METHANE, CARBON DIOXIDE AND AMMONIA EMISSIONS FROM SIMULATED ANAEROBIC/FACULTATIVE LAGOONS

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1.0 INTRODUCTION

For many years, lagoons have been widely used to treat agricultural and animal wastes. Compared to activated sludge and other advanced treatment systems, lagoons are simple to build, economical to operate, and easy to manage. However, lagoons are open to the atmosphere, and can contribute large amounts of nitrogen (Jones et al., 2000) methane (CH_4) and carbon dioxide (CO_2) (Sharpe and Harper, 1999) into the air.

Agricultural ammonia (NH_3) emissions are linked to various negative effect on ecosystem and human health, such as acidification, eutrophication, particulate matter formation, and loss of biodiversity (Kurvits and Marta, 1998). Both methane and carbon dioxide are greenhouse gases and have been proven to be very effective at trapping infrared radiation. These gases also persist for relatively long periods in the atmosphere, which can lead to global warming (El Fadel and Massoud, 2001).

Some researchers indicate high ammonia emission from lagoons, while others believe that ammonia gas emission is not as significant as dinitrogen gas emission (Jones et al., 2000; Aneja et al., 2000; Harper and Sharpe, 1998). Current estimates of methane emissions are based on biogas production from anaerobic digesters and covered lagoons (Hashimoto, 1983; Hill, 1984; Safley and Westerman, 1988). However, most of the digesters have higher volumetric loading rate, which would increase biogas production. No literature was found with quantified carbon dioxide emission from anaerobic and facultative lagoons.

This thesis reports the results of studies that were conducted to address the gaseous emissions under varying conditions such as mixing, heating, lighting, etc. in a

pilot facility built to simulate anaerobic/facultative lagoons. The first objective was to measure methane, carbon dioxide and ammonia emissions from simulated anaerobic/facultative lagoons during mid-summer in Oklahoma. The second objective was to investigate the influence of different physical and operational factors on these emissions. The final objective was to find possible pathways for the gas formation. These research objectives address a significant area of concern in biological treatment of animal waste. The negative impact of the mentioned gases is directly connected with the amount of gases produced.

2.0 LITERATURE REVIEW

2.1 TYPES OF LAGOONS

Lagoons typically are designed as one of three distinctive types: aerobic, anaerobic and facultative.

A lagoon is aerobic when sufficient dissolved or free oxygen is available to allow aerobic bacteria to flourish. Usually, these lagoons have a depth range from 2 to 5 feet for easier oxygen diffusion and light penetration, which is necessary for the survival of the aerobic bacteria and the growth of algae (Hamilton et al., 2002). Aerobic lagoons typically produce a minimal amount of odor and are biologically lightly loaded, i.e. the organic matter added per unit volume of lagoon per unit time is very low. However, they have much larger surface areas than other types of lagoons.

Anaerobic lagoons are generally deep and have small surface areas compared to organic loading rate. The primary characteristic of anaerobic lagoon is that they contain anaerobic bacteria that thrive and grow without free oxygen. Anaerobic bacteria are very efficient and effective at decomposing most kinds of organic matter. However, they frequently give off unpleasant odors due to the fact that decomposition progresses primarily to the point of volatile organic compounds, CH_4 and NH_3. Most manure handling systems in use on livestock farms today use either earthen storage ponds or anaerobic lagoons. The primary design criterion for anaerobic lagoons is pounds of organic matter per unit volume per day (volumetric loading rate or VLR). As this loading rate increases, the likelihood of objectionable odors increases. Lagoons are designed

based on a given loading rate. The optimal pH for strictly anaerobic lagoons is about 6.5. When pH is below this level, methane producing bacteria are inhibited by free hydrogen ion concentration; therefore, loading of anaerobic lagoons should be done carefully, because overloading organic matter can stimulate the facultative acid-producing bacteria (USDA, 1992).

A facultative lagoon is a hybrid system with both aerobic and anaerobic features. The anaerobic digestion of organic matter is maintained in the bottom zone of the lagoon. The top zone may be dilute enough to allow dissolved oxygen to be present and maintain an aerobic layer. This results in clarification of this surface layer and keeps odor release to a minimum. The intermediate zone favors the growth of facultative bacteria which are capable of operating, growing and thriving in either aerobic or anaerobic conditions as the lagoon characteristics change (Tchobanoglous and Burton, 1979). Typically, facultative lagoons generate minimal odor due to the presence of photosynthetic bacteria, which reduce odors (Hamilton et al., 2002).

2.2 ENVIRONMENTAL CONCEQUENCES OF CH_4, CO_2 AND NH_3 EMISSIONS

Atmospheric ammonia (NH_3), methane (CH_4), and carbon dioxide (CO_2) are very important constituents of the global environmental system.

NH_3 is known to affect ecosystems at relatively low concentrations, due to its high water solubility. The lifetime of released ammonia in the atmosphere is 1-5 days. In the atmosphere, NH_3 can react with acidic species to form ammonium sulfate, ammonium nitrate or ammonium chloride, or it may be deposited to the earth's surface close to its

source (Aneja et al., 2001). There are several environmental concerns associated with atmospheric NH_3 and its deposition, such as the biological transformation of NH_4^+ to NO_3^- in soils (nitrification) and plant uptake release acidity into the soil, contributing to acidification. NH_3 deposition can also give rise to eutrophication, where nutrient enrichment changes ecosystems. Among all contributors to ammonia emission, agriculture is a major source. Recent measurements of ammonia in the UK have shown that ammonia loss from agriculture is 406 x 10^3 tons of NH_3-N per year (Sutton et al., 1995). In North Carolina, which is the currently the second largest pork producing state in US, emission of ammonia from swine waste was 65,540 tons per year, which corresponds to 20.6% of total nitrogen emission in North Carolina as estimated in 1995 (Walker et al.,2000). Moreover, Harper and Sharp (1998) measured ammonia flux near the surface of three lagoons in the south-eastern US Coastal Plain and found ammonia emission in the range 4.9-10.5 kg NH_3-N ha^{-1} day^{-1}.

Carbon dioxide (CO_2) is the most important anthropogenic greenhouse gas, which is expected to contribute about 50% of total global warming over the next 50 years. The second important greenhouse gas, expected to contribute 18% of total expected global warming, is methane (Milich, 1999). As greenhouse gas builds up in the atmosphere, it traps energy from the sun like a layer of insulation.

Methane emissions from domesticated animals and animal wastes in the US are about 8,400,000 tons $year^{-1}$, or about 30% of the total US annual anthropogenic emissions. Swine waste contributes about 1,100,000 tons $year^{-1}$. Daily methane emission from anaerobic swine lagoons ranged from 20 to 115 kg CH_4 ha^{-1} day^{-1} (Sharpe et al., 2001a). The average concentration of CO_2 in the atmosphere reached 360 parts per

million by volume (ppmv) in 1994 compared to 280 ppmv almost 100 years ago (Schlosser et. al., 2002). In contrast, the concentration of methane in atmosphere is 1.75 ppmv (Shively et al., 2001). However, one molecule of CH_4 traps about 30 times more heat than CO_2 (Alberto et al., 2000). Microbial decomposition accounts for 86% of the carbon dioxide produced, followed by animal carbon dioxide production at 10% (Shively et al., 2001).

2.3 COSTITUENTS OF SWINE WASTE

The composition of swine waste is complex. The largest constituents are carbohydrates, fats and proteins. Carbohydrates (particularly hemicellulose, cellulose and lignin) are primarily found in undigested feed materials. Fats are usually in form of lipids. These are mainly from vegetable feeding. The VFA content ranges from 8,000 to 14,000 mg/l (Masse et al., 2000). Proteins are mainly from undigested feed and microbial biomass. Major components of swine waste are presented in table 2.1.

Table 2.1. Some representative analyses of swine waste.

Solids (%)	
Lipids	13.72
Cellulose	15.48
Hemicellulose	22.31
Lignin	8.12
Crude protein	19.88
Ammonia N	2.15
P	1.67
K	0.93
Ash	13.96
Starch	0.00
Supernatant (% wt/vol)	
Total N	0.25
Ammonia N	0.21
P	0.014
K	0.06

Adapted from Hobson et al.,1981

Variations in manure slurry characteristics are possible due to differences in pig metabolism and diets. (Masse et al., 2000)

2.4 MICROBIAL AND CHEMICAL DEGRADATION OF ORGANIC MATTERS

Manure degradation is a multi-step chemical and biological process. Once manure is deposited to the lagoon, settable solids fall to the bottom, where they are converted to sludge, soluble liquids and gases. Soluble solids enter directly into the liquid portion of the treatment system (Hamilton et al., 2002). This can be represented by the scheme shown in Fig. 2.1.

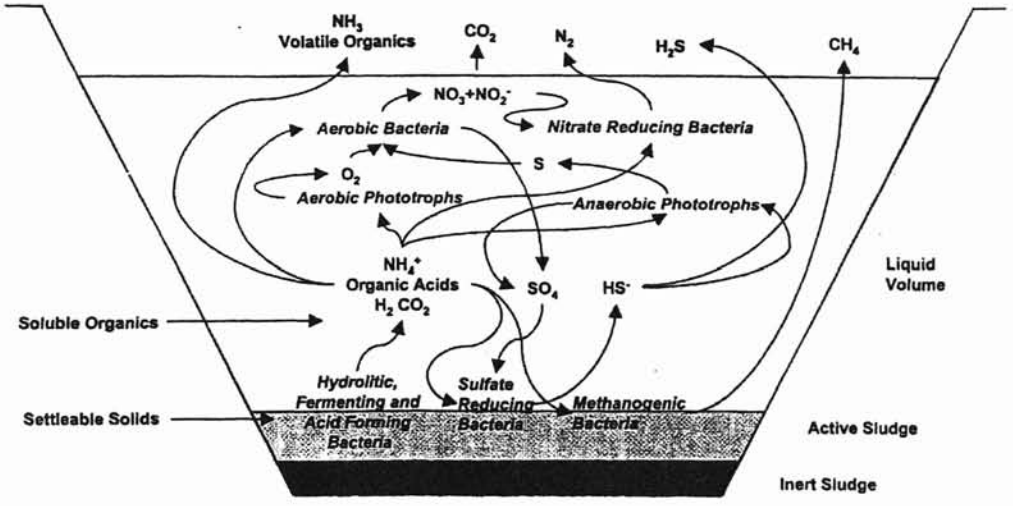


Fig. 2.1 Degradation of manure in uncovered anaerobic/facultative lagoons (Hamilton et al., 2002)

The microbiological degradation of organic materials in an anaerobic environment can only be accomplished by organisms which are able to use molecules other than oxygen as hydrogen acceptor. This anaerobic decomposition ultimately results

in biogas production which consists of methane (50-70%) and carbon dioxide (25-45%) and small amounts of hydrogen, nitrogen and hydrogen sulfide (Price and Cheremisinoff, 1981). There are four metabolic stages in anaerobic digestion of biological materials (Le Mer and Roger, 2001; Veeken et al., 2000):

Hydrolysis – complex insoluble organic material is solubilized by enzymes excreted by hydrolytic microorganisms. For example cellulose hydrolysis (equation 1).

(1) $\text{Cellulose} + H_2O \xrightarrow[\text{Cellulase}]{\text{Endogluconase}} \text{Cellodextrin} + \text{Cellobiose}$

$\text{Cellodextrin} + P_i \xrightarrow[\text{phosphorylase}]{\text{Cellodextrin}} \text{Glucose-1-P} + \text{Cellobiose}$

$\text{Cellobiose} + P_i \xrightarrow[\text{phosphorylase}]{\text{Cellobiose}} \text{Glucose} + \text{Glucose-1-P}$

Acidogenisis - soluble organic components including the products of hydrolysis are converted into organic acids, alcohols, hydrogen and carbon dioxide. For example, glucose fermentation, where the final products are butyric acid, carbon dioxide and hydrogen (equation 2).

(2) $C_6H_{12}O_6 \longrightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$

Acetogenisis – the products of acidogenesis are converted into acetic acid, hydrogen and carbon dioxide. For example, butyrate can be converted to acetic acid, and hydrogen (equation 3). With propionate as a substrate, carbon dioxide is produced in addition to acetate and hydrogen (equation 4).

(3) $CH_3CH_2CH_2COOH \longrightarrow 2CH_3COOH + 2H_2$

(4) $CH_3CH_2COOH \longrightarrow CH_3COOH + CO_2 + H_2$

Methanogenesis – methane is produced from acetic acid (equation 5), hydrogen, and carbon dioxide (equation 6) as well directly from other substrates such as formic acid (equation 7) and methanol.

(5) $CH_3COOH \longrightarrow CH_4 + CO_2$

(6) $4H_2 + CO_2 \longrightarrow CH_4 + 2H_2O$

(7) $4CH_2O_2 \longrightarrow CH_4 + 3CO_2 + 2H_2O$

During anaerobic digestion, various organic substances are depolymerized, then gradually decomposed and changed to VFA. Carbohydrates and fats are the main sources of fermentation acid and hydrogen (Hobson et al., 1981). The microorganisms responsible for fermentation of carbohydrates are described in the table 2.2.

Table 2.2 Metabolic Products Released During the Anaerobic Fermentation of Carbohydrates

Organism	Substrate	Products*
Bacteroides succinogenes	Cellulose	F,A,S
Bacteroides fibrisolvens	Cellulose	F,L,H_2,CO_2
	Hemicellulose	F,B,L, H_2,CO_2
	Pectin	F,A,B,L,M, H_2,CO_2
Bacteroides ruminicola	Hemicellulose	F,A,P,S
	Pectin	F,A,P,B,L,S
Ruminococcus flavefaciens	Cellulose	F,A,S,H_2,CO_2
Neocallimastix frontalis	Cellulose	F,A,L,E,CO_2
Rumen spirochetes	Pectin	F,A,M,L,S
Rumen treponemes	Pectin	F,A,S,M
Lachnospira multiparus	Pectin	F,A,L,M,E, H_2,CO_2
Acetivibrio cellulolyticus	Cellulose	A,E, H_2,CO_2
Clostridium thermocellum	Cellulose	A,E, H_2,CO_2
Clostridium papyrosolvens	Cellulose	F,A,L,E

*F = formate; A = acetate; P = propionate; B = butyrate; S = succinate; L = lactate ; M = methanol;E = ethanol; Adapted from Chynoweth and Isaacson, 1987

The most important source of carbohydrates in faeces, especially in animal excreta, is a residue of vegetables matter from the feedstuffs passing through the gut (Hobson et al., 1981). These are cellulose, hemicellulose and lignin.

The degradation of cellulose can be initiated by two extracellular enzymes. These are β-1,4-endoglucanase and β-1,4-exoglucanase. The endogluconase hydrolyzes cellulose molecules randomly within the polymer, producing smaller and smaller cellulose molecules. Exogluconase consequently hydrolyzes two glucose subunits from the reducing end of the cellulose molecule, releasing the disaccharide cellobiose. A third enzyme, β-glucosidase (or cellobiose) then hydrolyzes cellobiose to glucose (Maier et al., 2000). Degradation of hemicellulose is similar to the cellulose degradation process, except that many more extracellular enzymes are involved, because the molecule is more heterogeneous. An example of a hemicellulose polymer is a pectin molecule. The final product of hemicellulose is also glucose. Biodegradation of lignin is slower and less complete than degradation of other organic polymers. This is because it is constructed as a highly heterogeneous polymer, and, in addition, contains aromatic residues rather than carbohydrates residues. Biodegradation of lignin occurs only under aerobic conditions, because reactive oxygen is needed to release lignin residues; however, once residues are released, they can be degraded under anaerobic conditions (Maier et al., 2000).

Glucose, which is the final product in cellulose, hemicellulose and lignin degradation, can undergo further breakdown. Glycolysis, Krebs cycles, and glucose fermentation are three major pathways for aerobic and anaerobic degradation of glucose.

The first pathway, glycolysis, involves 10 enzyme reactions and produces two molecules of pyruvic acid and two molecules of ATP from one molecule of glucose (Bergquist and Pogosian, 2000) (equation 8)

(8) $C_6H_{12}O_6 + 2ADP + 2Pi \xrightarrow{glycolysis} 2CH_3COCOOH + 2ATP$

No oxygen is required for glycolysis to occur. The end product of glycolysis, pyruvate or pyruvic acid, participates in many reactions in cells. The two pyruvic acid molecules formed in glycoslysis may be used by homofermentative lactic acid bacteria to produce lactic acid by a simple expedient of enzymatic addition of two hydrogens. Or, it may be converted into ethanol, acetate and carbon dioxide by other enzymes (Anderson, 1973). This is presented in the Fig. 2.2

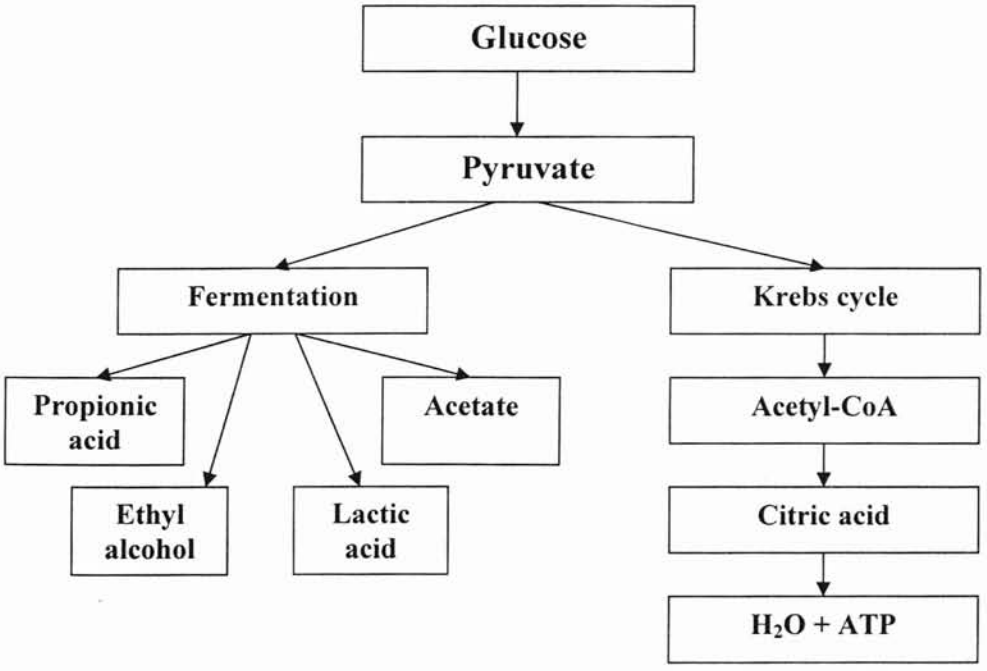


Figure 2.2 Pathways of glucose degradation

In aerobic respiration, pyruvate connects glycolysis to the citric acid cycle by an enzyme converting the pyruvate to acetyl-coenzyme A (acetyl-CoA) and carbon dioxide while transferring two hydrogen ions to NAD^+. The two electrons and hydrogen ions accepted by NAD^+ reduce this molecule to NADH + H^+. Coenzyme A (CoA) is a sulfur containing carrier molecule that transports the acetyl group to the citric acid cycle (Bergquist and Pogosian, 2000). (equation 9)

(9) $CH_3COCOOH + CoA\text{-}SH + NAD^+ \longrightarrow 2CH_3CO\text{-}S\text{-}CoA + CO_2 + NADH$

Anaerobic fermentation is not as efficient as aerobic respiration in gaining energy from sugars. In most fermentations, the net yield is the two molecules of ATP. The end products of anaerobic fermentation are alcohols and CO_2. The overall reaction for glucose in an alcoholic fermentation is described in equation 10.

(10) $C_6H_{12}O_6 + 2ADP + 2Pi \longrightarrow 2C_2H_5OH + 2CO_2 + 2ATP$

Anaerobic homofermentative organisms can produce lactic acid. These bacteria are *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. The overall reaction for homofermentatation of glucose is described as equation 11.

(11) $C_6H_{12}O_6 + 2ADP + 2Pi \longrightarrow 2C_3H_6O_3 + 2ATP$

Heterofermentative microorganisms produce mixtures of acids, alcohols, and gases as the end products of fermentation. The produced alcohols from anaerobic fermentation may be converted to acetate in the absence of H_2. For instance, methanol is converted to acetate, by means of CO_2 (equation 12)

(12) $4CH_3OH + 2CO_2 \longrightarrow 3CH_3COOH + 2H_2O$

This reaction is strictly dependent on the presence of Na^+. The bacteria that accomplish this transformation are *B.methylotrophicum* and *Sporomusa acidovorans* (Drake, 1994).

Fats are found in swine waste, as they are constituents of vegetable matter. Swine waste fats are mostly lipids, compounds of glycerol, and long chain fatty acids. Besides residue from food lipids, faecal waste also contain lipids of the intestinal bacteria, and these can amount 5-10% of the bacterial weight (Hobson et al., 1981). Lipids are large molecules and cannot be transported across the membrane of bacteria. Consequently, lipids are broken down to acid by extracellular enzymes called lipases. The result of this digestion is a hydrophilic glycerol molecule and long-chain fatty acids of various chain length. Fatty acids are then degraded by a β-oxidation mechanism. First, a fatty acid is activated by the addition of coenzyme-A to the end. This activation requires energy in the form of ATP, but is performed only once per molecule. The β-carbon is then oxidized from CH_2 to C=O by three reactions. An enzyme called *β-ketothiolase* splits the fatty acid into acetyl-Co-A and adds another coenzyme-A to the previously oxidized β-group on the fatty acid. The fatty acid is now two carbons shorter and an Acetyl-CoA has been generated. The overall reaction for LCFA oxidation can be represented as an equation 13 (Anonymous, 1992).

(13) $CH_3(CH_2)_nCH_2CH_2COOH+2H_2O \longrightarrow CH_3(CH_2)_nCOOH+CH_3COOH + 4H$

Hobson (1981) indicated that the acids found in the digesters are predominantly acetic, with little or no propionic or butyric acids. No lactic or succinic acids were found. This was explained by high populations of lactic and succinic acid fermenting bacteria present in the digester. For instance, the concentration of bacteria fermenting lactic acid to acetic and propionic was about 3×10^7 cells per ml of swine waste digester sludge. Thus, any lactic or succinic acid from the fermentation of sugars would be immediately

used up (Hobson et. al., 1981). A well functioning anaerobic digester usually has a VFA concentration below 800 mg/l.

2.5 METHANOGENESIS

Methane production is a slow process. It is a rate limited step of anaerobic degradation (Henze and Harremoës, 1983). Generally, it is assumed that methane production can only occur in absence of oxygen. However, some methanogens were able to survive in aerobic phase, even in pure culture. The methanogens *Methanosarcina barkeri, Methanobacterium bryantii, Methanothrix soehngenii, Methanobacterium therma-tropicum and Methanobrevibacter arboriphilus* all exibit tolerance to low oxygen level (Zitomer, 1998). There are four types of strictly anaerobic bacteria known to produce methane (Price and Cheremisinoff, 1981):

1. *Methanobacterium*, a nonspore-forming rod;
2. *Methanobacillus*, a spore-forming rod;
3. *Methanococcus*, a nonspore-forming coccus; and
4. *Methanosarcina*, a nonspore-forming coccus in packets of eight

Each specie within the four groups is very restrictive to its carbon source. Some of the methanogenic bacteria and their substrates are presented in the table 2.3

Table 2.3 Methanogenic bacteria and their substrates.

Species	Substrates
Methanobacterium thermoautotrophicum	H_2, CO_2
Methanobacterium wolfei DSM 2970	H_2, CO_2
Methanococcus vannielii DSM 1224	H_2, CO_2, formate
Methanococcus jannaschii JAL-1	H_2, CO_2
Methanosarcina barkeri MS	H_2, CO_2, methanol, acetate
Methanosarcina mazei S-6	Methanol, acetate, TMA*
Methanothrix soehngenii Opfikon	Acetate
Methanothrix concilii GP6	Acetate
Methanolobus tindarius Tindari 3	Methanol, TMA*
Methanobrevibacter ruminantium M1	H_2, CO_2, formate

Adapted from (Chynoweth and Isaacson, 1987) TMA* - trimethylamine

The main substrates for methanogenesis are acetic acid, hydrogen and carbon dioxide (Henze and Harremoës, 1983). Approximately 70 % of the digester methane comes from acetate and the rest of it is from CO_2 and H_2 (Chynoweth and Isaacson, 1987). The bacteria producing methane from hydrogen and carbon dioxide are fast growing, as compared to the acetic acid utilizing bacteria (Henze and Harremoës, 1983). The formation of methane from propionic and byturic acids has been demonstrated in cultures obtained from diluted digesters. However, such cultures contained a mixture of bacteria. Degradation of propionic acid is unfavorably affected by hydrogen, so if methane

formation from hydrogen is stopped, propionic acid will tend to accumulate in digesters (Hobson et al., 1981).

Many methanogens metabolize formic acid as an energy source. Formate may be oxidized by formate dehydrogenase to CO_2 and protons. The mechanism of methane formation from acetate proceeds through the following steps. First, acetate is cleaved by decarboxylation and then the methyl group is reduced to methane with its hydrogen intact. Very little of the methyl group of acetate is converted to CO_2. Conversely, almost none of the carboxyl group of acetate is reduced to methane by axenic strains of *Methanosarcina* or *Methanothrix soehngenii.* Coenzyme M is probably involved in the final reduction step of methane production from acetate (Chynoweth and Isaacson, 1987).

Methanogenesis from CO_2 and H_2 is achieved through seven biochemical steps. CO_2 is bound to coenzyme methanofuran (MF) and subsequently reduced to formil-MF. This reaction is most probably driven by an electrochemical ion gradient across the membrane. The reaction is catalyzed by formil-MF dehydrogenase and is not yet fully understood. The enzyme contains a pterin cofactor as well as iron-sulfur centers. The formyl group is then transferred from formyl-MF to H_4MPT raising formyl-H_4MPT. The methenyl group is reduced via methylene intermediate. The produced methyl- H_4MPT combines with coenzyme M (CoM) with following methane formation (Drake, 1994).

Methanogenic organisms can tolerate extreme salinity and pH conditions, and generally show increased production with increased temperatures (Milich, 1999; Hill et al., 2001). Henze and Harremoës (1983) stated that acid production rate is high compared to the methane production rate, which means a sudden increase in easily degradable organics will result in increased acid production with accumulation of the acids. This

(14) $C_5H_4O_3N_4 + 1.5O_2 + 4H_2O \longrightarrow 5CO_2 + 4NH_3$

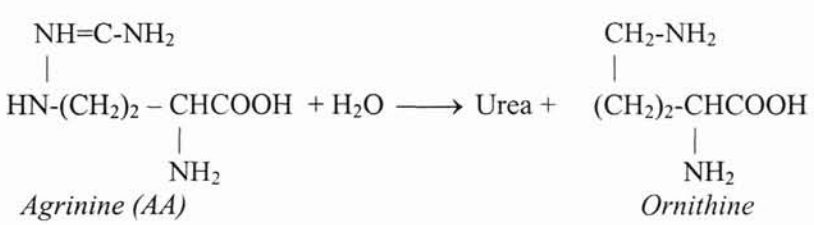
However, this reaction requires oxygen, and, therefore, should be only considered in aerobic lagoon systems (Arogo and Westerman, 2000).

Misselbrook et al., (2001) stated that ammonia emission is primarily from urea content of urine deposits, with possibly a small proportion from faeces. After urine and faeces are mixed, urea is hydrolyzed to NH_3 by the enzyme urease, which is produced by microorganisms present in faeces.

(15) $CO(NH_2)_2 + H_2O \xrightarrow{Urease} 2NH_3 + CO_2$ (Francis et al. 2002).

Important parameters influencing NH_3 emission from urea are urea concentration of the urine, urease activity, pH, temperature, air velocity, and contact area (Erisman and Monteny, 1998; van der Peet-Schwering et al., 1999). Some scientists believe that this reaction occurs very rapidly and can be assumed to occur mostly in buildings, just after the manure is produced (Arogo and Westerman, 2000). However, urea could be formed in lagoons through amino acid hydrolysis (equation 16) (Cantarow and Schepartz, 1962).

(16)



Ammonia may be produced from dietary amino acids and by catabolism of amino acids, particularly glutamine and glutamate, amines and nucleic acids (Cantarow and Schepartz, 1962). Anaerobic production of ammonia from proteins is relatively slow process compared to urea degradation (Arogo and Westerman, 2000).

The reaction of glutamine hydrolysis is described below (equation 17)

(17) $H_2NCOCH_2CH_2CHNH_2COOH + H_2O \xrightarrow{glutaminase} HOOCCH_2CH_2CH(NH_2)COOH + NH_3$

Tyrosine and tryptophan, which are largely present in feces and urine can contribute in ammonia emission equations 18 and 19 (Cantarow and Schepartz, 1962).

(18) Tyrosine $\xrightarrow{-CO2}$ Tyramine $\xrightarrow{+2H}$ CH_3NH_2 + Cresol $\longrightarrow$ Phenol

$\downarrow$ **$-NH_2$**

p-Hydroxyphenylpropionic acid $\longrightarrow$ p-Hydroxyphenylacetic acid $\xrightarrow{-CO2}$ Cresol

(19) Tryptophan $\xrightarrow{-NH2}$ Indolepropionic acid $\xrightarrow{-CO2}$ Ethylindole $\longrightarrow$ Sckatole

$\downarrow$ $-CO_2$

Indole ethylamine $\xrightarrow{+2H}$ Indole $+C_2H_5NH_2$

Amides also may undergo hydrolysis in either acidic (equation 20) or basic conditions (equation 21) (Solomons, 1992)

(20) $R\text{-}C(=O)\text{-}NH_2 + H_3O^+ \longrightarrow R\text{-}COOH + NH_4^+$

(21) $R\text{-}C(=O)\text{-}NH_2 + NaOH + H_2O \longrightarrow R\text{-}COONa + NH_3$

Amides hydrolysis (basic)

However, chemical reactions 20 and 21 are unlikely to occur in lagoons since, they require extreme pH($7.0 > pH > 8.0$) and high temperature($t > 40$) (Solomons, 1992).

2.7 NITRIFICATION AND DENITRIFICATION PROCESSES

2.7.1 AUTOTROPHIC NITRIFICATION

Autotrophic nitrification is the oxidation of NH_4 or NH_3 to NO_3^- via NO_2^-. This is an aerobic biological process, which uses two groups of microorganisms called Nitrobacteriaceae (Wrage et al., 2001). The oxidation of ammonia, via hydroxylamine, to nitrite includes three steps (reactions 22, 23 and 24).

(22) $NH_3 + O_2 + 2e + 2H^+ \xrightarrow{AMO} NH_2OH + H_2O$

(23) $NH_2OH + H_2O \xrightarrow{HAO} NO_2^- + 5H^+ + 4e$

(24) $2NO_2^- + O_2 \xrightarrow{nitrobacter} 2NO_3^-$

The first step is oxidation of ammonia to hydroxylamine by ammonia monooxygenase (AMO). Here, two electrons are needed for the reduction of one of the atoms of O_2 to water. The second reaction is catalyzed by hydroxylamine oxidoreductase (HAO). Hydrazine is an inhibitor of this enzyme. Finally, nitrite is oxidized to nitrate by *Nitrobacter* (Wrage et al., 2001; Jones et al., 2000). The highest activity of *Nitrobacter* was observed at pH=7.9 and t=37°C (Grunditz and Dalhammar, 2000).

2.7.2 DENITRIFICATION

Denitrification is the stepwise reduction of NO_3^- to N_2. In heterotrophic denitrification, organic compounds are used as electron donors. This is the most common form of denitrification (Jones et al., 2000). This conversion proceeds in four steps

(reactions 25, 26, 27 and 28) (Richardson and Watmough, 1999; Jones et al., 2000). The reactions are carried out by many organism including: *Bacillus, Achromobacter, Pseudomonas, Micrococcus, Propioni-bacterium*. Most of these bacteria are facultative and able to use NO_3^- in place of oxygen as an electron acceptor in respiration in low oxygen or anaerobic conditions (Wrage et al., 2001).

(25) $NO_3^- + 2H^+ + 2e^- \xrightarrow{\text{Nitrate reductases}} NO_2^- + H_2O$

(26) $NO_2^- + 2H^+ + e^- \xrightarrow{\text{Nitrite reductase}} NO + H_2O$

(27) $2NO + 2H^+ + 2e \xrightarrow{\text{Nitric.oxide reductase}} N_2O + H_2O$

(28) $N_2O + 2H^+ + 2e \xrightarrow{\text{Nitrous.oxide reductase}} N_2 + H_2O$

Heterotrophic denitrifiers can use VFA or alcohols as a source of carbon (C). For example, in reaction (29) acetic acid is used as a substrate.

(29) $NO_3^- + CH_3COOH \xrightarrow{*} NO_2^- + CO_2 + H_2O$ (Tchobanoglous and Burton, 1979)
* *Bacillus, Achromobacter, Pseudomonas, Micrococcus*

2.7.3 CHEMODENITRIFICATION

Chemodenitrification is the chemical decomposition of intermediates from the oxidation of NH_4^+ to NO_2^-. These non biological reactions are known to occur in anoxic systems, usually at low pH (5.6-6.25) (Wrage et al., 2001; Jones et al., 2000). For example,in the van-Slyke reaction (30), amino groups react with nitrous acid (HNO_2^-) to produce dinitrogen gas (N_2) (Jones et al., 2000; Cantarow and Schepartz, 1962).

(30) $CO(NH_2)_2 + H_2O \xrightarrow{HNO2} 0.5N_2 + NH_2\text{-}COOH + 3H^+$

In ammonium nitrite (NH_4NO_2) decomposition, one mole of ammonium reacts with one mole of nitrous acid to form ammonium nitrite, which then decomposes to form dinitrogen gas, water, and hydronium ion (Jones et al., 2000) (equation 31).

(31) $NH_4NO_2 \longrightarrow N_2 + H_2O + H_3O^+$

Chemical denitrification is also possible at neutral pH in the range of 6-8. For example, in reaction (32) NO_2^- decomposition is by Fe^{2+} (Jones et al., 2000).

(32) $M_{red} + NO_2^- + 2H^+ \longrightarrow M^{ox} + H_2O + N_2O$,

where M is a metal cation, in our case Fe^{2+}.

Chemodenitrification is closely linked with nitrification, so it is often difficult to determine whether NO and N_2O are developed through nitrification from NH_4^+ or dinitrification from NO_2^- (Wrage et al., 2001).

2.7.4 ANAMMOX

Anaerobic ammonia oxidation (ANAMMOX) is a process where ammonia is oxidized to dinitrogen gas using ammonia as an electron donor and nitrite as an electron acceptor (Jetten et al., 2001; Richardson and Watmough, 1999). ANAMMOX is more favorable energetically than oxic nitrification (reactions 33 and 34) (Jetten et al., 2001):

(33) Nitrification: $NH_4^+ + O_2 \longrightarrow NO_2^-$ ΔG=-275 kJ/mol

(34) ANAMMOX: $NH_4^+ + NO_2^- \longrightarrow N_2$ ΔG=-357 kJ/mol

Jetten et. all., (2001) proposed the mechanism of dinitrogen gas formation through the hydrazine step. These reactions are 35, 36 and 37 (Richardson and Watmough, 1999).

(35) $NO_2^- + 5H^+ + 4e \longrightarrow NH_2OH + H_2O$

(36) $NH_2OH + NH_3 \longrightarrow N_2H_4 + H_2O$

(37) $N_2H_4 \longrightarrow N_2 + 4H^+ + 4e$

ANAMMOX is detectable in the temperature range, 20 to 43 ^{0}C and the pH range, 6.4 to 8.3 (Schmidt et al., 2000). Moreover, Thamdrup and Dalsgaard, (2002) reported that ANAMMOX is active at temperatures between 6 and 43 ^{0}C. However, in completely anaerobic conditions, these reactions are not favorable, because of the absence of nitrite (NO_2^-) and nitrate (NO_3^-).

The current literature has data about VFA, carbon dioxide and methane emission in digesters, peatlands and swamps. However, there is no such data from animal waste treatment lagoons. Also, there is a huge gap in the literature for ammonia and methane emission quantification in lagoon systems. This is mainly because of the difficulties with analytical methods. In this work we will try to fill the gaps in this area.

3.0 METHODS AND MATERIALS

3.1 EXPERIMENTAL APROACH

This study focused on the facultative lagoon emission of methane, carbon dioxide and ammonia gasses. The analytical methods, experimental procedures and chemicals used are described below.

3.2 REAGENTS-GRADE MATERIALS AND LABORATORY PROTOCOLS

The water (> 18 MΩ cm purity) used in all the experiments was produced by a Mill-Q purification system (Millipore Corp., CA) using deionization and reverse osmosis technology. The chemicals used in this experiment are acetic, propionic acids (both reagent grade, purchased from Spectrum Quality Products, Inc., Gardena, CA), butyric acid (99+%, Lancaster Synthesis, Pelham, NH); lactic acid (85%, EM Science, Gibbstown, NJ); Methansulfonic acid (99%, ACROS, NJ); ammonia standard (1000 ±5ppm as N, ORION Research, Inc., Beverly, MA); sodium hydroxide (98.3%, Fischer Scientific, Fair Lawn, NJ); COD (100-4500 mg COD/L range, Bioscience , Inc., Bethlehem, PA). The gasses used were: methane (99%, MATHESON TRI-GAS Inc., Twinsburg, OH); carbon dioxide (100%, MATHESON TRI-GAS Inc., Twinsburg, OH) and the gaseous mixture of carbon dioxide (5%), carbon monoxide (5%), nitrogen (5%), oxygen (5%), methane (4%), and hydrogen (4%) (MATHESON TRI-GAS Inc., Twinsburg, OH). Potassium hydrogen phthalate (99.97%, Scientific, Fair Lawn, NJ).

All glassware was washed with detergent, followed by triple-rinsing with tap water, Mili-Q water, and drying for 12 hours at room temperature (24^0C) before use.

3.3 BIOLOGICAL REACTORS

To study methane, carbon dioxide, ammonia emission processes, lagoon simulators were designed to reproduce the same environmental conditions as in anaerobic, aerobic and facultative lagoons. The design was developed taking into account heating, cooling, lighting, aeration, mixing, feeding and ventilation Fig 3.1 (Hamilton, 1998).

The columns were used to recreate the same thermal and chemical conditions present in commercial lagoons during midsummer in Stillwater, OK (35N). These conditions were established by an extensive survey of the lagoon at the OSU Swine Research Center during the Summer of 1997. The columns are 12 inches in nominal diameter, and 12 feet in depth (Fig 3.2). Two sets of two columns are coupled with the same lighting and heating systems to achieve two replicated sets of conditions from four columns. Column sets were connected by tubing at 0.5 and 4.5 ft to circulate of liquid using a recycling pump. The circulation was done on the daily basis at 7:00 am (lagoon time). Water baths were used to control the temperature as described in Hamilton (1998). The lights, designed to have the same intensity and wavelength of sunlight (Hamilton, 1998), were turned on at 7:00 am and turned off at 19:00 every day. An air conditioning system and wind tunnels (Cumba, 2000) controlled atmospheric conditions on one column of each two column set. The cross-section area of the wind

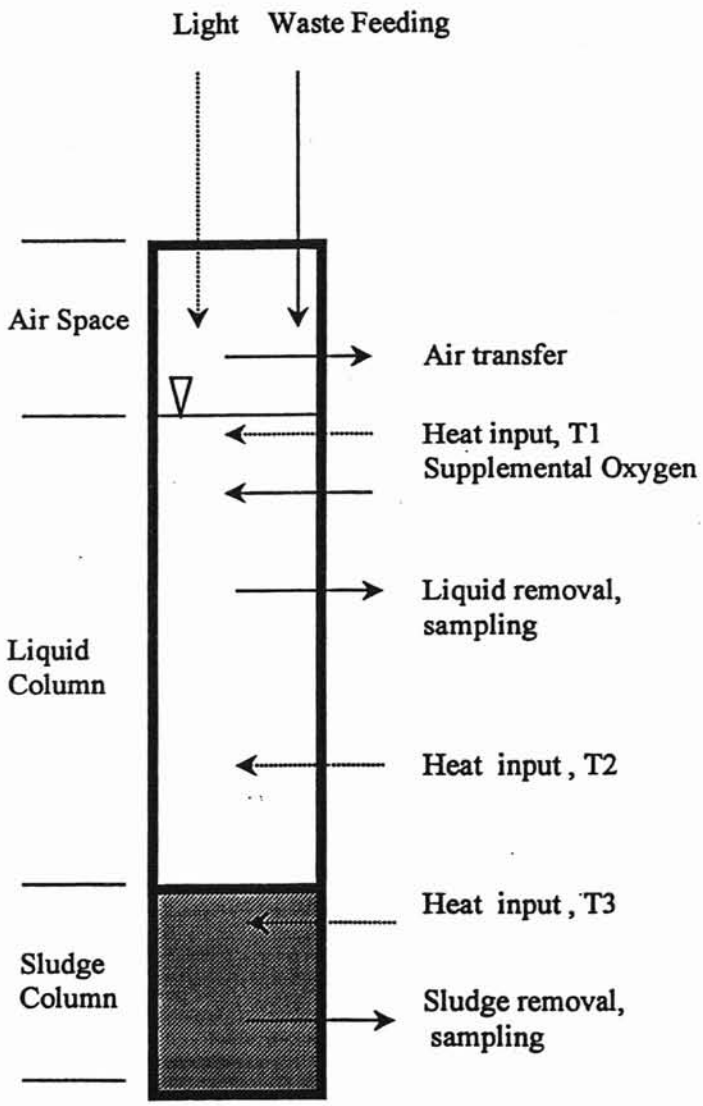


Figure 3.1 Pilot plant conceptual drawing (Hamilton, 1998)

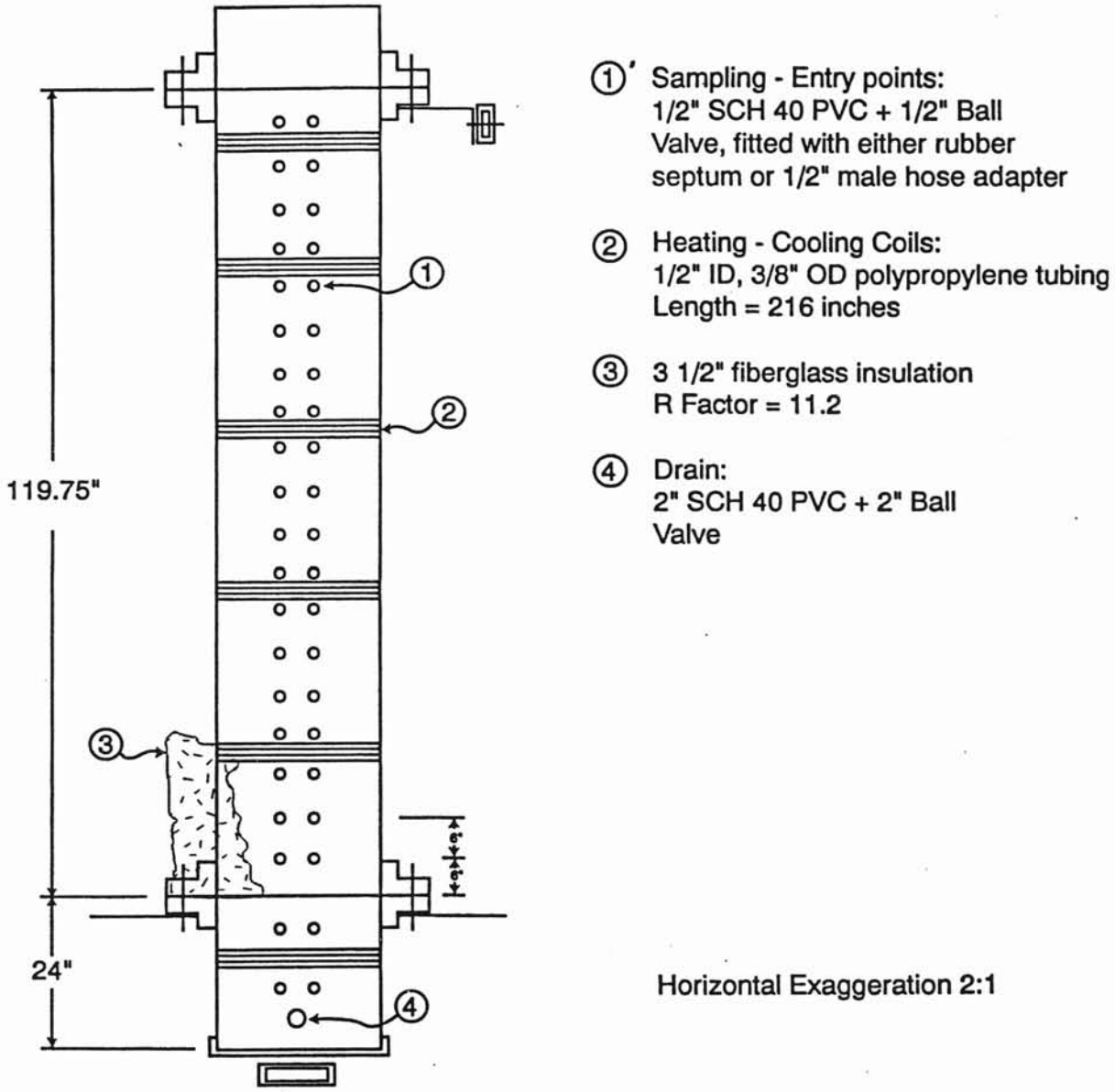


Figure 3.2 Pilot facility construction details (Hamilton, 1998)

tunnel was 0.0214 m^2 and the average wind speed through this cross-section area was 1.609 km/h. Therefore, the volumetric flow rate was 34.43 m^3/hour.

In August of 1998, the first inoculum for the columns was taken from the lagoon (Oklahoma State University swine facility). Then, in September 2001, the columns were partially drained of effluent, leaving the sludge layer intact. New liquid from the same lagoon was collected and transferred to the columns.

The columns were fed manually on a daily basis, as close to 12:00 as possible, with manure collected from pigs fed a fortified corn-soybean meal. The manure was collected from the Oklahoma State University swine facility. The amount of manure fed to the columns corresponded to the loading rate 1.5 lb.VS/10^3 ft^3. The characteristics of manure used on August 10, 2002 are shown in table 3.1.

Parameters	Values
TS, mg/l	122,360
VS, mg/l	95,545
pH	6.77
COD, g/l	102
TKN (dry), %	4.67
TKN, mg/l	10,968
NH_4^+, mg/l	4,807
TC, %	47.36
Acetate, mg/l	4,941
Propionate, mg/l	5,033
Butyrate, mg/l	8,376
Lactate, mg/l	2,181

Table 3.1 Raw manure characteristics (08/10/2002)

65 ml of raw manure was diluted with 685 ml of RO water to make a 750 ml mixture fed every day.

To determine if an equilibrium had been reached, samples from the reactors were monitored for: total solids, volatile solids, total and volatile suspended solids, pH, ammonia nitrogen, and total Kjeldahl nitrogen. After equilibrium was achieved (no major changes in the monitored parameters) in July of 2002, volatile fatty acids, NH_3, CH_4, and CO_2 were analyzed in addition to monitored parameters.

3.4 STANDARD CHARACTERIZATION METHODS

Lagoon simulator performance was evaluated by regularly monitoring and recording the following parameters for influent and effluent: total suspended solids (TSS), total solids (TS), volatile suspended solids (VSS), volatile solids (VS), total Kjeldahl nitrogen (TKN) according to APHA et al., (1998). The pH and redox potential were measured by Accumet 1001 pH/mV/Ion meter purchased from Fischer Scientific Inc. equipped with DO (Cole-Palmer) electrode. COD was measured by low range (100-4500 mg COD/l) accu-TEST ^{TM}, Chemical OD system EPA approved MICRO-COD (Bioscience Inc., Bethlehem, PA). Potassium hydrogen phthalate (KHP) served as a COD standard. For electro-conductivity (EC) measurement, a YSI 30 salinity, conductivity, temperature meter (Yellow Springs Instruments Company Inc., OH) was used.

3.5 METHANE AND CARBON DIOXIDE EMISSION

The release of methane and carbon dioxide from the lagoon simulator was measured by closed chamber system. The atmosphere immediately above the liquid surface was covered by a chamber and sampled 30 minutes after closure or at regular intervals over 30 min period after closure. For a constant net emission of methane and carbon dioxide, we have found that the concentration of carbon dioxide within the closed chamber is linear over a period of up to 1.5 h, and for methane up to 1h. Therefore, the time we have chosen was below 1 h.

The manual chamber was a modernized version of chamber used by Ball et al., (1999) for soil analysis. It was a 0.2 m-tall, 0.09 m diameter polypropylene (Nalgene) cylinder, pushed into piece of Styrofoam 0.15x0.15 m giving a headspace of $1.3x10^{-3}$ m^3. A piece of tubing was inserted into the top of the cylinder as an outlet. During sample collection, the tubing outlet was closed by a clamp. Samples were taken using a 5 ml glass syringe (VICI precision sampling, Baton Rouge, LA), by inserting the syringe needle to the tubing located on the top of the cylinder. After the sample was collected, the manual chamber was removed from the water surface to release accumulated gases and flushed with the air by an air compressor (GAST DOA, Benton Harbor, MI) for approximately 1.0 min. The sampled gas was immediately analyzed using an SRI 8610 C gas chromatograph (SRI, CA) fitted with the thermal conductivity (TCD) and helium ionization (HID) detectors. Separation was performed using a steel packed column 8'x1/8"x0.085 OD, SS, packed with HaySep Q, 80/100 mesh. Helium was used as a carrier gas with a constant pressure of 20 psi. The GC column was held at 32 ^{0}C for 4.0

min and then temperature was ramped to 72 ^{0}C for 8.0 min at 5.0 ^{0}C /min. The detector temperature was 150 ^{0}C. The GC was calibrated before and after each set of measurements in triplicate. For this purpose 99% methane and 100% carbon dioxide were used. One ml of the gas was diluted in a syringe with 39, 19, 9, 4, 3, 1 and 0 ml of carbon dioxide (for methane calibration) or methane (for carbon dioxide calibration). This gave 2.5, 5, 10, 20, 25, 50 and 100% of measured gas. Data were collected using SRI GC Peak Simple for Windows software data handling program. The average of the three measures was used.

3.6 AMMONIA GAS EMISSION

Ammonia gas emitted from columns equipped with wind-tunnels was measured with a single point meter (SPM) (Zellweger Analytics, IL). The SPM was equipped with a MDA continuous Chemcassette EP Monitoring System for aliphatic amines/ammonia (Zellweger Analytics, IL). The meter was connected to the wind tunnel. The gas was measured continuously. Flow rate on the SPM was set at 1.0 l/min; therefore, the sampled portion was 0.174% of the total air flow.

3.7 VOLATILE FATTY ACIDS MEASURMENT

Volatile fatty acid content was analyzed by ion chromatography. A Dionex DX 600 Ion Chrotatograph (IC) (Dionex) equipped with a conductivity detector ED50, AC11-HC IonPack column 4x250 mm, ASRS supressor and ATC-1 trap column and 25

μl loop was used. Eluents used were deionized water (A), 5mM NaOH (B) and 50mM NaOH (C). Total flow was 2.0 ml./min. During the sample run, eluents were mixed in the following proportions:

Run time, min	Eluent proportion, %
0-2	93% A, 7% B
2 -6	100% B
6 -9	50% B , 50% C
9 -18.99	hold 50% B , 50% C
19 -26	93%A, 7%B

Table 3.2 Ion Chromatography Eluent Concentration Gradient

Acetic, lactic, propionic and buturic acids were used for VFA standards. Triplicate standards (1, 2, 3, 4, 5, 10, 15, and 20 $mg \cdot l^{-1}$) were prepared in de-ionized water. Standards were filtered through OnGuard-H cartridges (Dionex) and analyzed by IC. The liquid samples were collected every hour from the lagoon simulator column at depth of 0.3, 1.22 and 3.0 m At a depth of 3.0 m, sludge had accumulated. 2.0 ml of sludge and 4.0 ml of column liquid were transferred to the separate 10.0 ml test tubes, and the aliquots of 8.0 and 4.0 ml respectively of reverse–osmosis pretreated water were added. The diluted samples were centrifuged at 3,400 rpm using ultra-centrifuge (IEC Centra GP8R, Need ham MA) for 15.0 min. The centrate was removed, filtered out through OnGuard-H cartridges (Dionex) and stored at 4.0 ^{o}C prior to analysis by the IC.

3.8 AMMONIUM MEASUREMENT

Ammonium content of the lagoon simulators was analyzed by Ion chromatograph (IC). A Dionex DX-600 Ion Chrotatograph, equipped with a conductivity detector ED50, CS12-A IonPack column 3x150 mm, CS12A 4-mm and CTC-1 trap column, and 25 µl loop was used. The eluent used was 33 mN Methansulfonic acid. The total flow rate was 1.0 ml/min The IC was calibrated with a minimum of seven calibration standards (20, 30, 40, 50, 100, 150 and 200 mg/L) of standard ammonia solution (1000±5 ppm. as N, ORION Research, Inc.,Beverly, MA). Three measurements were made for each sample or standard. The average of the three measurements was used for analysis.

4.0 RESULTS AND DISCUSSION

As mentioned in chapter 1, key objectives of this research were to measure methane, carbon dioxide and ammonia emissions from a simulated facultative lagoon. The lagoon simulators described in chapter 3 were used. Parameters used to determine an equilibrium were TS, VS, pH, EC, TKN and NH_4^+.

Since the major analyses for CH_4, CO_2 and VFA were conducted in the column A, most other analyses given in this section are taken from column A.

4.1 TEMPERATURE

Daily temperature trend at 0.5 ft. depth for all columns is shown in Fig. 4.1.

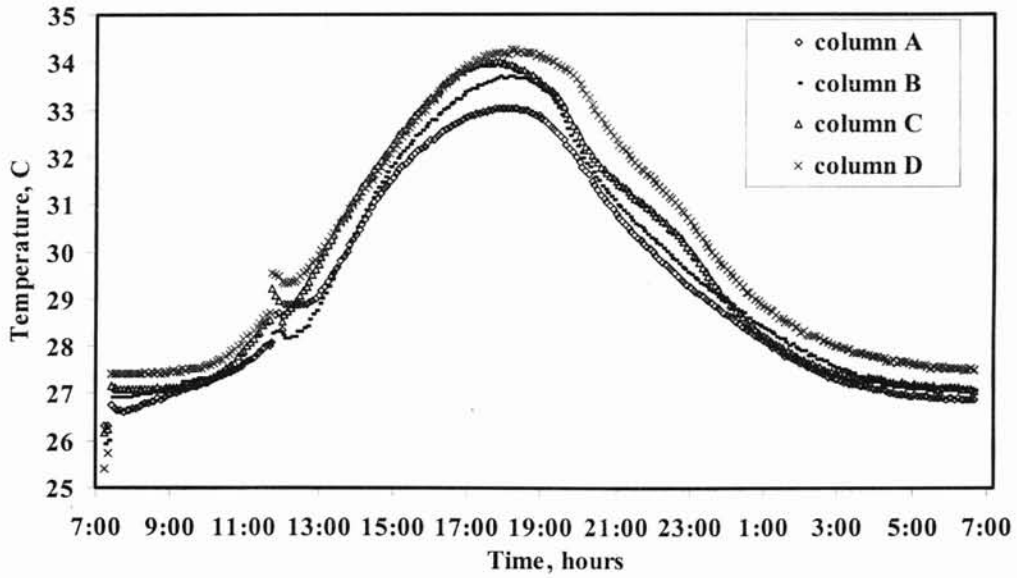


Figure 4.1. Daily temperature trend at 0.5 ft for all columns

The upper water baths began their heating cycle and lights switched on, creating a rise in temperature that is first noticeable in figure 4.1 at 9:00. On all columns, there is a spike around 12:00, which may be due to water baths compensating for a drop in temperature caused by feeding of the mixture. At 12:00, all columns were fed. After manure was deposited to the column, the temperature decreased at 0.5 ft. The sensor, which controls the water baths, was situated at 0.5 ft, in the middle of the column. The hot water ran through the upper coil, heating the liquid around it. The thermocouple, which reads the temperature shown in figure 4.1, was situated close to the wall of the column. The thermocouple picked up this local heating as a rise in the temperature right after the feeding.

The maximum temperature at 0.5 ft was reached about 19:00. This corresponds to the time when the light is turned off and water baths began their cooling cycle. The temperature at 0.5 ft. in all columns differs by 1-2 ^{0}C. This may be due to the fact that the columns C and B were equipped with Plexiglas wind tunnels. The Plexiglas absorbs and reflects radiation heat from the lamps, causing a relative decrease in the temperature. Columns A and D, did not have tunnels, and the lights radiated heat directly to the liquid surface.

Temperatures were not measured above 0.5 ft. Given the fact that this is the layer most affected by radiant heat from the lamps, it is difficult to speculate on actual liquid temperature above 0.5 ft.

During the course of experiments, the temperature profile for entire column was approximately the same as presented in Fig. 4.2. from 09/09/2002. The major temperature changes were above 2 ft. The mixing of the liquid at 7:30 seems to have no effect on the

temperature. This is due to the fact that the recycling pump was turned on (for 5 min.) at a time when the temperature at 4.5 ft was equal to the temperature at 0.5 ft.

There are very little daily temperature fluctuations below 2 ft. The lowest temperature was below 9.5 ft, and remained constant throughout the day. The highest temperature was at 0.5 ft and was equal to 34 ^{0}C at 19:00.

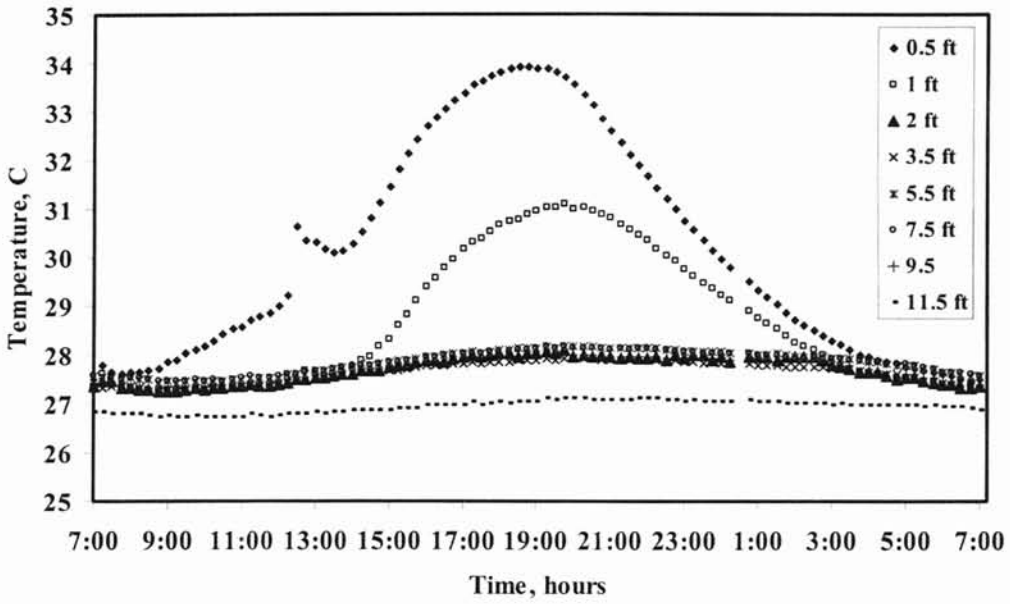


Figure 4.2. Temperature profile column A, 09/09/2002

4.2 TS & VS

TS and VS profile of column A are shown in Fig. 4.3. There is no major difference in solids concentrations between 0.5, 4.0 and 8.0 ft. TS and VS concentrations at these depths were about 3.5 g/l (TS) and 2.5 g/l (VS), respectively. Depths below 10 ft contained accumulated sludge; thus, TS and VS concentrations at 10 ft were much higher than for 0.5, 4.0 and 8.0 ft.

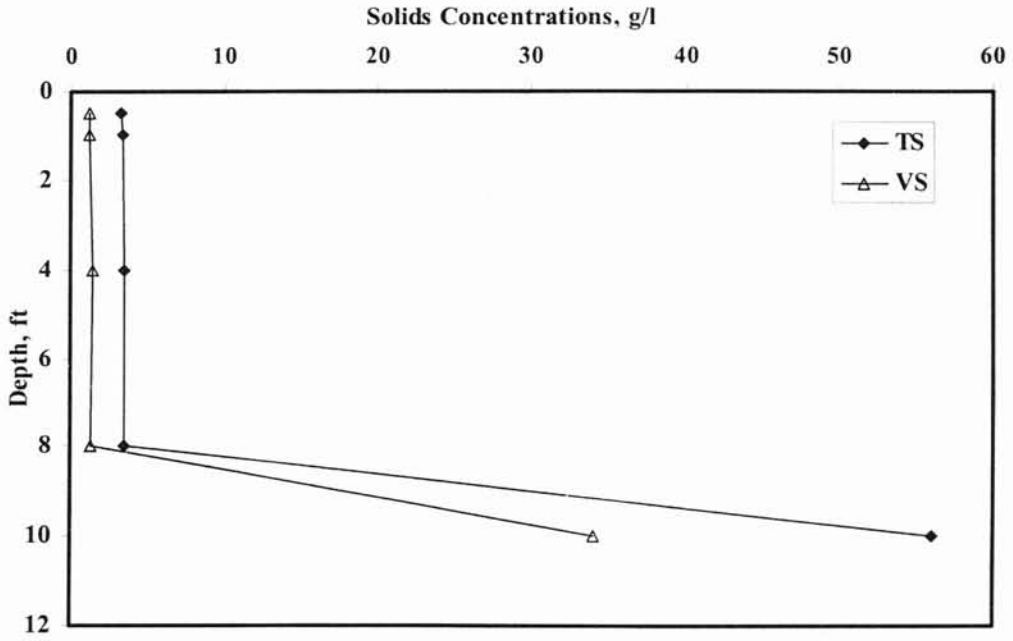


Figure 4.3. TS&VS profile of column A, 3/19/2002

The daily TS and VS trends for the period from 3/19/2002 to 9/7/2002 are presented in Fig. 4.4 and Fig. 4.5.

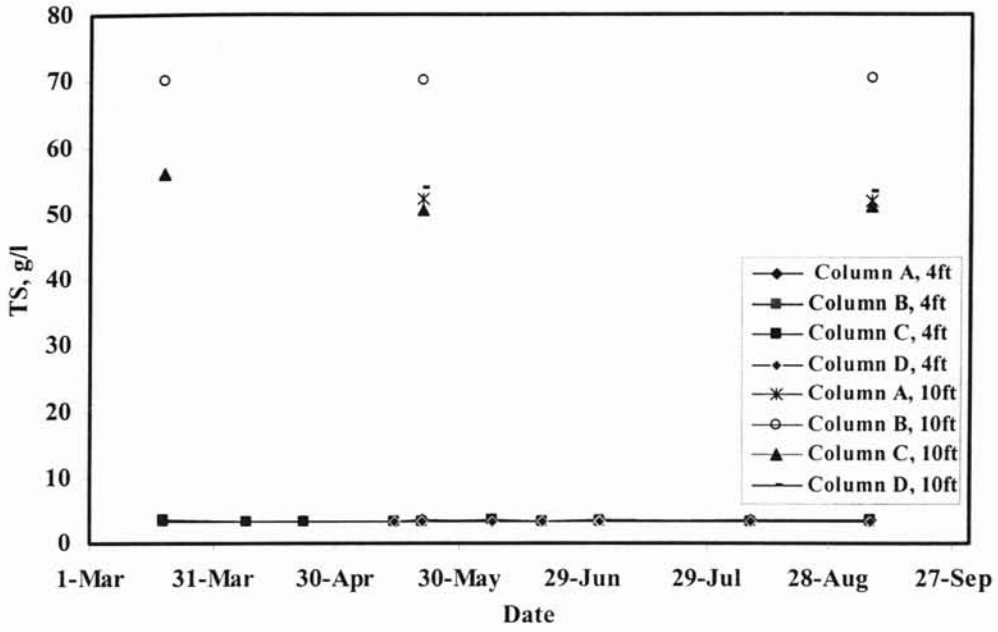


Figure 4.4. Daily Total Solids trend for all columns

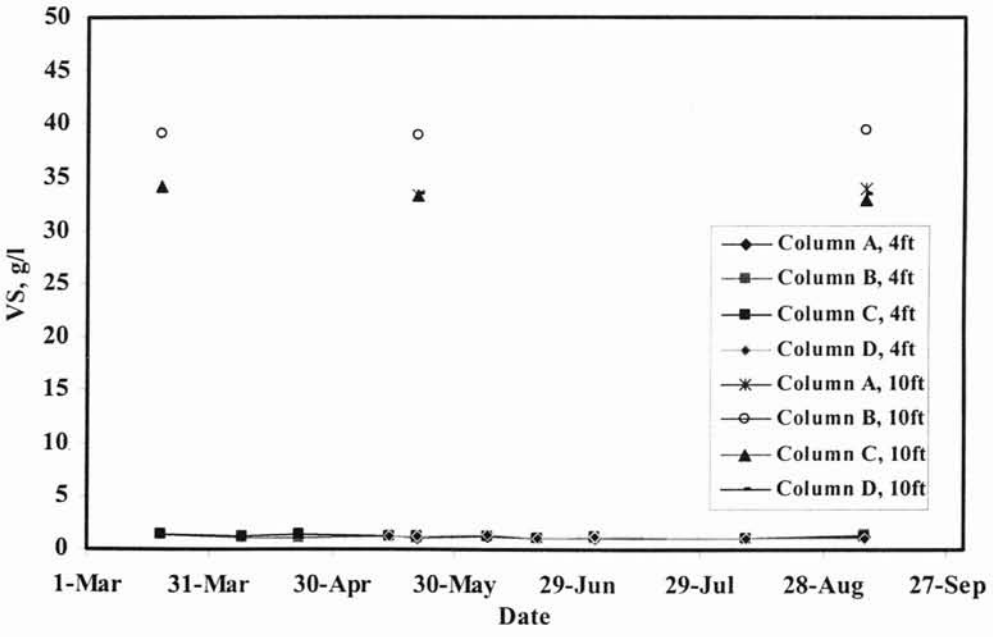


Figure 4.5. Daily Total Volatile Solids trend for all columns

At 4 ft depth, there is a little change in TS and VS concentrations during the investigated time. The solids concentration at this depth is approximately the same for all columns. However, there is a significant difference in solid concentrations between the group of columns A, C, D and column B at the depth below 10 ft. Column B apparently had more sludge at a depth of 10 ft. TS and VS concentrations at 10 ft in column B were 70 g/l and 39 mg/l, respectively, compared to 50-55 g/l and 34-35 g/l for columns A,C and D. Even though the liquid between columns A and B, was circulated, the solid concentration in column B was higher than in column A. This is due to the fact that recycling occurred at the depth above 8 ft; therefore, sludge was not disturbed below a depth of 8 ft.

4.3 pH

The pH profile for all columns taken on 08/17/2002 is shown in Fig. 4.6. There is a slight decrease in pH with depth from 0.5 to 10 ft; however, pH in the sludge zone (below 10 ft) is considerably lower than in the liquid zone above 10 ft.

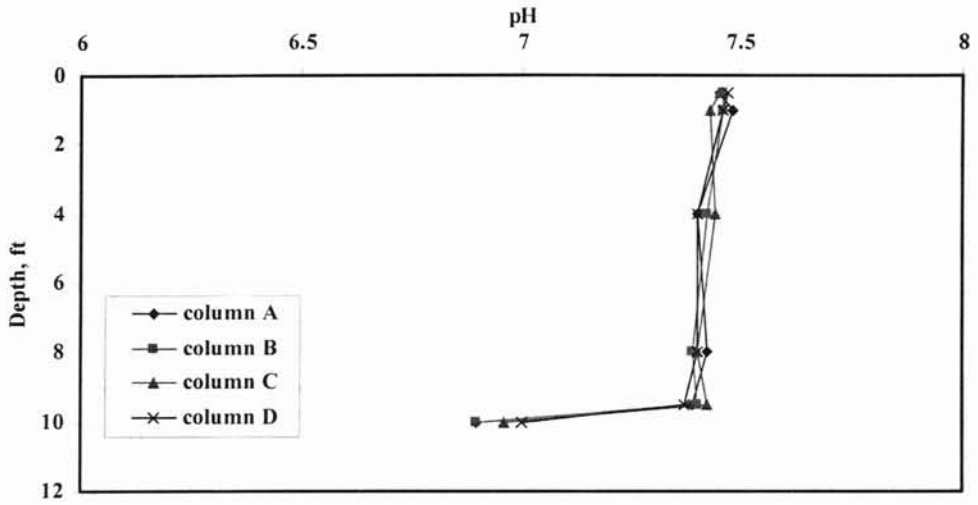


Figure 4.6. pH profile for all columns, 08/17/2002

During the tested period from February 3 to September 29, pH in the sludge level at 10 ft and below was constantly lower than at 1 and 4 ft, and was in the pH range of 6.9-7.2 (Fig. 4.7-4.10). Figures 4.7 through 4.10 show that pH in all columns was relatively stable throughout the experimental period.

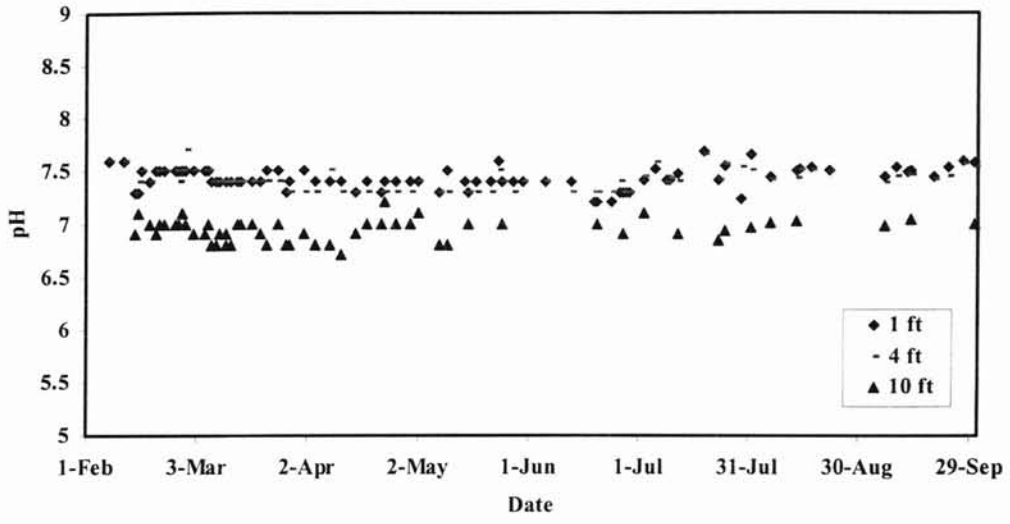


Figure 4.7. pH trend of column A

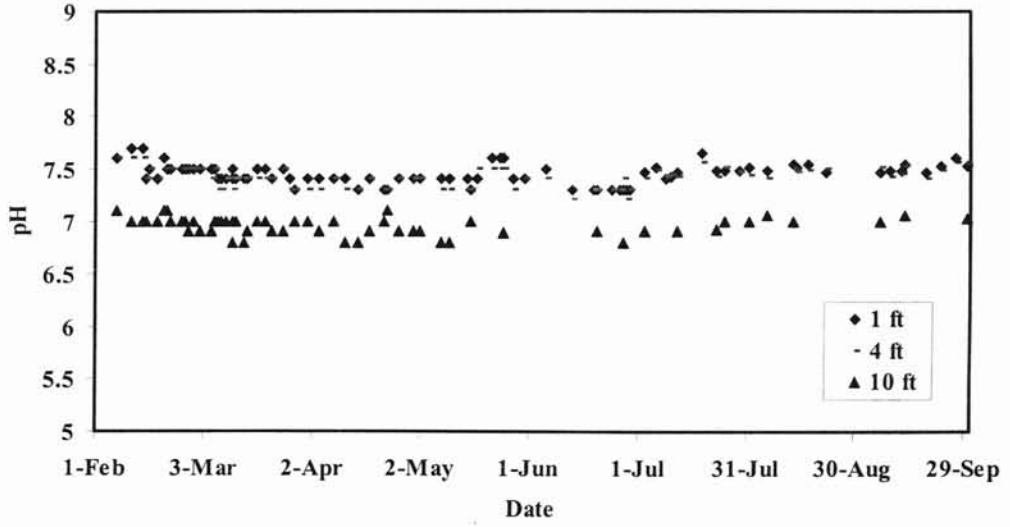


Figure 4.8. pH trend of column B

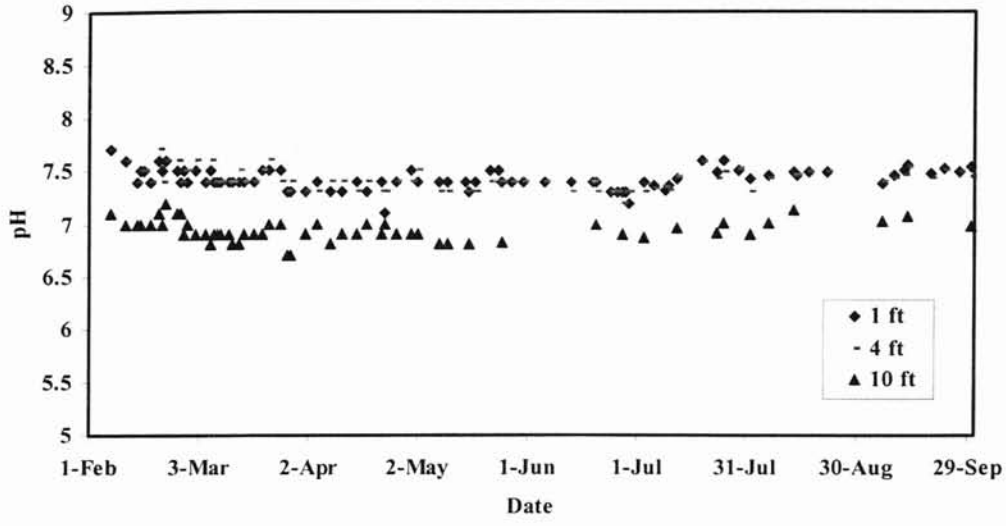


Figure 4.9. pH trend of column C

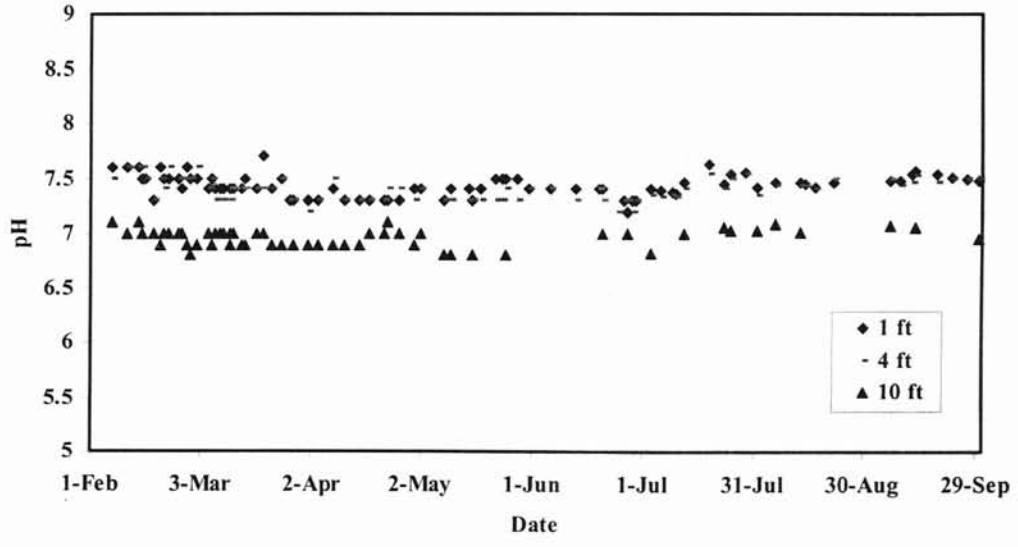


Figure 4.10. pH trend of column D

4.4 EC

The EC profile for all columns on 08/6/20 is shown in Fig. 4.11.

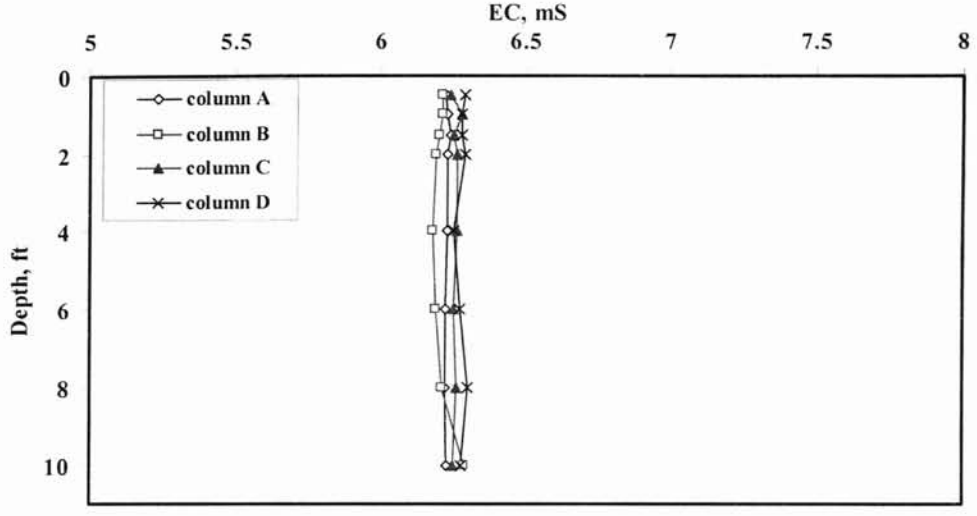


Figure 4.11. EC profile for all columns, 08/06/2002

There is little variation in EC for the liquid portion of the columns. EC values in each individual column were relatively constant from 0.5 ft to 10 ft. In all columns, EC trends could be divided into two parts, before and after July 1 (Fig. 4.12-4.14). After July 1, the EC values became more uniform. This may be explained by the fact that the recycling pump was plugged and liquid circulation was not reliable before July 1. After July 1, the tubing in the pump was cleaned out (algae was removed), the liquid started circulating and the EC values tended to be more homogenous. Thus, we can conclude that the amount of ions during the time was approximately the same after July 1.

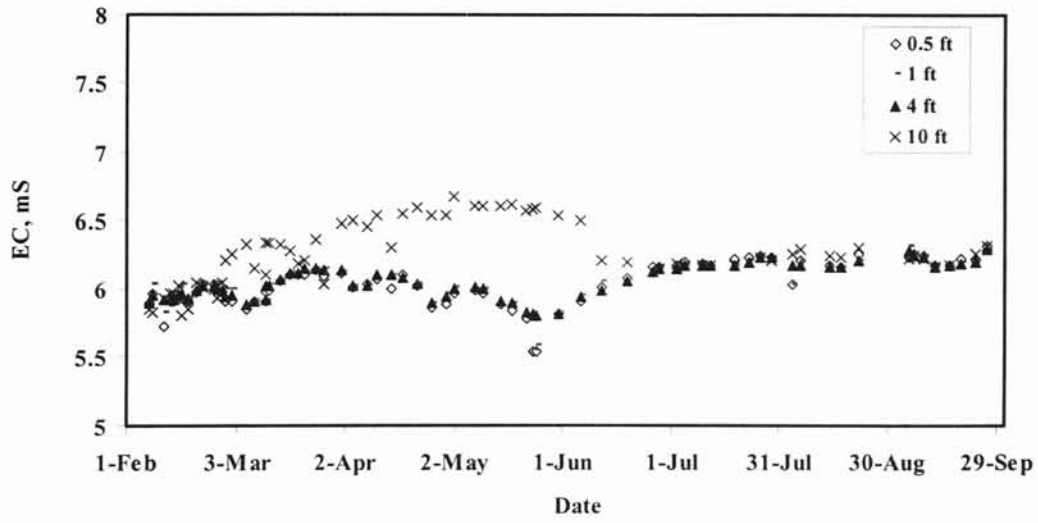


Figure 4.12. EC trend of column A

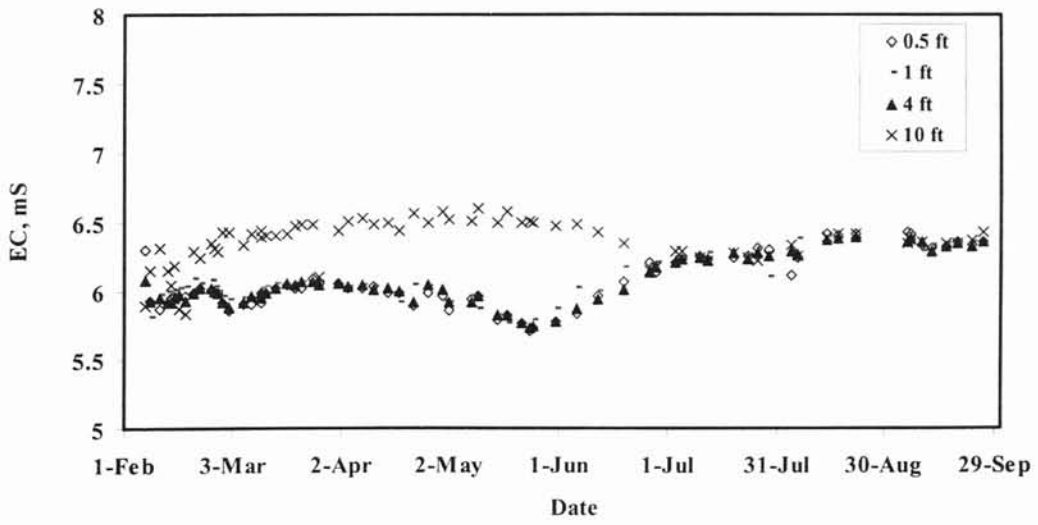


Figure 4.13. EC trend of column B

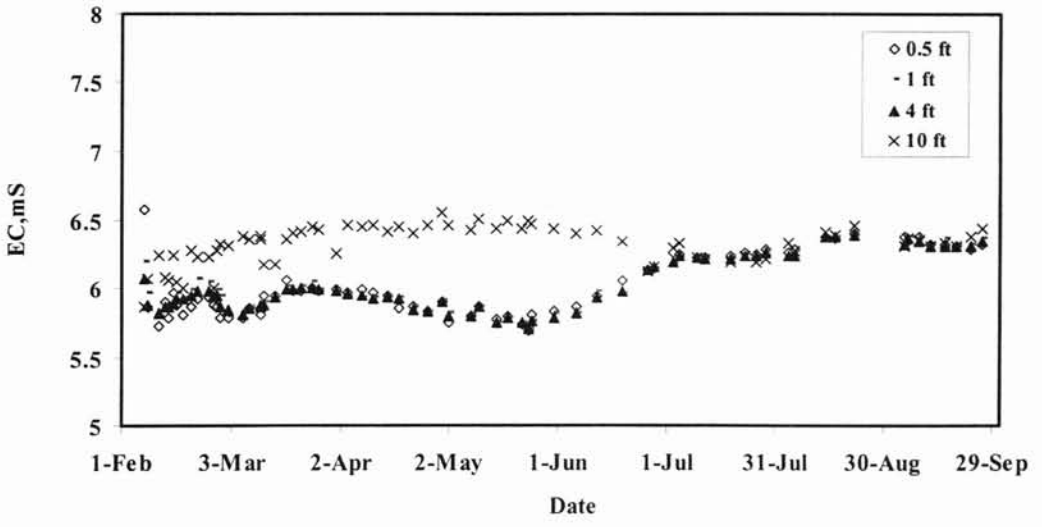


Figure 4.14. EC trend of column C

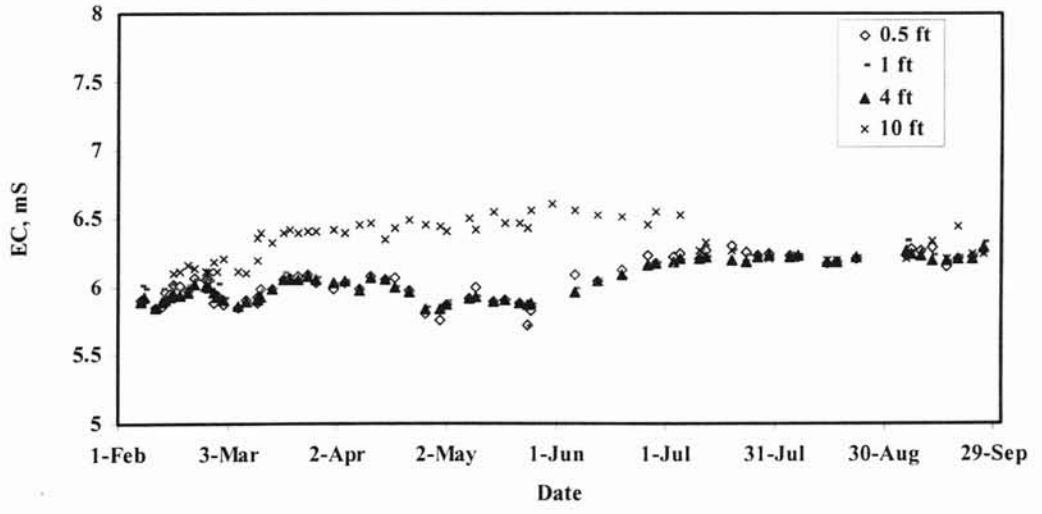


Figure 4.15. EC trend of column D

4.5 TKN

The TKN profile for columns B and C is presented in Fig. 4.19

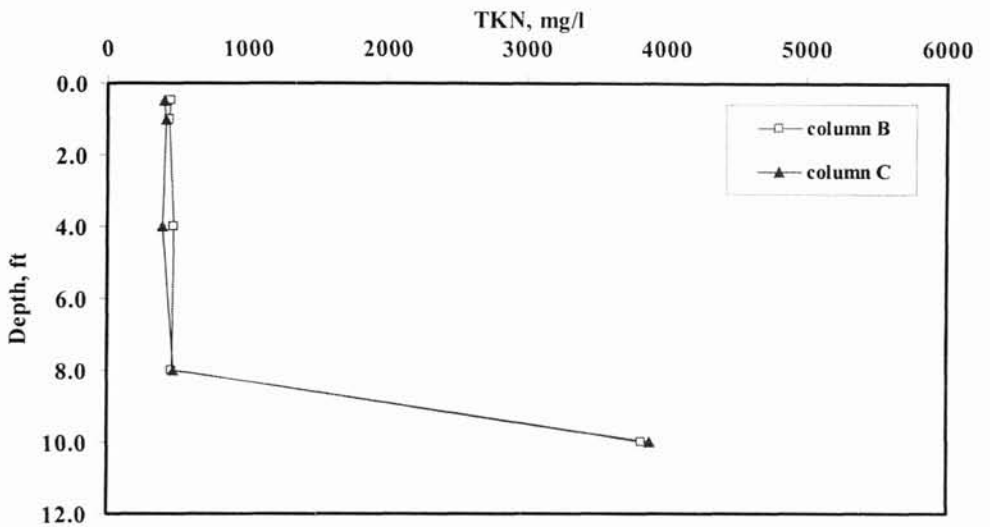


Figure 4.16. TKN profile for columns B and C, 07/17/2002

The TKN profiles of columns B and C are very close. From 0.5 to 8 ft, TKN values are constant and approximately equal to 350 mg/l. Below 8 ft. TKN of both columns increased to 4000 mg/l. Consequently, TKN of sludge is ten times higher than TKN of the liquid. The TKN data for columns B and C were collected from 02/21/2002 to 09/27/2002 and shown in Fig. 4.17 and 4.18. In both columns at the depth above 10 ft, TKN values are relatively constant throughout the period of observation.

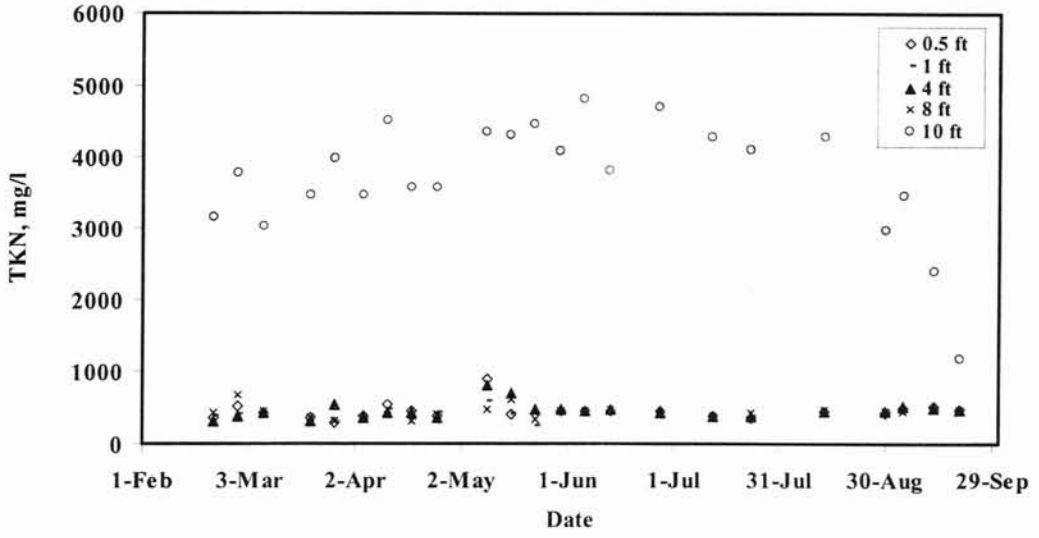


Figure 4.17. TKN trend of column B

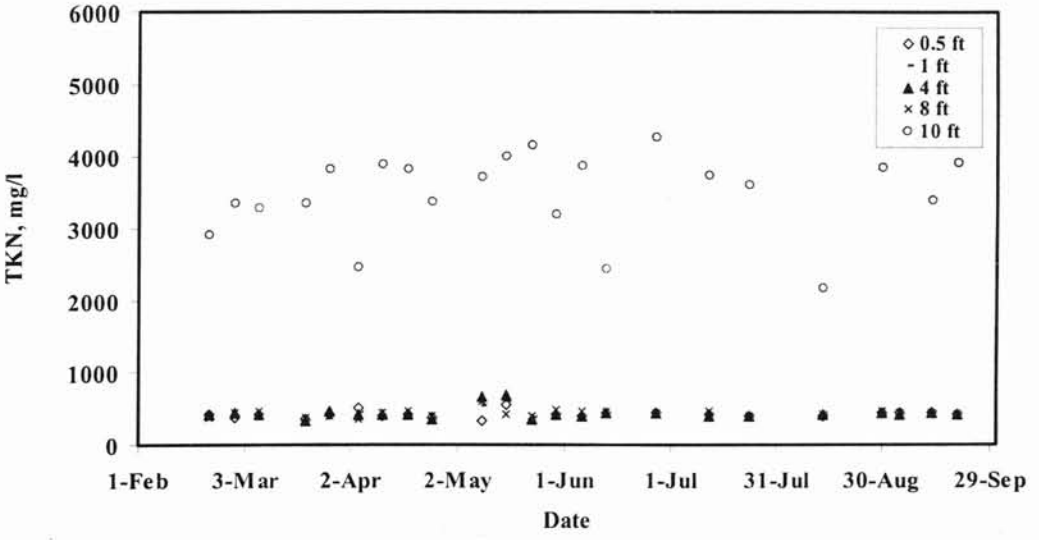


Figure 4.18 TKN trend of column C

4.6 AMMONIUM

The ammonium profile for all columns is presented in Fig. 4.19. The ammonium concentrations in all columns above 10 ft are very close. At the depth of 10 ft, there is an increase in the ammonium concentrations for all columns. Also, columns A and B had lower concentration of ammonium than columns C and D in the sludge zone. The ammonium trends for each individual column are presented in Fig. 4.20-4.23. In the liquid part of the columns the ammonium concentration was in the range of 220-450 mg/l compared to 250- 450 mg/l of the liquid TKN; therefore, in liquid zone TKN is approximately 90% ammonium. In the sludge zone (below 10 ft), ammonium concentrations were 750-1500 mg/l compared to the TKN values of 3000-4500 mg/l; therefore, ammonium accounts for only 25-33 % of the total nitrogen in the sludge zone.

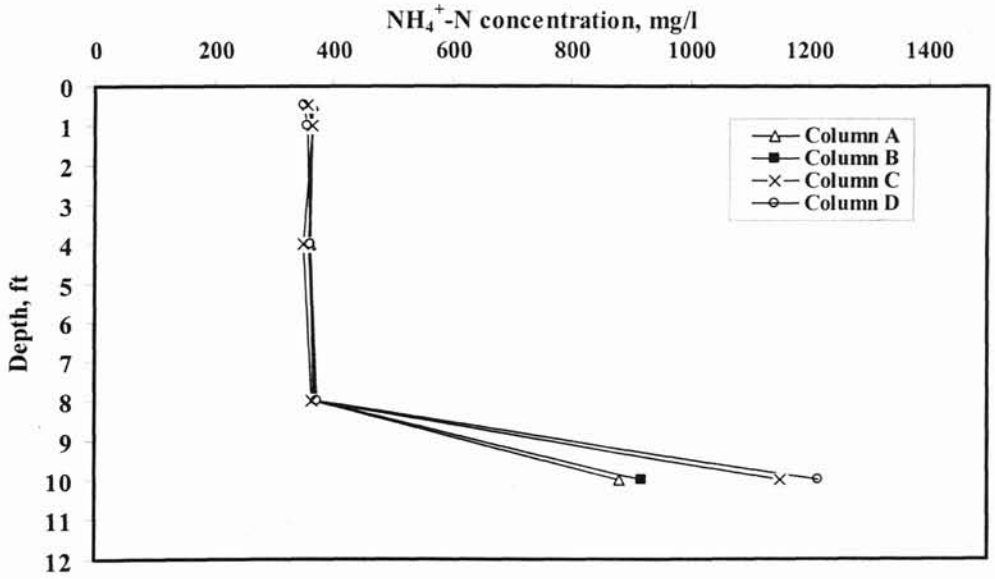


Figure 4.19. Ammonium profile for all columns, 07/11/2002

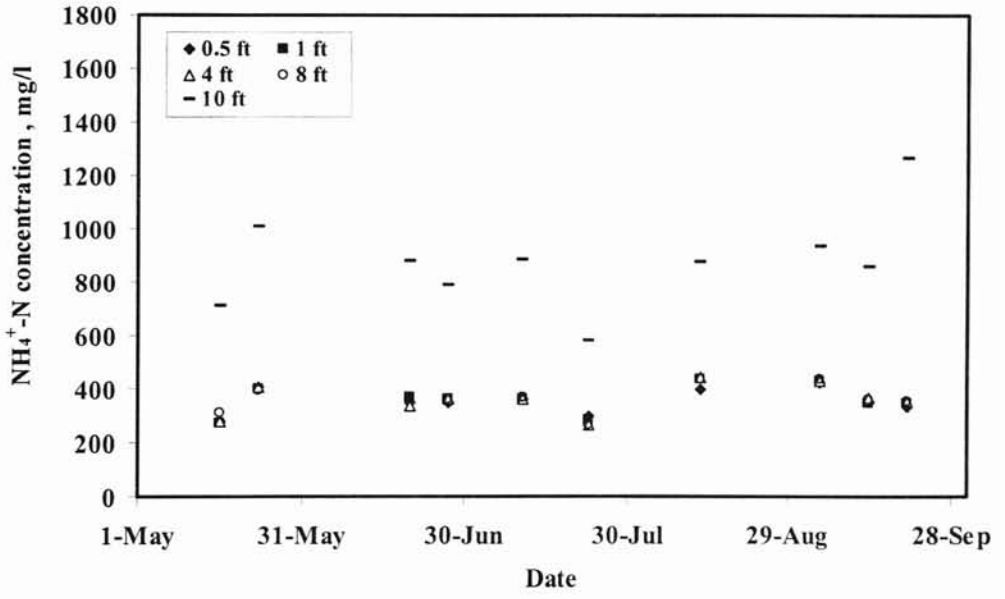


Figure 4.20. Ammonium trend of column A

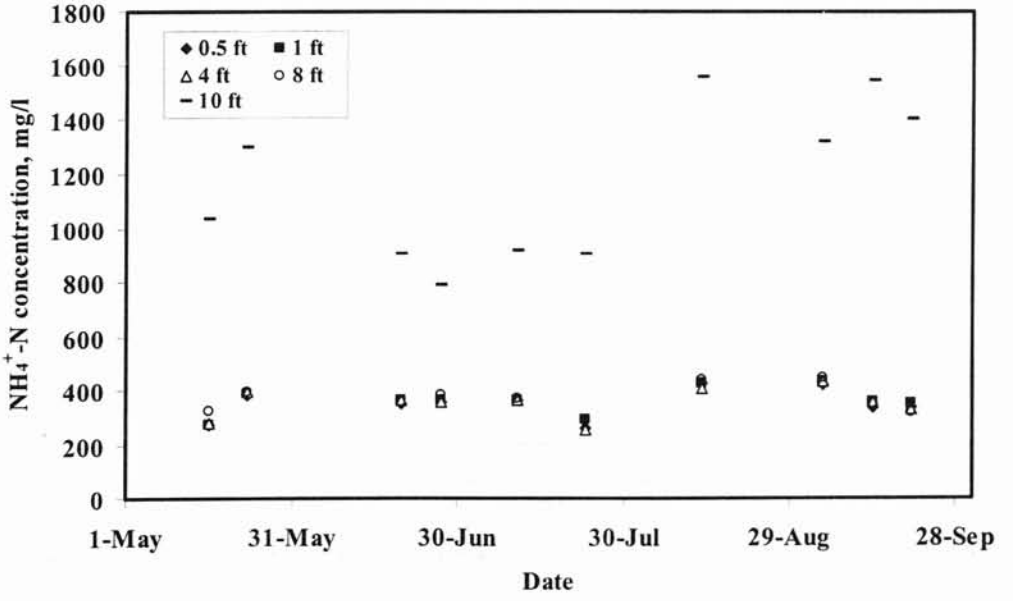


Figure 4.21 Ammonium trend of column B

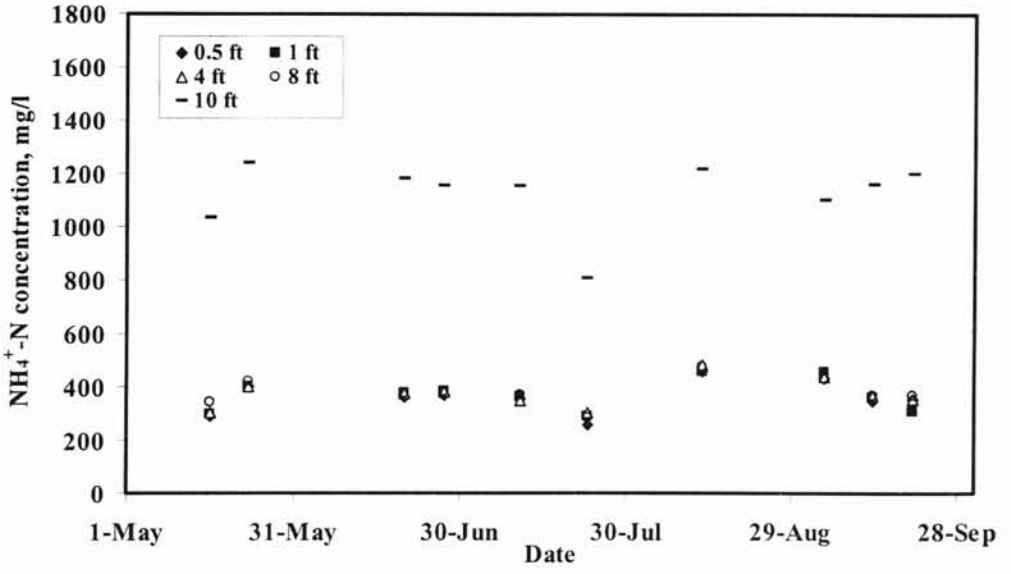


Figure 4.22. Ammonium trend of column C

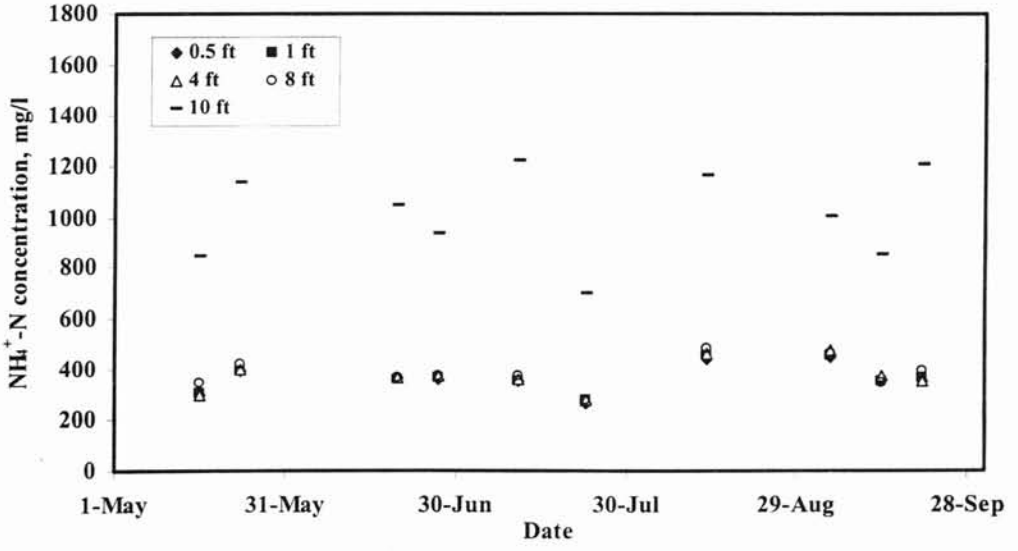


Figure 4.23. Ammonium trend of column D

4.7 AMMONIA EMISSION

Columns B and D were equipped with wind tunnels for this study. Ammonia emission was measured on column B. Fig. 4.24. shows the daily ammonia emission trend from column B.

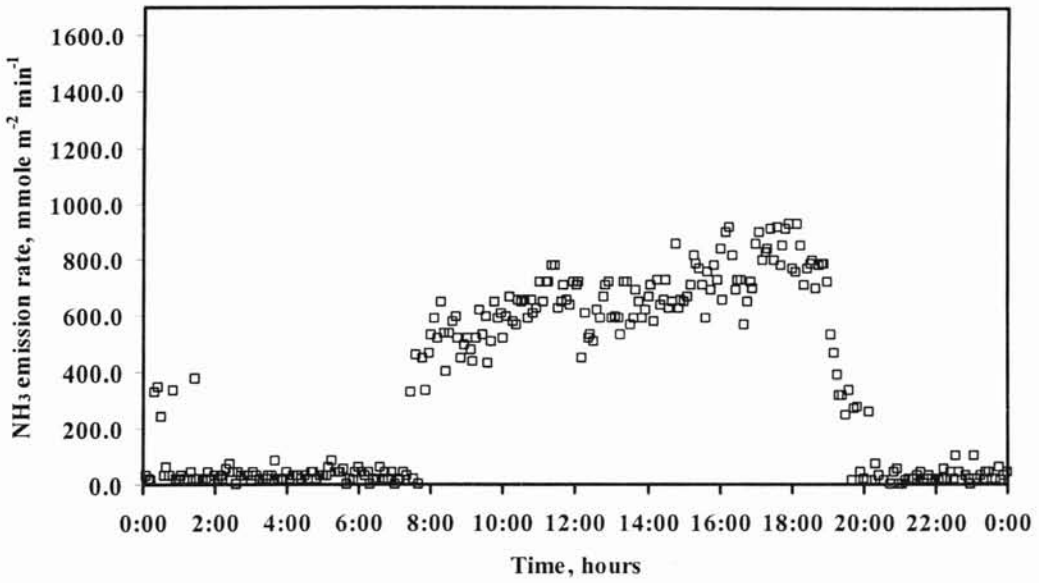


Figure 4.24 Ammonia emission from column B, 6/22/2002

Ammonia emission started around 7:30, about 30 minutes after the lamps were turned on, and increased from 380 mmole m^{-2} min^{-1} to 600 mmole m^{-2} min^{-1} until 12:00. At about 12:00, emission slightly decreased. This can be explained by the fact that the wind tunnels extensions were removed, for approximately 5-10 min., so that algae could be removed from the top of the columns. This drop was observed in all ammonia trends of our study as shown in Fig. 4.25.

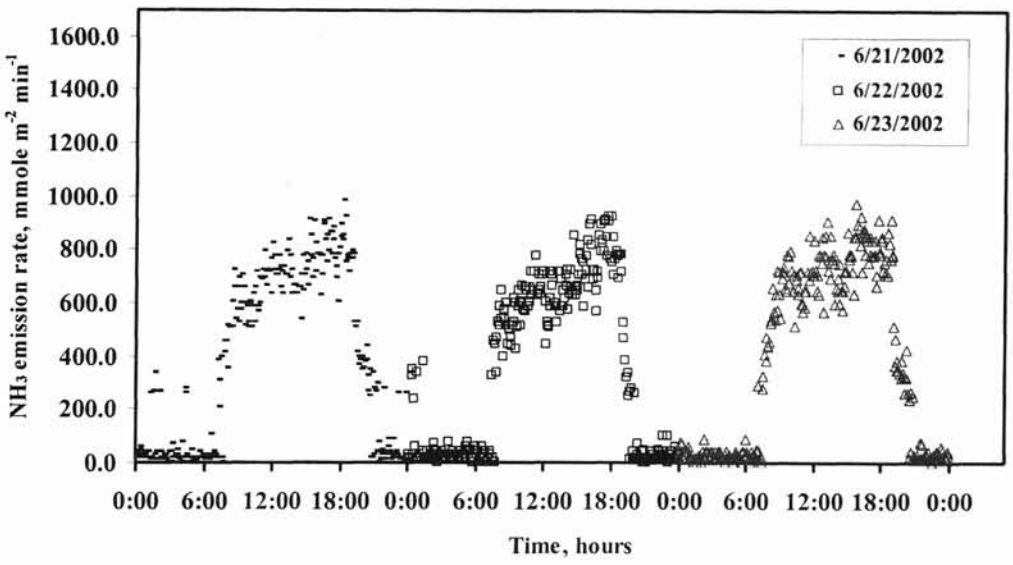


Figure 4.25 Daily Ammonia emission trend from column B, 6/21/2002-6/23/2002

Maximum ammonia emission occurs around 18:00, which corresponds to the highest temperature in the liquid in the upper 1 ft of column liquid (Fig 4.1 and 4.2). At 19:00, the emission started decreasing, and from 21:00 until 7:30 of the following morning, very little ammonia emission occurred. This trend is repeated on a daily basis (Fig. 4.25).The observed phenomenon can be explained by the temperature dependent solubility of ammonia in liquid. Increase in temperature decreases the NH_3 solubility in a liquid (Stephen and Stephen, 1963). Increase in temperature also increases biological activity. Using the regression equation derived from the ammonia solubility data adopted from Stephen and Stephen (1963), we can conclude that the maximum increase in the temperature from 27.5 ^{0}C (the lowest temperature, at 6:00) to 34^{0}C (the highest temperature, at 18:00) would decrease the ammonia solubility 12.2%. However, this would not explain why the emission began to decrease at 20:00. The temperatures in

column B at 20:00 were 31 ^{0}C, at 1 ft and 34 ^{0}C at 0.5 ft which are higher than the morning temperature at 7:30 (27.5 ^{0}C), at which emission was started. Unfortunately, the temperature directly on the surface in the liquid, where ammonia emission phenomena occur, was not measured; so, the exact effect of temperature is not known.

Some researchers (Aneja et al., 2001; Sommer, 1997; Dewes, 1996) found temperature to be a strong factor controlling ammonia volatilization. Aneja et al., (2001) observed similar diurnal emission pattern in anaerobic lagoons, with the maximum emission rate corresponding to the highest liquid temperature.

The total mass of ammonia (NH_3-N) released from the simulated lagoon was in the range 300-400 mg/day, which corresponds to a daily flux of 65.2-86.9 kg/ ha day. Mass of TKN loaded per day was 713 mg; therefore, 42-56% of the nitrogen added to the column is accounted for the ammonia emission. These results are higher than those reported by Harper and Sharpe (1998). The reported range for ammonia emission was 4.9-10.5 kg/ha day for anaerobic/facultative lagoons in North Carolina at the volumetric loading rate of 0.02-0.03 kg VS /m^3 day. Our volumetric loading rate was 0.024 kg VS/m^3 day, which is in the middle of their range. Consequently, volumetric loading rate is not the only factor affecting ammonia emission.

Higher emission rates from the simulated lagoons may be due to: feeding and sampling location, and oxygen concentration at the lagoon's effluent. Since we measured ammonia emission at the same location where we fed, our emission was higher than if the emission was measured away from the feeding place (less nitrogenous materials are available there). In addition, this difference could be explained by the fact that lagoons which were used in the North Carolina studies could have sufficient oxygen to allow

ammonia oxidation and dinitrification to dinitrogen gas. Their dinitrogen gas emission was much higher than ammonia emission and was 8.9-120 kg N_2-N/ha day. Presumably they used the same lagoons to measure methane and ammonia emissions. If so, their reported values for dissolved oxygen in methane emission study was > 0.5 mg/l (Sharpe and Harper, 1999), which is sufficient for denitrifying organisms (Jones et al., 2000). In our columns, no oxygen was detected throughout the depth. Redox potential was much lower than –200 mV, which means the column is completely anaerobic (Maier et al., 2000). Therefore, no significant ammonia conversion to dinitrogen gas was possible. This would lead to higher ammonia emission.

4.8 VFA

The VFA measurements were taken on 7/17/2002 and 8/10/2002 during 19-20 hour periods, every hour at the depths of 1, 4 and 10 ft. On a third occasion (9/01/2002) VFA concentrations were measured at random times at the same depths. All studies were performed in the column A. The major detected acids were acetic and lactic. However, no lactic acid was detected on 7/17/2002. Propionic and butyric acids concentrations were very close to detection limit. The chromatograms in each study contained a large variety of other acids, which were not identified. The same general trend was observed every day. At 1 ft., acetate concentration on July 17 was in the range of 0-30 mg/l, 0-14 mg/l on August 10 and 0-20 mg/l on September 1 (Fig. 4.26).

From 8:00 to 12:00 the acetate concentrations were very low (close to detection limit). However, once the columns were fed, at 12:00, acetate concentration started to increase. A possible explanation for this is that acidogenic bacteria immediately began converting easily-degradable products to acetate. In the time period from 6:00 to 14:00 the acetate concentration profiles were similar. However, on August 10 the acetate concentration reached its maximum at approximately 14:00 and remained constant until 2:00. Then it began to decrease. On July 17 and September 1, the maximum acetate concentration was at 18:00. At 20:00, acetate concentration began decreasing and 23:00 it was about 4 mg/l.

A similar acetate concentration trend was observed at a depth of 4 ft (Fig 4.27).

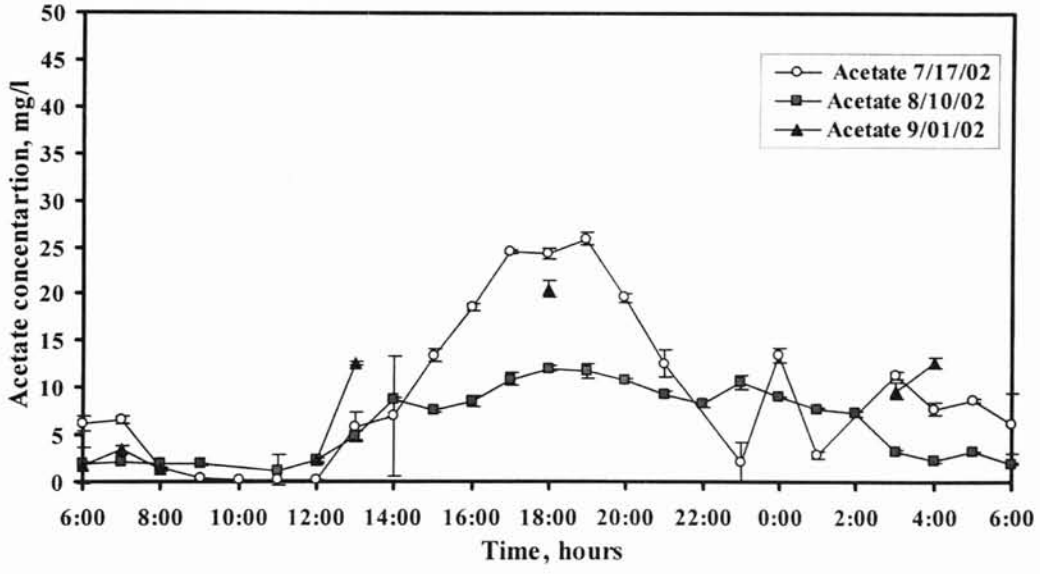


Figure 4.26 Acetate concentration trend, column A, 1 ft, 7/17/2002, 8/10/02 & 9/01/2002

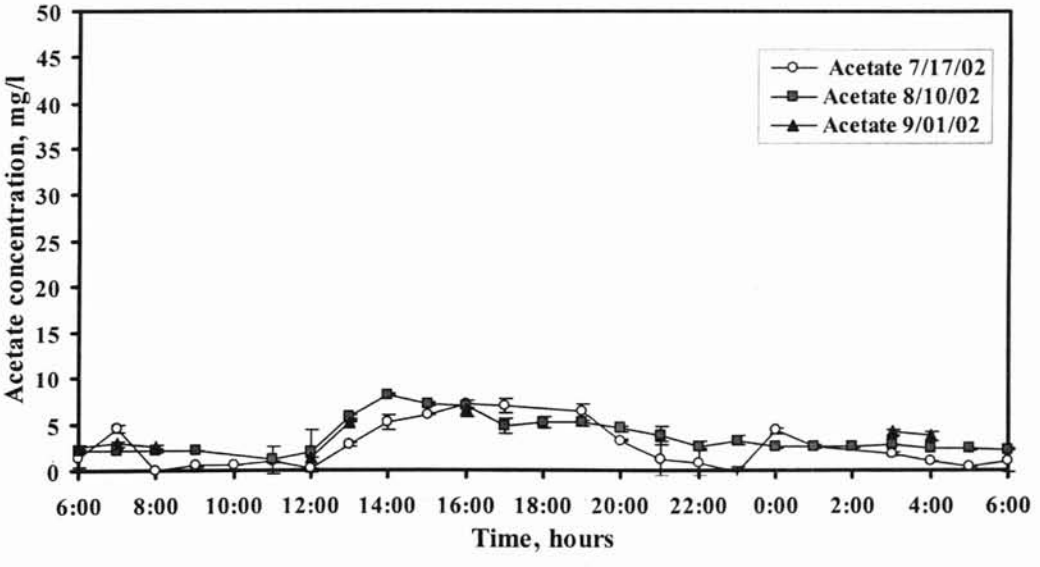


Figure 4.27 Acetate concentration trend, column A, 4 ft, 7/17/2002, 8/10/02 & 9/01/2002

However, acetate concentrations were three times lower than at a depth of 1 ft. As the material fed at the top of the column settles into the column, less and less of the easily degradable material is available for acidogenic microorganisms.

The acetate concentrations at 10 ft are shown in Fig 4.28. Acetate concentration is relatively constant, and within the range of 2-16 mg/l. Compared to 1 and 4 ft, acetate concentration appears to be less dependent on the feeding cycle. This could be explained by the fact that acidogenesis and acetogenesis in the sludge are more complex than in the upper layers. In other words, in upper part of the lagoon, acetate is created from easily degradable, soluble materials added with feeding. In the lower zone, acetate is created from slowly degradable materials (lignin, cellulose) that settle to the bottom. As was discussed in chapter 2, cellulose and lignin degradation involves several steps before it is converted to the acetate.

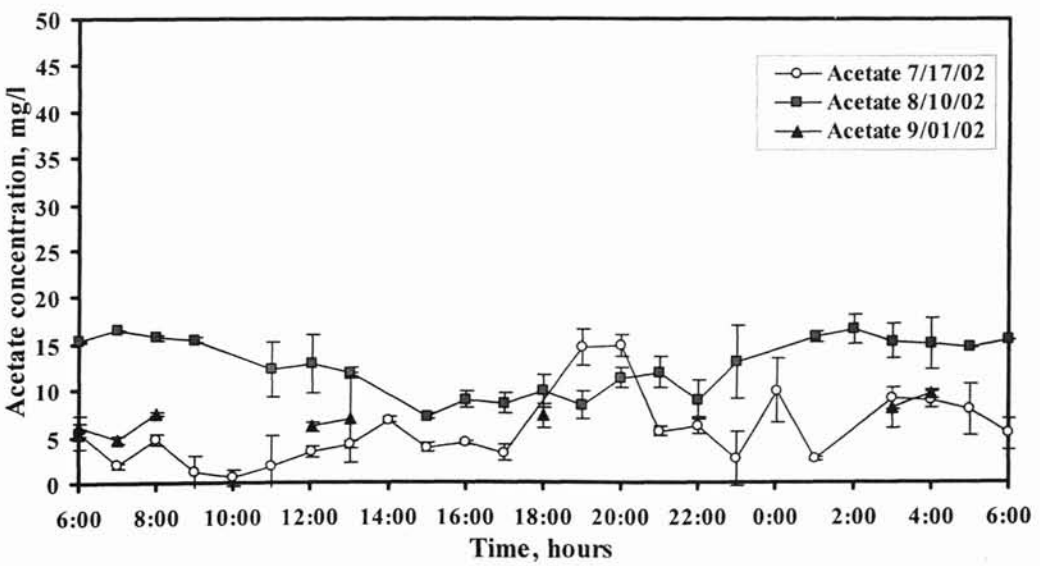


Figure 4.28 Acetate concentration trend, column A, 10 ft, 7/17/2002, 8/10/02 & 9/01/2002

As it was mentioned before, no lactic acid was detected on July 17. However, when the experiments were performed again on August 8 and September 1, lactic acid was detected at all measured depths and was in the range of 4-33 mg/l (Fig 4.29).

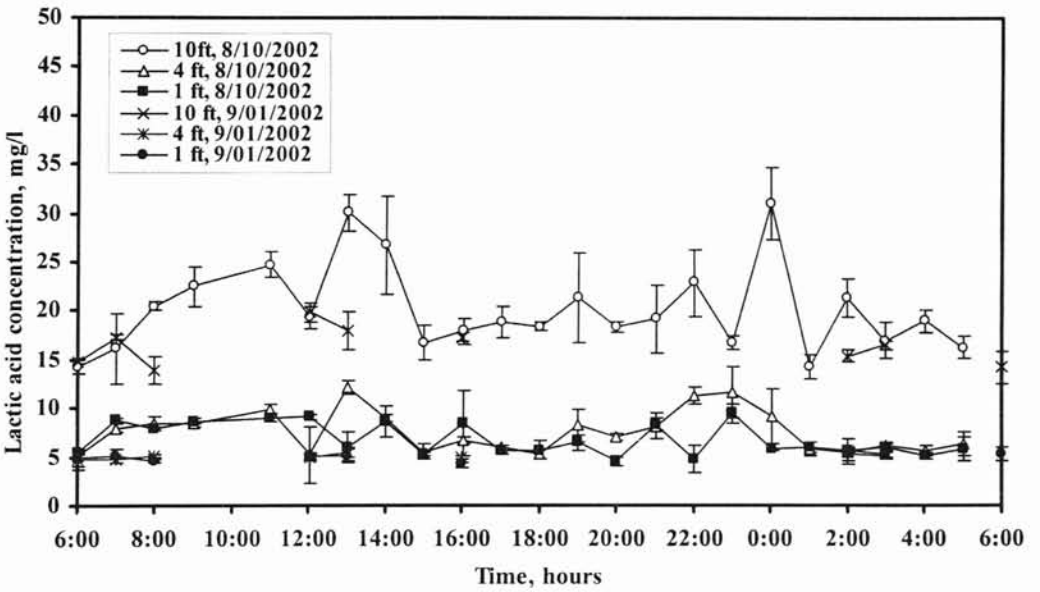


Figure 4.29 Lactic acid concentration trend, column A, 8/10/2002 & 9/01/2002

The concentration of lactic acid at 10 ft was in the range of 15-30 mg/l and always higher at 10 ft than in the upper depths. Below 10 ft, columns have large storage of sludge, which consist of 47% TC (Table 3.1). This is mainly lignin, cellulose and pectin, which are great substrates for lactic acid producing bacteria (Table 2.2). At 1 and 4 ft the lactate concentrations were very close and in the range of 4-12 mg/l. Feeding does not appear to affect lactic acid production. This trend was repeated on September 1.

As it was discussed in the literature review, no lactic acid was observed in swine manure digesters. Moreover, there was not any literature data found on lactic acid production in swine lagoons. To confirm whether the columns had VFA concentrations similar to a full-sized lagoon or not, VFA concentrations were measured from Oklahoma State Swine Research Center lagoon. The data was collected on 8/21/2002 at 9:30. Results are shown in Fig 4.30

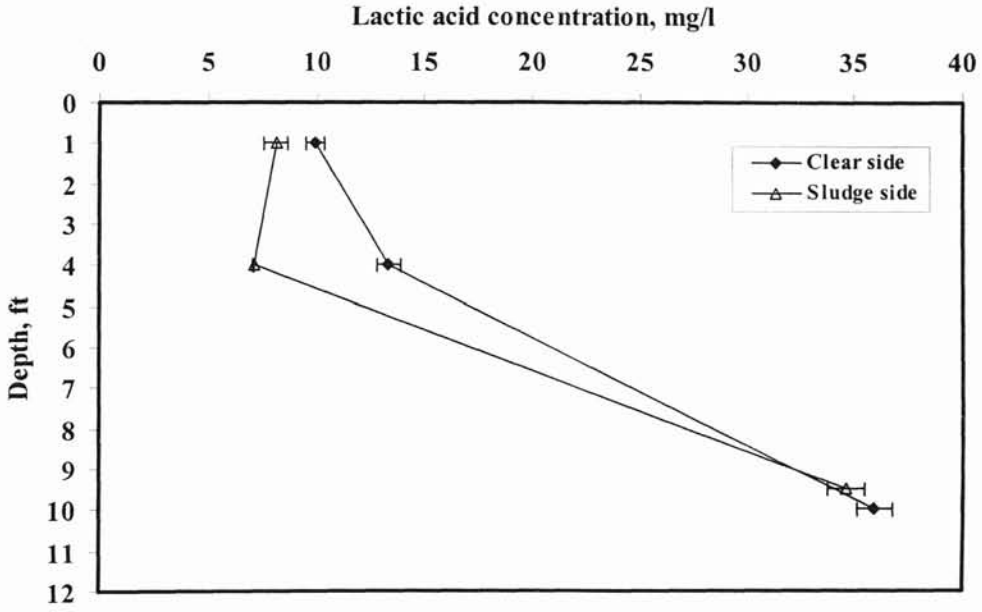


Figure 4.30 Lactic acid concentration profile in OSU swine lagoon, at 9:30 on 8/21/2002

The samples were taken from two connected lagoons. One of them had more accumulated sludge ("sludge side"), so it was only possible take the sample no deeper than at 9.5 ft. Second lagoon had less accumulated sludge ("clear side"); so, there wasn't

any problem in taking sample at 10 ft. As in the column A, both sides had more lactate in the deeper layers than in the upper parts. Concentration at 1 ft. of the depth in the "sludge side" was about 8 mg/l compared to 10 mg/l in the "clear side" (same level). At 4 ft, the concentration in the "sludge side" was 7 mg/l, which is a little bit lower than at 1 ft. However, in the clear side it was 13 mg/l, which is a little bit higher than at 1 ft. of this lagoon.

Even though the "clear side" had less sludge than the "sludge side", the lactic acid concentrations at 9.5 and 10 ft. in those lagoons were very close (34 and 35 mg/l respectively). In column A, lactic acid concentration at 10 ft was lower than in the lagoons. This could be due to columns operation differences (mixing, feeding etc.).

In the upper layers of column A on August 10, and upper layers of the lagoon ("clear side"), the lactic acid concentration was in the same range of 7-8 mg/l. No acetic, propionic or butyric acids were detected in the OSU swine lagoon on 8/21/2002 at 9:30. This time of sampling corresponds to the time between when the liquid in the columns was mixed and fed. Very little of acetic, propionic or butyric acids were detected (close to the detection limit) in the upper layers of the columns at that time (Fig. 4.26.; Fig. 4.27). However, at the lower zone (below 10 ft) of the column, acetate concentrations were detected at 5-15 mg/l (Fig. 4.28) and no acetate was detected in the full-sized lagoons at this depth. This difference can be explained by the fact that the samples were taken in the middle of the lagoon. The lagoons were fed 6-8 m. from the place where the samples were taken. Supposedly, most of the acetate would be available there after the lagoons were fed. In addition acetate could be immediately converted to methane by methanogenic bacteria. Butyrate could be immediately converted to acetate by acetogenic

bacteria and butyrate could undergo conversion to lactic or acetic acids. All mentioned acids finally converted as carbon dioxide and methane gases.

4.9 METHANE AND CARBON DIOXIDE EMISSION

Daily CH_4 and CO_2 emission from the column A are presented in Fig. 4.31 and 4.32.

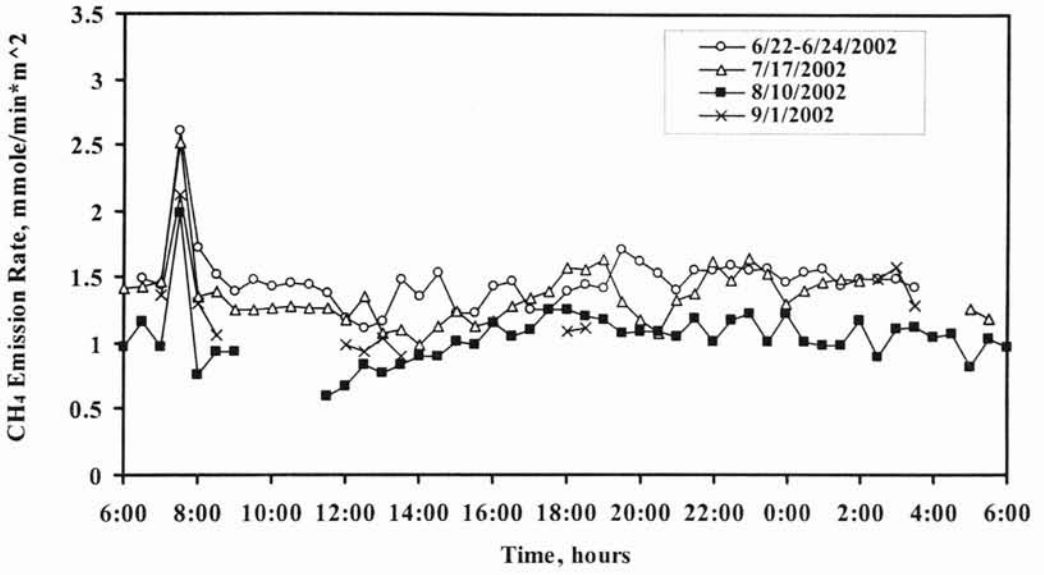


Figure 4.31 Daily CH_4 emission trend from column A

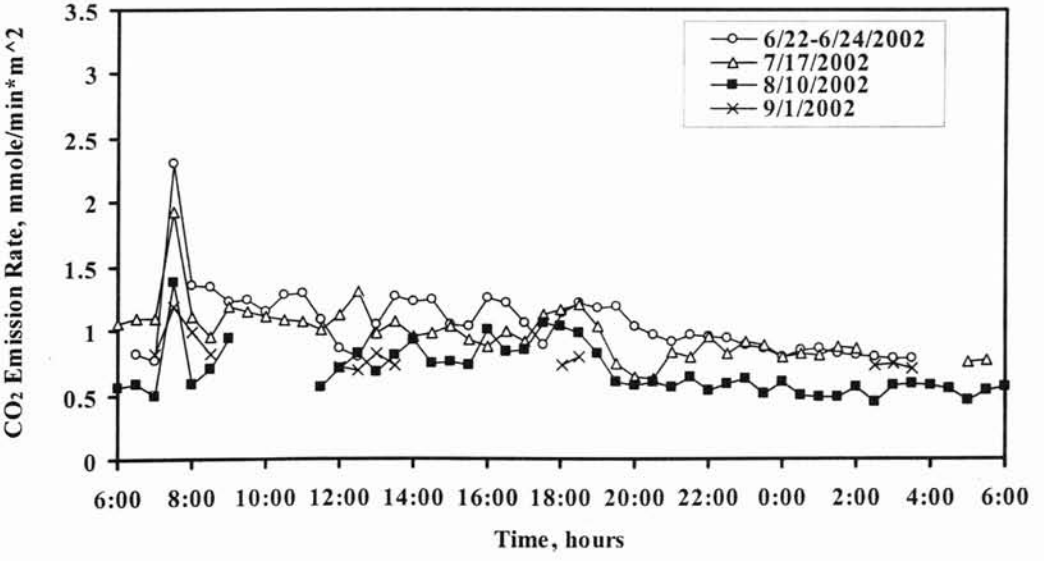


Figure 4.32 Daily CO_2 emission trend from column A

The data was collected in the following sequences:

- on June 22, data were taken every 30 min from 6:00 to 18:00, and on June 24 from 16:00 to 6:00. Then, they were combined in to one plot;
- on July 17 and August 10, data were taken continuously for 19-20 hours at 30 min. intervals;
- on September 1, data were taken randomly, at 30 min intervals.

Although the data were collected in the different times, methane and carbon dioxide plots follow the same trends. However, CH_4 emission rate was always higher than CO_2 emission rate, and was in the range of 0.6 – 1.8 mmole/min*m^2, with short maximum at 7:00 of 2-2.7 mmole/min*m^2. The CO_2 daily emission rate range was 0.5-1.4 mmole/min*m^2, and 1.4-2.4 mmole/min*m^2 at 7:00. The increase in the emission rates at 7:00 for both gases can be explained by the fact that at this time the recycling pump was operating and mixing the liquid, which is highly saturated with gases. The gas-liquid equilibrium was shifted and gas bubbles were released from the liquid. The emission of both gases went down slightly lower than it was before mixing, and was constant until 12:00. Acetic acid concentrations also dropped after 7:00 (Fig. 4.26, Fig. 4.27) indicating a burst of biogas production from acetate where the upper layer is mixed.

At 12:00, the methane emission rate began to increase, reached its maximum almost immediately and stayed at the same level during the night (Fig. 4.31). The CO_2 emission rate had the same trend until 19:00. However, after 19:00 carbon dioxide emission started declining and reached its minimum at 7:00. These points correspond to turning on and off the light. Therefore, the conclusion could be made that photosynthetic

bacteria are involved in this process. However, photosynthetic organisms, for example algae, use carbon dioxide in photosynthetic activity during the day to produce new cells and release carbon dioxide during the night (Tchobanoglous and Burton, 1991). This does not correspond to our data.

To better understand methane- carbon dioxide correlation, CH_4/CO_2 ratio graph was plotted and presented in Fig. 4.33.

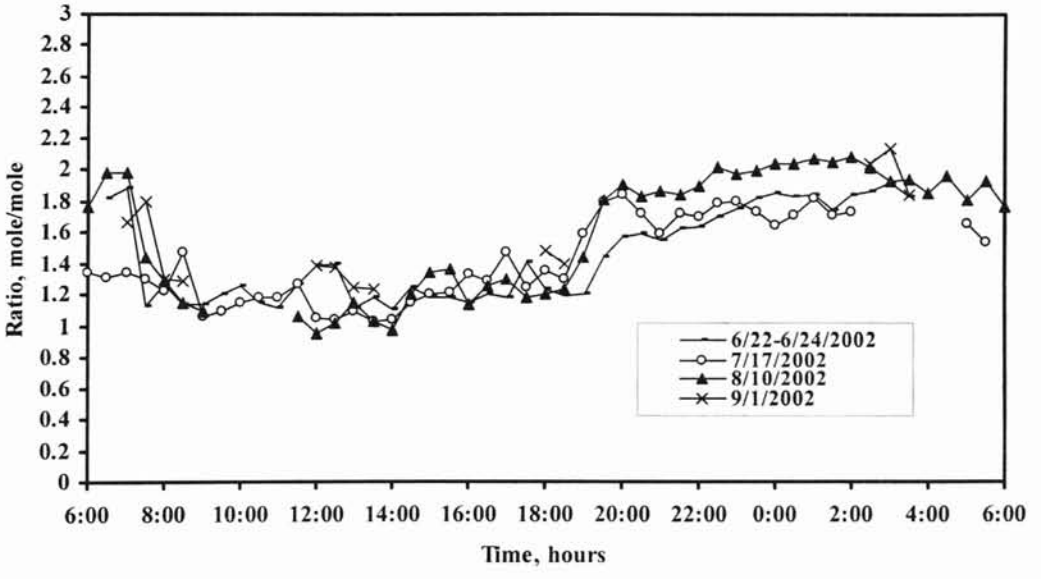


Figure 4.33 Daily CH_4/CO_2 ratio for column A

Diurnal CH_4/CO_2 ratio may be divided into two sections. First of all, from 7:30 to 19:00, the ratio of CH_4/CO_2 was close to 1.0 (1-1.4). From 19:00 to 6:00, the ratio increased to 1.6-2.0. Methane production was almost twice that of carbon dioxide during the night. As it was discussed in chapter 2, methane could be produced either from direct microbial degradation of low molecular weight acids or from microbial conversion of carbon dioxide. Methane emission increased once the columns were fed. After manure

was applied to the liquid, the microbes immediately began to convert easily digestible material to acetate and propionate. The newly created acetate as well as the VFAs presented in the fed are immediately converted to CH_4 and CO_2. After, the major part of acetate is consumed, the methane production switches to microbiological carbon dioxide conversion to methane. Correlation of CH_4/CO_2 ratio and acetate concentrations on 7/17/2002 is shown in Fig. 4.34.

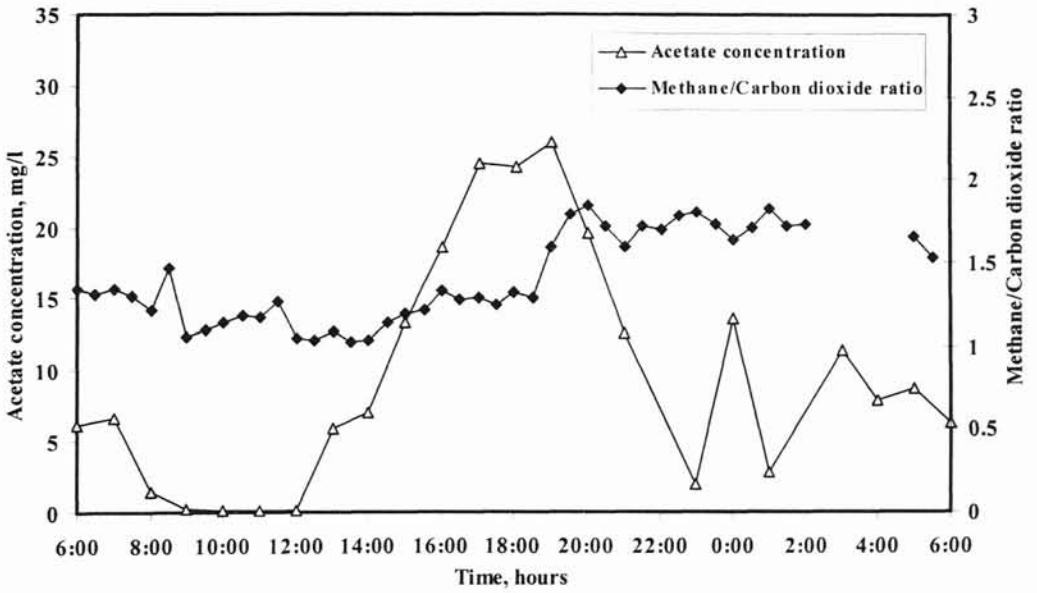


Figure 4.34 CH_4/CO_2 ratio and acetate concentration, column A, 7/17/2002

At 14:00 methane and carbon dioxide emission rates began to increase. This corresponds to increase in the acetate concentration. However, the methane/carbon dioxide emission ratio was constant. At 19:00 acetate concentration started to decrease and as a result methane and carbon dioxide emission also went down (Fig. 4.31, Fig. 4.32), but the methane/carbon dioxide ratio started to increase (Fig. 4.34). At 21:00 methane emission began to increase, but carbon dioxide emission remained at the low

level, which resulted in elevation of methane/carbon dioxide ratio. This can be explained by the fact that methanogenic bacteria started using carbon dioxide as a carbon source instead of acetate. The same phenomenon was observed on 8/10/2002 (Fig. 4.35).

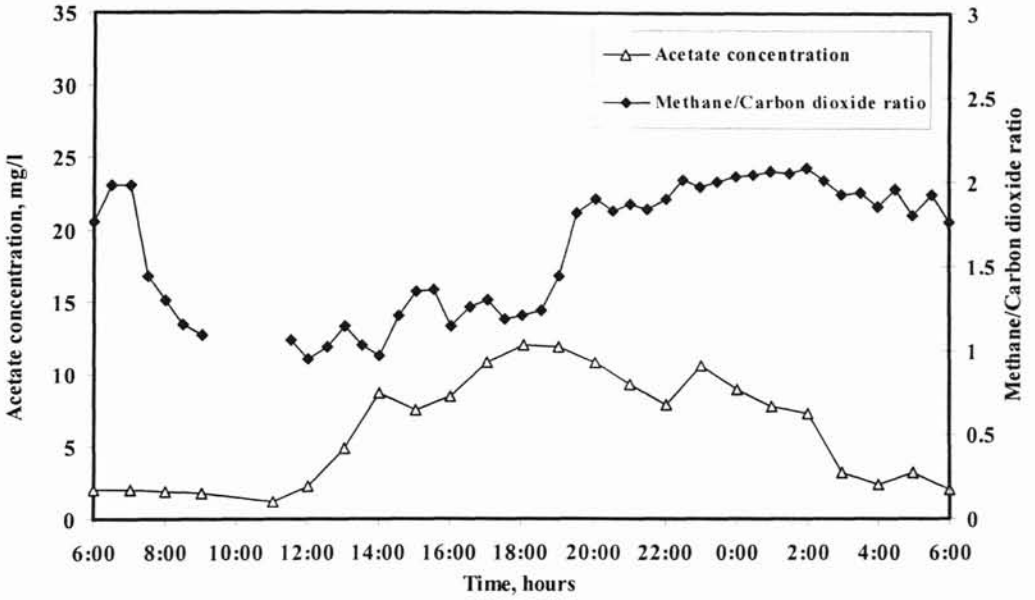


Figure 4.35 CH_4/CO_2 ratio and acetate concentration, column A, 8/10/2002

On 8/10/2002, acetate concentration was lower than on 7/17/2002 (Fig. 4.34) and remained constant from 14:00 to 2:00. As a result, the methane production was also lower than in other days and stayed constant during the night (Fig. 4.31). However, on 7/17/2002 the acetate concentration was twice as high. In general, methane and carbon dioxide emissions are well correlated with acetate data.

Daily carbon dioxide emission was in the range of 350-530 kg/ha day. As it was stated before, no literature data was found on carbon dioxide emission from anaerobic lagoons; thus, these values can not be compared.

Daily methane emission was in the range 190-280 kg/ha day. As in the case of ammonia emission, this is higher than methane emission measured by Sharpe et al., (2001a). Sharpe et al., (2001a) measured emission of CH_4 from anaerobic lagoon in Southeastern US in the range of 20-115 kg CH_4 /ha day. Such difference may be explained by the same hypotheses described in ammonia emission (Chapter 4.7). These factors are presence of oxygen in the lagoon's effluent, and feeding and sampling locations.

It is known that presence of oxygen in solution even as low as 0.18 mg/l inhibits methanogenic processes. Methanogenic reaction occurs only at the Eh range of -200 mV (Le Mer and Roger, 2000). The dissolved oxygen in the studied lagoons by Sharpe and Harper (1999) was always higher than 0.18 mg/l, consequently methanogenic activity was suppressed. However, in our lagoon simulators Eh was always lower than –200 mV at all depths. This may explain differences in the methane emission.

Safley and Westernman (1988) stated that most agricultural lagoons are loaded at one or to points resulting in higher loading rates in these areas. Consequently, methane emission may vary, depending on the areas of the lagoon. The gaseous emissions were measured from the same place were the lagoons simulators were fed.

The theoretical biogas (CH_4 and CO_2) production may be calculated from the COD of the feed. The COD of the raw manure used as a feeding material on 8/10/02 was 102 g/l (Table 3.1). Based on the loading rate, the volume of the manure fed on this day was 0.065 l; therefore, the theoretical methane and carbon dioxide production should be 4.64 l (Tchobanoglous and Burton, 1979). The actual CH_4 and CO_2 gas production on 8/10/2002 was 3.79 l, which is approximately 82% from the theoretical gas production.

Thus, we can conclude that our daily COD removal rate from gas production is about 82%. The rest of this COD, 18% may be accounted as slowly degradable or non degradable materials, which accumulate in the sludge layer.

5.0 CONCLUSIONS

The major aim of this study was to measure methane, carbon dioxide and ammonia emissions from an anaerobic/facultative lagoon simulator. The ammonia emission from the lagoon simulator was 65.2-86.9 kg/ha day, methane was 190-280 kg/ha day and carbon dioxide was 350-530 kg/ha day. The emission of all gases was higher than those reported in the literature. Acetic, lactic and very little of propionic, butyric acids were found in the lagoon simulator. At the time of the day lagoons (loaded at the lower loading rate than the columns) were sampled, VFAs were similar in the columns and full-sized lagoons. TKN, ammonium and pH values of the lagoon simulators were close to the values found in the full-sized lagoons.

Based on the results of this study, the following conclusions can be drawn:

- the columns have fair representation of full size lagoons
- emissions of methane, carbon dioxide and ammonia depend on change of temperature, mixing of the liquid, lighting, loading rate and other physical factors.
- emission of biogas (methane and carbon dioxide) depends on VFA concentration, particularly acetic acid
- practical biogas emission was very close to theoretical (82%)
- the studied column had high COD removal rate of 82%

Recommendations for future research in this area may be provided based on our experimental methodology. To better understand emission of CH_4, CO_2 and NH_3, measurements should be made over a wide range of lagoon sizes, covering the range of yearly lagoon temperatures, redox potentials and other parameters.

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VITA 2

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