5-10-2003

Manipulation of Microbial Metabolic Pathways for the Reduction of Sulfide Production

Charles David Zuller

MANIPULATION OF MICROBIAL METABOLIC PATHWAYS FOR THE REDUCTION OF SULFIDE PRODUCTION

By

Charles David Zuller

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Biological Engineering
in the Department of Agricultural and Biological Engineering

Mississippi State, Mississippi

May 2003

MANIPULATION OF MICROBIAL METABOLIC PATHWAYS FOR THE REDUCTION OF SULFIDE PRODUCTION

By

Charles David Zuller

Approved:

Timothy N. Burcham Professor and Gil Parker Chair of Excellence (Director of Thesis)	Alex Thomasson Graduate Coordinator of the Department of Agricultural and Biological Engineering
William T. French Assistant Professor of Chemical Engineering (Committee Member)	A. Wayne Bennett Dean of the College of Engineering
Thomas P. Cathcart Professor of Agricultural and Biological Engineering (Committee Member)	

Name: Charles David Zuller

Date of Degree: May 10, 2003

Institution: Mississippi State University

Major Field: Biological Engineering

Major Professor: Dr. Timothy N. Burcham

Title of Study: MANIPULATION OF MICROBIAL METABOLIC PATHWAYS FOR THE REDUCTION OF SULFIDE PRODUCTION

Pages in Study: 79

Candidate of the Degree of Master of Science

A bacterial additives treatment experiment was conducted to assess the microbial and biochemical changes in stored swine manure. Nitrate salt was added to a slurry of swine waste collected from a waste storage pit to identify the effects of varying levels of nitrate upon the microbial community and the resulting metabolic changes. This research was an attempt to reduce the formation of odorous sulfur-containing compounds and to increase the formation of odorless nitrogen gas by manipulating the metabolic pathways in anaerobic decomposition of the organic matter within manure. Sulfide production from swine wastewater was reduced approximately 45 percent with the addition of 1500 mg/l or more of nitrate to the wastewater.

DEDICATION

I dedicate this research to Susie, my long-suffering wife, who put up with my longing to return to graduate school after 20 years.

ACKNOWLEDGMENTS

The author expresses his sincere gratitude to the Dr. Timothy Burcham and to the other members of the committee for their support, advice, and their encouragement throughout the process of this study. Dr. Todd French and his graduate assistants provided much needed assistance in data acquisition, analysis, and procedures. Finally, Dr. Lewis Brown provided valuable input and direction during the course of the research effort.

The author is much indebted to the USDA Natural Resources Conservation Service (NRCS) for their financial support and to Kim Harris, NRCS Mississippi State Conservation Engineer, for his patience through this long process.

TABLE OF CONTENTS

CHAPTER Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Statement of the Problem

Because of economic and demographic factors, farms have become far fewer and much larger during the last quarter-century than at any other time in the history of the United States (Barker et al., 1996). Animals are now raised in controlled environments in facilities that house thousands of animals per building. Economies-of-scale and standardized treatments have produced a consistent, high-quality product that consumers expect. Although these agribusiness changes have greatly improved efficiency and reduced labor costs, they have resulted in a concentration of animal wastes -- often on land areas too small to adequately utilize the wastes produced. In addition, suburban encroachment into traditional agricultural production areas has created conflicts between suburban families and farmers over waste treatment and odor production. The public associates intensive use of total confinement of livestock with increased risks to the health of humans and animals due to waste and odor accumulations (Gustafson and Veenhuizen, 1999). As a result, Concentrated Animal Feeding Operations (CAFOs) have become defendants in numerous lawsuits, leading to changes in regulations at the local, state, and federal levels.

For decades, water quality has been regulated by state and federal laws; air quality regulations for farms are now being discussed. An economical method of odor reduction will be required.

Background

Since 1980, very large livestock operations have replaced the traditional family farms that once supplied the nation with poultry, swine and cattle. The U.S. Environmental Protection Agency (EPA) estimates there are approximately 238,000 animal feeding operations (AFOs) of all sizes currently in operation (Associated Press, 2002). Although the total number of hog farms fell from 600,000 to 157,000 since 1982, the number of CAFOs has increased 50 percent to 4500. Of this 4500, about 50 large pork producers now supply approximately 45 percent of the total pork production (Kratz, 1998). The CAFOs, some having capacity of more than 1 million animals, often concentrate operations in areas with insufficient land resources for proper waste utilization. While animal waste and related by-products can be applied as fertilizer on farm fields for grain and forage production, excessive application rates can result in water pollution and odor problems (Associated Press, 2002).

Although water quality is regulated by state and federal agencies, odor emissions from livestock production is not regulated by federal statutes and often not by the air pollution control agencies of the states. The available legal recourse for the public has been private or public suits based upon the common-law

doctrine of nuisance. Nuisance litigation is based upon the concept of right to enjoyment of property. Each case has to be considered separately, with a judge and/or jury attempting to determine what constitutes an unreasonable odor level and material harm. Actual and punitive damages can be awarded for odors caused by negligence and irresponsible actions. These lawsuits have been expensive undertakings for all parties involved (Miner, 1995).

Since 1990, several states have tried to regulate CAFOs. In 1995, Vermont banned land application of animal wastes on frozen ground. In 1998, Maryland passed Senate Bill 178 requiring nutrient management plans based on nitrogen and phosphorus as limiting nutrients. In the same year, Mississippi passed Senate Bill 2895, a moratorium on issuing permits for new swine facilities or expansion of existing facilities. North Carolina also took similar action that year. Minnesota passed Section 713 of Chapter 116 of the Minnesota Statutes 2002 requiring testing of animal feeding operations for air emissions of sulfide and ammonia. Other states, such as Nebraska, have tried to limit or exclude CAFOs completely (State Environmental Resource Center, 2003).

Federal agencies have also become involved. To address the effects of animal feeding operations on water quality, such as runoff from land applications of manure, the United States Department of Agriculture (USDA) and the EPA issued the final Unified National Strategy for animal feeding operations (AFO Strategy) on March 9, 1999. This plan called for the development and

implementation of comprehensive nutrient management plans (CNMPs) on all AFOs by the year 2009. AFOs above a certain size would be considered CAFOs and require additional permits. CAFOs are defined to be point sources of pollution according to the Clean Water Act (CWA) Section 502(14) and subject to the National Pollutant Discharge Elimination System (NPDES). This program is administered by the EPA to regulate point source discharge of pollutants into the waters of the United States (U.S. Environmental Protection Agency, 1999).

Believing improper management of manure from CAFOs continues to cause serious acute and chronic water quality problems throughout the United States, the EPA recently revised the NPDES permitting requirements for CAFOs (Section 122) and the Effluent Limitations Guidelines and Standards (ELGs) for CAFOs (Section 412) on December 15, 2002. This revision resulted in more AFOs being considered CAFOs, thereby tripling the number of operations requiring NPDES permits from 4,500 to 15,500. The EPA estimates the 15,500 CAFOs produce 300 million tons of manure annually (U.S. Environmental Protection Agency, 2002).

Civil lawsuits and fines continue to be issued for improper operations. In November 1998, ConAgra in Idaho agreed to pay $1 million in civil penalties and more than $1 million in injunctive relief for violations of the Clean Water Act. In April 1999, Continental Grain Company in Missouri was required to pay

neighbors $5.2 million for odor nuisance. Buckeye Egg Farm in Ohio agreed in January 2001 to pay a $1 million fine and invest $366,000 in manure treatment equipment, due to permit violations. In September 2001, rural neighbors of Buckeye Egg Farm were awarded $19.7 million in a civil nuisance judgment. In February 2002, Cargill Pork Inc. in Missouri pleaded guilty to violating the Clean Water Act and agreed to pay a $1 million fine (Sierra Club, 2002). In August 1999, Premium Standard Farms in Missouri agreed to pay a $25 million fine for violating the state's Clean Water Act (Johnson, 1999). In April 2001, Tyson Foods in Marion, Kentucky was prosecuted under the city's nuisance ordinance even though the broiler houses were outside the city limits (Associated Press, 2001).

Animal waste treatment and utilization have always been important parts of swine production. Recently, odor has become a great concern, with limited success in reducing it. Although agricultural facilities have traditionally been considered exempt from air quality regulations, farms could be redefined as industrial facilities and required to meet strict air pollution standards (Labance, Heinemann, and Beyer, 1999). Additionally, as state regulatory agencies impose strict odor abatement requirements, currently permitted facilities may also be required to obtain air quality permits. Minnesota Department of Health (MDH) and Minnesota Pollution Control Agency (MPCA) have taken leadership roles, setting maximum acceptable sulfide standards (30-50 ppb) at property boundaries

(Minnesota Pollution Control Agency, 2002). While detectors for sulfides are readily available, they are significantly affected by weather conditions, wind speed, temperature, and relative humidity (Tengman and Goodwin, 2000). An economical method of reducing sulfide production is required to avoid potential air quality violations.

Objectives of Study

Commercial additives to manure have been well researched (Liao and Bundy, 1994; Zhu et al., 1997; Heber et al., 1997; Johnson, 1997; Lorimor, 1997). However, current literature indicates no examination of microbial changes over time within the pit and the effects of adding nutrients to indigenous bacteria. Although the inhibitory effects of nitrate upon microorganisms have been well documented (Kluber and Conrad, 1998; Lens et al., 2000; Fdz-Planco et al, 2001), the effects of varying levels of nitrate upon the microbial community and the resulting metabolic changes need further research.

Objectives of this research were to reduce the formation of odorous sulfur-containing compounds and to increase the formation of odorless nitrogen gas by manipulating the metabolic pathways in anaerobic decomposition of the organic matter within manure. This was accomplished by adding nitrate salts, in varying concentrations, to the swine wastewater. The nitrate affects indigenous bacterial metabolic activities and subsequent gas production.

This research documents the effects of adding nitrate salts on:

- the relationships among populations of methanogens, sulfidogens, and denitrifiers;
- the relative amounts and types of gases produced; and
- the relative amounts and types of biochemicals produced.

CHAPTER II

LITERATURE REVIEW

Livestock facilities produce odors, gases, and other by-products during the microbial decomposition of animal wastes. The magnitude and types of by-products generated are dependent upon the microbial community and the environmental conditions (Schmidt and Jacobson, 1995).

Odors from Swine Facilities

Sense of Smell

An odorant is a compound that creates an odor. An odor is a mental impression of the odorant as detected and interpreted by the olfactory system. Detection of the odor consists of physiological reception and psychological interpretation. Reception within the human central nervous system occurs in the olfactory system, consisting of the olfactory epithelium, the olfactory bulb, and the olfactory cortex (Pearce, 1997). The olfactory epithelium, located in the upper nasal cavity, contains receptor cells that connect via neurons to the olfactory bulb. Psychological interpretation occurs within the olfactory bulb (preliminary perception and interpretation of electrical stimuli) and within the olfactory cortex (final perception and interpretation of the odor). Response to an

odor is based upon concentration, intensity, odor quality, and prior exposure, resulting in a mental judgment of the odor. Because of the many factors involved, the psychological interpretation varies greatly from person to person, resulting in differing opinions as to the objectability of an odor. Livestock odor may become an annoyance and affect the well being of some nearby residents, while having little effect on others.

Sources of Odors

Odors from swine facilities are a major concern to the public as a nuisance and potential safety hazard. Odorants also represent a lost resource. For example, nitrogen is a required nutrient for agricultural production but can be lost to the atmosphere as ammonia. Odorants can be produced from multiple sources within the farm enterprise (Nicolai, 1996), such as the animals, the livestock facilities, the waste treatment facilities, and the land application operations. The animals generate odors if they are dirty or manure covered. The building and livestock facilities generate odors from wet and dirty floors, spoiled feed, or manure pits under the floor. Waste treatment facilities, such as storage basins or lagoons, generate gases from microbial decomposition of manure and other organic matter. Land applications of animal wastes may generate odorants from the oxidation of volatile organic compounds that were formed during anaerobic decomposition. Weather, incorporation of the wastes, and topography

play important roles in the offsite movement of odorants associated with land applying wastes.

Within the swine facility, wastes may be collected for several days using a partially slatted floor over a manure pit with a basin-and-plug system. To minimize odors, the pit is typically filled with 10 cm of wastewater prior to collecting wastes (Barker, 1996). Initially the pit can be aerobic, but these systems can quickly become anaerobic (and odorous) due to the loading rate.

Formation of Odors

Over 200 organic compounds are potentially involved in odor production from animal wastes (Mackie, Stroot, and Varel, 1998). Odorous gases, such as ammonia and hydrogen sulfide, and nonodorous gases, such as carbon dioxide and methane, are by-products of bacterial digestion of organic matter in manure. Compounds may have their own odors, or they may combine with other compounds to produce different odors (Lee Wilson and Associates, 1996). The main classes of odor-causing chemicals are volatile fatty acids, indoles and phenols, ammonia and volatile amines, and volatile sulfur-containing compounds. Use of slotted floors and liquid manure storage pits or slurry alleys within the livestock facility can allow gases to spread throughout the building. In addition, use of wastewater from a lagoon to flush the pits can allow odors from the lagoon to enter the building. However, using fresh water to flush would increase the volume of wastes for disposal and could increase odor production due to

sulfates from some water sources. Finally, land application of the wastes to fields allows the oxidation of volatile compounds that were created during the waste treatment process within the lagoon.

Hydrogen sulfide is the principal odorant associated with anaerobic decomposition of manure (Gostelow, Parsons, and Stuetz, 2001). Hydrogen sulfide is a by-product of the reduction of sulfate, using organic matter as an electron donor. Sources of the sulfate may be from the water and/or from proteins within the organic matter. The optimum reduction-oxidation (redox) potential (Barnes et al., 1985), E_h, is between –200 and –300 (Boon, 1995). A low pH tends to exacerbate odors, whereas a high pH tends to reduce odor emissions from sulfides. Other sulfur-containing compounds can be created from reactions between hydrogen sulfide and unsaturated ketones. Finally, sulfur-containing gases such as mercaptans can be significant odorants, even in trace amounts.

Nitrogen-containing gases (such as amines, ammonia, indole, and skatole) can also be significant odorants. Sources for this nitrogen can be protein and amino acids within the swine's feed and urine. Formation of volatile fatty acids (VFA), aldehydes, alcohol, and ketones are from fermentation of carbohydrates within the wastes. Ammonia may form because of the hydrolysis of urea and uric acid in the urine and because of ammonification. Because of the pH-dependent relationship between ammonia and ammonium, a high pH tends to increase

ammonia odors but decreases sulfide odors. Conversely, a low pH tends to decrease ammonia odors but increases sulfide odors.

Environmental Effects of Odors

Environmental effects of agribusiness odors are controversial. Workers within livestock facilities and neighbors downwind are sometimes subjected, for short durations, to odors in excess of the U. S. Department of Labor, Occupational Safety and Health Administration (OSHA) acceptable levels for industry (U. S. Department of Labor, Occupational Safety and Health Administration, 2001). Odors can have psychological and physiological responses in humans (Schiffman, Miller, and Suggs, 1995). Animal health and reproductive activities can be impaired by high levels of gases (Gustafson and Veenhuizen, 2001). Some gases like hydrogen sulfide and ammonia can cause structural damage to the facilities by accelerating oxidation of metal components. In addition, greenhouse gases can be generated, with unknown long-term effects on the ecosystem (Meadows, 1995).

Measurement of Odors

Odors are difficult to quantify because the response to odor is subjective, involving both psychological and physiological responses that form a mental impression of the odor (Gostelow, Parsons, and Stuetz, 2001). An individual's sensitivity to a specific odor varies because of prior exposure to that odor. While

it is possible to measure the concentration of some specific odorants in manure, the concentration of the air emission may have little to do with the perceived odor downwind. Perceived odor may be the result of a combination of many odorants.

Many methods have been developed in an attempt to quantify odor production. In several states and localities, human panels measure odor detection threshold (ODT) to form a basis for odor regulations. However, because of varying sensitivities, the threshold measurements from different panels may vary by a factor of 100 (Koster, 1986). Iowa State University has proposed a standard method combining olfactometry and chemical analysis (Jacobson, Clanton, and Nicolai, 1999). In addition, some mathematical models are used to predict odor potential based upon physical characteristics such as terrain and wind speed. An attempt to automate the process of odor detection and measurement has led to development of a semiconductor gas sensor (Kato et al., 1996). Attempts by state agencies to regulate odors have involved widely varying methods of detection and acceptability (McGinley, Mahin, and Pope, 2000). The methods have included using a sensory panel determination, a scentometer, an olfactometer, an electronic nose, chemical tests or combinations of methods (Hamilton and Arogo, 1999).

Indicators of Odors

Considerable research has been conducted to identify a principal compound within manure that would be an indicator of its odor. Air sampling and analysis for pollutants are costly, complex, and time consuming. Ammonia and methane may present the greatest risk to the ecosystem (Hartung and Phillips, 1994). Volatile ammonia, released from the urine and feces, varies depending upon factors such as temperature and pH. At high concentrations, ammonia may be representative of the manure odor (Schulz and Barnes, 1990) but not at low concentrations. Methane, a greenhouse gas, is produced in the greatest volume of all gases during anaerobic decomposition. Methane production may vary by a factor of three throughout a 24-hour period (Hatfield, Zahn, and Prueger, 1999). Hydrogen sulfide, a strong odorant, is very volatile, quickly forming other sulfur-containing compounds. Some gases found in trace amounts, like amines and mercaptans, also have very strong odors (Fulhage, 1993). Volatile organic compounds (VOC), like p-cresol and toluene, produce intense odors (Liao, Liang, and Singh, 1997) but vary greatly with aeration, time, and manure thickness (Liao, Liang, and Singh, 1998). Currently, a high correlation ($r^2 = 0.88$) has been found between odor and a mixture of 19 VOC in ambient air. This correlation has resulted in a patented synthetic wastewater with the appropriate odors (Zahn and DiSpirito, 1998).

Treatments of Odors

Improved Management

Attempts to mitigate odor production within the facility include improved management, ventilation, manure additives, and feed additives. Management remains the key to controlling odors. Odor-reducing management practices include site selection, structural design, waste treatment and disposal, and daily maintenance activities. Manure removal practices can affect odor production by the frequency and type of cleaning used. A scrape-and-wash system (Schulte, Kottwitz, and Gilbertson, 1985) can leave behind a film of urine, from which ammonia may be generated. A flush system can leave solids in corners. A pit beneath the barn can generate odors, depending upon length of storage of the wastes.

Ventilation

Ventilation of the buildings can provide relief to the occupants but can increase odor problems downwind. Exhausted air may include odorous gases in addition to dusts, which are also potential carriers of odors.

The use of slotted floors over collection pits within the swine facility allows for efficient manure collection and storage while reducing odor production. Adequate ventilation helps dry floor surfaces and is required for adequate odor reduction. Using the pit recharge method (Barker, 1996), waste slurry is periodically drained from the pit into a lagoon; the pit is then recharged with

effluent from the lagoon. This periodic draining removes settable solids and reduces odors, while enhancing lagoon performance due to regular loading (Barker et al., 1996).

Manure Pit Treatments

However, the manure pit can also be one of the greatest sources of odors within the swine facility (Nicolai, 1996). Volatile compounds generated from reduced sulfur compounds and reduced nitrogen compounds can be very offensive. The quantity of gases produced in the pit is a function of manure temperature, pH, loading rate, and type of feed ration. Treatment of the manure pit to reduce odor production must either enhance the aerobic conditions, enhance the anaerobic conditions, or modify the microbial activities (Zhu and Jacobson, 1999).

Methods of odor reduction within the pit have included physical, chemical, and biological treatment. Physical treatment includes adequate ventilation to control odor buildup within the pit either by exhausting the gases or by attempting to maintain high dissolved oxygen content. Air within the swine facility can be exhausted through the pit, thereby effectively removing odors from the facility. Although mechanical aeration can be very expensive to operate and maintain, increased oxidation of the manure within deep pits can cause some reduction of odor production.

The loading rate, temperature, and characteristics of the manure (solids content, pH, and ammonia concentration) affect anaerobic conditions. Inhibiting microbial activities can decrease gas emissions but increase the potential for pollution upon surface application due to reduced waste stabilization occurring within the pit.

In addition, the method of manure removal from the pit can affect odor production. Because sources tend to be dirty surfaces such as the floor, the slats, and the animal, the rate of ammonia released from manure increases for storage times longer than 24 hours. While frequent clean out of feces helps maintain low ammonia gas levels, swine urine is the main contributor to ammonia production, requiring half-hourly clean-outs to completely remove this odor source. Clean-out also requires a flush system because scraping always leaves behind a film of urine on the surface, from which emission takes place. Since ammonia is highly soluble in water, its presence usually is less noticeable where liquid manure systems are used rather than solid floors.

The liquid method of removal may use either a flush or a static system. Ammonia emissions from a pit with storage over 14 days appear to peak at day three and decline to a constant level by day 14 (Heber et al., 1999). The magnitude of the ammonia emissions may be reduced by recharging the pit, prior to waste deposition, with 10 cm of liquid after draining the pit. Manure solids are submerged, thereby trapping some gas release. Hydrogen sulfide emissions from

the pit are low initially but can increase with time and swine weight. Ammonia emissions may be due to mass transfer across a liquid-gas interface subject to Henry's law relating emission to concentration, air velocity, and temperature. Hydrogen sulfide emissions may be due to gas-bubble formation. Thus, flushing the pits with lagoon water could increase bubble formation, causing hydrogen sulfide peaks. In addition, the frequency and type of manure removal can affect the sulfide emissions (Heber et al., 1999).

Using Additives

Additives are chemical and biological treatments added to the feed or manure. Manure additives studied to date have had limited success in odor reduction, possibly because of the difficulty in maintaining a suitable environment or supporting proprietary varieties of bacteria. In an aerobic environment, oxygen is reduced while the organic matter is oxidized, producing reduced nitrogen gases and carbon dioxide. In an anaerobic environment, sulfate and carbon dioxide can be reduced while the organic matter is oxidized, producing methane, carbon dioxide, and hydrogen sulfide. In an anoxic environment, nitrate is reduced by facultative organisms while oxidizing the organic matter, producing non-odorous carbon dioxide, water, and nitrogen gas.

Feed additives are used to improve the digestive efficiency of conversion of feed into weight gain (Swine Odor Task Force, 1995). A lower concentration of degradable proteins in the feces could lead to a reduction in ammonia production.

Protein in the feed could be reduced without loss of feed efficiency by the addition of amino acids, but synthetic amino acids are expensive.

For odor reduction, chemical or biological compounds may be added to manure in the pit. Chemical agents are strong oxidizing agents or germicides used to reduce odors by inhibiting microbial decomposition, masking the odors, or absorbing some of the volatile compounds. Biological compounds, such as enzymes and bacteria, are used to reduce odors and suppress gases produced during biochemical digestive processes.

Ohio State University applied chemical additives to manure to change the pH of the manure. Lime was used to raise the pH to 12, thereby destroying odor-producing organisms (Gustafson and Veenhuizen, 1999) but increased the ammonia volatilization within the manure pit. Acid was used to lower the pH to about six, resulting in reduced ammonia volatilization by increasing nitrogen fixation but increased the volatilization of hydrogen sulfide.

The University of Minnesota conducted research to evaluate the effectiveness of various chemical additions to swine and dairy manure in reducing hydrogen sulfide gas emission (Clanton et al., 2001). Among the oxidizing agents tested, hydrogen peroxide and potassium permanganate were found to have the best cost-benefit ratio.

The University of South Carolina created a slurry of swine manure and peat for odor removal (Rizzuti et al., 1998). A temporary reduction of odor was achieved, depending upon the type of peat and degree of saturation of the peat.

The University of Kentucky experimented with lowering protein in the swine diet, while raising supplemental amino acids and other dietary additives (Cromwell et al., 1998). A lower concentration of degradable proteins in the feces led to a reduction in ammonia emissions without a performance penalty; thus, protein in the feed could be reduced with the addition of amino acids. However, synthetic amino acids are expensive, and little reduction in the hydrogen sulfide emissions occurred. An economical method of reducing manure production, while increasing feed efficiency, could be achieved if the costs of synthetic amino acids decrease.

Iowa State University evaluated several commercial additives to manure (Lorimor, 1997) for their effectiveness in reducing odor. Changes in the water quality analysis were compared to odor reduction using olfactometry. The results were mixed with some additives able to reduce odor and ammonia volatilization. Any changes in the hydrogen sulfide concentration were not reported. Purdue University conducted similar research using other commercial additives (Heber et al., 1997) for finish houses with long-term, under-floor manure storage.

Taiwan University (Liao and Bundy, 1994) reported some bacterial additives to manure were able to slightly reduce methane and carbon dioxide emissions, but little reduction in ammonia or hydrogen sulfide emissions occurred.

The total costs of using additives are complicated by such factors as initial and maintenance dosing, the type of manure removal system, pit conditions, the type of operation, the number of animals, and even the pH of the drinking water (Johnson, 1997).

Waste Stabilization

Swine manure is a complex organic compound that can be approximated by the formula $C_{272}H_{445}O_{148}N_{23}S$ (Liao and Bundy, 1994). It also typically contains an interdependent population of bacteria, such as sulfate-reducing bacteria (SRB), denitrify bacteria, and methanogenic bacteria. Waste stabilization occurs as the microorganisms use oxidation-reduction (redox) reactions to produce energy for growth and cellular maintenance. Microbes, especially bacteria, are able to utilize almost any redox reaction that produces energy. Electrons are transferred from electron donors (oxidation) and transferred to terminal electron acceptors (reduction). The amount of energy released is dependent upon the terminal electron acceptor used (Rittmann and McCarty, 2001).

Anaerobic treatment is usually the preferred method of animal waste stabilization. Compared to aerobes, anaerobic bacteria are slower growing, diverting less energy to biomass, and producing less sludge disposal problems. In

addition, the slower growth rate translates into fewer nutrient requirements, requiring little or no addition of limiting nutrients to the reactor. Finally, the energy requirements for anaerobic treatment are significantly less because no aeration is required (Rittmann and McCarty, 2001). Anaerobic digestion of swine manure leads to the production of biomass, methane, carbon dioxide, hydrogen sulfide, ammonia, and other volatile or soluble compounds. Sulfide is produced by sulfate reduction. Ammonia, produced from the digestion of proteins, can inhibit methanogenesis at high pH values. Methanogenesis, sulfate-reduction, and denitrification are affected by many factors in the solution, such as pH, redox potential, organic matter loading, temperature, and dissolved oxygen content (Yu et al., 2001). Finally, the type and concentration of electron acceptors available affect the processes that are occurring.

Under aerobic conditions, oxygen (O_2) is the most common electron acceptor for microbes; under anaerobic conditions, nitrate, sulfate, and carbon dioxide may be used by microbes for electron acceptors (Rittmann and McCarty, 2001). Methanogens, obligate anaerobes, use the electron equivalents in the organic matter to transform carbon to its most reduced state (-4) as methane (CH_4), using ATP for energy transfer. In fermentation, organic matter is used as both the electron acceptor and the electron donor. Because microbes try to obtain the maximum energy possible, the order of preference for electron acceptors (based only on maximum potential energy production) ranges from

oxygen (most energy), nitrate, sulfate, carbon dioxide (methanogenesis), to fermentation (least energy). Anaerobic microorganisms cannot use oxygen as electron acceptors; they are at a competitive disadvantage with the aerobes if oxygen is not a limiting factor. When oxygen is unavailable, anaerobes have a competitive advantage. Although facultative bacteria prefer oxygen, they can live in either the presence or absence of oxygen. Thus, population shifts occur as the concentrations of different electron acceptors change (Rittmann and McCarty, 2001).

Electron Transfer

Cellular respiration produces energy from the decomposition of organic matter, releasing free energy. For example, glucose may be broken down to carbon dioxide and water, releasing energy. Similarly, energy can be stored as microbes create glucose during photosynthesis. Some interspecies electron transfer can occur between acetate-fermenting methanogens and hydrogen-utilizing SRB. Hydrogen or formate can act as electron carriers between methanogens and some syntrophs, a specialized group of microorganisms able to metabolize some organic acids. The organic acids are oxidized into simpler compounds that the methanogens are able to utlize (Kluber and Conrad, 1998).

Microbial Populations

By a process of selection, the microbes best able to become established within an environment multiply while carrying out biochemical reactions. Competition for common electron donors can cause changes to the composition of the microbial population because the less competitive microorganisms may be excluded from the environment. Due to metabolic diversity, microbes may find or create niches within the environment, ensuring their survival (Rittmann and McCarty, 2001).

Methanogens

Methanogenic bacteria (methanogens), under low redox potential conditions, produce methane during energy metabolism. Methanogens are slow-growing strict anaerobes of the domain Archaea (White, 2000) that obtain energy by converting certain substrates, such as hydrogen and formate (hydrogen oxidizers), and acetate (acetate fermenters) to methane gas. In addition, some methanogens can use some of the simpler alcohols like methanol, ethanol, or propanol as energy sources. These energy substrates provide electron donors, while carbon dioxide, used as an electron acceptor, is reduced to methane. Methanogens reduce carbon in the organic matter and produce by-products of carbon dioxide and methane. Methane, having a low solubility in water, evolves as a gas (Rittmann and McCarty, 2001). Some methanogens are capable of autotrophic growth, creating organic carbon (cellular biomass) from carbon dioxide. Because

of the limited range of usable substrates, methanogens rely upon synergistic interactions with fermentative bacteria to convert many organic compounds into usable substrates such as volatile fatty acids, hydrogen, and carbon dioxide. Syntrophs require a very low concentration of hydrogen to make oxidation of the fatty acids thermodynamically favorable. Methanogens are able to maintain a low concentration of hydrogen and formate, encouraging oxidation of the fatty acids. In addition, a low concentration of hydrogen favors interspecies electron transfer, since hydrogen or formate can act as the electron carriers between methanogens and syntrophs (Kluber and Conrad, 1998).

Nitrogen and sulfur sources are required for cell synthesis. Autotrophic methanogens may use ammonia as the sole nitrogen source. Heterotrophic methanogens may utilize nitrogenous compounds like urea or dinitrogen. The sulfur sources include organic and inorganic sulfur compounds. However, high concentrations of nitrogen or sulfur sources can inhibit methanogenesis.

Acetate reduction to methane and carbon dioxide by methanogens is often limited by the availability of any alternative electron acceptor like sulfate or nitrate. Methanogens require anoxic or anaerobic environments, possibly due to the oxygen sensitivity of many of the enzymes involved during methanogenesis. In addition, the presence of oxidized sulfur-containing compounds inhibits methanogens. In anoxic environments, methanogenesis is inhibited by the presence of nitrate or sulfate due to competitive inhibition from other

microorganisms. SRB are more competitive than the methanogens for reduced substrates possibly due to the more positive reduction potential of sulfate compared to carbon dioxide. In addition, the SRB can drive the hydrogen concentration below the level that methanogens can use it. In a sulfate-rich environment, organic carbon sources are oxidized to carbon dioxide and sulfate is reduced to sulfide. If sulfate becomes limited, methanogenesis begins. A pH between 6.5 and 7.6 is favored. Intermediary organic acids created during anaerobic decomposition, as well as carbonic acid due to high levels of carbon dioxide, tend to lower the solution pH (Kluber and Conrad, 1998).

Sulfate Reducers

Obligate anaerobic bacteria from genera such as *Clostridium, Proteus*, *Desulfovibrio*, and *Desulfotomaculum* reduce sulfate to sulfide (Carpenter, 1972). The bacteria use sulfate as an electron acceptor for the oxidation of organic matter. Plant or animal proteins are oxidized into amino acids. Then sulfur compounds in the amino acids cysteine, cystine, and methionine are reduced to sulfide. *Desulfuromonas* can oxidize acetate as an energy substrate and generate sulfide by reducing elemental sulfur. Sulfate reducers require organic carbon sources (electron donors) such as acetate, propionate, pyruvate, lactate, or molecular hydrogen. Sulfate reduction may be inhibited by the presence of oxygen or nitrate. Since sulfide has some antimicrobial properties, sulfide producers can

inhibit the metabolism of other microbes, as well as compete for common substrates (Fukui, Suh, and Urushigawa, 2000).

The Sulfur Cycle

All organisms require sulfur; it is an essential component of proteins, found in three amino acids -- cysteine, cystine, and methionine (Carpenter, 1972). Similar to the nitrogen cycle, plants uptake a nutrient (sulfate) and reduce it to a usable form (sulfhydryl) for assimilation into proteins. Some heterotrophic saprophytes can decompose (dissimilate) plant or animal proteins, yielding amino acids after a series of intermediate steps. Hydrogen sulfide may be produced as the amino acids are reduced.

Autotrophic bacteria, such as species of *Thiobacillus, T. thioparus and T. thiooxidans*, can oxidize sulfur to sulfate. Also, H_2S can serve as a hydrogen donor in photosynthesis, releasing elemental sulfur. Species of *Clostridium, Proteus*, and *Desulfovibrio* can reduce sulfates to H_2S (Atlas and Bartha, 1997).

Sulfate Reduction

Sulfur compounds can function like oxygen in respiratory processes, acting as terminal electron acceptors (Atlas and Bartha, 1997). Sulfate reducers, organisms capable of dissimilatory sulfate reduction, include the genera *Desulfovibrio* and *Desulfotomaculum, Desulfobacter*, *Desulfobulbus, Desulfococcus, Desulfonerma,* and *Desulfosarcina.* Sulfate is reduced to sulfide by:

$$5H_2 + SO_4^{2-} \rightarrow H_2S + 4H_2O \qquad (2\text{-}1)$$

Some species of *Bacillus, Pseudomonas,* and *Saccharomyces* can also produce small amounts of sulfide from sulfate (Atlas and Bartha, 1997). While most sulfate reducers cannot metabolize acetate, *Desulfuromonas acetoxidans* can use acetate to anaerobically reduce elemental sulfur (S°) to H_2S:

$$CH_3COOH + 2H_2O + 4S^0 \rightarrow 2CO_2 + 4H_2S \quad (2\text{-}2)$$

Sulfate reduction can occur over a wide range of pH, pressure, temperature, and salinity conditions. Only relatively few compounds can serve as electron donors for sulfate reduction. Although hydrogen and sulfate can serve as their only source of energy for growth, sulfate reducers lack the enzyme systems to assimilate CO_2 and require organic carbon sources. The most common electron donors are pyruvate, lactate, and molecular hydrogen. Sulfate reduction is inhibited by the presence of oxygen, nitrate, or ferric ions. The rate of sulfate reduction is often carbon limited. *Desulfovibrio desulfuricans* and *Desulfobulbus propionicus* are able to grow with nitrate as electron acceptor, which is reduced to ammonia. These strains can oxidize sulfide while reducing nitrogen compounds to ammonia (Atlas and Bartha, 1997).

Two different studies resulted in different findings from adding nitrate salts to wastewater high in sulfates. In one study (Fdz-Polanco et al., 2001), SRB converted nitrate to ammonium. Ammonium was then oxidized, forming nitrogen gas, while the sulfates were reduced to elemental sulfur.

$$SO_4^{2-} + 2NH_4^+ \rightarrow S^0 + N_2 + 4H_2O \quad (2\text{-}3)$$

In the second study (Lens et al., 2000), ammonium concentration remained constant, while sulfide concentration decreased. Nitrate was reduced to nitrite and hydrogen sulfide was oxidized to sulfate. Most nitrogen compounds remained in solution during the 90-day study.

$$8\ NO_3^- + H_2S + HS^- \rightarrow 8\ NO_2^- + 2\ SO_4^{2-} + 3\ H^+ \quad (2\text{-}4)$$

Purple Sulfur Bacteria

Lagoons with purple coloring tend to be less of an odor nuisance, than typical non-purple lagoons (Schulte and Koelsch, 2000). Purple sulfur bacteria (PSB), creating a hue ranging from pink to rose red, may be the active force in odor reduction. The PSB are anoxygenic phototrophs, using solar energy to oxidize hydrogen sulfide to sulfate under anoxic conditions. Light energy is captured during photophosphorylation and this energy is used for oxidation processes. In an animal waste treatment lagoon, photosynthetic bacteria such as PSB may be the driving force for nutrient conversion (Sund et al., 2001) with sulfur conversion being driven by the PSB.

Carbon dioxide is used as the carbon source during photosynthesis, while sulfide is oxidized to sulfate. PSB have an optimum temperature range of 20-35°C, with a preferred pH of 6.8-7.5 (Schulte and Koelsch, 1998). Lagoons with PSB exhibit a diurnal trend, with sulfide oxidized to sulfur compounds during the day and the SRB reducing the sulfate to sulfide for energy. During the night, the PSB activity decreases, thus sulfide concentration increases. This increase in

sulfide can retard the reduction of sulfate, causing an increase in sulfate concentration (Sund et al., 2001).

Chromatium weissei tend to be the dominate phototrophic bacteria (Camacho, Vicente, and Miracle, 2000). *Chromatium* are able to oxidize sulfide to elemental sulfur and store the sulfur intercellularly, providing a competitive advantage. Stored sulfur can be used as an electron donor for cellular reactions when photosynthesis has depleted the available sulfide during the day. In addition, carbon fixed by the phototrophs can provide organic carbon for the heterotrophs like the denitrifiers. PSB tend to be concentrated near the surface of the lagoon where sunlight is readily available, as is sulfide as is evolves as a gas (Schulte and Koelsch, 1998).

Denitrifiers

Nitrate can be reduced to nitrogen gas or to ammonium, depending upon the environment. Although denitrification occurs along the same metabolic path, ammonification can occur along many pathways.

Denitrification

Denitrification is the dissimilatory reduction of nitrate and/or nitrite into molecular nitrogen by facultative aerobes, under anoxic conditions. Facultative aerobes, autotrophs and heterotrophs, can use either oxygen or oxidized forms of nitrogen as an electron receptor. These aerobes are chemotrophs, using organic compounds or inorganic compounds (like hydrogen or sulfur) for electron

donors. Denitrifiers include organisms of the genera *Enterobacter*, *Bacillus*, and *Pseudomona*. The process of denitrification involves several steps, requiring microbial enzymes for catalysts. It is the sequential reduction of nitrate to nitrite, nitrite to nitric oxide, nitric oxide to nitrous oxide, and nitrous oxide to nitrogen gas. The dissolved oxygen (DO) concentration can affect denitrification by repression of genes involved in the reduction process and by the buildup of intermediaries in the reduction process. In addition, a shortage of electron donors can limit the number of electrons available to complete the reduction process because of the competition for electrons between nitrite-reducers and nitrate-reducers. Some denitrifiers are able to obtain energy from fermentation if neither nitrate nor oxygen is available. Finally, the denitrification process produces strong bases, raising the pH of the solution (Rittmann and McCarty, 2001).

Ammonification

In a sulfide-free environment, nitrate reduction via ammonification is the main nitrate reduction pathway. Adding nitrate to sulfate-rich wastewater does not favor nitrate reduction to ammonia, but rather denitrification. Sulfate reduction is inhibited by the intermediate buildup of nitrous oxide. Once denitrification begins methane production halts. Simultaneously, redox potential increases as a buildup of nitrite occurs, and sulfide concentration decreases (Percheron, Bernet, and Moletta, 1999). Denitrification may occur using the

sulfide as an electron donor. Reduction of nitrate to ammonium is more thermodynamically favorable than denitrification when organic matter is not the limiting factor, but available electron acceptors are limited (Zhang and Scherer, 2000).

Microbial Interactions

Methanogens have to compete with other microorganisms for electron donors, such as organic matter. Wastewater containing high levels of sulfate tends to yield odorous sulfur-containing compounds from sulfate reduction and from metabolism of proteins in the organic matter. Methane production is inhibited by faster growing SRB because of enzyme inhibition and competition for common substrates, such as hydrogen and acetate (Fukui, Suh, and Urushigawa, 2000). Sulfate is used as an electron acceptor and reduced to sulfide. Because sulfate reduction yields more energy than methanogenesis and is thermodynamically favorable, methanogenesis usually begins after complete sulfate reduction has occurred. However, some interspecies electron transfer may occur between acetate-cleaving methanogens (acetoclastic methanogens) and hydrogen-utilizing sulfate reducers (Rittmann and McCarty, 2001).

The degradation of animal wastes containing both nitrogen and sulfur compounds can lead to many microbial interactions. Nitrate may be reduced either to nitrogen gas or to ammonium. The SRB and denitrifiers may compete for organic carbon sources, such as acetate; the SRB may also utilize organic

compounds created by denitrification. Oxidation or reduction of sulfur-containing compounds may occur, depending upon the type and concentration of electron donors and acceptors available. *Thiobacillus denitrificans* can use nitrate as an electron acceptor and sulfide as an electron donor; the sulfide is oxidized to sulfate or elemental sulfur and the nitrate is reduced to nitrogen gas (Carpenter, 1972). In the presence of nitrate, some SRB like *Desulfovibrio desulfuricans* and *Desulfobulbus propionicus* can reduce either nitrate or nitrite to ammonium, while oxidizing sulfide to sulfate (Lens et al., 2000). Methanogenesis may be inhibited due to the buildup of toxic compounds (such as nitrite, nitrous oxide, and sulfide), the competition for hydrogen, and/or the increase in pH of the solution (Kluber and Conrad, 1998).

Nitrate can be reduced to nitrogen gas (denitrification) or to ammonium (ammonification), forming nitrite as an intermediate compound either way. In the presence of high concentrations of sulfate, denitrification is favored; a buildup of nitrite or sulfide can inhibit further denitrification. Similarly, high concentrations of nitrate can retard sulfate reduction and high concentrations of sulfide can inhibit both denitrification by *Pseudomona* and ammonification by *Desulfovibrio.* The inhibition appears to be concentration dependent (Percheron, Bernet, and Moletta, 1999).

Gas Production

Over 136 gaseous compounds may be generated in swine housing facilities (Hartung and Philips, 1994). The gases produced from anaerobic decomposition of animal wastes are predominately methane, carbon dioxide, ammonia and hydrogen sulfide. Methane, an odorless greenhouse gas produced from the degradation of organic acids, usually escapes to the atmosphere. Carbon dioxide, another odorless greenhouse gas, is produced mainly from animal respiration, with some production from stored feces. Hydrogen sulfide comes from the anaerobic bacterial decomposition of sulfur-containing amino acids in urine and in feces. Ammonia is produced by bacterial and enzymatic decomposition of nitrogen-containing compounds in freshly deposited or stored animal wastes, especially in urine. Ammonia emissions represent a considerable economic loss in fertilizer value as well as a possible safety hazard and public nuisance. Ammonia has a very pungent odor and often leads to odor complaints. Hydrogen sulfide, produced by the degradation of sulfur-containing amino acids, has a "rotten-egg" odor that is considered very offensive by some people. Many other gases, present in trace quantities may also produce undesirable odors (Fulhage, 1993).

Ammonia is formed during bacterial degradation of proteins in feces, specifically the hydrolysis of urea by urease. Ammonia is highly water-soluble, remaining in solution in the ionized form as ammonium. Only the un-ionized

form, ammonia, is volatile. Its release is a function of the difference in partial pressure between ammonia in the liquid phase and ammonia in the ambient air. The proportion of un-ionized ammonia to the total ammonia concentration is a function of pH as seen in Figure 2.1 and expressed in Equation 2-5.

$$NH_4^+ \leftrightarrow NH_3 + H^+ \quad (2\text{-}5)$$

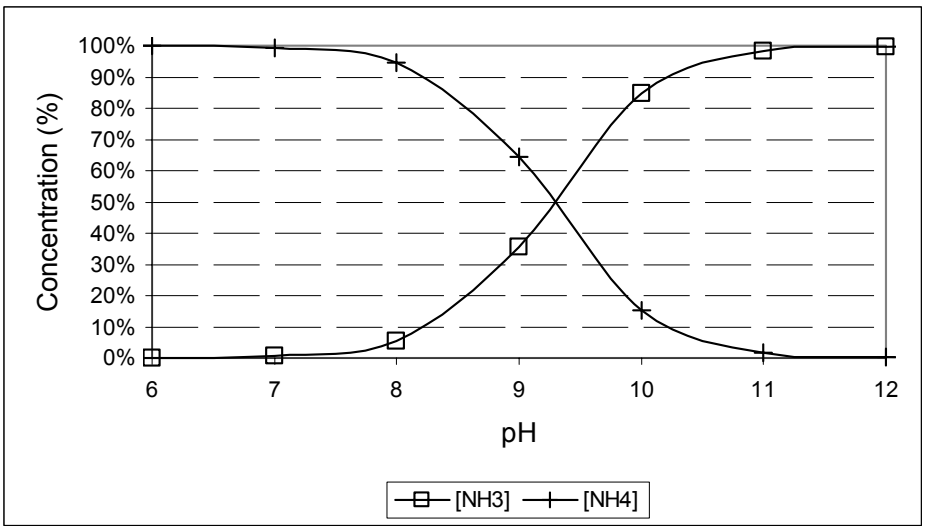

Figure 2.1 Ammonia Ionization over a Range of pH

Figure 2.1 was generated using Equation 2-6 for equilibrium calculations.

$$\frac{[H^+][Ac^-]}{[HAc]} = K_A \quad (2\text{-}6)$$

For ammonium, K_A = 5.56 x 10-10 (Sawyer, McCarty, and Parkin, 1994).

Ammonia-N may compose less than 40% of the total nitrogen within the swine manure (Zhu et al., 1997); therefore, most of the nitrogen may be in non-volatile compounds, such as nitrate, nitrite, and other organic compounds.

Simply reducing the ammonia emissions may not reduce the odor emissions of the swine manure.

Hydrogen sulfide, the predominant odorant often associated with the anaerobic decomposition of organic matter by microorganisms, is formed when sulfate is available as a terminal electron acceptor:

$$SO_4^{2-} + \text{organic matter (anaerobic bacteria)} \rightarrow H_2S + H_2O + CO_2 \quad (2\text{-}7)$$

Hydrogen sulfide, being sensitive to the pH, dissociates as follows:

$$H_2S \leftrightarrow S^{2-} + HS^- \leftrightarrow S^{2-} + H^+ \quad (2\text{-}8)$$

$pK_{A1} = 7.04$ $pK_{A2} = 12.89$ (Sawyer, McCarty, and Parkin, 1994)..

The odorant is H_2S in molecular form, which is capable of transmission to the atmosphere. At a pH of 7, approximately 50% of the sulfide is in this form. Acidic conditions tend to enhance odor emissions, while basic conditions tend to suppress odor emissions. At a pH greater than 8, the amount of H_2S is insignificant. The relationships among H_2S, HS^-, and S^{2-} are shown in Figure 2.2 as generated using Equation (2-6).

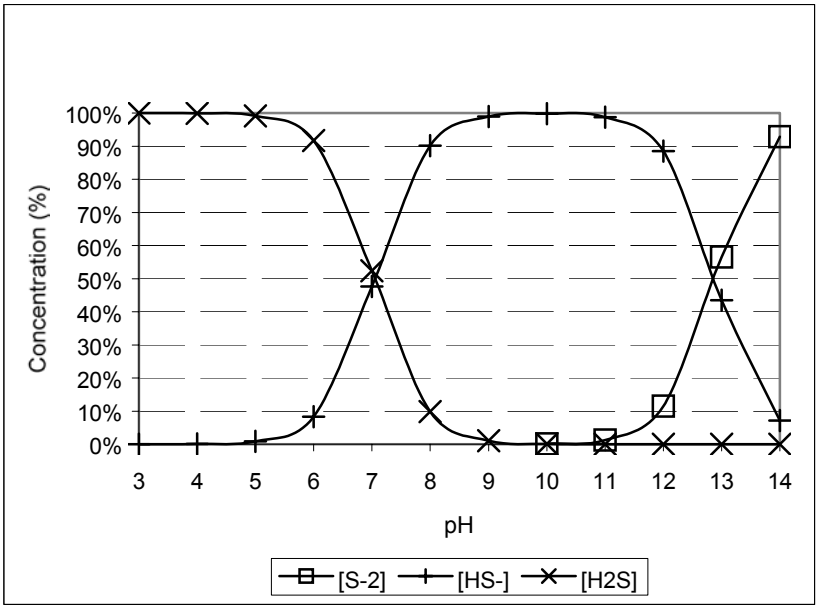

Figure 2.2 Hydrogen Sulfide Ionization over a Range of pH

When measuring gas production from stored swine manure (Clanton, Morey, and Schmidt, 1999), the principal sulfur-containing gases were hydrogen sulfide, carbonyl sulfide, methyl mercaptan, dimethyl sulfide, carbon disulfide, and dimethyl disulfide. Of the sulfur-containing compounds, only the hydrogen sulfide concentration had any correlation with odor perception, possibly because of the sensitivity of the detection equipment. Human odor perception does not correlate linearly with the concentration of odorants (Sweeten, 1995) but is a logarithmic relationship.

Ammonium and hydrogen sulfide are easily measured with commercially available detectors but do not necessarily correlate well with odor complaints

from neighbors. Meeting ammonia emission limits might be as difficult for poultry and dairy operators as meeting hydrogen sulfide emission levels is for pork producers.

Addition of Nitrate

Addition of nitrate to an anaerobic system can result in the complete, but largely reversible inhibition of methanogenesis (Kluber and Conrad, 1998). Nitrate reduction can cause the temporary accumulation of intermediates, like nitrite and nitrous oxide, and a decrease in the partial pressure of hydrogen. Hydrogen, serving as an electron donor for denitrification, would no longer be available for methanogenesis. Nitrate addition also can result in the transient accumulation of sulfate, possibly due to the oxidation of reduced sulfur compounds. After denitrification is complete, sulfate may be reduced because the sulfate reducers are more competitive than methanogens for hydrogen. Once the partial pressure of hydrogen has increased sufficiently, methanogenesis may begin, but at lower rate of production. Although the redox potential may increase, the inhibitory effect of nitrate may not be primarily due to the increase in the redox potential but due to the toxicity of the denitrification products on methanogens. Also, the denitrifiers are more competitive than the methanogens for the available hydrogen. Denitrifiers use hydrogen so efficiently, the partial pressure may drop below the methanogens threshold and may no longer support

methanogenesis. Finally, the temporary accumulation of sulfate may result in competitive inhibition of methanogenesis (Percheron, Bernet, and Moletta, 1999).

CHAPTER III

METHODS AND MATERIALS

Three batches of swine slurry were collected from the manure pit of a local swine farrowing facility in Mississippi. The slurry was a mixture of manure, urine, and flush water from a purple-colored lagoon. Samples were treated with various concentrations of nitrate, while the control sample did not receive nitrate. Relative population counts were performed and the samples were stored in sealed glass bell jars with septa, seen in Figure 3.1, and incubated at 25°C for seven days. The samples were maintained at quiescent conditions to allow stratification of material and ranges of oxidation-reduction potential (ORP) to occur, simulating conditions in the manure pit.

Plate counts for total viable anaerobic bacteria were estimated using the spread plate method 9215C (Clesceri, Greenberg, and Trussell 1999) and Bacto Anaerobic Agar in Petri dishes placed in an anaerobic container. Viable sulfide producers were estimated using Bacto Peptone Iron Agar in Petri dishes placed in an anaerobic container. Viable denitrifiers were estimated using a Bacto Nitrate Broth in Duram fermentation tubes for most-probable-number (MPN) using multiple-tube fermentation method 9221B (Clesceri, Greenberg, and Trussell

1999). Numbers of viable sulfate reducers able to oxidize lactate were estimated using MPN dilutions of Sulfate API Broth in multiple test tubes.

Figure 3.1 Glass Bell Jar

The headspace gases (Chen, Liao, and Lo, 1994) were sampled every day for seven days and analyzed using gas chromatography (GC) techniques to detect methane, nitrogen, and carbon dioxide production. The area units under each peak were compared to known standards and the concentrations determined. Quantitative analyses were used to measure the changes in concentration of the

gases produced. After seven days of incubation, microbial counts were taken again. Samples for biochemical analysis were collected daily.

Serial Dilutions

Dilutions of the samples were performed in 180 ml serum bottles containing either 99 mL of sterile physiological saline (0.85% NaCl) or 90 mL of sterile physiological saline. To dilute the sample 100 fold, 1 ml of sample was transferred to the serum bottles containing 99 mL of sterile physiological saline and shaken 5 times to mix.

Plate Counts

One hundred microliters of sample were spread on a 95-mm diameter by 15-mm deep Petri dish containing appropriate media for the population under study. Duplicates were made for each dilution sample. The plates were placed in an anaerobic container and the oxygen was removed using a vacuum pump. Finally, the atmosphere in the container was replaced with sterile nitrogen gas. The anaerobic container was placed in an incubator at 25°C for 72 hours. The number of colony-forming units (#CFU) was counted and recorded only for plates containing between 30 and 300 colonies. The cell density was estimated based on the following formula:

Cell density = # CFU/(Volume plated x dilution factor). (3.1)

Total Anaerobic Population

Anaerobic populations were estimated using media containing Bacto Anaerobic Agar, as seen in Figure 3.2.

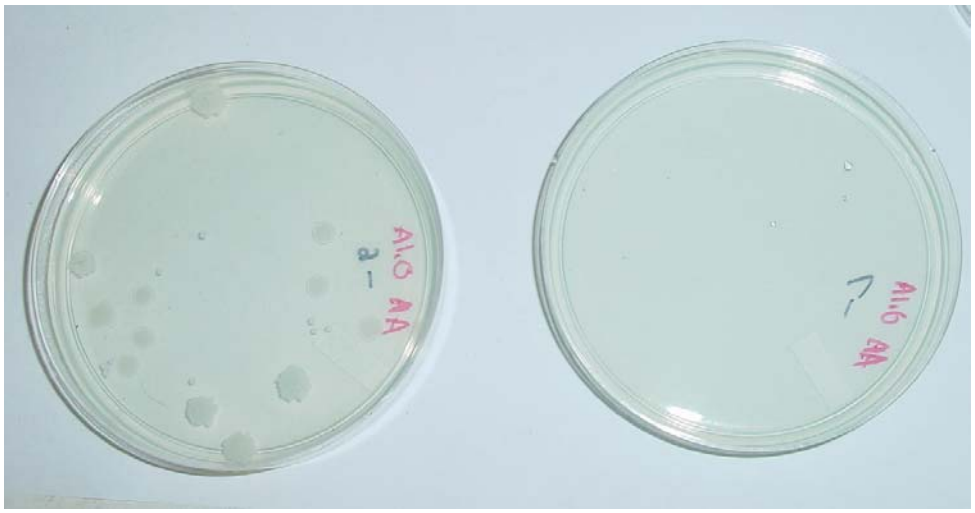

Figure 3.2 Total Anaerobic Bacteria

Sulfide Producers Population

Populations of sulfide producers were estimated using media containing Bacto Peptone Iron Agar, as seen in Figure 3.3

Figure 3.3 Sulfide Producers

Denitrifiers

Test tubes with Duram fermentation tubes were filled with 9 mL of Bacto Nitrate Broth and then sterilized by autoclaving. Into each tube, 1 mL of sample or sample dilution was inserted. MPN determinations were made using tubes arranged into a 3-3-3 configuration. The tubes were placed in an anaerobic container and the oxygen was removed using a vacuum pump. Finally, the atmosphere in the container was replaced with sterile nitrogen gas. The anaerobic container was placed in an incubator at 25°C for 48 hours. The tubes were then examined for the presence of gas in the Duram tubes, as seen in Figure 3.4. MPN of denitrifiers was estimated using a statistical table, based on the number of tubes showing positive for gas production.

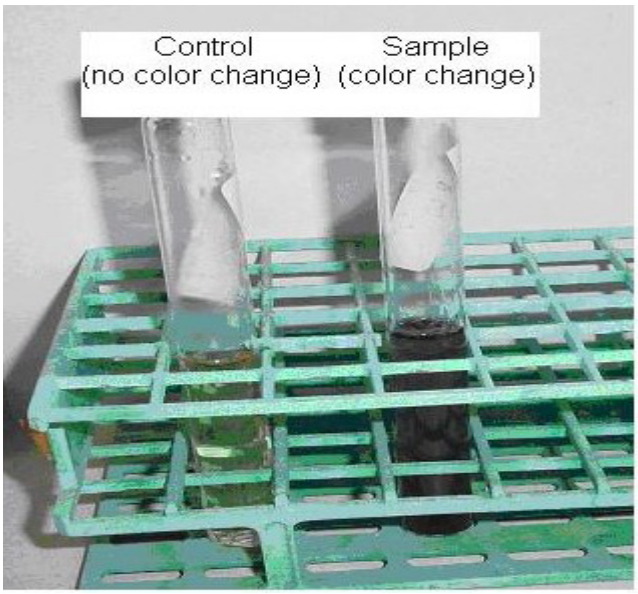

Figure 3.4 Denitrifiers

Sulfate Reducers

Test tubes were filled with 9 mL of Sulfate API Broth and then sterilized by autoclaving. Into each tube, 1 mL of sample or sample dilution was inserted. MPN determinations were made using tubes arranged into a 3-3-3 configuration. The tubes were placed in an anaerobic container and the oxygen was removed using a vacuum pump. Finally, the atmosphere in the container was replaced with sterile nitrogen gas. The anaerobic container was placed in an incubator at 25°C for 48 hours. The tubes were then examined for the presence of a black color, as seen in Figure 3.5. The MPN of sulfate reducers was estimated using a statistical table based upon the number of tubes showing positive for growth.

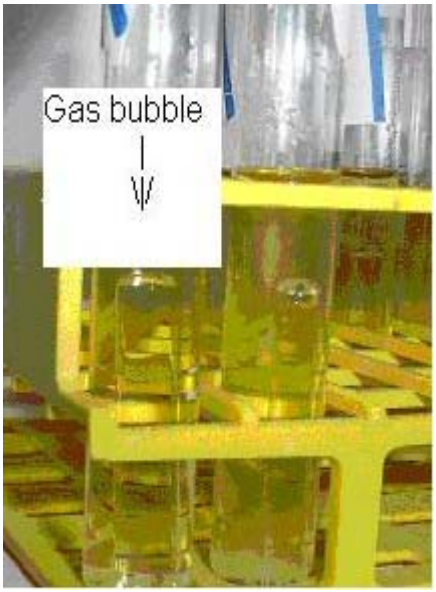

Figure 3.5 Sulfate Reducers

Gas Chromatography

During the 7-day incubation, a gas sample (50 µl) was periodically withdrawn from the headspace of the anaerobic jug with a Hamilton gas-tight syringe for gas analysis at the Microbiological Laboratory at Mississippi State University. A Fisher Gas Partioner Model 1200 Gas Chromatograph with dual columns and dual detectors was used for analysis of carbon dioxide, nitrogen, and hydrocarbon. Column 1 was a 20' x 1/8" aluminum column packed with 37.5% DC-200/500 mesh chromosorb P-AW. Column 2 was a 6' x 3/16" aluminum column packed with 60/80 mesh molecular sieve, 13x. The column temperature was 100°C, and the injector temperature was 65 °C. Helium was used as the carrier gas at a flow rate of 35 ml/min. The GC produced a chromatograph that was compared against a chromatograph of compounds of known concentrations.

Qualitative and quantitative analyses were used to measure the change in concentration of methane, nitrogen, and carbon dioxide production.

Biochemical Analysis

Samples (20 ml) were periodically taken from the anaerobic jugs for biochemical analysis in the Environmental Laboratory of Mississippi State University. Biochemical analysis included ammonia, COD, ortho phosphorus, nitrate, nitrite, sulfate, sulfite, TKN, TKP, and the pH. The instrumentation and testing methods were used as described in Table 3.1 from the Hach methods manual (Hach, 1999) and Standard Methods (Clesceri, Greenberg, and Trussell 1999).

Table 3.1

BIOCHEMICAL TESTING METHODS

Instrument	Parameter	Method
Hach DR/4000 Spectrophotometer (Model: 48100)	Ammonia (NH_3)	Hach Method 10031 (Salicylate Method)
Hach DR/4000 Spectrophotometer (Model: 48100) and Hach COD reactor (Model: 45600)	COD	Hach Method 8000 with Hach Company COD digestion vial type - high range (0-1500 ppm)
Hach DR/4000 Spectrophotometer (Model: 48100)	Ortho Phosphorus	Hach Method 8048 (PhosVer 3 Ascorbic Acid Method)
Hach DR/4000 Spectrophotometer (Model: 48100)	Nitrate	Hach Method 8039 Cadmium Reduction Method
Hach DR/4000 Spectrophotometer (Model: 48100)	Nitrite	Hach Method 8507 Ferrous Sulfate Method
Hach DR/4000 Spectrophotometer (Model: 48100)	Sulfate	Hach Method 8051 SulfaVer 4 Method
Hach DR/4000 Spectrophotometer (Model: 48100)	Sulfide	Hach Method 8131 Methylene Blue Method
Orion Ross® pH electrode (model 8156) with Orion pH SensorLink software	pH	Standard Methods, 17th Edition, Procedure 4500 - H
Lachat Quick Chem 8000	TKN	TKN 10-107-06-2-D
Lachat Quick Chem 8000	TKP	TKP 10-115-01-1-C

Statistical Analysis

Most Probable Number Method

The most probable number method (MPN) was used to estimate the microbial concentrations. MPN technique uses the principle of dilution to extinction (Clesceri, Greenberg, and Trussell, 1999). Broths were prepared with appropriate carbon and nutrient sources. Indicators were used to provide for positive or negative reactions. In one test, iron compounds were used to indicate the presence of H_2S, which would react and form a black color. In another test for detection of a gas, Duram fermentation tubes are placed into each test tube to trap gases formed. The presence of a bubble denoted a positive test for gas production.

Test tubes containing 9 mL of broth solution were prepared with 1 mL from each sample dilution placed into each of three broth tubes. The tubes were stoppered and incubated for 48 hours at 25°C. Tests were done in triplicate.

Results for each dilution were reported as a fraction, with the number of positive tubes over the number of negative tubes. Concentration of total bacteria was reported as the "most probable number" per 100 mL, where MPN is based on the application of the Poisson distribution. Standard MPN tables were used to determine the MPN index and 95% confidence limits for various combinations of positive and negative results.

Statistical Analysis Software

Statistical analysis was performed using SAS, version 8 (SAS Institute Inc., 2002). Pairwise correlations between the variables were used to test for collinearity, where the effectiveness of a variable can depend upon other which other variables are in the model. Correlations were tested using the Pearson product moment correlation coefficient to examine the relationship between any two variables. Strength of the linear relationship was indicated by a number ranging between -1 and +1.

Finally, multiple linear regression analysis in observational studies method (Schabenberger, 2001) was used, because factorial experiments can be complicated by significant interactions among the variables. Using multiple linear analyses, the variables that potentially influenced the outcome were examined by using known treatments under known conditions to determine which variables affected the outcome. A small subset of variables was found that could predict the outcome with sufficient accuracy and precision. A linear function was then found that included the significant regressor variables, as determined by the R-square method, the sum of squares reduction test, stepwise regression, and the C(p) method.

The R-square method, a brute force method, was used to fit all models and arranges them according to the number of variables and best fit. The models

were ranked by the best one-variable model, the best two-variable models, the best three-variable models, etc.

Next, the sum of square reduction method was used to test the hypotheses. One or more variables were dropped from the model to test if dropping the variable would alter the quality of the model significantly.

Stepwise regression, combining forward selection and backward elimination, was then used. One variable was added to the model (forward step), then removal of any of the variables (backward elimination) was attempted. If no variable could be removed without significantly worsening the model, another variable was added, and so forth.

The C(p) method was used for selection of the combination of the least number of variables with the lowest C(p) value. This statistic was introduced by Mallow to judge a mode by checking for models with too few regressor variables and models with too many regressor variables.

CHAPTER IV

RESULTS AND DISCUSSION

The goal of this study was to determine if the addition of nitrate to wastewater would reduce the formation of odorous sulfur-containing compounds. By manipulating the metabolic pathways during the stabilization of the waste, odorless nitrogen gas could be formed. Changes in microbial populations, changes in amounts and types of gases produced, and changes in amounts and types of biochemicals produced were recorded. Correlating the changes with the amount of nitrate added should indicate the inhibition due to nitrate salts. Inhibition was calculated as the percentage difference between the treated sample and the untreated control. Also, the chemical oxygen demand (COD) and total Kjeldahl nitrogen (TKN) ratio was calculated to indicate the amount of nitrate being sequestered by the synthesis of heterotrophic biomass. If the COD:TKN ratio was large enough, little nitrate would be available for denitrification or ammonification.

Inhibition of Microbial Populations

Effects of varying levels of nitrate addition upon microbial populations were examined, as seen in Figure 4.1. As expected, nitrate addition inhibited bacterial populations, except for denitrifiers (nitrate-reducing bacteria).

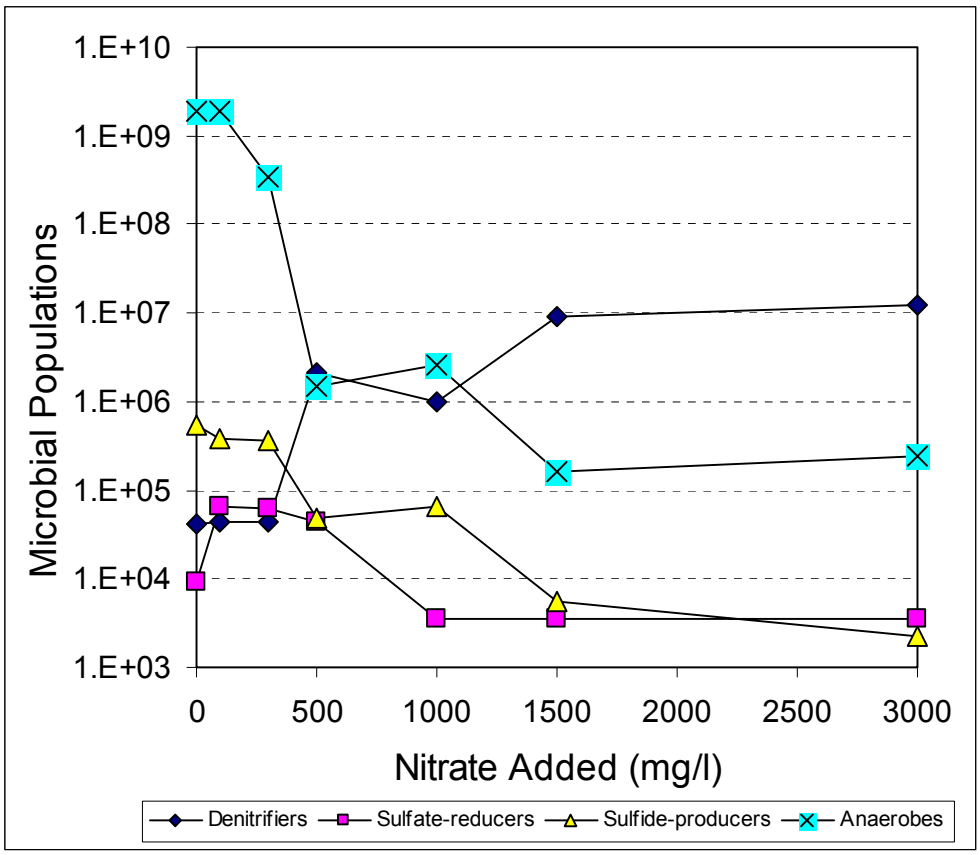

Figure 4.1 Nitrate Effects on Microbial Populations

Addition of nitrate enhanced the metabolic rate of denitrifiers. Some additional inhibition of sulfate-reducers and sulfide-producers at nitrate treatment levels between 1000 mg/l and 3000 mg/l was observed. Changes in the relative composition of the microbial community seemed to occur at nitrate treatment rates of approximately 300, 500, and 1500 mg/l. The changes could have been because of combinations of inhibition and competition for common substrates between different microbial populations. Table 4.1 provides details.

Table 4.1

NITRATE EFFECTS ON MICROBIAL POPULATIONS

	Nitrate Added (mg/l)	0.0	100.0	300.0	0.0	500.0	1000.0	0.0	1500.0	3000.0
Anaerobic	Sample 1 (mg/l)	1.9E+09	1.5E+09	3.0E+08	2.0E+06	1.3E+06	2.1E+06	3.7E+06	1.0E+05	2.6E+05
Anaerobic	Sample 2 (mg/l)		2.3E+09	3.8E+08		1.6E+06	3.0E+06		2.3E+05	2.2E+05
	Mean (mg/l)	1.9E+09	1.9E+09	3.4E+08	2.0E+06	1.5E+06	2.6E+06	3.7E+06	1.7E+05	2.4E+05
	Variation from Control (%)	-----	0.0%	-82.1%	-----	-27.5%	27.5%	-----	-95.5%	-93.5%
NRB	Sample 1 (mg/l)	4.2E+04	3.4E+04	4.2E+04	1.1E+06	2.1E+06	3.1E+06	2.4E+06	9.1E+06	9.3E+06
NRB	Sample 2 (mg/l)		5.3E+04	4.5E+04		2.1E+06	3.2E+06		9.3E+06	1.5E+07
	Mean (mg/l)	4.2E+04	4.4E+04	4.4E+04	1.1E+06	2.1E+06	3.2E+06	2.4E+06	9.2E+06	1.2E+07
	Variation from Control (%)	-----	3.6%	3.6%	-----	90.9%	186.4%	-----	283.3%	406.3%
SPB	Sample 1 (mg/l)	5.5E+05	4.0E+05	3.3E+05	6.4E+04	4.4E+04	6.0E+04	6.0E+03	5.0E+03	3.0E+03
SPB	Sample 2 (mg/l)		3.6E+05	3.8E+05		5.5E+04	6.8E+04		6.0E+03	1.5E+03
	Mean (mg/l)	5.5E+05	3.8E+05	3.6E+05	6.4E+04	5.0E+04	6.4E+04	6.0E+03	5.5E+03	2.3E+03
	Variation from Control (%)	-----	-30.9%	-35.5%	-----	-22.7%	0.0%	-----	-8.3%	-62.5%
SRB	Sample 1 (mg/l)	9.3E+03	1.1E+04	6.3E+04	3.8E+05	4.3E+04	3.6E+03	4.6E+04	3.6E+03	3.6E+03
SRB	Sample 2 (mg/l)		2.1E+04	6.1E+04		9.3E+04	3.6E+03		3.6E+03	3.6E+03
	Mean (mg/l)	9.3E+03	1.6E+04	6.2E+04	3.8E+05	6.8E+04	3.6E+03	4.6E+04	3.6E+03	3.6E+03
	Variation from Control (%)	-----	72.0%	566.7%	-----	-82.1%	-99.1%	-----	-92.2%	-92.2%

Nitrate-reducing bacteria (NRB) benefited from the availability of nitrate for electron acceptors and increased in population over 4-fold. In addition, the toxic effects of nitrate and byproducts of denitrification inhibited other microbial populations, reducing competition for common substrates.

Although sulfate-reducing bacteria (SRB) were stimulated at nitrate treatment rates of 300 mg/l or less, they were inhibited by over 80 percent at nitrate treatment rates above 500 mg/l. Sulfide-producing bacteria (SPB), anaerobic bacteria capable of respiring sulfide, exhibited less inhibition at nitrate treatment rates between 300 and 1000 mg/l. This reduced inhibition may have been the

result of competition for common substrates between sulfate reducers and sulfide producers.

At nitrate treatment rates in excess of 1500 mg/l, anaerobic bacteria were inhibited more than 90 percent. SRB and SPB were also inhibited by over 90 and 60 percent, respectively, by nitrate treatment rates of 3000 mg/l. The effects of nitrate and its by-products of denitrification were toxic to most anaerobic bacteria at nitrate treatment rates of 3000 mg/l. These effects are also reflected in the changes shown in Figure 4.1.

Microbial Gas Production

Effects on gas production from swine slurry by varying levels of nitrate addition are shown in Table 4.2. Maximum methane production occurred with a nitrate treatment level of 500 mg/l. Methane production was reduced more than 85 percent at nitrate treatment rates of 1500 mg/l, reflecting the inhibition of the total anaerobic populations seen in Figure 4.1. Carbon dioxide production was inhibited at all levels of nitrate addition. This may have reflected the inhibition of the microbial populations seen in Figure 4.1. Nitrogen gas production from the treated samples varied less than 25 percent from the control, regardless of the nitrate treatment level, indicating denitrification was occurring despite changes in microbial populations and other biochemical concentrations

Table 4.2

NITRATE EFFECTS ON GAS PRODUCTION

	Nitrate Added (mg/l)	0.0	100.0	300.0	0.0	500.0	1000.0	0.0	1500.0	3000.0
CO_2	Sample 1 (ppm)	84.0	59.5	63.0	203.0	161.0	108.5	140.0	59.5	98.0
CO_2	Sample 2 (ppm)		73.5	56.0		143.5	80.5		94.5	98.0
	Mean (ppm)	84.0	66.5	59.5	203.0	152.3	94.5	140.0	77.0	98.0
	Variation from Control (%)	-----	-20.8%	-29.2%	-----	-25.0%	-53.4%	-----	-45.0%	-30.0%
CH_4	Sample 1 (ppm)	220.4	208.8	116.0	208.8	220.4	150.8	139.2	11.6	11.6
CH_4	Sample 2 (ppm)		150.8	116.0		232.0	116.0		23.2	11.6
	Mean (ppm)	220.4	179.8	116.0	208.8	226.2	133.4	139.2	17.4	11.6
	Variation from Control (%)	-----	-18.4%	-47.4%	-----	8.3%	-36.1%	-----	-87.5%	-91.7%
N_2	Sample 1 (ppm)	1151.3	1246.7	1261.3	1107.3	1239.3	1224.7	1327.3	1004.7	1342.0
N_2	Sample 2 (ppm)		1217.3	1268.7		1210.0	1496.0		1364.0	1408.0
	Mean (ppm)	1151.3	1232.0	1265.0	1107.3	1224.7	1360.3	1327.3	1184.3	1375.0
	Variation from Control (%)	-----	7.0%	9.9%	-----	10.6%	22.8%	-----	-10.8%	3.6%

Biochemical Production

Effects on biochemical production from swine slurry by varying levels of nitrate addition are shown in Table 4.3. Ammonium and sulfate concentrations increased by more than 20 and 25 percent, respectively, by the addition of 300 mg/l nitrate. Sulfide production was decreased more than 15 percent at nitrate treatment rates between 100 and 300 mg/l. At nitrate treatment rates between 300 and 500 mg/l, sulfide production increased, while the sulfate concentration decreased. Sulfate production was decreased greater than 40 percent at nitrate treatment rates between 500 and 1000 mg/l.

.

Table 4.3

NITRATE EFFECTS ON BIOCHEMICAL PRODUCTION

	Nitrate Added (mg/l)	0.0	100.0	300.0	0.0	500.0	1000.0	0.0	1500.0	3000.0
NH_4-N	Sample 1 (mg/l)	170.0	167.0	233.0	234.0	259.0	227.0	268.0	270.0	253.0
NH_4-N	Sample 2 (mg/l)		154.0	176.0		240.0	228.0		279.0	274.0
	Mean (mg/l)	170.0	160.5	204.5	234.0	249.5	227.5	268.0	274.5	263.5
	Variation from Control (%)	-----	-5.6%	20.3%	-----	6.6%	-2.8%	-----	2.4%	-1.7%
NO_3-N	Sample 1 (mg/l)	33.6	35.8	36.9	26.2	24.0	27.0	15.9	21.7	22.0
NO_3-N	Sample 2 (mg/l)		34.8	35.0		27.2	24.0		19.3	24.9
	Mean (mg/l)	33.6	35.3	36.0	26.2	25.6	25.5	15.9	20.5	23.5
	Variation from Control (%)	-----	5.1%	7.0%	-----	-2.3%	-2.7%	-----	28.9%	47.5%
Sulfate	Sample 1 (mg/l)	318	376	409	814	484	478	384	249	239
Sulfate	Sample 2 (mg/l)		361	396		453	415		247	259
	Mean (mg/l)	318.0	368.5	402.5	814.0	468.5	446.5	384.0	248.0	249.0
	Variation from Control (%)	-----	15.9%	26.6%	-----	-42.4%	-45.1%	-----	-35.4%	-35.2%
Sulfide	Sample 1 (mg/l)	1.88	1.49	1.36	2.00	2.12	2.29	1.66	0.90	0.84
Sulfide	Sample 2 (mg/l)		1.70	1.71		2.20	1.91		0.96	0.99
	Mean (mg/l)	1.9	1.6	1.5	2.0	2.2	2.1	1.7	0.9	0.9
	Variation from Control (%)	-----	-15.1%	-18.4%	-----	8.2%	5.0%	-----	-43.9%	-44.8%
TKN	Sample 1 (mg/l)	334.6	334.1	306.1	277.9	311.1	291.2	292.9	298.1	281.6

Inhibition of sulfate production may have occurred because of changes in the composition of the microbial community. Relative changes in bacterial populations, shown in Figure 4.1, at about nitrate treatment rates of 500 mg/l were mirrored in changes in inhibition, shown in Table 4.2, at about the same nitrate treatment rate. At nitrate treatment rates in excess of 1000 mg/l, sulfide production was inhibited approximately 45 percent.

COD:Nitrate Ratio

Table 4.4 shows the variation in TKN in relation to a ratio between COD and the amount of nitrate added to the sample. This was an attempt to determine

the metabolic pathway taken. TKN includes organic nitrogen and ammonia; therefore, an increase in TKN, compared to the control, could indicate nitrate being reduced to ammonium instead of nitrogen gas.

Table 4.4

NITRATE EFFECTS ON METABOLIC PATHWAYS

	Nitrate Added (mg/l)	0.0	100.0	300.0	0.0	500.0	1000.0	0.0	1500.0	3000.0
COD	Sample 1 (mg/l)	1466.0	1197.0	1294.0	2464.0	2152.0	2468.0	1536.0	1172.0	1071.0
COD	Sample 2 (mg/l)		1484.0	1385.0		2109.0	2337.0		1368.0	1252.0
	Mean (mg/l)	1466.0	1340.5	1339.5	2464.0	2130.5	2402.5	1536.0	1270.0	1161.5
	Variation from Control (%)	-----	-8.6%	-8.6%	-----	-13.5%	-2.5%	-----	-17.3%	-24.4%
COD/NO3		-----	13.4	4.5	-----	4.3	2.4	-----	0.8	0.4
TKN	Sample 1 (mg/l)	334.6	334.1	306.1	277.9	311.1	291.2	292.9	298.1	281.6
TKN	Sample 2 (mg/l)		295.8	307.5		308.8	298.9		303.3	307.7
	Mean (mg/l)	334.6	315.0	306.8	277.9	309.9	295.0	292.9	300.7	294.6
	Variation from Control (%)	-----	-5.9%	-8.3%	-----	11.5%	6.2%	-----	2.6%	0.6%
NH_4-N	Sample 1 (mg/l)	170.0	167.0	233.0	234.0	259.0	227.0	268.0	270.0	253.0
NH_4-N	Sample 2 (mg/l)		154.0	176.0		240.0	228.0		279.0	274.0
	Mean (mg/l)	170.0	160.5	204.5	234.0	249.5	227.5	268.0	274.5	263.5
	Variation from Control (%)	-----	-5.6%	20.3%	-----	6.6%	-2.8%	-----	2.4%	-1.7%

In examining the effects of the COD:nitrate ratio on denitrification, nitrate was reduced to nitrogen gas or nitrogen oxides except at a nitrate treatment rates between 300 and 500 mg/l. Table 4.4 shows an increase in ammonium production of about 20 percent for a nitrate treatment rate of 300 mg/l. The increase in ammonium may have been the result of the NRB and SRB reducing nitrate to ammonium, while oxidizing sulfide to sulfate.

Inhibition of Sulfide Gas Production

Sulfide gas produced during the test was undetectable (< 100 ppb). Although this may have been due to experimental error, it may also have been a result of purple sulfur bacteria present in the lagoon being sampled. Sulfide may have been oxidized to elemental sulfur (Sund et al., 2001; Schulte and Koelsch, 2000). The sulfide in solution measured between 2.25 and 3.22 mg/l.

Statistical Analysis

The Statistical Analysis Software (SAS Institute Inc., 2002) Pearson Correlation procedure (CORR) was used to test relationships between variables. The correlations between the nitrate treatment levels (Add_NO3) and variables of specific interest are shown in Table 4.5. The correlation coefficient between Add_NO3 and inhibition of sulfide producers was 0.75. The correlation coefficient between Add_NO3 and inhibition of sulfate reducers was 0.66. The correlation coefficient between Add_NO3 and inhibition of methane production was 0.69.

Table 4.5

PEARSON CORRELATION COEFFICIENTS

	Ortho P	Total P	COD	Sulfide	Sulfide Producers	Anaerobes	Denitrifiers	Sulfate Reducers	CO2	N2	CH4	Add_NO3	Sulfate	Nitrate	Nitrite	Ammonium	TKN
Ortho P	1.00	0.19	-0.26	-0.34	-0.58	-0.02	0.17	-0.08	0.34	0.02	-0.21	-0.27	0.27	0.48	0.04	-0.12	-0.20
Total P	0.19	1.00	0.23	-0.26	-0.53	0.22	0.27	-0.08	0.12	-0.25	-0.27	-0.30	0.16	0.31	0.58	-0.23	0.15
COD	-0.26	0.23	1.00	-0.17	-0.27	0.68	0.68	-0.19	0.13	0.06	-0.05	-0.23	0.26	0.47	0.74	-0.22	-0.33
Sulfide	-0.34	-0.26	-0.17	1.00	0.70	-0.40	-0.51	0.27	-0.19	0.70	0.75	0.66	0.31	-0.49	0.09	-0.21	0.29
Sulfide Producers	-0.58	-0.53	-0.27	0.70	1.00	-0.42	-0.73	0.37	-0.15	0.14	0.70	0.75	-0.13	-0.88	-0.31	0.15	0.24
Anaerobes	-0.02	0.22	0.68	-0.40	-0.42	1.00	0.81	-0.21	0.12	-0.25	-0.20	-0.42	-0.22	0.52	0.43	-0.02	0.13
Denitrifiers	0.17	0.27	0.68	-0.51	-0.73	0.81	1.00	-0.31	0.14	-0.02	-0.41	-0.53	0.16	0.86	0.47	-0.16	-0.17
Sulfate Reducers	-0.08	-0.08	-0.19	0.27	0.37	-0.21	-0.31	1.00	0.46	-0.16	0.45	0.66	0.24	-0.54	-0.31	0.41	-0.18
CO2	0.34	0.12	0.13	-0.19	-0.15	0.12	0.14	0.46	1.00	-0.28	0.40	0.14	0.29	0.01	0.02	0.11	-0.19
N2	0.02	-0.25	0.06	0.70	0.14	-0.25	-0.02	-0.16	-0.28	1.00	0.35	0.14	0.58	0.20	0.34	-0.46	-0.08
CH4	-0.21	-0.27	-0.05	0.75	0.70	-0.20	-0.41	0.45	0.40	0.35	1.00	0.69	0.29	-0.55	0.07	-0.08	0.23
Add_NO3	-0.27	-0.30	-0.23	0.66	0.75	-0.42	-0.53	0.66	0.14	0.14	0.69	1.00	0.30	-0.69	-0.22	0.09	0.10
Sulfate	0.27	0.16	0.26	0.31	-0.13	-0.22	0.16	0.24	0.29	0.58	0.29	0.30	1.00	0.29	0.49	-0.29	-0.50
Nitrate	0.48	0.31	0.47	-0.49	-0.88	0.52	0.86	-0.54	0.01	0.20	-0.55	-0.69	0.29	1.00	0.50	-0.34	-0.26
Nitrite	0.04	0.58	0.74	0.09	-0.31	0.43	0.47	-0.31	0.02	0.34	0.07	-0.22	0.49	0.50	1.00	-0.50	-0.11
Ammonium	-0.12	-0.23	-0.22	-0.21	0.15	-0.02	-0.16	0.41	0.11	-0.46	-0.08	0.09	-0.29	-0.34	-0.50	1.00	-0.09
TKN	-0.20	0.15	-0.33	0.29	0.24	0.13	-0.17	-0.18	-0.19	-0.08	0.23	0.10	-0.50	-0.26	-0.11	-0.09	1.00

Varying correlations among pairs of variables indicated the potential for interaction between variables. Multivariate analysis of variance was used to determine the relationship among the multiple variables. Sum of square reduction (R-square) methods were used to determine the least combination of variables that produced a high R-square value with a low C(p), while minimizing the number of variables required to inhibit sulfide production. The results are shown in Table 4.6.

Table 4.6

R-SQUARE SELECTION METHOD

Number in Model	R-Square	C(p)	Variables in Model
2	0.90	34.41	CO_2, CH_4
3	0.96	10.46	Time, CO_2, CH_4
4	0.98	7.13	Ortho P, Sulfide Producers, CO_2, CH_4
5	0.99	4.60	Ortho P, Sulfide Producers, Anaerobes, CO_2, CH_4
6	1.00	3.89	Nitrite, Ortho P, Sulfide Producers, Anaerobes, CO_2, CH_4

A compact linear model, with only four variables and a correlation of 0.98, was derived. Although this model would be of little use in calculating nitrate requirements for inhibition of sulfide production, it does indicate the variables with the most effect on sulfide production. Ortho P (available phosphorus), methane, and carbon dioxide factors could indicate the competition for substrates between the SRB and methanogens.

SAS multiple linear regression (REG) procedures were used to test whether a linear model could be used to determine the relationship between multiple variables and inhibition of sulfide production. Including variables for nitrate treatment levels and the nitrate in solution produced the model shown in Table 4.7 with an R-square of 0.98 and an adjusted R-square of 0.96. The p-values are included in the table. The nitrate treatment level (Add NO_3) had the least influence of the variables included in the model. Sulfide production was primarily a function of the competition for substrates (between the sulfide-producing bacteria and the methanogens) and the inhibition caused by addition of nitrate.

Table 4.7

MULTIPLE LINEAR REGRESSION METHOD

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Type I SS	Type II SS
Intercept	1	-0.51	0.11	-4.77	0.01	0.29	0.05
Sulfide Producers	1	0.70	0.20	3.40	0.02	0.33	0.02
CO_2	1	1.27	0.31	4.08	0.01	0.00	0.04
N_2	1	-0.56	0.14	-3.88	0.01	0.24	0.03
CH_4	1	-0.62	0.14	-4.55	0.01	0.03	0.04
Add NO_3	1	-5×10^{-5}	0.00	-2.21	0.08	0.02	0.01
Nitrate	1	0.45	0.11	3.89	0.01	0.03	0.03

CHAPTER V

CONCLUSIONS

Nitrate reducers, sulfate reducers, and methanogens were able to coexist, despite the possibility of substrate competition. Sufficient organic matter from the swine wastes was available to support the populations, allowing the creation of niches for the bacteria. In addition, the concentrations of sulfur-containing compounds may not have been high enough to significantly inhibit methanogenesis. Finally, sulfide could be one of the sulfur sources required for cellular growth of the methanogens.

The addition of nitrate stimulated the production of nitrogen gas less than 25 percent; hence, denitrification was not greatly limited by the availability of electron acceptors. The significant increase in the nitrate-reducing bacteria (NRB) population was probably because of competitive advantage. Toxic levels of nitrate may have inhibited other bacteria present.

Competitive interactions occurred between the three main bacterial groups. Nitrate addition inhibited both sulfate reduction and methane production. The inhibition may have been due to competition for common substrates and/or the toxic byproducts of nitrate reduction. The manner of inhibition was difficult to establish, since nitrate could be reduced to nitrite, NO, and/or N_2O. Each of

these compounds could have different inhibitory effects. Nitrate could provide a nitrogen source for cell synthesis at one concentration but could be an inhibitor at a higher concentration.

Under anaerobic conditions, the bacteria able to use a terminal electron acceptor varied due to a changing environment. Varying the electron acceptor altered the metabolic pathway, the byproducts of respiration, and the energy produced. These changes affected the relative populations of the different bacteria due to competition and inhibition as the concentrations of electron donors and acceptors varied.

A linear model was developed relating the sulfide concentration to the amount of nitrate added, the nitrate in solution, the sulfate-reducing bacteria (SRB) population, and the carbon dioxide, methane and nitrogen gases produced. Sulfide production from an exponentially growing population was reduced approximately 44 and 45 percent with the addition of 1500 and 3000 mg/l nitrate, respectively. The addition of 1000 mg/l nitrate had little influence on sulfide production. Based upon the research, the linear model is:

$$\Delta \text{ sulfide} = 0.7(\Delta \text{ SRB}) + 1.27(\Delta \text{ } CO_2) + 0.45(\Delta \text{ } NO_3) - 0.56(\Delta N_2) - 0.62(\Delta CH_4) - 5x10\text{-}5(\text{Add } NO_3) - 0.51$$

CHAPTER VI

ENGINEERING SIGNIFICANCE

Adding nitrate to swine wastes before land application has the potential to reduce odor complaints as well as help maintain soil fertility. Using a cost of $240 per metric ton of nitrate (bulk rate), 1500 mg/l nitrate would cost approximately $200 per hectare, assuming an irrigation application depth of 50 mm. While this nitrate requirement might be uneconomical for odor control only, it could be incorporated as part of a fertility program. Prior to land application, nitrate added to the effluent would significantly reduce the sulfide concentration. Because sulfides are precursors of odor, reducing the sulfide concentration would reduce one potential odor source. For producers whose land application rates are limited by phosphorus levels, this method would help meet the crop requirements for nitrate as well as provide some odor reduction. For other producers with an odor problem due to a temporary imbalance in lagoon operation, the additional costs could be cheaper than fines and lawsuits.

If other states follow Minnesota's example of setting maximum acceptable sulfide standards (30-50 ppb) at property boundaries, nitrate addition may offer an economical approach to compliance. Except for odor, Mississippi currently uses the EPA primary and secondary standards for air quality, as defined by EPA

40 CFR part 50 (Mississippi Department of Environmental Quality, 2002). A multimedia permit is required. Both air and water quality issues must be addressed in the waste treatment plan. Odor is currently addressed on a nuisance basis. An operation's profits must to be balanced against the costs of waste treatment and odor mitigation.

This research could be also be used to optimize methane production from swine wastes. Concentrations of nitrate between 300 and 500 mg/l stimulated methanogenesis while decreasing the SRB population. Additional research is required to determine the required dosage rate.

Finally, the effects on odor reduction by purple sulfur bacteria (PSB) in lagoons need additional research. Challenges in establishing PSB in operating lagoons have yet to be explored, yet offer many opportunities for odor reduction.

REFERENCES CITED

Associated Press. "Bush issues new factory-farm rules." www.myinky.com/ecp/gleaner_news/article/0,1626,ECP_4476_1616600,00.html. December 18, 2002. The Henderson Gleamer. 455 Klutey Park Plaza, Henderson KY. January 28, 2003.

Associated Press. "Poultry suit gets go-ahead." www.thegleaner.com/daily/2001-04-19/news1.html. April, 19, 2001. The Henderson Gleamer. 455 Klutey Park Plaza, Henderson KY. January 28, 2003.

Atlas, R.M. and R. Bartha. Microbial Ecology. The Benjamin/Cummings Publishing Company, Inc., Redwood City, CA. (1997) 328-331.

Barker, J. C. "Swine Production Facility Manure Management: Pit Recharge-Lagoon Treatment (EBAE-128-88)." North Carolina Cooperative Extension Service. March, 1996.

Barker, J., S. Curtis, O. Hogsett, and F. Humenik. Safety in Swine Production Systems. North Carolina Cooperative Extension Service: Pub. No. PIH-104, 1996.

Barnes, D., P. A. Fitzgerald, R. M. McFarland, H. Swan, and T. Schulz. "Redox Potentials/Basis of Measurement and Application." Effluent and Water Treatment Journal. Vol. 2. (1985): 232-236.

Boon, A. "Septicity in Sewers: Causes, Consequences, and Containment." Water Science Technology. 31 (1995): 237-253.

Camacho, A.C., E.Vicente, and M.R. Miracle. "Spatio-Temporal Distribution And Growth Dynamics Of Phototrophic Sulfur Bacteria Populations In The Sulfide-Rich Lake Arcas." Aquatic Science. 62 (2000) 334-349.

Carpenter, P.L. Microbiology. W.B. Saunders Company, Philadelphia, PA. (1972): 257-278.

Chen, A., P. H. Liao, and K. V. Lo. "Headspace Analysis of Malodorous Compounds from Swine Wastewater under Aerobic Treatment." Bioresource Technology 49 (1994): 83-87.

Clanton, C., R. V. Morey, and D. R. Schmidt. "Sulfur Compounds in Gases Emitted from Stored Manure." http://www.bae.umn.edu/annrpt/1999/research/lvstk1.html. August 1999. Minnesota Department of Agriculture. June 4, 2001.

Clanton, C., D.R. Schmidt, R. Nicolai, L. Jacobson, P. Goodrich, and K. Janni. "Chemical Addition to Manure for Hydrogen Sulfide Control: A Laboratory Study." http://www.bae.umn.edu/annrpt/2000/research. December 2002. Minnesota Department of Agriculture. January 4, 2003.

Clesceri, L.S., A.E. Greenberg, and R.R. Trussell. Standard Methods for the Examination of Water and Wastewater, 19th Edition, APHA, Port City Press, Baltimore, MD. (1999): 9-77 - 9-80.

Cromwell, G.L., L.W. Turner, J.L. Taraba, R.S. Gates, M.D. Lindermann, S.L. Traylor, W.A. Dozier, and H.J. Monegue. "Manipulation of Swine Diets to Reduce Odor and Harmful Gaseous Emissions from Manure." Department of Animal Sciences and Department of Biosystems and Agricultural Engineering, University of Kentucky, Lexington, Kentucky. 1998.

Fdz-Polanco, F., M. Fdz-Polanco, N. Fernandez, M. A. Urueña, P. A. Garcia, and S. Villaverde. "New Process for Simultaneous Removal of Nitrogen and Sulphur under Anaerobic Conditions." Water Resources. 35, 4 (2001): 1111-1114.

Fukui, M., J. Suh, and Y. Urushigawa. "*In Situ* Substrates for Sulfidogens and Methanogens in Municipal Anaerobic Sewage Digesters with Different Levels of Sulfate." Water Resources. 34, 5 (2000): 1515-1524.

Fulhage, C. D. Gases and Odors from Swine Wastes. University of Missouri Extension: G01880. 1993.

Gostelow, P., S. A. Parsons, and R. M. Stuetz. "Odour Measurements for Sewage Treatment Works." Water Resources. 35 (2001): 579-597.

Gustafson, R. J. and M. A. Veenhuizen. "Reducing Noxious Gas Emissions from Swine Manure Storages. http://nppc.org/Research/98reports/98-Gustafson-1991.htm. August 9, 1999. National Pork Producers Council. June 4, 2001.

Hach. DR/4000 Spectrophotometer Procedures Manual. http://www.hach.com/dr4k_manual/index.htm. December 1999. Hach Company, P.O. Box 389, Loveland, Colorado. June 2, 2001.

Hamilton, D.W. and J. Arogo. "Measuring Farmstead Odors." Oklahoma Cooperative Extension Service F-1740. Oklahoma State University. 1999.

Hartung, J. and V. R. Phillips. "Control of Gaseous Emissions from Livestock Buildings and Manure Stores." Agricultural Engineering Resources. 57 (1994): 173-189.

Hatfield, J. L., J. A. Zahn, and J. H. Prueger. "Release and Dispersion of Volatile Organic Compounds and Trace Gases from Swine Manure Systems in Iowa, Arkansas, and North Carolina." http://nppc.org/Research/98reports/98-Hatfield-1911.html. August 9, 1999. USDA-ARS National Soil Tilth Laboratory Ames, Iowa. June 2, 2001.

Heber, A., T. Lim, J. Ni, D. Kendall, B. Richert, and A. Sutton. "Odor, Ammonia and Hydrogen Sulfide Emission Factors for Grow-Finish Buildings (#99-122)." http://danpatch.ecn.purdue.edu/~odor/NPPCfinal99122.pdf. September 6, 1999. Purdue University. February 26, 2001.

Heber, A., R. Duggirala, J. Ni, M.L. Spence, B.L. Haymore, V.I. Adamchuk, D.S. Bundy, A.L. Sutton, and K.M. Kenner. "Field Performance of Pit Additive Tested in Commercial Grow-Finish Houses." http://www.ansc.purdue.edu/swine/swineday/sday97/psd17-97.htm. 1997. Purdue University. February 26, 2001

Jacobson, L., C. Clanton, and R. Nicolai. "Development of a Standard Method of Odor Quantification Using Olfactometry and Chemical Analysis."

http://www.bae.umn.edu/annrpt/1998/research/lvstk6.html. April 23, 1999. University of Minnesota. June 21, 2001.

Johnson, D. "Pork Producer Settles Suit as Pollution Rules Tighten." http://query.nytimes.com/search/article-page.html?res=9F03EEDF1139F935A2575BC0A96F958260. August 16, 1999. The New York Times. 229 West 43rd Street, New York, NY. Jan. 28, 2003.

Johnson, J. "Final Report: Evaluation of Commercial Manure Additives." Agricultural Utilization Research Institute. PO Box 251, Waseca, MN. 1997.

Kato, Y., T. Murakawa, K. Nishida, M. Kitagawa, T. Higuchi, T. Higuchi, and J. Endo. "Research and Development of Odor Measurement Using a Semiconductor Gas Sensor." Air and Waste Management Association. 46 (1996): 260-266.

Kluber, H.D. and R. Conrad. "Effects of Nitrate, NO, and N_2O on Methanogenesis and Other Redox Processes in Anoxic Rice Field Soil." FEMS Microbiology Ecology. 25 (1998): 301-318.

Koster, E.P. "Limitations Imposed on Olfactometric Measurements by the Human Factor." Odor Prevention and Control of Organic Sludge and Livestock Farming. (1986) 86-93.

Kratz, G. "Will Corporate Farms Hog Market?" http://www.desnews.com/dnews.env. (September 16, 1998). Deseret News, 30 E. 100th S. Salt Lake City, UT. January 28, 2003.

Labance, S. E., P. H. Heinemann, and D. M. Beyer. "Evaluation of Microporous Covers for the Reduction of Mushroom Substrate Preparation Odors." Applied Engineering in Agriculture. 15(5) (1999): 559-566.

Lee Wilson and Associates. "Swine Cafo Odors: Guidance for Environmental Impact Assessment Region 6 U. S. Environmental Protection Agency." http://www.epa.gov/Region06/6en/xp/odor.pdf . September 30, 1996. 1445 Ross Avenue, Dallas, Texas. June 6, 2000.

Lens, P. N. L., J. Sipma, L. W. Hulshoff Pol, and G. Lettinga. "Effect of Nitrate on Acetate Degradation in a Sulfidogenic Staged Reactor." Water Resources. 34, 1 (2000): 31-42.

Liao, C.-M. and D.S. Bundy. "Bacterial Additives to the Changes in Gaseous Mass Transfer from Stored Swine Manure." Journal of Environmental Science and Health. B29 (6) (1994): 1219-1249.

Liao, C.-M., H.-M. Liang, and S. Singh. "Exposure Assessment Model for Odor Causing Vocs Volatilization from Stored Pig Slurry." Journal of Environmental Science and Health. B33(4) (1998): 457-486.

Liao, C.-M., H.-M. Liang, and S. Singh. "Swine Manure Cleanup Criteria Calculation for Odor Causing Volatile Organic Compounds Based on Manure-to-Ventilation Air Exposure Pathway." Journal of Environmental Science and Health B32(4) (1997): 449-468.

Lorimor, J. "Manure Odor Reduction from Pit Additives." ISU Extension Publication #: none, electronic format only. Dept. of Agricultural & Biosystems Engineering, Iowa State University. 1997.

Mackie, R.I., P.G. Stroot, and V.H. Varel. "Biochemical Identification and Biological Origin of Key Odor Components in Livestock Wastes." Journal of Animal Science. 76 (1998): 1331-1342.

McGinley, C.M., T.D. Mahin, and R.J. Pope. "Elements of Successful Odor / Odour Laws." St. Croix Sensory Inc. / McGinley Associates, P.A. 13701 - 30th Street Circle North, Stillwater, MN. 2000.

Meadows, R. "Livestock Legacy." Environmental Health Perspective. 103,12 (1995): 1096-1100.

Miner, J. "A Review of the Literature on the Nature and Control of Odors from Pork Production Facilities." The Odor Subcommittee of the Environmental Committee of the National Pork Producers Council. September 1, 1995

Minnesota Pollution Control Agency. "Feedlots: Livestock Odor FAQ." www.pca.state.mn.us. September 8, 2002. MPCA, 520 Lafayette Road, St. Paul, MN. January 10, 2003.

Mississippi Department of Environmental Quality. "Regulation APC-S-4: Ambient Air Quality Standards." June 27, 2002. MDEQ, P.O. Box 20305, Jackson, MS. March 11, 2003.

Nicolai, R. E. "Managing Odors from Swine Waste." http://www.bae.umn.edu/extens/aeu/aeu8.html. April 16, 1996. University of Minnesota. May 14, 2001.

Pearce T. C. "Computational Parallels Between the Biological Olfactory Pathway and Its Analogue `The Electronic Nose': Part I. Biological Olfaction." BioSystems. 41, (1997): 43-67.

Percheron, G., N. Bernet, and R. Moletta. "Interactions between methanogenic and nitrate reducing bacteria during the anaerobic digestion of an industrial sulfate rich wastewater." FEMS Microbiology Ecology. 29 (1999): 341-350.

Rittmann, B. E. and P. L. McCarty. Environmental Biotechnology: Principles and Applications. New York: McGraw Hill, 2001, 569-572.

Rizzuti, A.M., A.D. Cohen, P.G. Hunt, and M.B. Vanotti. "Testing of Peats for Removal of Odors from Liquid Swine Manure." Journal of Science and Health. A33 (8) (1998): 1719-1739.

SAS Institute Inc. http://www.sas.com. January10, 2002. 100 SAS Campus Drive, Cary, NC. December 10, 2002.

Sawyer, C.N., P.L. McCarty, and G.F. Parkin. Chemistry for Environmental Engineering. Fourth Edition. McGraw-Hill, Inc. New York, NY. (1994).

Schabenberger, O. "Stat 5616, Spring Semester, 2001." February 10, 2001. http://home.nc.rr.com/schabenb/sasresources.htm. November 28, 2002.

Schiffman S., E. Miller, and M. Suggs. "The Effect of Environmental Odors Emanating from Commercial Swine Operations on the Mood of Nearby Residents." Brain Research Bulletin. 37, 4 (1995): 369-375.

Schmidt, D. and L. Jacobson. "Odor and Odor Policy Criteria." http://www.bae.umn.edu/extens/aeu/aeu11.html. November, 1995.

Extension Publication AEU-11. University of Minnesota. December 10, 1999.

Schulte, D.D. and R.K. Koelsch. "Improving Odor Control in Swine Lagoons by Better Understanding and Management of Purple Sulfur Bacteria." Transactions of the ASAE. 48, 6 (2000): 1853-1859.

Schulte, D., D. Kottwitz, and C. Gilbertson. "Nitrogen Content of Scraped Swine Manure." Proceedings of the 5th International Symposium on Agricultural Wastes. (1985): 321-328.

Schulz, T. J. and D. Barnes. "The Stratified Facultative Lagoon for the Treatment and Storage of High Strength Agricultural Wastewaters." Journal of Water Science and Technology. 22, 9 (1990): 43-50.

Sierra Club. "The RapSheet on Animal Factories." www.sierraclub.org/factoryfarms/rapsheets. August 10, 2002. Sierra Club, 85 Second St., Second Floor, San Francisco, CA. January 28, 2003.

State Environmental Resource Center. "Innovative State Legislation - Issue: CAFOs." www.serconline.org/cafos.html. 2003. State Environmental Resource Center, 106 East Doty Street, Suite 200, Madison, Wisconsin. Jan. 28, 2003.

Sund, J.L., C.J. Evenson, K.A. Strevett, R.W. Nairn, D. Athay, and E. Trawinski, "Nutrient Conversion by Photosynthetic Bacteria in a Concentrated Animal Feeding Operation Lagoon System." Journal of Environmental Quality. 30 (2001): 648-655.

Sweeten, J. "Odor Measurement Technology and Application – A State-of-the-Art Review." Proceedings of the 7th International Symposium on Agricultural and Food Processing Wastes, 1: (1995): 214-229.

Swine Odor Task Force, "Options for Managing Odor." http://www.ces.ncsu.edu/whpaper/SwineOdor.html. March 1, 1995. North Carolina Agricultural Research Service, North Carolina State University. December 15, 2000.

Tengman, C.L. and R.N. Goodwin. "Hydrogen Sulfide Concentrations Downwind from Agitated Swine Manure Pits." Presented at 2000 ASAE Annual International Meeting, Paper No. 004078. ASAE, 2950 Niles Road, St. Joseph, MI. 2000.

U. S. Department of Labor, Occupational Safety and Health Administration. Chemical Sampling Information, Hydrogen Sulfide . http://www.osha-slc.gov/dts/chemicalsampling/data/CH_246800.html. Revised 02/29/2000. United States Department of Labor, Occupational Safety and Health Administration. June 7, 2001.

U. S. Environmental Protection Agency, Region 6. "Environmental Assessment on Proposed Reissuance of EPA's NPDES General Permits for CAFOs in Texas, New Mexico and Oklahoma." http://www.epa.gov/Region06/6wq/npdes/genpermt/assessmt.pdf. July 7, 1998. United States Environmental Protection Agency. June 4, 2001.

U. S. Environmental Protection Agency. "National Pollutant Discharge Elimination System Permit Regulation and Effluent Limitation Guidelines and Standards for Concentrated Animal Feeding Operations (CAFOs)." http://www.epa.gov/npdes/regulations/cafo_final_rule_section1.pdf. December 16, 2002. United States Environmental Protection Agency. February 4, 2003.

U. S. Environmental Protection Agency. "Unified National Strategy for Animal Feeding Operations." http://www.epa.gov/npdes/pubs/exexsum.pdf. March 9, 1999. United States Environmental Protection Agency. June 4, 2001.

White, D. The Physiology and Biochemistry of Prokaryotes. Second Edition. Oxford University Press. New York, NY (2000) 66-321.

Yu, K., Z. Wang, A. Vermoesen, W. Patrick, and O. Van Cleemput. "Nitrous Oxide and Methane Emissions from Different Soil Suspensions: Effect of Soil Redox Status." Biological Fertilization of Soils. 34 (2001): 25-30.

Zahn, J. and A. DiSpirito. "Composition and Use of a Swine Odor Standard in Quantification of Swine Odors." June 16, 1998. US5766551, US Patent and Trademark Office, Washington, D.C.

Zhang, Y. and H.W. Scherer. "Mechanisms of Fixation and Release of Ammonium in Paddy Soils after Flooding." Biological Fertilization of Soils. 31 (2000): 517-521.

Zhu, J. and L.D. Jacobson. "Correlating Microbes to Major Odorous Compounds in Swine Manure." Journal of Environmental Quality. 28 (1999): 737-744.

Zhu, J., D.S. Bundy, L. Xiwei, and N. Rashid. "Controlling Odor and Volatile Substances in Liquid Hog Manure by Amendment." Journal of Environmental Quality. 26 (1997): 740–74

APPENDIX

Table A.1

BIOCHEMICAL ANALYSIS DATA, PART 1

DATE	SAMPLE NO	ADD NO3 (mg/l)	COD (mg/l)	AMMONIA (mg/l)	NITRATE (mg/l)	NITRITE (mg/l)	TKN (mg/l)	SULFATE (mg/l)	SULFIDE (ug/l)
10/08/01	A	0	3109	221	35.1	1.21	278.93	353	1151
10/10/01	1	0	3652	237	34.8	0.58	286.15	340	1550
10/10/01	2	100	3634	225	37.2	0.62	276.22	326	1300
10/10/01	3	100	4902	220	36.5	0.91	291.15	390	1450
10/10/01	4	300	3145	220	36	0.65	291.56	330	1480
10/10/01	5	300	2983	257	35.5	0.72	331.90	370	1580
10/11/01	6	0	1071	203	34.5	1.21	284.56	322	1105
10/11/01	7	100	1862	200	34	1.05	274.48	360	1557
10/11/01	8	100	1560	260	34.6	1.20	296.44	370	1372
10/11/01	9	300	1685	248	34.5	0.79	283.64	400	1492
10/11/01	10	300	1829	278	34.8	1.15	379.62	390	1392
10/12/01	11	0	1267	254	35	0.55	311.35	345	1274
10/12/01	12	100	1030	246	35	0.56	282.52	306	1541
10/12/01	13	100	1020	233	37	0.86	288.85	420	2062
10/12/01	14	300	1490	210	35	0.59	282.44	328	1147
10/12/01	15	300	1600	242	35	0.69	288.51	368	1736
10/15/01	16	0	1466	170	33.6	1.16	334.57	318	1882
10/15/01	17	100	1197	167	35.8	1.01	334.11	376	1491
10/15/01	18	100	1484	154	34.8	1.26	295.81	361	1704
10/15/01	19	300	1294	233	36.9	0.82	306.11	409	1360
10/15/01	20	300	1385	176	35	1.22	307.48	396	1713
10/25/01	B	0	3987	293	21.8	0.98	323.32	369	3224
10/26/01	21	0	2760	260	21.2	1.10	309.59	278	1627
10/26/01	22	500	2793	252	24.3	1.30	319.07	400	1867
10/26/01	23	500	2523	225	32.5	1.90	316.80	499	1583
10/26/01	24	1000	2856	229	38.3	1.80	302.63	348	1809
10/26/01	25	1000	2462	227	32.9	1.30	304.11	504	2150
10/29/01	26	0	2125	229	23.8	1.01	294.91	671	1428
10/29/01	27	500	1515	243	23.9	1.27	379.04	495	2133
10/29/01	28	500	1446	222	25.7	1.03	288.68	643	1656
10/29/01	29	1000	1511	260	24	1.35	305.63	468	1884
10/29/01	30	1000	1609	224	29.1	1.47	295.08	491	2379
10/31/01	31	0	2840	272	22	1.02	306.08	1263	1831
10/31/01	32	500	2056	258	23.6	0.96	317.99	422	1947
10/31/01	33	500	2573	230	25.1	1.13	298.29	453	2124
10/31/01	34	1000	2340	279	24.5	1.27	316.48	476	2124
10/31/01	35	1000	2524	221	22.7	1.12	308.38	451	2029
11/02/01	36	0	2464	234	26.2	1.40	277.85	814	1998
11/02/01	37	500	2152	259	24	1.47	311.05	484	2120
11/02/01	38	500	2109	240	27.2	1.32	308.80	453	2202
11/02/01	39	1000	2468	227	27	1.72	291.17	478	2285
11/02/01	40	1000	2337	228	24	1.13	298.89	415	1911
11/08/01	C	0	3691	259	19	0.99	320.57	389	2806
11/09/01	41	0	1846	225	17.1	1.34	270.00	337	2070
11/09/01	42	1500	1751	197	32.3	1.26	268.46	324	856
11/09/01	43	1500	2083	236	35.1	1.60	285.92	387	1273
11/09/01	44	3000	2729	228	39.8	2.20	272.39	349	1173
11/09/01	45	3000	2421	217	26.4	2.40	245.90	375	1127
11/12/01	46	0	3295	246	19.8	1.12	267.10	374	1695
11/12/01	47	1500	3510	225	29	1.25	257.54	262	856
11/12/01	48	1500	3363	251	25.3	0.82	279.70	260	1052
11/12/01	49	3000	3995	225	19.8	2.35	248.74	272	1083
11/12/01	50	3000	3824	259	24.3	0.82	287.60	280	1083
11/14/01	51	0	2991	243	26.9	1.97	281.32	679	2660
11/14/01	52	1500	2853	253	35.7	1.88	307.64	1000	1677
11/14/01	53	1500	1688	223	49	1.71	280.37	904	1871
11/14/01	54	3000	2305	219	29.1	1.96	250.84	1017	2234
11/14/01	55	3000	2368	248	29.1	2.07	282.27	930	1660
11/16/01	56	0	1536	268	15.9	1.01	292.93	384	1658
11/16/01	57	1500	1172	270	21.7	1.47	298.08	249	895
11/16/01	58	1500	1368	279	19.3	0.79	303.29	247	964
11/16/01	59	3000	1071	253	22	0.82	281.56	239	844
11/16/01	60	3000	1252	274	24.9	0.98	307.68	259	987

Table A.2

BIOCHEMICAL ANALYSIS DATA, PART 2

DATE	SAMPLE NO	ADD NO3 (mg/l)	V. ACIDS (mg/l)	ORTHO-P (mg/l)	Total P (mg/l)	DO (mg/l)	ORP (mv)	pH
10/08/01	A	0	3252	74.60	139.4674	0.11	-127.5	7.35
10/10/01	1	0	1567	45.23	143.0741	0.19	-120.5	7.90
10/10/01	2	100	2597	31.72	138.1088	0.18	-119.8	7.90
10/10/01	3	100	4562	37.50	145.5744	0.32	-115.2	7.90
10/10/01	4	300	4671	35.50	145.7785	0.30	-114.6	7.90
10/10/01	5	300	1224	36.53	165.9487	0.27	-110.8	8.00
10/11/01	6	0	2785	51.50	142.2812	0.37	-114.5	8.13
10/11/01	7	100	1025	56.30	137.2412	0.22	-110.4	8.53
10/11/01	8	100	2457	50.25	148.2213	0.17	-112.2	8.42
10/11/01	9	300	678	44.76	141.819	0.24	-98.9	8.46
10/11/01	10	300	625	47.34	189.8085	0.23	-90.7	8.45
10/12/01	11	0	3150	44.73	155.6756	0.63	-110.6	8.91
10/12/01	12	100	925	30.69	141.2621	0.25	-124.2	9.15
10/12/01	13	100	1241	38.10	144.4266	0.16	-112.2	8.93
10/12/01	14	300	2465	34.65	141.2186	0.15	-117.3	9.02
10/12/01	15	300	1287	37.04	144.2551	0.16	-114.7	8.99
10/15/01	16	0	781	53.26	167.2867	0.10	-110.6	8.97
10/15/01	17	100	802	55.92	167.0545	0.03	-117.2	9.11
10/15/01	18	100	741	50.44	147.904	0.04	-115.5	9.10
10/15/01	19	300	1931	44.76	153.0562	0.40	-119.2	9.13
10/15/01	20	300	661	47.34	153.7406	0.90	-117.0	9.09
10/25/01	B	0	2658	92.10	104.72	0.06	-33.9	7.56
10/26/01	21	0	1497	71.30	95.94	0.04	-38.1	7.65
10/26/01	22	500	2622	71.00	98.83	0.07	-54.0	7.93
10/26/01	23	500	4756	74.80	94.65	0.05	-60.1	8.03
10/26/01	24	1000	4551	76.80	95.92	0.06	-25.8	7.90
10/26/01	25	1000	1099	74.90	88.56	0.04	-72.8	8.25
10/29/01	26	0	2661	83.10	88.49	0.13	-91.2	8.56
10/29/01	27	500	999	78.40	108.97	2.26	-104.1	8.78
10/29/01	28	500	2094	70.70	79.96	1.07	-98.2	8.69
10/29/01	29	1000	568	75.60	95.92	0.04	-100.9	8.73
10/29/01	30	1000	554	73.80	84.47	0.03	-110.3	8.89
10/31/01	31	0	3371	74.61	96.22	1.21	-93.2	8.60
10/31/01	32	500	848	73.85	102.61	0.03	-93.9	8.61
10/31/01	33	500	1173	70.11	83.35	0.03	-97.1	8.67
10/31/01	34	1000	2335	76.56	81.91	0.03	-102.7	8.76
10/31/01	35	1000	1174	73.07	82.97	0.04	-108.7	8.87
11/02/01	36	0	658	73.70	80.14	0.03	-84.5	7.53
11/02/01	37	500	761	72.80	75.74	0.03	-95.9	7.72
11/02/01	38	500	733	67.80	73.78	0.04	-94.3	7.70
11/02/01	39	1000	1896	32.60	62.23	0.06	-102.6	7.83
11/02/01	40	1000	503	37.60	82.59	0.09	-110.1	7.96
11/08/01	C	0	4222	42.50	83.26	0.03	-83.7	8.42
11/09/01	41	0	3097	45.10	36.79	0.18	83.2	8.40
11/09/01	42	1500	12900	48.60	35.68	0.26	-98.5	8.66
11/09/01	43	1500	3467	45.40	35.74	0.70	-101.3	8.71
11/09/01	44	3000	5900	43.10	38.28	0.71	-102.2	8.73
11/09/01	45	3000	8226	37.60	39.42	0.79	-101.5	8.71
11/12/01	46	0	5266	34.90	32.30	1.89	-96.6	8.66
11/12/01	47	1500	3511	31.90	33.39	3.00	-114.4	8.97
11/12/01	48	1500	550	34.50	32.40	3.33	-112.3	8.93
11/12/01	49	3000	8728	39.30	32.11	3.05	-114.8	8.98
11/12/01	50	3000	1229	41.10	32.27	2.84	-114.1	8.96
11/14/01	51	0	667	41.50	32.17	2.61	-102.6	8.77
11/14/01	52	1500	1523	48.50	32.89	3.67	-113.8	8.96
11/14/01	53	1500	3895	38.50	30.79	3.28	-109.0	8.88
11/14/01	54	3000	1821	39.90	33.39	3.02	-106.9	8.84
11/14/01	55	3000	413	44.90	32.40	3.29	-112.5	8.94
11/16/01	56	0	400	36.20	32.11	1.39	-92.2	8.58
11/16/01	57	1500	264	39.60	32.27	3.86	-107.6	8.85
11/16/01	58	1500	455	41.30	32.17	3.14	-102.5	8.76
11/16/01	59	3000	1828	38.20	32.89	4.23	-104.2	8.79
11/16/01	60	3000	379	39.70	30.79	3.19	-109.2	8.88

Table A.3

GAS CHROMATOGRAPHY DATA

Gas	Time (days)	Control (ppm)	100 A (ppm)	100 B (ppm)	300 A (ppm)	300 B (ppm)	Control (ppm)	500 A (ppm)	500 B (ppm)	1000 A (ppm)	1000 B (ppm)	Control (ppm)	1500 A (ppm)	1500 B (ppm)	3000 A (ppm)	3000 B (ppm)
CO2	1	94.5	66.5	70.0	52.5	66.5	59.5	59.5	38.5	38.5	21.0	119.0	70.0	101.5	77.0	91.0
	3	91.0	66.5	70.0	56.0	52.5	129.5	126.0	140.0	70.0	38.5	87.5	84.0	80.5	80.5	94.5
	4	88.8	60.4	71.5	58.7	50.5	122.5	101.5	140.0	28.0	38.5	94.5	77.0	87.5	91.0	101.5
	5	77.0	59.5	73.5	59.5	49.0	112.0	147.0	129.5	105.0	80.5	98.0	87.5	63.0	87.5	94.5
	6	79.9	58.5	75.5	60.2	55.6	150.5	140.0	129.5	108.5	70.0	147.0	87.5	105.0	115.5	105.0
	7	84.0	59.5	73.5	63.0	56.0	203.0	161.0	143.5	108.5	80.5	140.0	59.5	94.5	98.0	98.0

Gas	Time (days)	Control (ppm)	100 A (ppm)	100 B (ppm)	300 A (ppm)	300 B (ppm)	Control (ppm)	500 A (ppm)	500 B (ppm)	1000 A (ppm)	1000 B (ppm)	Control (ppm)	1500 A (ppm)	1500 B (ppm)	3000 A (ppm)	3000 B (ppm)
N2	1	1562.0	1591.3	1591.3	1591.3	1620.7	476.7	821.3	447.3	836.0	718.7	1246.7	1232.0	1408.0	1305.3	1378.7
	3	1502.7	1625.8	1578.6	1559.2	1578.9	1019.3	1158.7	1320.0	1085.3	968.0	1034.0	1349.3	1305.3	1173.3	1386.0
	4	1488.7	1606.0	1554.7	1554.7	1584.0	1078.0	1129.3	1400.7	557.3	880.0	1114.7	1151.3	1364.0	1408.0	1415.3
	5	1235.2	1587.6	1521.2	1550.9	1550.8	1056.0	1349.3	1283.3	1488.7	1576.7	1114.7	1408.0	1004.7	1393.3	1408.0
	6	1356.7	1503.3	1510.7	1532.7	1598.7	1173.3	1217.3	1254.0	1415.3	1298.0	1342.0	1422.7	1466.7	1459.3	1437.3
	7	1151.3	1246.7	1217.3	1261.3	1268.7	1107.3	1239.3	1210.0	1224.7	1496.0	1327.3	1004.7	1364.0	1342.0	1408.0

Gas	Time (days)	Control (ppm)	100 A (ppm)	100 B (ppm)	300 A (ppm)	300 B (ppm)	Control (ppm)	500 A (ppm)	500 B (ppm)	1000 A (ppm)	1000 B (ppm)	Control (ppm)	1500 A (ppm)	1500 B (ppm)	3000 A (ppm)	3000 B (ppm)
CH4	1	34.8	23.2	23.2	11.6	23.2	11.6	11.6	11.6	11.6	0.0	116.0	11.6	23.2	23.2	34.8
	3	45.7	32.1	30.2	22.9	25.5	58.0	58.0	81.2	46.4	23.2	92.8	34.8	23.2	23.2	34.8
	4	69.6	46.4	46.4	34.8	34.8	69.6	81.2	127.6	23.2	34.8	104.4	11.6	23.2	23.2	23.2
	5	78.9	57.5	67.8	40.1	42.2	92.8	127.6	150.8	92.8	46.4	104.4	23.2	11.6	11.6	23.2
	6	116.0	92.8	92.8	58.0	69.6	139.2	150.8	174.0	127.6	81.2	150.8	23.2	23.2	34.8	11.6
	7	220.4	208.8	150.8	116.0	116.0	208.8	220.4	232.0	150.8	116.0	139.2	11.6	23.2	11.6	11.6