2008. Carboxymethyl chitosan as a matrix material for platinum, gold, and silver nanoparticles. *Biomacromolecules*. 9(10): 2682-2685.

Liu, T., Zhao, L., Sun, D., and Tan, X. 2010. Entrapment of nanoscale zero-valent iron in chitosan beads for hexavalent chromium removal from wastewater. *Journal of hazardous materials*. 184(1): 724-730.

Lopez-Serrano, A., Olivas, R. M., Landaluze, J. S., and Camara, C. 2014. Nanoparticles: a global vision. Characterization, separation, and quantification methods. Potential environmental and health impact. *Analytical Methods*. 6(1): 38-56.

Luna-delRisco, M., Orupõld, K., and 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.

Massé, D., Croteau, F., Patni, N., and Masse, L. 2003. Methane Emission from Dairy Cow and Swnie Manure Slurries Staorage at 10ºC and 15ºC. *Canadian Biosystems Engineering*. 45(6): 1-6.

Mishchuk, N., Ralston, J., and Fornasiero, D. 2012. The analytical model of nanoparticle recovery by microflotation. *Advances in Colloid and Interface Science*. 179: 114-122.

Mishra, S., and Singh, H. B. 2015. Biosynthesized silver nanoparticles as a nanoweapon against phytopathogens: exploring their scope and potential in agriculture. *Applied Microbiology and Biotechnology*. 99(3): 1097-1107.

Monticelli, O., Russo, S., Campagna, R., and Voit, B. 2005. Preparation and characterisation of blends based on polyamide 6 and hyperbranched aramids as palladium nanoparticle supports. *Polymer*. 46(11): 3597-3606.

Mortezaali, A., and Moradi, R. 2014. The correlation between the substrate temperature and morphological ZnO nanostructures for H2S gas sensors. *Sensors and Actuators A: Physical*. 206(0): 30-34.

Muñoz, C., Paulino, L., Monreal, C., and Zagal, E. 2010. Greenhouse gas (CO_2 and N_2O) emissions from soils: A review. *Chilean Journal of Agricultural Research*. 70(3): 485-497.

Muraviev, D. N., Macanás, J., Parrondo, J., Muñoz, M., Alonso, A., Alegret, S., Ortueta, M., and Mijangos, F. 2007. Cation-exchange membrane as nanoreactor: Intermatrix synthesis of platinum–copper core–shell nanoparticles. *Reactive and Functional Polymers*. 67(12): 1612-1621.

Myakonkaya, O., Eastoe, J., Mutch, K. J., and Grillo, I. 2011. Polymer-induced recovery of nanoparticles from microemulsions. *Physical Chemistry Chemical Physics*. 13(8): 3059-3063.

Pesch, G. R., Du, F., Schwientek, U., Gehrmeyer, C., Maurer, A., Thoming, J., and Baune, M. 2014. Recovery of submicron particles using high-throughput dielectrophoretically switchable filtration. *Separation and Purification Technology*. 132: 728-735.

Porel, S., Singh, S., Harsha, S. S., Rao, D. N., and Radhakrishnan, T. P. 2005. Nanoparticle-embedded polymer: in situ synthesis, free-standing films with highly monodisperse silver nanoparticles and optical limiting. *Chemistry of Materials*. 17(1): 9-12.

Predicala, B., Alvarado, A., and Asis, D. 2012. Use of Zinc Oxide Nanoparticles to Control Hydrogen Sulphide, Ammonia and Odour Emissions from Pig Barns. In *9th International Livestock Environment Symposium American Society of Agricultural and Biological Engineers Valencia Conference Centre Valencia, Spain July 8 - 12, 2012.* Paper Number: ILES12-1507, ASABE. Retrieved on 8/20/2013 from http://elibrary.asabe.org/azdez.asp?JID=1&AID=41577&CID=iles2012&T=2.

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

Rahman, S., and Newman, D. 2012. Odor, ammonia, and hydrogen sulfide concentration and emissions from two farrowing-gestation swine operations in North Dakota. *Applied engineering in agriculture*.

Rashad, A. M. 2013. Effects of ZnO_2, ZrO_2, Cu_2O_3, CuO, $CaCO_3$, SF, FA, cement and geothermal silica waste nanoparticles on properties of cementitious materials - A short guide for Civil Engineer. *Construction and Building Materials*. 48: 1120-1133.

Rejinold, N. S., Baby, T., Chennazhi, K. P., and Jayakumar, R. 2015. Multi Drug Loaded Thermo-Responsive Fibrinogen-graft-Poly (N-vinyl Caprolactam) Nanogels for Breast Cancer Drug Delivery. *Journal of Biomedical Nanotechnology*. 11(3): 392-402.

Shalumon, K. T., Anulekha, K. H., Nair, S. V., Nair, S. V., Chennazhi, K. P., and Jayakumar, R. 2011. Sodium alginate/poly (vinyl alcohol)/nano ZnO composite nanofibers for antibacterial wound dressings. *International journal of biological macromolecules*. 49(3): 247-254.

Su, Z., Qin, S., Tang, D., Yang, H., and Hu, C. 2006. Theoretical study on the reaction of methane and zinc oxide in gas phase. *Journal of Molecular Structure: Theochem*. 778(1): 41-48.

Vesperinas, A., Eastoe, J., Jackson, S., and Wyatt, P. 2007. Light-induced flocculation of gold nanoparticles. *Chem. Commun.*(38): 3912-3914.

Vidor, F. F., Wirth, G. I., and Hilleringmann, U. 2014. Low temperature fabrication of a ZnO nanoparticle thin-film transistor suitable for flexible electronics. *Microelectronics Reliability*. 54(12): 2760-2765.

Wang, X., Wang, J., and Wang, H. 2014. Improvement of output performance of solar cells using small nanoparticles. *Journal of nanoparticle research*. 16(6): 1-5.

Wu, L., Li, F., Xu, Y. Y., Zhang, J. W., Zhang, D. Q., Li, G. S., and Li, H. X. 2015. Plasmon-induced photoelectrocatalytic activity of Au nanoparticles enhanced TiO_2 nanotube arrays electrodes for environmental remediation. *Applied Catalysis B-Environmental*. 164: 217-224.

Wu, S.-J., Liou, T.-H., and Mi, F.-L. 2009. Synthesis of zero-valent copper-chitosan nanocomposites and their application for treatment of hexavalent chromium. *Bioresource Technology*. 100(19): 4348-4353.

Xu, J., and Bhattacharyya, D. 2007. Fe/Pd nanoparticle immobilization in microfiltration membrane pores: Synthesis, characterization, and application in the dechlorination of polychlorinated biphenyls. *Industrial & engineering chemistry research*. 46(8): 2348-59.

Zhang, J., Han, B., Liu, J., Zhang, X., He, J., Liu, Z., Jiang, T., and Yang, G. 2002. Recovery of silver nanoparticles synthesized in AOT/$C_{12}E_4$ mixed reverse micelles by antisolvent CO_2. *Chemistry-A European Journal*. 8(17): 3879-3883

CHARACTERIZATION OF ALGINATE-nZnO BEADS APPLIED TO SWINE MANURE FOR CONTROLLING GASEOUS EMISSION

Abstract

Gaseous emission from livestock manure is a major problem as it may directly impact human health, livestock welfare, and the environment. Recently, application of nanoparticles (NPs) has evolved as a potential option to minimize gaseous emission from agricultural and non-agricultural sources. However, researchers are applying NPs as bare form, which may accumulate in the soil, air and water; thus posing an adverse effect on plant, soil, human health and the environment. Therefore, a study was conducted with NPs entrapped in polymeric beads in order to treat manure while preventing environmental exposure to NPs and to NPs for reuse. The objectives of the study were to improve the effectiveness of zinc oxide NPs (nZnO) entrapped alginate (alginate-nZnO) beads and their application method, explore the option of their reuse, and characterize them to understand the reaction mechanism involved in controlling gaseous emissions. An experiment was carried out taking 1 L Erlenmeyer flasks with a working volume of 500 ml, where alginate-nZnO beads containing 3 g L^{-1} of nZnO were applied freely as well as inside a porous nylon bag. The experiment was carried out for 33 days, headspace gas was collected to determine H_2S, CH_4, and CO_2 concentration every 2-6 days. After the experiment, the treated alginate-nZnO beads were taken out and used for treating a new set of swine manure following the same procedure. Treated and control manure samples were also collected for bacterial growth analysis using the plate count method, and methanogen activity was estimated using RT-PCR methods. In addition, the alginate beads were taken for Scanning Electronic Microscopy (SEM), Energy Dispersive Spectroscopy (EDS) and X-ray Photoelectron Spectroscopy (XPS) analysis to assess physiochemical changes during the treatment. The

performance of alginate-nZnO beads in bags was found to be as effective as freely applied beads on reducing gaseous emission. The performance of reused beads was comparable with fresh beads in term of gasses emissions. The bacterial count showed that nZnO (bare as well as entrapped from) had an inhibitory effect on bacteria under both aerobic and anaerobic conditions. Both EDS and XPS analysis confirmed the presence of ZnS in treated beads, which could have been formed by reacting nZnO with H_2S. So, in manure, the reduction of gaseous emission is likely due to the chemical conversion and the inhibitory effect of nZnO on microbes responsible for gaseous production.

Introduction

Livestock industry, especially swine production is one of the major agricultural sources of hydrogen sulfide (H_2S) and greenhouse gas (GHG) emission, which may affect human health, animal welfare, and the environment. Hydrogen sulfide is considered as a pollutant gas produced at different stages of livestock production and manure management practices (Barrasa et al., 2012; Moreno et al., 2010). Hydrogen sulfide is highly toxic, corrosive, and has a very unpleasant smell (Thu, 2002). The assimilatory and dissimilatory sulfate reduction process in manure generates H_2S (Peck et al., 1982; Schiff and Fankhauser, 1981). A Lower concentrations (<100 ppm) of H_2S may cause coughing, eye irritation, loss of appetite and vomiting; whereas higher concentrations (>100 ppm) are likely to cause nausea, unconsciousness and even death (ASABE, 2005). In addition, H_2S also contributes to acid rain and deterioration of production facilities due to its corrosive nature (Abdelmseeh et al., 2008; Likens et al., 1972). Similarly, methane (CH_4), nitrous oxide (N_2O) and carbon dioxide (CO_2) are the major GHGs emitted from livestock production systems at different stages of manure production and management practices that contribute to global warming. Methane and N_2O have higher global warming potential (25 and 298 times higher, respectively) than the CO_2 (USEPA, 2015). Anaerobic decomposition of manure generates CH_4; whereas the alternate aerobic and anaerobic conditions of manure generate N_2O, and chemical or thermal decomposition of manure produce CO_2 (Spellman and Whiting, 2010). Therefore, scientists are exploring or evaluating new technologies to reduce this gaseous emission from manure management practices.

The dietary modification (Boadi et al., 2004; Mirabelli et al., 2006); capture and control of emitted gas (Ndegwa et al., 2008; Ruokojärvi et al., 2001); and design of appropriate animal housing system (Amon et al., 2001) are some common methods to reduce gaseous emission from in house condition of livestock production system. Similarly, the use of additives in manure system (Ndegwa et al., 2008; Singh et al., 2010), suppression of gaseous emission with composting (Pattey et al., 2005), aeration (Amon et al., 2006), and acidification (Kai et al., 2008); and bio-treatments applications (Luostarinen et al., 2006) are treatment options to reduce gaseous emission from manure storage system. Similarly, the manure injection system (Montes et al., 2013), and quick incorporation of manure after application (Sommer and Hutchings, 2001) are some of effective methods to reduce gaseous emission during land application. Though, there are a lot of technologies developed for reducing H_2S and GHGs, they are mostly time-consuming, labor intensive, effective only in the short term and target only a specific gas. Thus, there is still a demand for an innovative technology, which could be effective for longer periods of time and for multiple gases. Recently, nanotechnology has been regarded as one of the potential mitigation options and has been explored in this study.

Application of nanoparticles (NPs) has shown promising results on mitigating the CH_4 and H_2S producted from the solid waste management sector (Yang et al., 2012), and wastewater treatment plants (Mueller and Nowack, 2008). Recently, researchers are trying to evaluate the performance of different NPs in controlling gaseous emission from livestock manure (Gautam et al., 2015; Luna-delRisco et al., 2011; Predicala et al., 2012). Among NPs, zinc oxide NPs (nZnO) is considered as a very effective NPs for reducing H_2S (Carnes and Klabunde, 2002; Ma et al., 2013; Sayyadnejad et al., 2008; Sekhavatjou et al., 2014) and CH_4 production (Gautam et al., 2015; Luna-delRisco et al., 2011). However, most of the research has measured the effect of bare NPs, which may have negative effects on soil, air and water sources. To overcome some of these issues, NPs may be after entrapment in a polymer.

Currently, Gautam et al. (2015) have adopted an indirect application method of NPs to manure systems where they entrap nZnO in polymeric beads and apply the NPs to liquid dairy manure to control GHGs (CH_4 and $CO_{2)}$ and H_2S gas. They applied alginate-nZnO beads in two different ways: loose alginate-nZnO beads in manure and alginate bead placed in porous (0.8 mm mesh size) nylon bags for easy recovery after use. They found a 99% reduction of H_2S concentration using nZnO entrapped alginate (alginate-nZnO) beads, which was almost equivalent to the effectiveness of bare nZnO (Gautam et al., 2015). Similarly, they found 51% reduction in CH_4 concentration on average. However, the reduction mechanism of these gases are not clear. Whether used beads could be reused for controlling the pollutant gas emissions was not evaluated either in the previous study.

There could be several reasons for the reduction of gaseous emission from manure with nZnO treatment. The reduction of H_2S could be due to the chemical conversion of H_2S to ZnS while reacting with nZnO. During the reaction, it is assumed that H_2S dissociate into H^+ and HS^-, and the HS^- diffused to into ZnO and converted to ZnS as in Equation 30 (Song et al., 2013; Steudel and Steudel, 2006).

$$ZnO_{(s)} + H_2S_{(g)} \rightarrow ZnS_{(s)} + H_2O_{(l)} \qquad (30)$$

The reduction of H_2S from the application of nZnO is likely to occur due to both physical sorption and chemical conversion (Awume, 2014). However, in the case of manure the ZnO is applied into the liquid phase, where the reaction mechanism might be different than the solid-gas interaction. Till now no investigation of a H_2S reduction mechanism in liquid manure treated with NPs has been carried out. Similarly, the mechanism controlling CH_4 production is unknown; as there is no reaction of ZnO with CH_4 gas in normal condition (Ebrahim and Jamshidi, 2001; Su et al., 2006). Therefore, the reduction of CH_4 must be biological and not due to chemical conversion. Rather, the reduction could be due to the inhibition of microbial growth including methanogens during the anaerobic decomposition process in manure (Yang et al., 2012).

There might be several techniques, which could be helpful for understanding the mechanism of gaseous reduction while treating the manure with NPs. Scanning Electron Microscopy (SEM) analysis, Energy Dispersive Spectroscopy (EDS) analysis, and X-ray Photoelectron Spectroscopy (XPS) analysis are some well-established characterization techniques used to study the morphology and chemical composition of materials (Bajpai et al., 2012; Finotelli et al., 2010; Shipochka et al., 2013). Hence, in this research, these characterization techniques were used to understand the reduction mechanism. In addition, microbial plate counting and polymeric chain reaction (PCR) were also conducted for the quantification of population dynamic of microorganism in manure. Therefore, the major objective of this research were i) to characterize both untreated and treated NPs entrapped alginate beads and ii) to understand the reduction mechanism of hydrogen sulfide (H_2S) gas by means of different characterization techniques, and iii) to study the effectiveness of reused beads for reducing target gases.

Materials and methods

Firstly, the alginate-nZnO beads were prepared. Then a simple experiment was carried out treating the swine manure with the prepared beads by applying them directly to manure or enclosed in a mesh bag. After finishing the experiment, the treated manure samples were taken for nutrient analysis as well as for plate counting of bacteria and real-time polymeric chain reaction (RT-PCR) analysis. The freely applied treated beads were taken for SEM and XPS analysis; whereas the treated beads kept in bags were used for treating the new set of manure. A detailed description of the procedure is given below.

Preparation of alginate-nZnO beads

The alginate-nZnO beads were prepared as described in Gautam et al., (2015) with some modification of preparation. In this study, separate batches of alginate-nZnO beads were prepared and applied individually to each reactor. For every batch, 1.5 g of nZnO (ZnO, US3580, US Research Nano-materials, Inc., Texas, USA) and 7.5 g of sodium alginate powder ($(C_6H_7O_6Na)_n$, S1118, Spectrum, Gardena, CA90248, New Brunswick, NJ 08901) were added to 500 mL of deionized water and stirred using a magnetic stirrer until a complete dissolution was obtained (around 36 h). Then the solution was kept in a sonicator (Bransonic® CPXH Ultrasonic Cleaning Bath, Brason, USA) for ~1 h to disperse the NPs uniformly in the solution. Then the nZnO containing sodium alginate solution was poured into 3.5% calcium chloride solution ($CaCl_2.2H_2O$, BDHO224, VWR international LLC 1310 Parkway, West Chester, PA 19380) as drop using syringe. To ensure all the nZnO were completely entrapped in alginate solution, the leftover in the flask was rinsed with 50 mL of sodium alginate solution (solution made from 1.5% sodium alginate only without NPs), after which the solution from the used syringe was dropped into the same batch to avoid loss of nZnO. The beads were kept in calcium chloride for around 9 h for complete hardening of the beads and to ensure porosity inside (Bezbaruah et al., 2009)

Experimental set up for treating manure

Liquid swine manure was collected from the swine research unit at North Dakota State University (NDSU). The experiments were carried out in 1 L Erlenmeyer flasks with a working volume of 500 mL by treating the swine manure with bare nZnO and alginate-nZnO beads at an application rate of 3 g L^{-1}. There were four treatments: i) bare nZnO ii) loose alginate-nZnO beads applied freely iii) alginate-nZnO beads placed loosely in a nylon bag, and iv) control where no bare NPs or beads were applied. After placing manure and treatment in a flask, anaerobic conditions inside the flask were maintained by purging the headspace with nitrogen gas for ~3 min and quickly closing the opening with a rubber stopper attached to a1 L Tedlar bag (SKC Gulf coast Inc., Texas, USA). This experiment was continued for approximately 33 days. All the experiments were performed at room temperature (~22±2°C) and atmospheric pressure (around 760 mm Hg). During the experiment, the headspace gas was collected in Tedlar bags for measuring H_2S concentrations at interval of every 2-10 days interval depending on the volume of gas accumulated in the bag. The H_2S gas concentration was measured using a Jerome meter (Jerome® 631-X, Arizona Instrument, Arizona, USA). Due to higher H_2S concentration, the headspace gas was diluted with nitrogen gas to bring the gas concentration within the Jerome meter's maximum detection limit of 50 ppm. Similarly, CH_4 and CO_2 concentrations produced in the headspace were measured using a gas chromatography (8610C, SRI instrument, California, USA), inbuilt with flame ionized detector (FID) detector. A detailed sampling and analyses have been described in Gautam et al. (2015).

Once the experiment was completed (after 33 days); the beads inside the bag were collected and washed with deionized water for a day for reusing them. All manure samples (initial samples and samples after 33 days incubation in flasks) were analyzed for nutrient content, VFAs, microbial growth and mRNA activity of methanogenic bacteria. Immediately after the first experiment, an additional set of similar experiments were carried with newly collected swine manure and the used alginate-nZnO beads for 34 days. Each treatment including control was replicated 3 times.

Characterization of alginate-nZnO beads and nZnO

Scanning electron microscopy (SEM) analysis

For the SEM analysis, the alginate beads were freeze dried (FreeZone® 4.5 Liter Freeze Dry Systems, Labconco Corporation, Kansas City, USA) for 5 days at – 40°C. After that, the sub-samples were collected for SEM analysis. The samples were affixed to cylindrical aluminum mounts using high-purity silver paint (SPI Products, West Chester, Pennsylvania) and coated with carbon in a high-vacuum carbon evaporative coater (Cressington 208c, Ted Pella Inc., Redding, California). Images were obtained with a JEOL JSM-7600F scanning electron microscope (JEOL USA, Inc., Peabody, Massachusetts) operated at 2.0 KV. The surface and the cross-sectional images were taken at different resolutions (10,000; 30,000; 60,000 and 90,000 times). Energy-dispersive spectroscopy (EDS) analysis was carried out using an ultra-dry silicon drift X-ray detector and NSS-212e NORAN^{TM} System 7 X-ray Microanalysis System (Thermo Fisher Scientific, Madison, Wisconsin).

X-ray photoelectron spectroscopy (XPS) analysis

The freeze-dried samples of alginate beads were outgassed overnight under the UV conditions to maintain an appropriate pressure in the analyzer chamber. XPS analysis was carried out using Thermo Scientific X-ray photoelectron spectrometer (Thermo Scientific^{TM} K-Alpha^{TM+} X-ray photoelectron spectrometer, USA). The X- ray radiation source was micro-focused Al K α.

Spectra acquisition formed under the ultra-high vacuum conditions and analysis was carried out on 400 um diameter of the sample. Data processing and peak fitting were performed using the Thermo Scientific™ Avantage software.

Analysis of microbial population dynamic

Plate count

The microbial population density analysis was carried out using the plate count method to determine the effect of NPs on aerobic and anaerobic microbes in manure. The McGarvey et al. (2004) procedure was used with some modifications. Firstly, the agar was prepared to culture the media for bacterial growth following the manufacturer's instructions. For this, 20 g of agarose (IBI Scientific, 9861 Kappa Court, Peosta, IA 52068, USA) and 37 g of brain heart infusion powder (Bacto™ Brain heart infusion; Becton, Dickinson and Company; Sparks, MD 21152 USA) were added to 1 L of deionized water, and boiled on heater plate to dissolve the agar and powder. The solution was then autoclaved (121˚C, 40 PSI for 70 min), cooled to ~50-60˚C after which 10 mL of media was transferred to each petri dish. Petri dishes were then covered and left to cool for ~40 min. 10 µL of each manure treatment: manure treated with bare nZnO, manure with alginate-nZnO beads, and control was transferred into separate petri dishes and spread uniformly across the agar surface with a sterile hockey stick. The experiment was carried out with a series of diluted samples (up to 5 different dilutions) and two replication of each treatment.

Diluted manure samples were poured into plates, closed, placed on a tray and incubated at 30˚C for 24 h to culture aerobic microbes. Following the same procedure, second set of plates were prepared using the same procedure but kept in an anaerobic chamber. The entire chamber was incubated at 30˚C for 24 h to culture anaerobic bacteria. In the anaerobic chamber, the vacuum was created by pumping out the headspace from the chamber and replacing with nitrogen gas. After incubation, the petri dishes were removed from the incubator and colonies of bacteria were counted in a counting chamber using a 10X magnifying glass. All bacterial plate-counting preparations were done inside a sterile environmental hood.

Real time polymeric chain reaction (RT-PCR) analysis

To investigate the effect of nZnO on the methanogenic community of bacteria, RT-PCR analysis was performed targeting the *mcrA* gene in methanogenic bacteria. A number of researchers have shown a positive correlation between *mcrA* gene copy numbers and methane production rates (Freitag and Prosser, 2009, Ma et al., 2012). The RT-PCR analysis consisted of several steps. The RNA extraction process was performed using a FastRNA® Pro soil- Direct Kit (MoBio Laboratories, Solana Beach, CA, USA) according to the manufacturers' instruction with some modifications. First, ribonucleic acid (RNA) was extracted from manure samples and purified, followed by conversion of messenger ribonucleic acid (mRNA) to complimentary deoxyribonucleic acid (cDNA). Any co-isolated, DNA was removed with a DNase-Free kit (Invitrogen). The cDNA synthesis reactions were performed using the SuperScript VILO cDNA Synthesis kit (Invitrogen). Resulting cDNA was frozen at -80°C for use in RT-PCR analyses.

MLF-for and mcrA-rev were the forward and reverse primers used for RT-PCR analysis. The primer sequences for MLF-for and mcrA-rev are given below in Table 38.

Table 38. Properties of RT-PCR primers targeting the mcrA gene.

Primers	Direction	Probe sequence	Length (bp)	References
MLF-for	Forward	GGTGGTGTMGGATTCACACARTAYGCWACAGC	32	(Luton et al., 2002))
mcrA-rev	Reverse	CGTTCATBGCGTAGTTVGGRTAGT	24	(Narihiro and Sekiguchi, 2011)

Statistical analysis

All experiments were carried out in triplicate and the average values are reported with standard deviations. The comparative study and analysis of manure characterization and gaseous emissions among the treatments were conducted using PROC ANOVA procedure in SAS 9.4 (2010) software. The hypothesis was tested at 95% ($P \leq 0.05$) significance level.

Results and discussion

Experiment with fresh alginate beads

Manure characterization

Moisture content (MC), pH, crude protein (CP), total nitrogen (TN), and ammonia (NH_3) of the initial and treated manure is presented in Table 39. There was no significant difference in the pH value among the initial, control and treated samples. The pH range of the manure samples were from 7.44 to 8.08. The dry matter content of the manure samples were quite high (11.65 to 13.20%), as thick manure slurry was taken from the manure pit. The ash content of the NPs treated manure samples were significantly higher compared to initial samples. The addition of nZnO and NPs entrapped alginate beads might have increased the ash content in manure treated with bare nZnO and freely applied alginate-nZnO beads. The CP and TN reduced significantly in control and treated manure sample compared to initial sample. Ammonium content was significantly higher in control, followed by NP treated manure and initial manure.

The total VFA ranged between 325 mM to 605 mM among the manure samples (Table 40). A significant difference in total VFA was observed between initial and treated manure. Total VFAs increased significantly by treating the manure. Total VFAs were found to be the highest in control (605 mM), followed by manure treated with alginate-ZnO beads enclosed in bags (548 mM), loosely applied alginate-nZnO (523 mM) and lowest in the untreated initial manure (332 mM). The acetic acid, which is the most prominent VFA for CH_4 formation was highest in the controls relative to other treatments. The lower value of acetic acid and TVFAs in bare nZnO and alginate-nZnO treated samples compared to controls indicated that the application of alginate-nZnO beads either promoted VFAs conversion to CH_4 or inhibited the production of VFAs. The previous study by Gautam et al. (2015) showed that although VFAs were lower in the manure treated with nZnO compared to control, CH_4 production in control treatment was comparatively higher. Therefore, it is expected that the application of nZnO in manure could have inhibited either hydrolysis; acidogenesis or acetogenesis processes in the anaerobic conversion pathway, and retarded the formation of VFAs, thus likely affecting the formation of CH_4.

Table 39. Characterization of swine manure before and after the completion of experiment

Treatments	pH	Mean % MC	Mean % Ash	Mean % CP	Mean % TN	Mean mM NH_3
[1]Initial	8.08 a ± 0.28	86.80 a ± 0.41	23.31 a ± 0.45	21.83 a ± 0.04	3.49 a ± 0.01	271.81 a ± 3.13
[2]Control	7.44 a ± 0.53	87.94 bc ± 0.17	24.47 ab± 0.45	19.46 b ± 0.17	3.11 b ± 0.03	349.12 b± 3.63
Bare nZnO	7.88 a ± 0.24	88.35 c ± 0.09	29.53 c ± 0.41	19.49 b ± 0.41	3.12 b ± 0.07	276.06 a ± 5.89
Loose beads	7.63 a ± 0.22	87.25 ab ± 0.09	25.76 b ± 0.34	18.31 c ± 0.32	2.93 c ± 0.05	291.74 c ± 6.46
Beads in bags	7.71 a ± 0.18	87.07 a ± 0.45	29.75 c ± 1.54	18.05 c ± 0.45	2.89 c ± 0.07	321.98 d ± 5.24

[1]Initial means the fresh manure collected from source before starting the experiment, and
[2]control means the manure kept in a flask for 33 days without treating with NPs or any beads.

Table 40. Comparison of mean volatile fatty acid (VFA) concentrations of swine manure before (initial) and after the completion of the experiment (control, beads in bags and loose beads).

Treatments	Acetic mM	Propionic mM	Isobutyric mM	Butyric mM	Isovaleric mM	Valeric mM	Total mM
Initial	129.72 a ± 3.32	33.70 a ± 1.37	29.95 a ± 1.68	43.70 a ± 2.30	72.52 a ± 5.14	15.38 a ± 0.94	324.96 a ± 14.14
Control	202.56 d ± 8.92	69.06 c ± 6.24	84.76 d ± 7.67	75.66 c ± 6.76	156.01 c ± 13.37	17.24 a ± 1.51	605.30 d ± 43.37
Bare nZnO	159.82 c ± 2.80	56.52 b ± 1.08	58.13 c ± 0.40	66.63 b ± 0.94	128.08 b ± 1.98	25.18 c ± 0.09	494.36 c ± 5.75
Loose beads	166.15 c ± 9.65	56.53 b ± 1.28	69.23 b ± 2.39	67.19 b ± 1.88	140.52 bc ± 6.99	22.89 b ± 0.03	522.50 bc ± 6.57
Beads in bags	184.29 b ± 5.30	59.48 b ± 1.17	74.14 b ± 2.93	71.52 bc ± 2.65	136.18 b ± 8.67	22.20 b ± 0.94	547.82 b ± 17.01

*Values followed by the same letter in row are not significantly different at $P \leq 0.05$

Total gas production

Gas production trends are shown in Figure 27. During the experimental period, the maximum production rate of was observed in the control (3540 mL L^{-1}). Manure treated with bare nZnO or alginate- nZnO beads showed a significant reduction ($P \leq 0.05$) in total gas production. The gas volume of approximately 1807 mL L^{-1} was observed from the manure treated with 3 g L^{-1} bare. Total gas production was reduced by 49, 34, and 31% in control, manure treated with freely applied alginate-nZnO beads, and manure treated with alginate-nZnO beads in the bag, respectively. There was no significant different (P=0.195) in the gas production from the manure treated with alginate-nZnO beads applied loosely (2353 mL L^{-1}) and by keeping in a nylon bag (2433 mL L^{-1}).

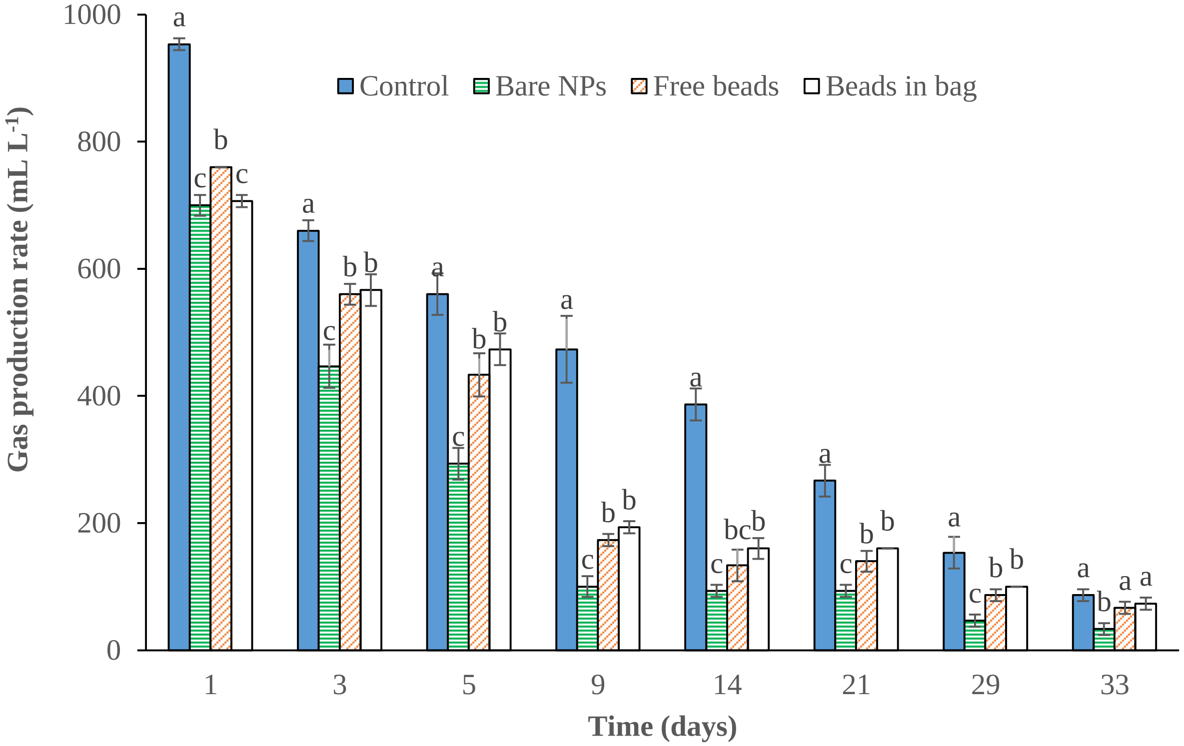


Figure 27. The gas productions per unit volume of liquid dairy manure treated with nZnO entrapped loose alginate beads, alginate beads in nylon bags, and control during different measurement periods. The error bars are the standard deviations calculated from three replications of each treatment.

Gautam et al, (2015) reported 43 and 20% reduction on total gas production from dairy manure treated with freely applied alginate-nZnO beads and alginate-nZnO beads in bags, respectively. There was 23% variation in the reduction of total gas production in between free beads application, and beads application by keeping in bags; however, the variation in the reduction of total gas production between these two application methods were only 3%. Compared to the previous study by Gautam et al. (2015), the modification of alginate-nZnO beads placement method increased the effectiveness of alginate-nZnO beads in a bag.

Methane production

The variation of CH_4 concentration was observed during the experiment period for all the treatments. For control, the initial concentration was almost 7% which increased gradually reaching approximately 21% on day 14 and remained almost constant during rest of the days of the experimental period. Control exhibited the highest CH_4 concentration whereas the manure treated with bare nZnO exhibited the lowest concentration in all the measurements taken throughout the experimental period (Figure 28). Manure treated with bare-nZnO treated manure, the initial concentration gradually increased from 6% to 14% on the 9th day, then the concentration gradually declined and reached ~6% towards the end of the experiment. Likewise, a similar trend of concentration was observed in both treatments associated with alginate-nZnO beads. Though, no significant difference in CH_4 concentration was observed until day 21 in the manure treated with loose alginate-nZnO beads and alginate-nZnO beads in bags; the CH_4 concentration was significantly reduced after manure was treated with loose alginate-nZnO beads. In contrast, the previous study by Gautam et al. (2015), measured a 32% difference; but this study showed only a 9% difference in the reduction in average CH_4 concentration between the treatments with freely applied beads and beads in the bag. The study showed that the modification in placement method increased the effectiveness of alginate-nZnO beads in a bag in reducing CH_4 concentration also.

Each treatment showed a significant difference in overall CH_4 production. Maximum CH_4 production of 449 mL L^{-1} of manure was observed in the control while the minimum CH_4 production (125 mL L^{-1}) was observed in manure treated with bare nZnO, account 33 days experimental period (Table 41). An approximately reduction of 72, 56 and 49% CH_4 production was observed from manure treated with bare nZnO, loose alginate-nZnO beads, and alginate-nZnO beads in the bag, respectively, compared to control.

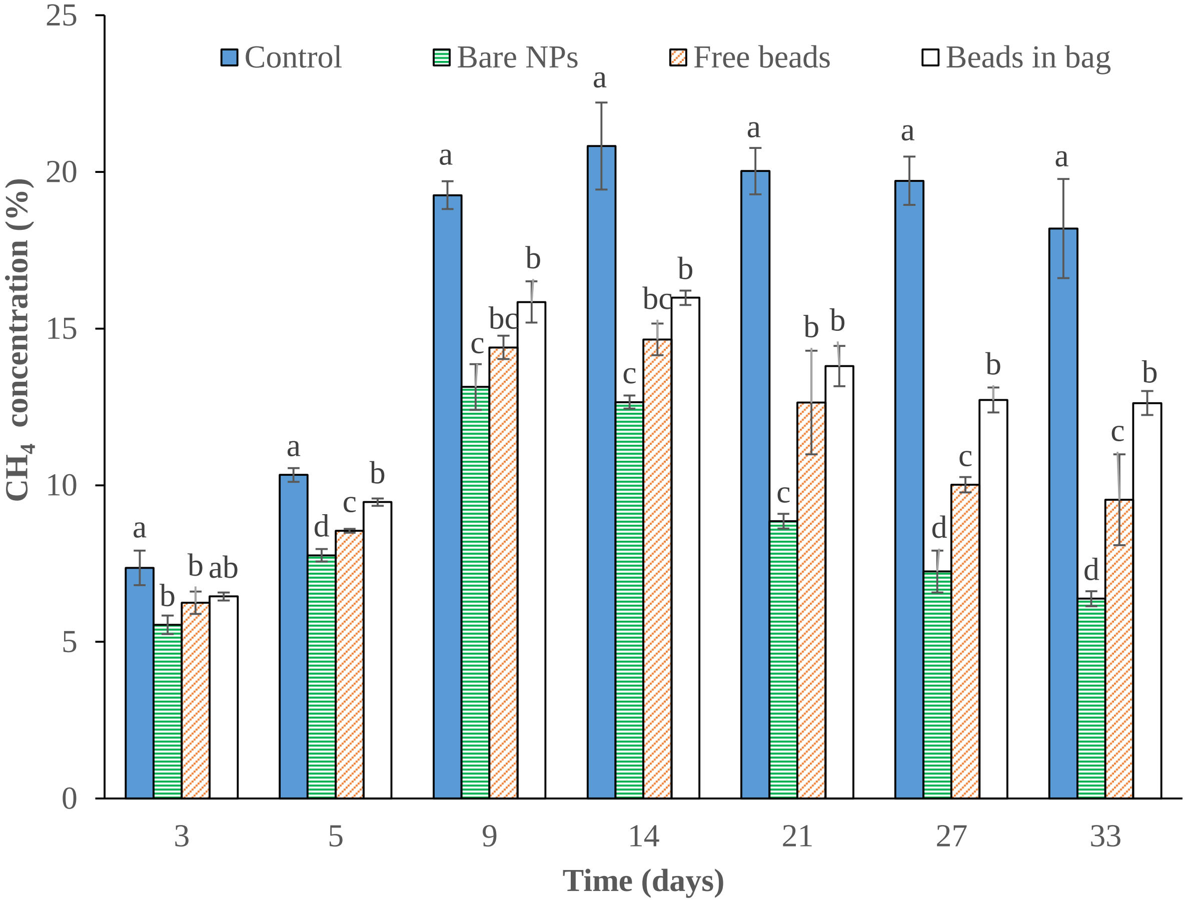


Figure 28. Methane concentration from the manure treated with alginate-nZnO beads applying freely and keeping in bags. The error bars are the standard deviations calculated from three replications of each treatment. In each measurement, the treatments with the same letter above error bar are not significantly different at $P \leq 0.05$.

Carbon dioxide production

In all treatments, the trend in CO_2 concentrations was fairly similar to that of CH_4 concentrations. After the first measurement, significantly lower ($P \leq 0.05$) CO_2 concentrations were observed in the manure samples treated with loose alginate-nZnO beads, alginate-nZnO beads in bags and bare nZnO compared to control (Figure 29). The control exhibited the maximum 2273 mL of CO_2 production per liter of swine manure. Similarly, the manure treated with loose alginate-nZnO beads, alginate-nZnO beads in bags and bare nZnO exhibited 1167, 1237 and 872 mL of CO_2 per liter of manure; respectively (Table 41). The reduction with those treatments was 49, 46 and 62%, respectively compared to control.

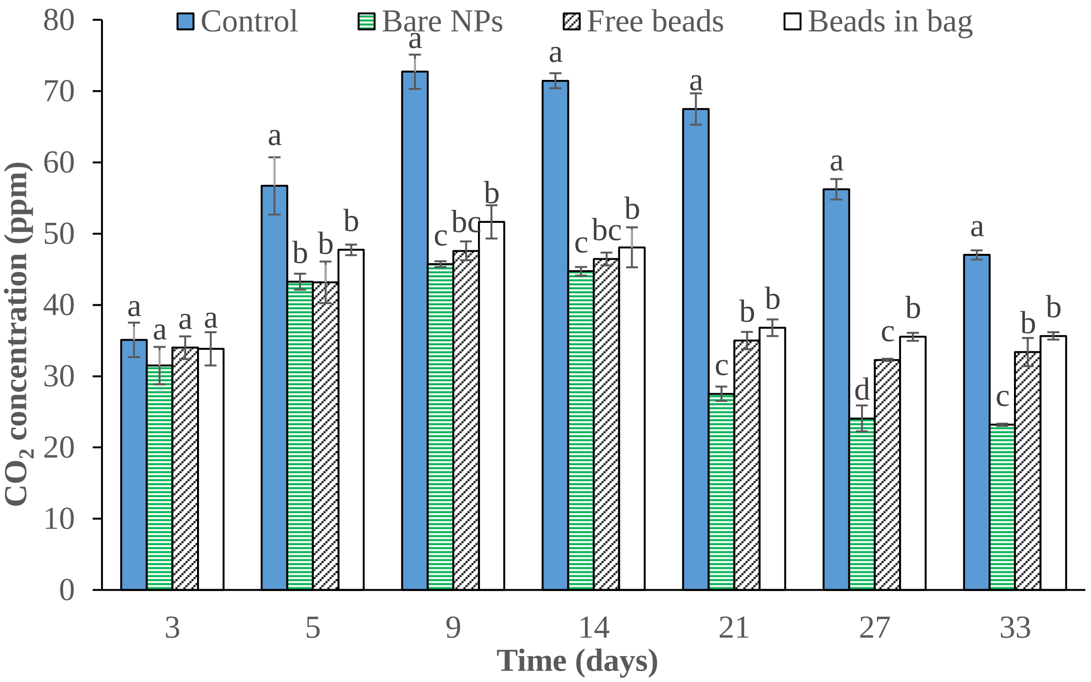


Figure 29. Carbon dioxide concentration from the manure treated with alginate-nZnO beads applied freely and contained in nylon bags. The error bars are the standard deviations calculated from three replications of each treatment.

Hydrogen sulfide production

Both bare nZnO, as well as the alginate-nZnO beads were effective in reducing H_2S production. In the case of manure treated with bare nZnO, loose alginate-nZnO and alginate-nZnO beads in the bag; the H_2S gas concentration gradually increased and reached a maximum (around 282 ppm, 395 ppm and 427, respectively) on day 5. Concentration then gradually decreased and remained almost stable after day 15. However, in the control manure, the concentration increased sharply and reached maximum (~22,000 ppm) on day 9, then decreased gradually (Figure 30).

Irrespective of nZnO application methods, nZnO proved to be very effective in reducing H_2S production. The H_2S production rate was 43.1 mL L^{-1} in case of control, whereas in case of manure treated with bare nZnO, loose alginate-nZnO and alginate-nZnO beads in bag; the H_2S gas production rate were 0.3, 0.4 and 0.5 mL L^{-1}; respectively (Table 41). No significant difference ($P \leq 0.05$) in H_2S production was observed in the manure treated with loose alginate-nZnO beads and alginate-nZnO beads in bag. In a previous study (Gautam et al., 2015), 96% reduction on H_2S production was reported from the liquid dairy manure treated with alginate-nZnO beads in bags; 99% reduction on H_2S production has been achieved from the swine manure treated with alginate-nZnO beads in bag. However in this study, the modification of alginate bead placement method increased the effectiveness of alginate-nZnO beads inside the bag and finally improve their performance in reducing H_2S concentration. Likewise, the reduction in H_2S production from the manure treated with alginate-nZnO beads (in both loose alginate-nZnO beads and alginate-nZnO beads in bag) was also very close to the reduction by the bare applications of nZnO (both ~99%). This was likely because the beads were porous (Deze et al., 2012; Stops et al., 2008); and the liquid portion of manure had sufficient time to come into contact with and react with entrapped NPs, thus resulting in improved performance.

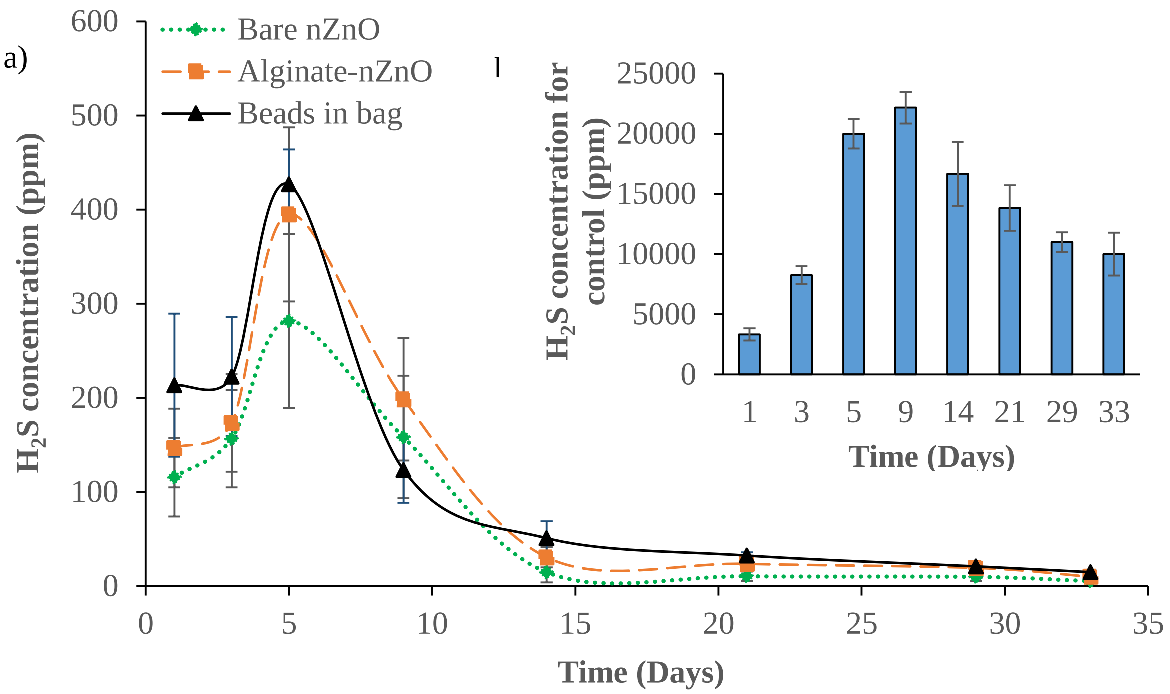


Figure 30. Hydrogen sulfide concentrations from a) the swine manure treated with free alginate-nZnO beads and nZnO beads in nylon bag and b) control. The error bars are the standard deviations calculated from three replications of each treatment.

Table 41. Volume of different gases produced per liter of swine manure.

Treatment	CH_4 (mL L^{-1})	CO_2 (mL L^{-1})	H_2S (mL L^{-1})
Control	448.6 a ± 13.1	2272.7 a ± 68.2	43.1 a ± 0.6
Bare nZnO	125.1 b ± 8.9	871.5 b ± 25.3	0.3 b ± 0.0
Loose alginate	196.5 c ± 4.2	1166.7 b ± 22.3	0.4 b ± 0.1
Alginate in bag	227.2 d ± 4.0	1237.3 c ± 49.0	0.5 b ± 0.1

Experiment with used alginate beads

Total gas production

The total gas production rate was relatively higher on day 1 and day 3 of experiment (approximately 1000 to1300 mL L^{-1} in both treatments); however, the production rate decreased gradually and reached up to 93 and 46 mL L^{-1} from control and manure treated with used alginate-nZnO beads, respectively, during the last measurement of the experiment. At the end of the experiment, the cumulative gas volume per liter manure was found to be 4133 and 3080 mL from the control and manure treated with used alginate-nZnO beads, respectively (Figure 31). The reduction in volume was significant ($P \leq 0.05$); and there was ~25% reduction in total gas production from the manure treated with used alginate-nZnO beads compared to the untreated manure control. The reduction was slightly lower than the value obtained from the fresh (untreated) alginate-nZnO beads (34% reduction).

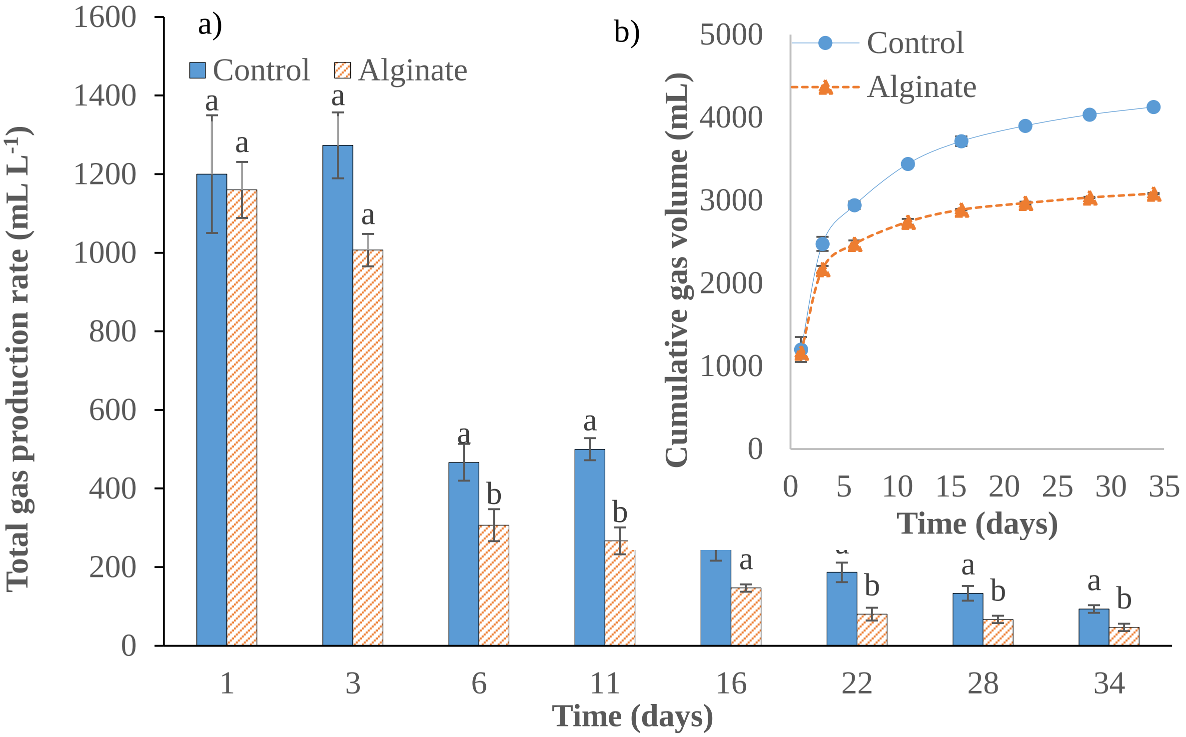


Figure 31. a) Total gas production b) cumulative gas volume per liter swine manure treated with used alginate-nZnO beads and control. The error bars are the standard deviations calculated from three replications of each treatment.

Methane production

Methane production varied widely during the experimental period. Initial CH_4 concentrations were nearly comparable (approximately 8%) from both control and manure treated with used alginate-nZnO beads. The CH_4 production gradually increased and reached maximum (approximately 13% for control and 11% for manure treated with used alginate-nZnO beads). Then, the CH_4 concentration gradually declined in both treatments and reached up to 6%

in control and 2% in manure treated with used alginate-nZnO beads at the end of the experiment (34 d) (Figure 32). Though until day 22, no significant difference in the concentration between the treatments was observed, after that the concentration decreased significantly in manure treated with used alginate-nZnO bead compared to control.

Considering 34 days of the experimental period, the overall CH_4 production rate in manure treated with used alginate-nZnO beads (432.1 mL L^{-1}) was significantly lower ($P \leq 0.05$) than control (258.8 mL L^{-1}). The reduction in the overall CH_4 production rate was 40.1% lower in the case of manure treated with used alginate-nZnO beads compared to control. The fresh (untreated) alginate-ZnO beads were able to show 56% reduction in CH_4 production, but the effectiveness of alginate-ZnO beads was found to be slightly reduced when they were reused for treating new sets of manure.

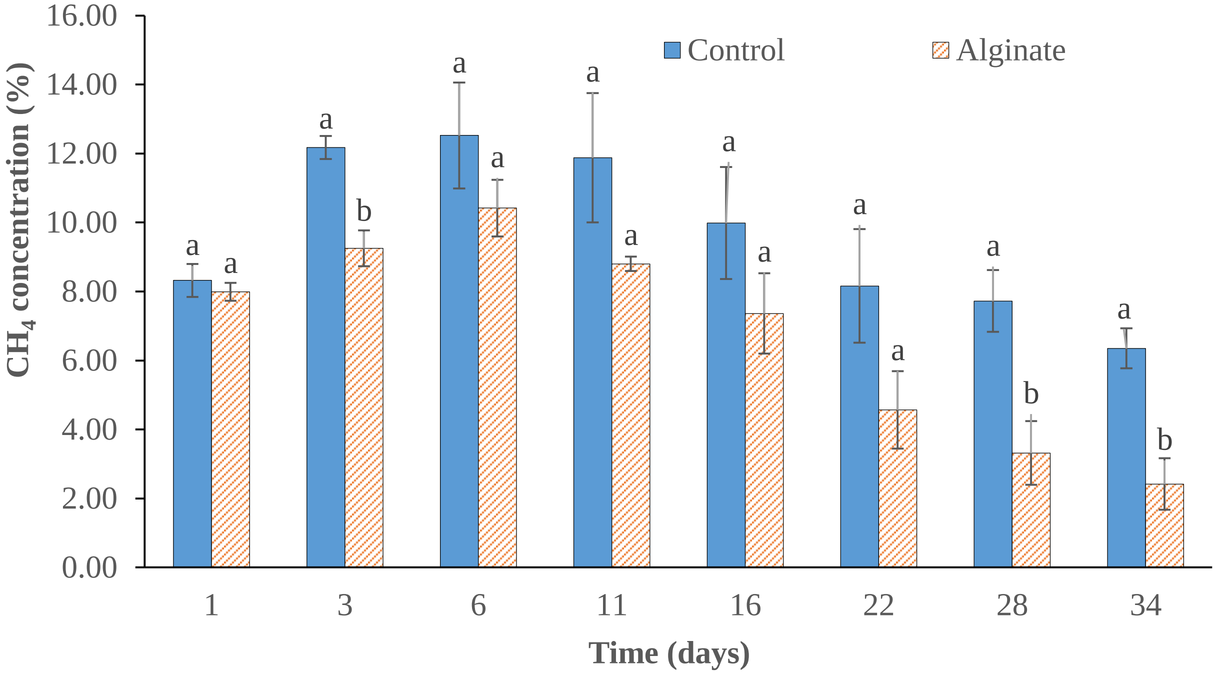


Figure 32. Methane concentration from the manure treated with used alginate-nZnO beads and control. The error bars are the standard deviations calculated from three replications of each treatment. In each measurement, the treatments with the same letter above error bar are not significantly different at $P \leq 0.05$.

Carbon dioxide production

Similar to CH_4, the CO_2 concentrations were also at their maximum (66% in control and 63% in manure treated with used alginate-nZnO beads) on day 3 of the experiment, then gradually declined to 34% in the control and 19% in manure treated with used alginate-nZnO beads (Figure 33). There was not significantly different in the concentration between treatments until day 11 of the experiment then the concentration reduced significantly in manure treated with used alginate-nZnO beads compared to control.

Overall, the CO_2 production rates in manure treated with used alginate-nZnO beads (1519.1 mL L^{-1}) was significantly lower ($P \leq 0.05$) than the control (2153.5 mL L^{-1}). Approximately, 29.4% reduction in total CO_2 production was observed using used alginate-nZnO beads. This reduction was comparatively lower than the CO_2 reduction from fresh alginate beads (49% reduction compare to control).

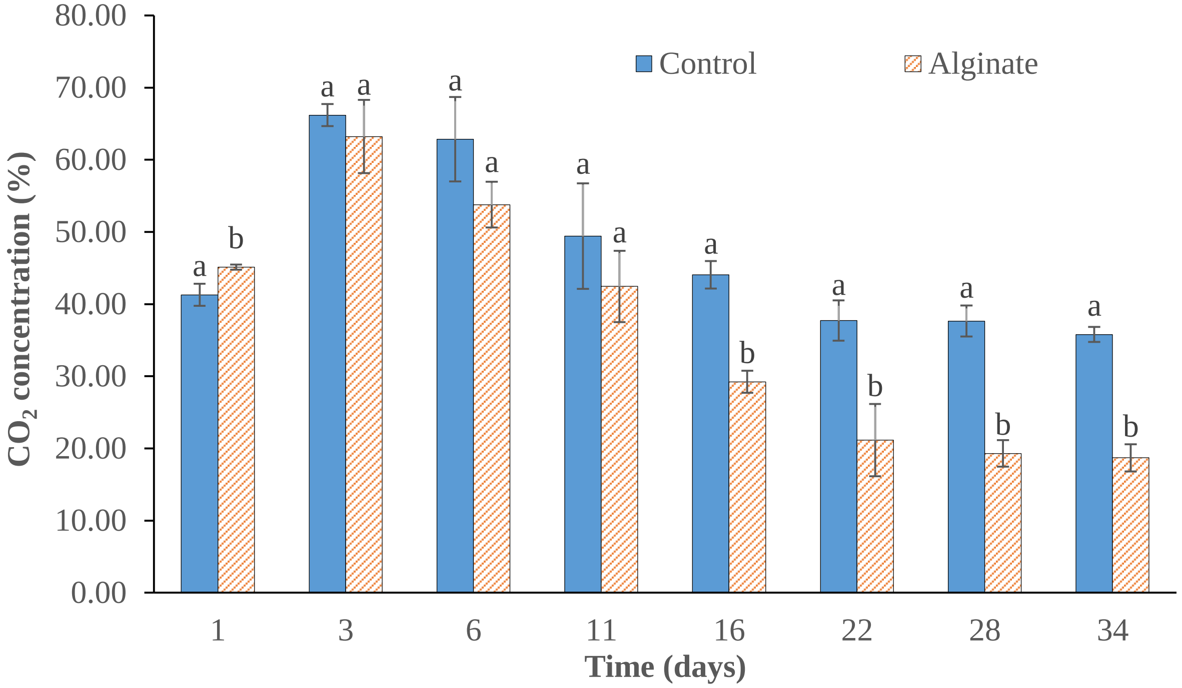
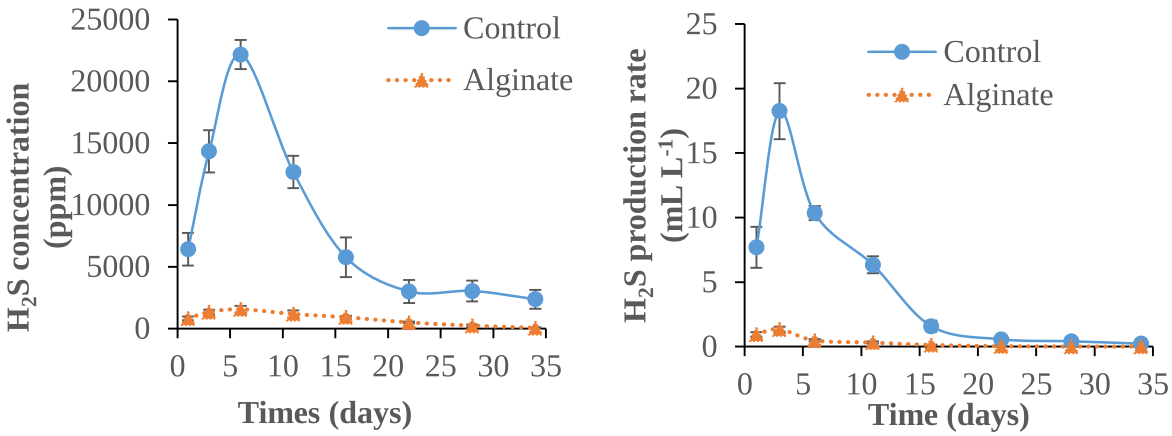


Figure 33. Carbon dioxide concentration from the manure treated with alginate-nZnO beads applied freely and kept in nylon bags. The error bars are the standard deviations calculated from three replications of each treatment.

Hydrogen sulfide concentration

Similar to the bare nZnO and fresh alginate-nZnO beads, the used alginate-nZnO beads also showed effective results in reducing H_2S from swine slurry. In the control, the H_2S concentration increased gradually and reached around 20000 ppm on day 7 of the experiment, after which the concentration declined gradually. However, in the manure treated with the used alginate-nZnO beads, the H_2S concentrations were below 1600 ppm in all the measurements (Figure 34).

Considering the entire 34 days of the experiment period, the total H_2S gas production rates were 45.4 and 3.3 mL L^{-1} from control and manure treated with used alginate-ZnO beads, respectively. Likewise, when the used alginate-nZnO beads were used, the reduction in total H_2S production was around 92.7%. It showed that the reactivity of the used alginate-nZnO beads was slightly reduced compared to fresh alginate beads [H_2S reduction was 99%, (Gautam et al., 2015)]; however, used alginate-nZnO beads also showed effective performance in reducing H_2S production.



a) b)

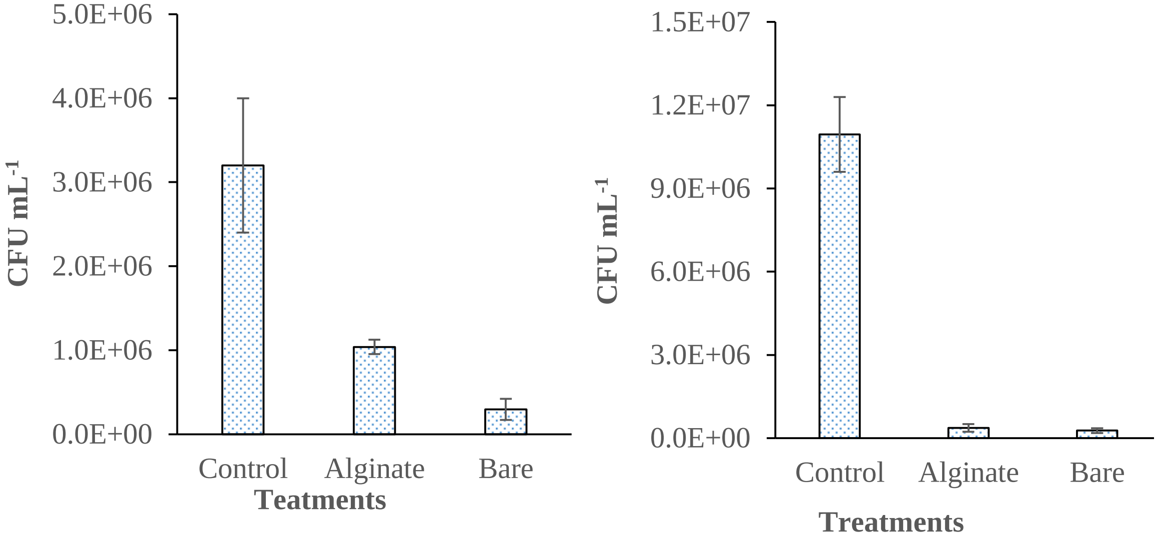
Figure 34. a) Hydrogen sulfide concentrations and b) production rate from the swine manure treated with used alginate-nZnO beads and control. The error bars are the standard deviations calculated from three replications of each treatment.

Analysis of microbial population in manure

Plate count

The average number of colonies of aerobic bacteria were $3.6x10^6$, $1.0x10^6$ and $3.0x10^5$ CFU mL^{-1} in control, manure treated with alginate-nZnO beads (3 g L^{-1} NPs) and manure treated with 3 g L^{-1} bare nZnO, respectively (Figure 35a). Similarly, under anaerobic condition, the colony count were $1.1x10^7$, $3.7x10^6$ and $2.7 x10^6$ CFU mL^{-1}, respectively on those treatments (Figure 35b). It shows that manure contained greater numbers of anaerobic microbes as compared to aerobic. In a companion experiment, the bacterial counts from the dairy manure were in the range of 0.4×10^6 to 7.2×10^7 CFU mL^{-1} and 0.1×10^7 to 5.1×10^7 CFU mL^{-1}, respectively, for aerobic and anaerobic bacteria (McGarvey et al., 2007). Similarly, Sengelov et al. (2003) has also reported the number of total bacteria in the range of 1.15×10^6 to 8.75×10^7 in swine slurry. Likewise, Cotta et al. (2003) reported the bacterial direct count of 6.6×10^9 to 1.0×10^{10} per mL while taking swine manure sample from storage pit.

Under both aerobic and anaerobic conditions, the application of bare nZnO resulted in the lowest bacterial counts (reduced by 91% and 98%, respectively under aerobic and anaerobic cases, compared to control). Manure treated with alginate-nZnO beads resulted in higher microbial survival (67% reduction compared to control) relative to bare nZnO treatment under aerobic condition. However, under anaerobic conditions, the reduction of the bacterial community was comparable to bare NPs. This kind of reduction in microbial community is likely to influence on overall gas production and concentration of gaseous components i.e. the manure treated with bare nZnO and alginate-nZnO beads are more likely to produce less gas and lower gas concentration of H_2S and CH_4 compared to control.



(a) (b)

Figure 35. Colony of bacteria measured under a) aerobic and b) anaerobic conditions.

RT-PCR analysis

The number of bacterial *mcrA* gene copies in the control was 9.97×10^8 copies mL^{-1} which was around six times higher than the gene copies in the initial sample. These values verifies that there was an active methanogens population in the initial manure sample, and that their populations increased during the experiment in manure treatments without additions of NPs or beads (control). In contrast, the manure treated with alginate-nZnO beads, resulted in significantly lower active gene copies (~1.57×10^3 copies mL^{-1}). However, in the case of the manure treated with bare nZnO, no amplification of the active *mcrA* gene was observed even with repeated. It showed that due to NPs application, either it inhibited all the methanogenic activity to below the detection limit for *mcrA* gene copy activity, and or the nano size of ZnO hindered the amplification process. Because the plate counts for bacteria were also below 1000 or less (Table 42) we assume that the activity of methanogens was inhibited and not the RT-PCR reactions.

Table 42. RT-PCR analysis of the manure before and after the experiment

Treatments	copies of gene (copies mL^{-1})
Initial	1.71×10^8
Control	9.97×10^8
Bare nZnO	NA
Alginate-ZnO beads	1.57×10^3

Note: The equation of the standard curve of measurement was $Y = -4.85 X + 55.54$ with R^2 value of 0.99 and the efficiency of the output was 60.80%

Characterization of nZnO and alginate-nZnO beads

SEM analysis

Figure 36a is the SEM micrograph of the bare nZnO, demonstrated that the particle size ranged from 30 to 120 nm. The majority of the particle sizes were in the range of 30-50 nm. The larger particle size (120 nm) is likely due to the agglomeration of NPs over time. Figure 36b demonstrates the cross-sectional view of an alginate bead without NPs. It shows the porous nature of the alginate bead. The porous nature of the alginate beads have also been described by other researchers (Stops et al., 2008; Torre et al., 2000). The round like structure in the micrograph is most likely the pore, which might have allowed the free movement of liquid inside the beads when applied in manure.

Figure 36c demonstrates the surface of the untreated alginate-nZnO bead, the small particles are nZnO, apparently covered with an alginate layer. From the micrograph, it can be assumed that nZnO were protected inside the beads and they were less likely to diffuse to media where beads were used. Likewise, when the cross section of those alginate-nZnO beads was observed, then nZnO was seen in an aggregate form in between alginate clusters (Figure 36d). The agglomeration of NPs is likely to lower the reactivity of ZnO.

The surface of the treated alginate-nZnO beads is illustrated in Figure 36e. Due to changes in the chemical composition of alginate beads resulting from the interaction with

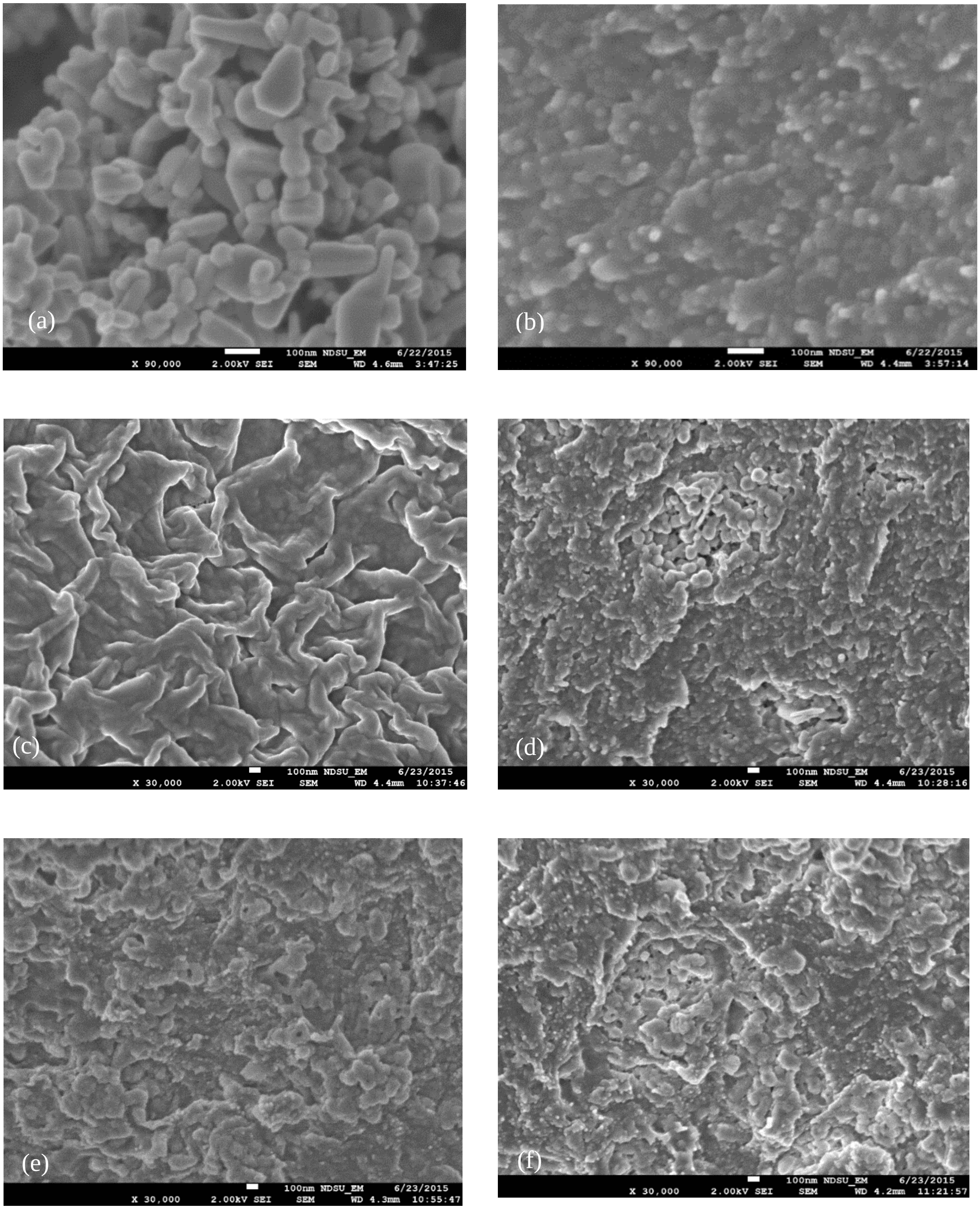
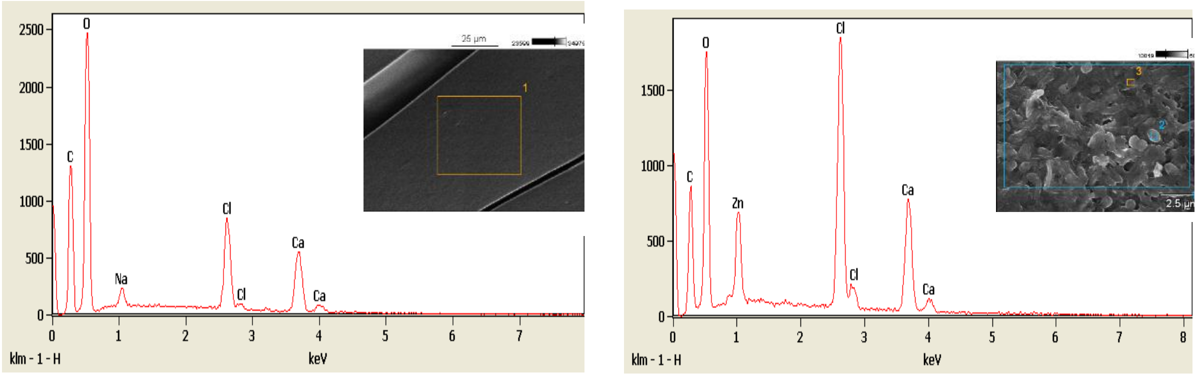


Figure 36. SEM micrographs of a) bare nZnO b) alginate beads without NP from inside c) fresh alginate-nZnO beads from outside d) from alginate-nZnO beads from inside e) treated alginate-nZnO beads from outside f) treated alginate-nZnO beads from inside.

elemental components in manure, some morphological changes on the structure were observed in alginate beads. The cross-sectional view of the beads showed the presence of nZnO as in similar fashion to untreated alginate-nZnO. The presence of nZnO in treated alginate beads illustrated that NPs were retained in the beads and less likely to escape to manure during treatment (Figure 36f).

For the EDS analysis, a certain portion (rectangular) of the alginate beads without NPs on its cross section was chosen (Figure 37a). The peaks of carbon, oxygen, calcium, sodium and chloride illustrated that those components existed inside the beads. In addition to these elemental peaks, a zinc peak was also present in case of alginate-nZnO bead (Figure 37b). These elements were either present in alginate powder or acquired during the bead preparation process.

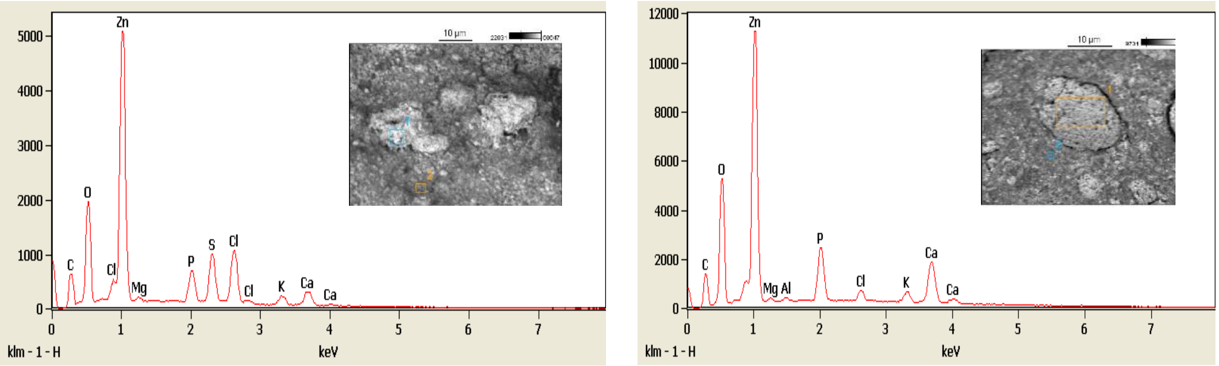


(a) (b)

Figure 37. EDS analysis of the alginate bead a) without NPs and b) with nZnO.

The EDS analysis of the treated alginate-nZnO beads showed that there was formation of a sulfur component inside the beads after the treatment. Besides sulfur, other elements like magnesium, phosphorous and potassium were also observed inside the alginate beads (Figure 38a). As the EDS analysis provides only the elemental information of the subject, it was not clear what form(s) of sulfur i.e., sulfate, sulfite, sulfide were present.

To further confirm the formation inside or outside, the EDS analysis of the agglomerated portion of the NPs inside the bead was carried out. The elemental peak of sulfur was observed only in the case when the outer portion of the agglomerated part was selected. When the middle section of the agglomerated part was selected, no sulfur peak was observed (Figure 38b). From these result, it can be presumed that the liquid manure may not be able to penetrate into the agglomerated portion of nZnO. This might be due to the strong adhesion force of nZnO among themselves or weak cohesive force between water molecule and nZnO. The presence of agglomerated portions of nZnO show that there remains a need to modify the entrapment method of NPs in alginate beads.



(a) (b)

Figure 38. EDS spectra of a) treated alginate beads with nZnO (area 1), b) agglomerated portion in a treated alginate bead (area 1).

XPS analysis of the treated alginate beads

Because SEM-EDS is not able to distinguish the chemical form of compounds in beads, XPS analysis of treated and untreated alginate-nZnO beads and alginate beads without NPs was carried out (Figure 39). XPS analysis revealed the presence in the treated alginate-ZnO bead of zinc, along with carbon, oxygen, sulfur, phosphorus, calcium, sodium, and magnesium. Based on the literature, the possible components in the alginate beads with their binding energy are presented in Table 43. However, the concern was mainly related with sulfur, zinc and their possible associated compounds (ZnS, $ZnSO_4$, Zn^{++}, SO_3^-, and SO_4^{--}).

Table 43. Binding energy of the different elements/compounds associated with alginate beads.

Element/compounds	Binding Energy
Calcium chloride ($CaCl_2$)	~200.8[1]
Sulfur-S 2p, $2p_{3/2}$	162.9 to 164.4[4]
Zinc sulfide-S 2P, $2P_{1/2}$, $2P_{3/2}$	~162.8[2], 161.7 to 162.4[4]
Zinc sulfide-Zn $2P_{3/2}$	1021.7 to 1022.0[4]
Zinc oxide	~1022[3]
Metal sulfide	~ 161.5[3]
Metal chloride	198.5 to 199[3]
Calcium chloride ($CaCO_3$)	~ 347.2[3]
Calcium phosphate ($Ca_3(PO_4)_2$	~ 347.4[3]
Oxygen-1S	530.8 to 533.5[4]
Metal oxide	529 to 530[3]
Carbon (C-C, C-O-C, O-C=O)	284.8 to 288.5[3]
Sodium-1S	1070.8 to 1071.8[4]
Sodium compound	1071 to 1071.5[3]
Phosphorous- $2P_{1/2}$, $2P_{3/2}$	129.4 to 130.9[4]
Nitrite and Nitrate	397 to 405[3]
Magnesium oxide and carbonate	1304.5 to 1305[3]

[1](Western-University, 2015), [2](Barreca et al., 2002), [3](ThermoScientific-XPS, 2013), [4](NIST, 2012)

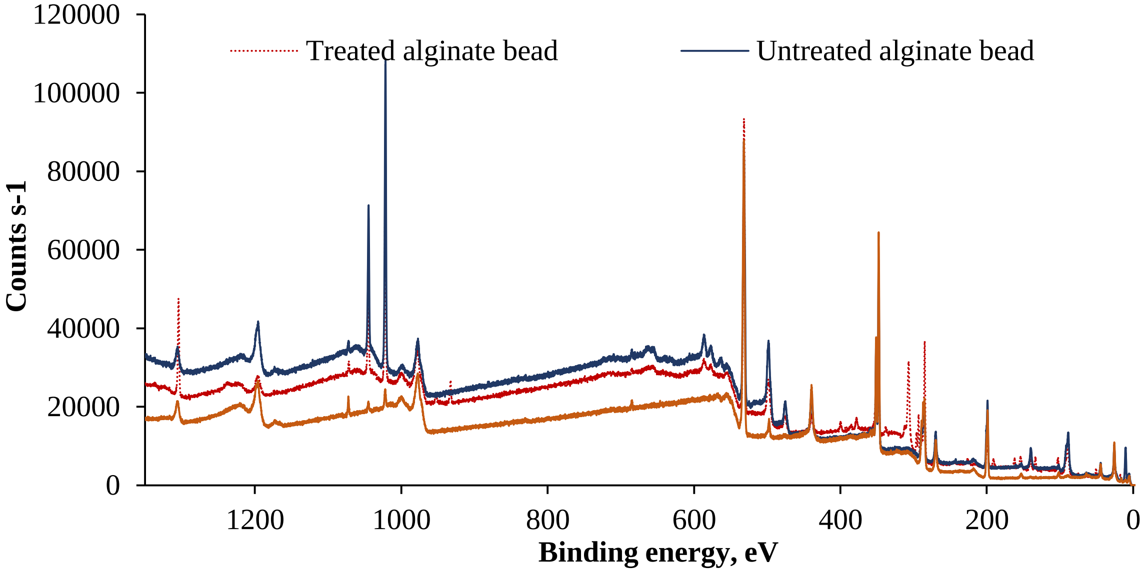


Figure 39. XPS analysis of the treated nZnO entrapped alginate bead.

The XPS spectra recorded from the treated alginate-nZnO beads is shown in Figure 40. The S 2p $_{3/2}$ and S 2p $_{1/2}$ peaks were detected in the treated alginate-nZnO beads samples (Figure 40). However, no sulfur peaks were detected in the untreated sample. The spectrum is displaced with two spin-orbital components resulting from least-squares fitting. The peak energy of S 2p $_{3/2}$ and S 2p $_{1/2}$ were 161.5 eV and 162.8 eV. Barreca et al., (2002) have reported the spectrum with two spin-orbital for S 2p $_{3/2}$ and S 2p $_{1/2}$ with peak energies of 161.7 eV and 162.8 e, respectively; and assigned as sulfur in ZnS. These peaks were assigned to the binding energy of sulfur in ZnS form, because the binding energies of other sulfur-related compounds are comparatively higher; such as elemental sulfur has a binding energy of 164.0 eV and chemisorbed SO_2 has a binding energy in between 163-165.5 eV (Liao et al., 2006; Rodriguez et al., 1999).

Figure 41 shows the XPS spectra of Zn 2p$_{3/2}$; taken from treated and untreated alginate-nZnO beads. The Zn 2p$_{3/2}$ peak obtained from untreated alginate-ZnO beads with its peak at 1021.5 eV appeared almost similar in shape compared to the peak (1021.9 eV) obtained from treated alginate-nZnO beads (Figure 41). The peaks located at 1021.5 and 1021.9 eV could be due to Zn–O bond and Zn–S bond, respectively. Mar et al. (1993) had got the similar peak at 1021.4 eV and assigned for Zn–O bond. Similarly, Laajalehto et al. (1994) has assigned the peak at 1022.0 eV for Zn-S bond. It showed that in treated beads, some of ZnO might have converted into ZnS while treated with manure.

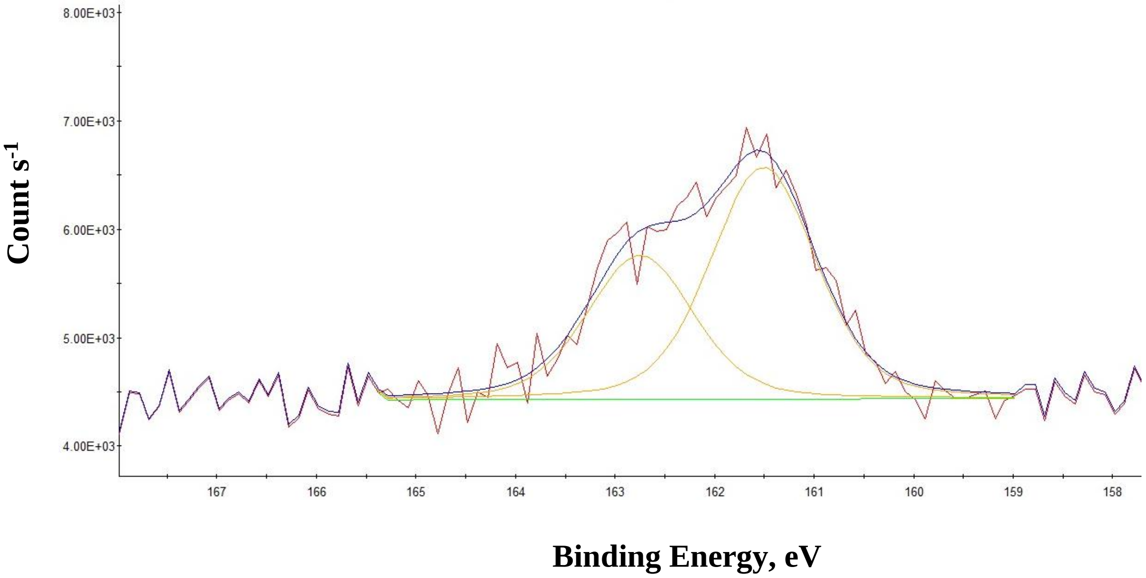


Figure 40. XPS spectra of S 2p positioning while analyzing treated alginate-nZnO beads, indicating the presence of ZnS.

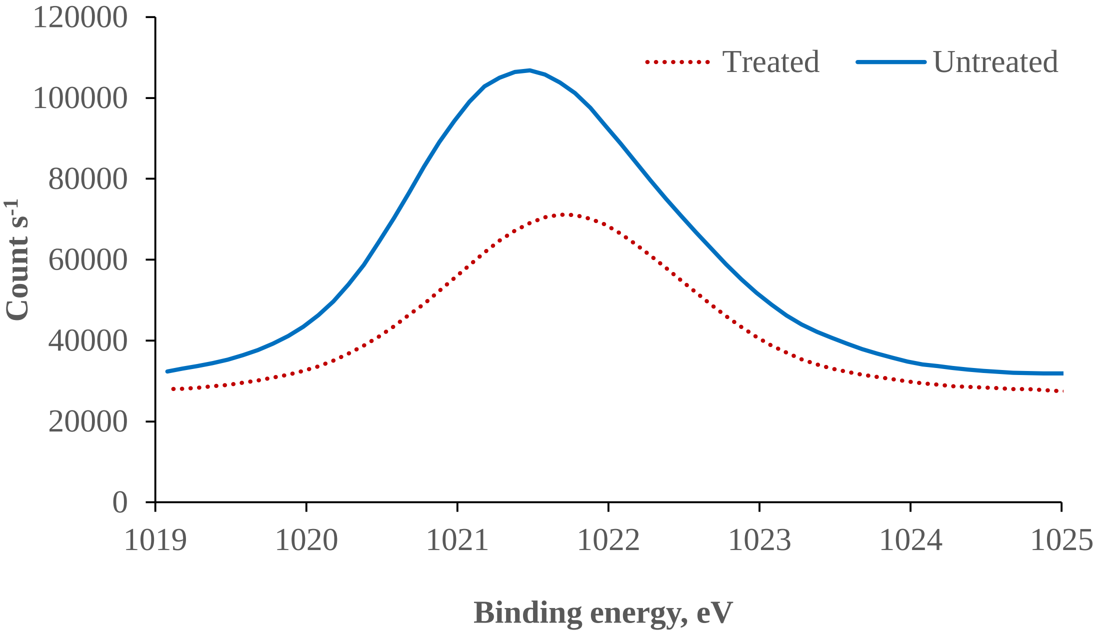


Figure 41. XPS spectra of Zn $2p_{3/2}$ on treated and untreated alginate beads.

Conclusions

It was demonstrated that the fresh alginate-nZnO beads reduced total gas production and CH_4, CO_2, and H_2S concentration. The total gas production was reduced by 34 and 49% when the manure was treated with alginate-nZnO beads and bare nZnO, respectively. The H_2S

concentration was reduced by 99% when alginate-nZnO beads or bare nZnO was added applied manure, compared to control. Total CH_4 production was reduced by 72 and 56% in manures treated with alginate-nZnO beads and bare nZnO compared to control. Manure treated with alginate-nZnO beads also showed a substantial reduction (49%) in total CO_2 production compared to the control. The effectiveness of alginate-nZnO beads was found to be 15, 16 and 13 % lower than bare nZnO in terms of reducing total gas, CH_4 and CO_2 production, rspectively, but showed almost comparable results in terms of reducing H_2S production. However, the application of beads provide the option of NPs recovery. The modification in placement method of alginate-nZnO beads increased the effectiveness. Specifically the performance of beads in bags were compared to freely applied alginate-nZnO beads. The used alginate-nZnO showed slightly low performance compared to fresh alginate beads, but used alginate-nZnO helped in reducing total gas volume, CH_4 and H_2S concentration from manure. It also provide an option of reusing the used alginate-nZnO beads, which may reduce the cost and time of this technology. Population growth of bacteria estimated using plate counts was reduced by approximately 66 and 98% under aerobic and anaerobic conditions respectively, when manure treated with alginate-ZnO beads. The survivability of bacterial was higher in the manure treated with alginate-nZnO beads relative to manure treated with bare nZnO, but nZnO in both direct and indirect applications showed inhibition of bacteria in manure. Similarly, RT-PCR analysis also illustrated the inhibition of methanogens in manure treated with alginate-nZnO beads. The SEM micrographs of the treated alginate-nZnO beads illustrated that nZnO was still retained in the beads after the treatment preventing their release to the environment. The EDS analysis showed that there was the formation of a sulfur component, and XPS confirmed that the sulfur component was zinc sulfide. Analysis exhibits that the sulfur component in the manure converted into ZnS after reacting with nZnO. Finally, it was concluded that the reduction of gaseous production from manure was due to the chemical conversion of produced gases and the inhibitory effect of nZnO on the microorganisms responsible for gaseous production.

References

Abdelmseeh, V. A., Jofriet, J., and Hayward, G. 2008. Sulphate and sulphide corrosion in livestock buildings, Part I: Concrete deterioration. *Biosystems Engineering*. 99(3): 372-381.

Amon, B., Amon, T., Boxberger, J., and Alt, C. 2001. Emissions of NH_3, N_2O and CH_4 from dairy cows housed in a farmyard manure tying stall (housing, manure storage, manure spreading). *Nutrient cycling in agroecosystems*. 60(3): 103-113.

Amon, B., Kryvoruchko, V., Amon, T., and 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.

ASABE. 2005. Manure storage safety.: American Society of Agricultural and Biological Engineers standard EP470. Retrived on 8/1/2015 from http://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs142p2_019839.pdf.

Awume, B. 2014. Control of Hydrogen Sulfide Emission Using Zinc Oxide Nanoparticles. Master thesis. Saskatoon: University of Saskatchewan, Department of Environment and Sustaninalbility. Reterived on 08/10/2015 from http://ecommons.usask.ca/bitstream/handle/10388/ETD-2014-07-1621/AWUME-THESIS.pdf?sequence=4.

Bajpai, S. K., Chand, N., and Chaurasia, V. 2012. Nano zinc oxide-loaded calcium alginate films with potential antibacterial properties. *Food and Bioprocess Technology*. 5(5): 1871-1881.

Barrasa, M., Lamosa, S., Fernandez, M. D., and Fernandez, E. 2012. Occupational exposure to carbon dioxide, ammonia and hydrogen sulphide on livestock farms in north-west Spain. *Annals of Agricultural and Environmental Medicine*. 19(1): 17-24.

Barreca, D., Gasparotto, A., Maragno, C., Tondello, E., and Spalding, T. R. 2002. Analysis of Nanocrystalline ZnS Thin Films by XPS. *Surface Science Spectra*. 9(1): 54-61.

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

Carnes, C. L., and Klabunde, K. J. 2002. Unique chemical reactivities of nanocrystalline metal oxides toward hydrogen sulfide. *Chemistry of materials*. 14(4): 1806-1811.

Cotta, M. A., Whitehead, T. R., and Zeltwanger, R. L. 2003. Isolation, characterization and comparison of bacteria from swine faeces and manure storage pits. *Environmental microbiology*. 5(9): 737-745.

Deze, E. G., Papageorgiou, S. K., Favvas, E. P., and Katsaros, F. K. 2012. Porous alginate aerogel beads for effective and rapid heavy metal sorption from aqueous solutions: Effect of porosity in Cu^{2+} and Cd^{2+} ion sorption. *Chemical Engineering Journal*. 209: 537-546.

Ebrahim, H. A., and Jamshidi, E. 2001. Kinetic study of zinc oxide reduction by methane. *Chemical Engineering Research and Design*. 79(1): 62-70.

Finotelli, P. V., Da Silva, D., Sola-Penna, M., Rossi, A. M., Farina, M., Andrade, L. R., Takeuchi, A. Y., and Rocha-Leão, M. H. 2010. Microcapsules of alginate/chitosan containing magnetic nanoparticles for controlled release of insulin. *Colloids and Surfaces B: Biointerfaces*. 81(1): 206-211.

Freitag, T. E., and 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. P., Rahman, S., Bezbaruah, A. N., and Borhan, M. S. 2015. Evaluation of Nano Zinc Oxide Entrapped Ca-Alginate Beads to Reduce Gaseous Emissions from Liquid Livestock Manure. *Applied Engineering in Agriculture*: (In press).

Kai, P., Pedersen, P., Jensen, J. E., Hansen, M. N., and Sommer, S. G. 2008. A whole-farm assessment of the efficacy of slurry acidification in reducing ammonia emissions. *European Journal of Agronomy*. 28(2): 148-154.

Laajalehto, K., Kartio, I., and Nowak, P. 1994. XPS study of clean metal sulfide surfaces. *Applied Surface Science*. 81(1): 11-15.

Liao, H.-C., Kuo, P.-C., Lin, C.-C., and Chen, S.-Y. 2006. Synthesis and optical properties of ZnO–ZnS core-shell nanotube arrays. *Journal of Vacuum Science & Technology B.* 24(5): 2198-2201.

Likens, G. E., Bormann, F. H., and Johnson, N. M. 1972. Acid rain. *Environment: Science and Policy for Sustainable Development*. 14(2): 33-40.

Luna-delRisco, M., Orupõld, K., and 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.

Luostarinen, S., Luste, S., and Rintala, J. 2006. Nitrogen removal from on-site treated anaerobic effluents using intermittently aerated moving bed biofilm reactors at low temperatures. *Water research*. 40(8): 1607-1615.

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

Ma, K., Conrad, R., and 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.

Ma, R., Levard, C. m., Judy, J. D., Unrine, J. M., Durenkamp, M., Martin, B., Jefferson, B., and Lowry, G. V. 2013. Fate of zinc oxide and silver nanoparticles in a pilot wastewater treatment plant and in processed biosolids. *Environmental science & technology*. 48(1): 104-112.

Mar, L. G., Timbrell, P. Y., and Lamb, R. N. 1993. An XPS study of zinc oxide thin film growth on copper using zinc acetate as a precursor. *Thin Solid Films*. 223(2): 341-347.

McGarvey, J. A., Miller, W. G., Sanchez, S., and Stanker, L. 2004. Identification of bacterial populations in dairy wastewaters by use of 16S rRNA gene sequences and other genetic markers. *Applied and environmental microbiology*. 70(7): 4267-4275.

McGarvey, J. A., Miller, W. G., Zhang, R., Ma, Y., and Mitloehner, F. 2007. Bacterial population dynamics in dairy waste during aerobic and anaerobic treatment and subsequent storage. *Applied and environmental microbiology*. 73(1): 193-202.

Mirabelli, M. C., Wing, S., Marshall, S. W., and Wilcosky, T. C. 2006. Race, poverty, and potential exposure of middle-school students to air emissions from confined swine feeding operations. *Environmental health perspectives*, 114(4): 591-596.

Montes, F., Meinen, R., Dell, C., Rotz, A., Hristov, A., Oh, J., Waghorn, G., Gerber, P., Henderson, B., and Makkar, H. 2013. Special topic-mitigation of methane and nitrous oxide emissions from animal operations: II. A review of manure management mitigation options. *Journal of animal science*. 91(11): 5070-5094.

Moreno, L., Predicala, B., and Nemati, M. 2010. Laboratory, semi-pilot and room scale study of nitrite and molybdate mediated control of H_2S emission from swine manure. *Bioresource Technology*. 101(7): 2141-2151.

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

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

Ndegwa, P. M., Hristov, A. N., Arogo, J., and Sheffield, R. E. 2008. A review of ammonia emission mitigation techniques for concentrated animal feeding operations. *Biosystems Engineering*. 100(4): 453-469.

NIST. 2012. National Institute of Standards and Technology, US Department of Commerce; NIST X-ray Photoelectron Spectroscopy Database. NIST Standard Reference Database 20, Version 4.1. Reterived on 08/10/2015 from http://srdata.nist.gov/xps/ElmComposition.aspx

Pattey, E., Trzcinski, M. K., and Desjardins, R. L. 2005. Quantifying the reduction of greenhouse gas emissions as a resultof composting dairy and beef cattle manure. *Nutrient Cycling in Agroecosystems*. 72(2): 173-187.

Peck, H., Van Beeumen, J., and LeGall, J. 1982. Biochemistry of Dissimilatory Sulphate Reduction *Philosophical Transactions of the Royal Society B: Biological Sciences*. 298(1093): 443-466.

Predicala, B., Alvarado, A., and Asis, D. 2012. Use of Zinc Oxide Nanoparticles to Control Hydrogen Sulphide, Ammonia and Odour Emissions from Pig Barns. In *9th International Livestock Environment Symposium American Society of Agricultural and Biological Engineers Valencia Conference Centre Valencia, Spain July 8 - 12, 2012.* Paper Number: ILES12-1507, ASABE. Retrieved on 8/20/2013 from http://elibrary.asabe.org/azdez.asp?JID=1&AID=41577&CID=iles2012&T=2.

Rodriguez, J. A., Jirsak, T., Chaturvedi, S., and Kuhn, M. 1999. Reaction of SO2 with ZnO(0001̄)–O and ZnO powders: photoemission and XANES studies on the formation of SO3 and SO4. *Surface Science*. 442(3): 400-412.

Ruokojärvi, A., Ruuskanen, J., Martikainen, P. J., and Olkkonen, M. 2001. Oxidation of gas mixtures containing dimethyl sulfide, hydrogen sulfide, and methanethiol using a two-stage biotrickling filter. *Journal of the Air & Waste Management Association*. 51(1): 11-16.

Sayyadnejad, M., Ghaffarian, H., and Saeidi, M. 2008. Removal of hydrogen sulfide by zinc oxide nanoparticles in drilling fluid. *International Journal of Environmental Science & Technology*. 5(4): 565-569.

Schiff, J., and Fankhauser, H. 1981. Assimilatory sulfate reduction. In *Biology of inorganic nitrogen and sulfur,* 153-168: Springer.

Sekhavatjou, M., Moradi, R., Hosseini Alhashemi, A., and Taghinia Hejabi, A. 2014. A New Method for Sulfur Components Removal from Sour gas Through Application of Zinc and Iron Oxides Nanoparticles. *International Journal of Environmental Research*. 8(2): 273-278.

Sengeløv, G., Agersø, Y., Halling-Sørensen, B., Baloda, S. B., Andersen, J. S., and Jensen, L. B. 2003. Bacterial antibiotic resistance levels in Danish farmland as a result of treatment with pig manure slurry. *Environment International*. 28(7): 587-595.

Shipochka, M., Stambolova, I., Blaskov, V., and Stefanov, P. 2013. XPS investigation on the surface of ZnO photocatalytic films obtained by polymer modified spray pyrolysis. *Bulgarian chemical communications*. 45: 105-109.

Singh, B. P., Hatton, B. J., Singh, B., Cowie, A. L., and Kathuria, A. 2010. Influence of biochars on nitrous oxide emission and nitrogen leaching from two contrasting soils. *Journal of environmental quality*. 39(4): 1224-1235.

Sommer, S. G., and Hutchings, N. 2001. Ammonia emission from field applied manure and its reduction: Invited paper. *European journal of agronomy*. 15(1): 1-15.

Song, H. S., Park, M. G., Kwon, S. J., Yi, K. B., Croiset, E., Chen, Z., and Nam, S. C. 2013. Hydrogen sulfide adsorption on nano-sized zinc oxide/reduced graphite oxide composite at ambient condition. *Applied Surface Science*. 276: 646-652.

Spellman, F. R., and Whiting, N. E. 2010. *Environmental management of concentrated animal feeding operations (CAFOs)*: CRC Press.

Steudel, R., and Steudel, Y. 2006. Interaction of Zinc Oxide Clusters with Molecules Related to the Sulfur Vulcanization of Polyolefins (“Rubber”). *Chemistry – A European Journal*. 12(33): 8589-8602.

Stops, F., Fell, J. T., Collett, J. H., and Martini, L. G. 2008. Floating dosage forms to prolong gastro-retention: The characterisation of calcium alginate beads. *International journal of pharmaceutics*. 350(1): 301-311.

Su, Z., Qin, S., Tang, D., Yang, H., and Hu, C. 2006. Theoretical study on the reaction of methane and zinc oxide in gas phase. *Journal of Molecular Structure: Thermochem*. 778(1): 41-48.

ThermoScientific-XPS. 2013. XPS Reference. Retrieved on 8/10/2015 from http://xpssimplified.com/periodictable.php.

Thu, K. M. 2002. Public health concerns for neighbors of large-scale swine production operations. *Journal of agricultural safety and health*. 8(2): 175-184.

Torre, M., Maggi, L., Vigo, D., Galli, A., Bornaghi, V., Maffeo, G., and Conte, U. 2000. Controlled release of swine semen encapsulated in calcium alginate beads. *Biomaterials*. 21(14): 1493-1498.

USEPA. 2015. Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990-2013, Washington DC, U.S.A.

Western-University. 2015. X-ray Photoelectron Spectroscopy (XPS) Reference Pages. Surface Science Western, Western University, Canada. Reterieved on 08/05/2015 from http://www.xpsfitting.com/search/label/Chlorine.

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

GENERAL CONCLUSIONS

The general conclusions of this study are given below:

- The field research with two levels of dietary crude protein (12 and 16%) coupled with bedding demonstrated little or no effects on manure composition and GHG (CH_4, CO_2, and N_2O) emissions from the feedlot pen surface.
- Similarly, another field experiment in the next year also demonstrated that the variation in fat levels (3 to 5.5% in the composite sample) in animal feed showed no or little effect on the manure characteristics, H_2S and GHGs (CH_4, CO_2, and N_2O) emissions.
- NPs application doses (0.1, 0.5, and 3.0 g L^{-1}) were effective in reducing total gas production and concentration reduction, but 3 g L^{-1} performed the best. Based on this experiment and published results, subsequent experiments were conducted with 3 g L^{-1} dose.
- Between nZnO and $nZrO_2$, Zirconia did not show any effect on the total gas production and gas concentrations from both dairy liquid manure and swine manure. Thus, no further study was conducted with Zirconia.
- Application of bare nZnO showed 82, 78, 40, and 99% reduction on total gas production, CH_4, CO_2, and H_2S concentrations, respectively, from swine manure. Similarly, 64, 67, and 99% reductions on total gas production, CH_4 and H_2S concentrations, respectively, were observed in the case of dairy manure. However, nZnO showed the minimal effect on pH, crude protein, nitrogen, ammonia, and volatile fatty acids content in the manures.
- Entrapped Zinc oxide nanoparticles in alginate (alginate-nZnO) beads reduced total gas production, and average H_2S and CH_4 concentration from dairy manure by 43, 99, and

51%, respectively. Whereas, beads placed in bags reduced these parameters by 96, 18, and 20%, respectively, compared to control.

- In another experiment with swine manure, freely applied alginate-nZnO beads reduced total gas production, and average H_2S and CH_4 concentration by 34, 99, and 32%, respectively, compared to control; while the alginate-nZnO placed in the bag showed almost comparable results in reducing total gas production and CH_4, CO_2, and H_2S concentration.
- The plate counting and RT-PCR analysis demonstrated that the reduction in gas production and gas concentration were likely due to inhibition of microorganism in manure from NPs.
- Similarly, EDS and XPS analysis demonstrated that the reduction of H_2S was also contributed due to the chemical conversion of H_2S to ZnS.

SUGGESTIONS FOR FUTURE WORKS

Dietary manipulation didn’t show any promising results, but the application of nZnO reduced both gas production, and gas concentration reduction from both swine and dairy manure. Thus, further research can be done in the following area:

- Fate and transport of NPs
- Further research on reduction mechanism of GHG and H_2S gases. Also, new polymers need to be developed to increase entrapped NPs effectiveness.
- Life Cycle Assessment (LCA) can be performed
- This study can be upgraded to a pilot scale study or applied to field scale to evaluate the effectiveness