The Effects of Time and Temperature on the Fate of Pathogens and Indicator Bacteria During Municipal Wastewater Sludge - Mesophilic Anaerobic Digestion, Air-Drying, and Composting

by

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Dedication

To my beloved country, Mexico, and especially to the people who have not yet been reached by the power of education.

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The fate of indicator bacteria and Salmonella and ova was investigated in field and laboratory-scale studies. The results of the field-scale studies indicated that the densities of fecal coliforms, fecal streptococci, and *Salmonella* were reduced by 6.1, 4.6, and 2.4 orders of magnitude, respectively, after mesophilic anaerobic digestion, air-drying and composting. Mesophilic anaerobic digestion produced EPA Part 503 Class B biosolids, i.e. fecal coliforms < $2x10^6$ MPN/g. The majority of samples of air-dried anaerobically digested sludge were Class A biosolids, i.e. fecal coliform < 1,000 MPN/g, or *Salmonella* < 3MPN/4g), and viable helminth ova < 1 ovum/4g. All samples of composted biosolids were Class A biosolids.

Laboratory-scale anaerobic digesters were operated at temperatures of 25°C and 35°C; hydraulic detention times of 7, 15, 30; and 45 days, and organic loadings of 0.9, 1.80, and 2.7 kg/m^3-d. Anaerobic digestion at minimum detention times of 30

days at 25°C or 15 days at 35°C produced EPA Part 503 Class B biosolids fecal coliform densities < 2x106 MPN/g TS and 38% destruction of volatile solids.

Analysis of variance (ANOVA) tests indicated that the individual effects of temperature, detention time, and organic loading and the interactions between time and temperature and between time and organic loading on the densities of fecal coliforms and *Salmonella* were statistically significant at the 95% confidence level. Temperature was the principal mechanism in reducing the densities of indicator bacteria in mesophilic anaerobic digestion.

Class A biosolids (*Salmonella* < 3 MPN/4 g TS) were produced by anaerobic digestion at 35°C, detention times of 30 and 45 days and organic loadings of 1.80 and 2.70 kg/m^3-d. Densities of fecal coliforms were $2.69x10^4$ MPN/g at 25°C and $6.61x10^4$ MPN/g at 35°C that are < $2x10^6$ MPN/g required for Class B biosolids.

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Chapter 1: Introduction

1.1 Problem Definition

One of the most important and challenging steps in municipal wastewater treatment systems is the stabilization and disposal of the sludge generated throughout the process. This sludge or "biosolids" generally contains high concentrations of organic matter and pathogenic entities such as bacteria, viruses, protozoa and helminths.

Approximately 6.9 million tons of biosolids were generated in the United States in 1998 (U.S. EPA, 1999). About 60 percent was beneficially used and 40 percent disposed in landfills or by incineration. Beneficial use of biosolids involves some type of land application, composting, and use as landfill cover. Land application of stabilized and disinfected biosolids for beneficial use has drawn considerable attention. Approximately 2.1 million tons (41%) of the 6.9 million tons of biosolids generated in 1998 were applied to the land. Almost 50% of the estimated 8.2 million tons biosolids that are predicted to be generated in 2010 will be applied to land (U.S. EPA, 1999). The Environmental Protection Agency (EPA) implemented strict regulations known as "Part 503" to protect public health and the environment from any potential risk of exposure to pathogens in the biosolids that are applied to the land.

The results of several studies reported the reduction of pathogenic organisms in wastewater treatment systems. However, a long-term evaluation of the effectiveness of conventional wastewater sludge treatment processes in reducing the number of such organisms is still lacking. Several technologies can be used to

stabilize biosolids: however, anaerobic digestion seems to be the process of choice. Anaerobic digestion was used to process sludge in 52 percent of the 117 Publicly Owned Treatment Works (POTWs) surveyed (Edgar et al, 1998). The destruction of organic matter in sludge during anaerobic digestion is well documented; however, little data are available to elucidate the individual or combined effects of operating variables such as time, temperature, and organic loading on the reduction of indicator and pathogenic bacteria.

This work focused on the reduction in the densities of total coliforms (TC), fecal coliforms (FC), fecal streptococci (FS), *Salmonella*, and helminth ova during thickening, anaerobic digestion, air-drying, and composting under field conditions. Furthermore, the effect of detention time, temperature, and volatile solids loading on the densities of TC, FC, FS, and *Salmonella* during mesophilic anaerobic digestion was evaluated using lab-scale reactors.

1.2 OBJECTIVES

The objective of this research was the development of a better understanding of the capabilities of several conventional sludge treatment processes and, specifically mesophilic anaerobic digestion, in reducing the densities of indicator and pathogenic organisms in municipal wastewater sludge. This research was divided into two phases: field-scale and lab-scale studies. The specific objectives of Phase I: field – scale studies were

- Evaluation of the effectiveness of belt thickening, anaerobic digestion, air-drying, and composting processes in the reduction of fecal coliforms, fecal streptococci, *Salmonella*, and helminth ova in a municipal sludge processing facility.

- Comparison of removal efficiencies of indicator organisms and pathogens in each sludge treatment process with the existing standards for the use or disposal of municipal wastewater sludge.

The objective of the lab-scale studies (Phase II) was an evaluation of the effect of temperature, hydraulic detention time, and volatile solids loading on the performance of mesophilic anaerobic digestion measured in terms of the densities of FC, FS, and *Salmonella*, expressed as the most probable number per gram of total solids (MPN/g TS).

Chapter 2: Literature Review

2.1 Indicator and Pathogenic Organisms found in Municipal Wastewater and Wastewater Sludge.

Municipal wastewater generally contains four major types of human pathogenic (disease causing) organisms: bacteria, viruses, protozoa, and helminths (parasitic worms). The actual species and densities of pathogens in municipal wastewater and eventually in the sludge produced during treatment depend on the health of the local community and may vary substantially at different times. The density of pathogens in sewage sludge also depends on the reductions achieved in the wastewater and sewage sludge treatment processes (U.S. EPA, 1992).

The pathogens in wastewater primarily are associated with suspended solids. Primary wastewater treatment processes concentrate the solids into sludge; therefore, the densities of pathogens in untreated wastewater sludge are higher than in the incoming wastewater. Biological wastewater treatment processes such as trickling filters, and activated sludge systems may reduce the number of pathogens in the wastewater; however, the densities of pathogens in the resulting biological sludge may pose a public health and environmental concern (U.S. EPA, 1992). Major pathogens of concern that may be present in wastewater and/or wastewater sludge are listed in Table 2-1. The characteristics of the most common indicator and pathogenic organisms are presented below:

Table 2-1: Major Pathogens in Municipal Wastewater and Sewage Sludge

Pathogen Class	Examples	Disease
Bacteria	*Shigella sp* *Salmonella typhi* *Salmonella sp.* *Vibrio cholerae* Enteropathogenic *Escherichia coli* *Yersinia sp.* *Campylobacter jejuni*	Bacillary dysentery Typhoid fever Salmonellosis (gastroenteritis) Cholera A variety of gastroenteritis diseases Yersiniosis (gastroenteritis) Campylobacteriosis (gastroenteritis)
Viruses	Hepatitis A virus Norwalk viruses Rotaviruses Polioviruses Coxsackie viruses Echoviruses	Infectious hepatitis Acute gastroenteritis Acute gastroenteritis Poliomyelitis “flu like” symptoms “flu like” symptoms
Protozoa	*Entamoeba histolytica* *Giardia lamblia* *Cryptosporidium sp.* *Balantidium coli*	Amebiasis (amoebic dysentery) Giardiasis (gastroenteritis) Cryptosporidiosis (gastroenteritis) Balantidiasis (gastroenteritis)
Helminths	*Ascaris sp.* *Taenia sp.* *Necator americanus* *Trichuris trichura*	Ascariasis (roundworm infection) Taeniasis (tapeworm infection) Ancylostomiasis Trichuriasis (whipworm infection)

National Research Council, 1996

2.1.1 Indicator Organisms

The analytical techniques for detection and enumeration of pathogenic bacteria (e.g., S*almonella*) in wastewater and accompanying sludge are time-consuming, require well-trained technicians, and usually are expensive. Therefore, other organisms have been used as indicators of pathogens. The best indicator

organisms of the potential presence of pathogens are the facultative enteric organisms, i.e., fecal coliforms, and fecal streptococci.

Fecal Coliforms

Fecal coliforms are the most important subgroup of the total coliforms organisms. Fecal coliforms are good indicators of pathogen density. Farrel, 1985 (as cited by U.S. EPA, 1993) showed that if the fecal coliform reduction in treated sewage sludge was 100-fold (2-logs), then a reduction of *Salmonella* and enteric viruses, respectively, would be about 1.5 logs and 1.3 logs. In fact, the EPA Part 503 "Standards for the Use or Disposal of Sewage Sludge" regulations have established pathogen reduction requirements in terms of fecal coliform densities.

Fecal Streptococci

Fecal streptococci are found mostly, but not exclusively, in the intestines of humans and other warm-blooded animals. Fecal streptococci, along with fecal coliforms have been used to differentiate human fecal contamination from that of other warm-blooded animals. Although fecal streptococci are not ideal as indicators of fecal contamination, these organisms are relatively easy to enumerate and survive longer than fecal coliforms (Lewis-Jones et al., 1991).

2.1.2 Pathogenic Bacteria

The pathogenic bacteria in wastewater and wastewater sludge have been classified into two categories based on their significance to human health: (1) of major concern, and (2) of minor concern (Kowal, 1983): The major concern category included: *Salmonella* sp., *Shigella* sp., *Vibrio cholerae*, *E. coli* (pathogenic strains), *Campylobacter jejuni*, *Leptospira* sp., and *Yersinia enterocolitica*. All of these

bacteria may have symptomless infections and human carrier states, and many have important nonhuman reservoirs as well. The minor concern organisms are *Aeromonas*, sp., *Clostridium perfringens*, and *Pseudomonas aeruginosa*. The significance for human health of *Salmonella*, one of the major pathogenic bacteria, is briefly considered.

Salmonella

Salmonella are pathogenic bacteria of major concern in wastewater and sewage sludge management, primarily when stabilized sludge or "biosolids" are considered for land disposal. *Salmonella* sp. are Gram-negative, flagellate and motile rods and are facultative anaerobes. Two serotypes of *Salmonella, S. typhi* and *S. paratyphi* (A, B, C), are most dangerous to people.

2.1.3 Viral Pathogens

Approximately 140 types of enteric viruses may contaminate water and wastewater. These viruses enter into the human body orally, multiply in the gastrointestinal tract, and are excreted in large numbers in the feces of infected individuals (Bitton, 1994). The human enteric viruses that may be present in wastewater and sludge include Enteroviruses, Reoviruses, Rotaviruses, Adenoviruses, Norwalk viruses, and Astroviruses. The average concentration of enteric viruses in raw sewage in the U.S. varies between 1,000 to 10,000 plaque-forming units [PFU] per liter (Akin and Hoff, 1978 as cited by Kowal, 1983). The two diseases principally associated with viruses in sludge are gastroenteritis and hepatitis.

2.1.4 Protozoa Parasites

Most protozoan parasites produce cysts that can survive outside their host under adverse environmental conditions. Encystment is stimulated by lack of nutrients, accumulation of toxic metabolites, and host immune response. The major waterborne pathogenic protozoa affecting humans are *Entamoeba histolytica, Giardia lamblia, Cryptosporidium, Acantamoeba castellani, Naeleria gruberi,* and *Balantidium coli*. Protozoa and protozoan cysts are reported to be more sensitive to the adverse effects of sewage sludge treatment processes than other pathogens (U.S. EPA, 1993).

2.1.5 Helminths

Helminths are taxonomically divided into nematodes (roundworms), cestodes (tapeworms), and trematodes (flukes). The term helminth is from the Greek for "worms." Helminths exist in at least two forms. The first is an actively growing form inside the host (i.e., the worm), which produces eggs or ova. The ova pass from the host in the feces and constitute, or develop into, a second form (the larvae), which is resistant to adverse conditions and infects a new host and establishes new growth.

The pathogenic helminths whose eggs are of major concern in wastewater and sludge include *Ascaris lumbricoides, Ascaris suum, Trichuris trichiura, Toxocara canis, Toxocara cati, Taenia saginata, Taenia solium,* and *Hymenolepis nana*. Reimers et al. (1981) found *Ascaris, Trichuris and Toxocara* ova to be the most frequently recovered from municipal wastewater sludge in southern United States. The characteristics of *Ascaris* and *Taenia* species, two of the major pathogenic helminths are considered below:

Ascaris species

Ascaris are classed as nematodes. The two most important species are the human roundworm *Ascaris lumbricoides* and the pig roundworm *Ascaris suum.* The roundworms of the genus *Ascaris* are significant for human health because their infections are very common worldwide and because their eggs are persistent in the environment and are likely to survive conventional wastewater treatment processes and sludge treatment processes. The prevalence of ascariasis in the U.S. was estimated to be about 4 million infections in 1972 (Warren, 1974 as cited by Kowal, 1983).

Ascaris lumbricoides is the largest intestinal roundworm and one of the most commonly occurring human helminths. The female roundworm is about 5 mm in diameter and 200 to 400 mm long, while the males are not as large. The female roundworm produces numerous eggs, which require 1-3 weeks for embryonation. Infection of humans occurs by ingestion of infective ova that are on food or fingers contaminated with infected human feces. Humid and warm, but not sunny conditions are ideal for egg survival and development, with an optimum temperature of about 25°C to 30°C reported (Lewis-Jones et al., 1991).

Ascaris suum, the swine roundworm, may produce Loeffler's syndrome. The life cycle of *Ascaris suum* resembles that of *Ascaris lumbricoides*, with a pig instead of a human host. The eggs of *Ascaris* ova are round to ovoid in shape and have an average size of 65 μm by 45 μm.

Taenia species

Taenia saginata and *Taenia solium* are the beef and pork worms, respectively. The infection arises from eating incompletely cooked meat (of the intermediate host)

containing the larval stage of the tapeworm, the cysticercus. Humans serve as the definitive host, harboring the self-fertile adult. The occurrence of *Taenia* in sewage sludges in the United States is extremely low (Reimers, et al. 1981).

2.2 PATHOGEN REDUCTION IN WASTEWATER TREATMENT SYSTEMS

The removal of indicator and pathogenic organisms in wastewater treatment works has been well documented. The following mechanisms have been identified to reduce the densities of pathogenic organisms in wastewater treatment systems: sedimentation, adsorption, antagonistic organisms, filtration, and floc-entrapment. The reduction of indicator and pathogenic organisms through different steps of the wastewater treatment process is summarized in Table 2-2.

Table 2-2: Removal of Pathogens by Various Wastewater Treatment Processes

Treatment	**Enteric Viruses**	**Bacteria**	**Protozoan cysts**	**Helminth eggs**
Primary Sedimentation	0-30	50-90	10-50	30-90
Trickling Filter**	90-95	90-95	50-90	50-95
Activated Sludge**	90-99	90-99	50	50-99
Oxidation Ditch**	90-99	90-99	50	50-99
Waste Stabilization Ponds (Three cells, 25 days retention)	≥ 99.99	≥ 99.99	100	100
Septic Tanks	50	50-90	0	50-90

Removals in % adapted by Gerba (1983) from Feachem et al (1980).
** With sedimentation, sludge digestion, and sludge drying.

2.3 REDUCTION OF PATHOGENIC ORGANISMS IN WASTEWATER SLUDGE TREATMENT SYSTEMS

Wastewater sludge is by far the largest residue by volume that is removed from the wastewater at treatment facilities. The treatment and disposal of sewage sludge is expensive and complex. Municipal wastewater sludge contains approximately 93 to 99 percent water (by weight) and 1 to 7 percent solids and dissolved substances in the wastewater or added during treatment. Unprocessed sewage sludge may contain large numbers of pathogenic organisms (e.g., bacteria, viruses, protozoa, and helminths).

The principal methods of biosolids processing and management include preliminary treatment, sedimentation, thickening, stabilization, conditioning, disinfection, dewatering, heat drying, thermal reduction (incineration), beneficial use, and ultimate disposal (Metcalf and Eddy, 1991). The reduction in the numbers of indicator bacteria and pathogen organisms during the processing of sewage sludge was investigated at the Hornsby Bend Biosolids Management Plant (HBBMP) in Austin, Texas. Therefore, only the processes that achieve significant pathogen reduction such as stabilization and disinfection will be reviewed in this section.

The stabilization and disinfection of sewage sludge is accomplished by physical, chemical, and biological processes. Stabilization refers to those processes that reduce the volatile solids content, pathogen levels, and odor. Disinfection processes emphasize the reduction of pathogen organisms to below detectable limits. It should be noted, however, that some stabilization methods also affect some disinfection. Major stabilization methods include anaerobic digestion, aerobic digestion, composting, alkaline (lime) stabilization, and air-drying. Disinfection

includes pasteurization, long-term storage, irradiation, heat drying, and heat treatment. The EPA Part 503 regulations for the Use or Disposal of Sewage Sludge (CFR, 1995) are summarized below.

2.3.1 Federal Regulations

The Environmental Protection Agency (EPA) was required by the Clean Water Act Amendments of 1987 to establish regulations for controlling the final use and disposal of sewage sludges. Current regulations are known as "Standards for the Use or Disposal of Sewage Sludges" and were promulgated on February 19, 1993 and subsequently published in the Code of Federal Regulations, Title 40, Part 503. The Part 503 regulations control sewage sludge applied to the land, placed on a surface disposal, or fired in a sewage sludge incinerator. Part 503 regulations are divided into five subparts (A to D). Subpart D includes the pathogen and vector attraction reduction requirements for biosolids that are applied to the land or placed on a surface disposal site. The EPA rule "Part 503" addresses pathogens, and divides stabilized sludge into "Class A" and "Class B." Class A biosolids criteria requires the highest microbiologically quality. This sludge can be applied on land without any pathogen-related restrictions at the site. On the other hand, site restrictions apply to Class B biosolids. The requirements to meet Class A and Class B biosolids are summarized below.

Class A sludge essentially is free of pathogens. There are six alternatives for demonstrating pathogen reduction. The objective of each alternative is the reduction of pathogen densities to below detectable limits, which are:

- *Salmonella* sp. < 3 MPN per 4 grams of total solids (TS) (dry weight basis);

- Enteric viruses < 1 PFU per 4 grams TS; and
- Viable helminth ova < 1 viable ovum per 4 grams TS.

Class “A” biosolids must meet either a fecal coliform limit of less than 1,000 MPN/g TS or less than 3 *Salmonella*/4g TS, and one of the following six alternative requirements (CFR, 1995; NRC, 1996):

1. Time and temperature requirements specified, depending on solids content of sludge;
2. Alkaline and temperature treatment requirements: pH > 12 for at least 72 hours. Temperature > 52°C for at least 12 hours, then air-dry sludge to ≥ 50% TS;
3. If the untreated sludge contains < 1 Plaque Forming Units (PFU)/4 g TS of enteric viruses and < 1 viable ova/4 g TS for helminth ova, only the limits for fecal coliforms and *Salmonella* must be satisfied until the next time samples of the sewage sludge are tested.

   If enteric viruses are ≥ 1 PFU/4g TS and/or helminth ova are present prior to pathogen treatment, process operating parameters to achieve < 1 PFU/ 4 g TS for enteric viruses and < 1 viable ova/4 g TS for helminth ova must be documented;
4. Products obtained from unknown processes can be considered as Class A biosolids if the enteric virus density is < 1 PFU/4 g TS and helminth ova is < 1 viable ova/4g TS;
5. Use of a process to further reduce pathogens (PFRP), i.e., composting, heat drying, heat treatment, thermophilic aerobic digestion, beta ray irradiation, gamma ray irradiation, and pasteurization; and
6. Use of a process equivalent to PFRP, as determined by the permitting Authority.

Class B: biosolids are described by the Part 503 regulations as a sludge having lesser sanitary quality than Class A biosolids at the time of application. This class of sludge is applied to land under a variety of restrictions. A stabilized sludge must meet one of the following three alternative requirements to qualify as a Class B biosolids:

1. Monitoring of a pathogen indicator. The geometric mean of fecal coliform in seven samples should be less than 2×10^6 Most Probable Number (MPN) or Colony Forming Units (CFU) per gram of total solids (TS).
2. Use of a process to significantly reduce pathogens (PSRP), i.e., aerobic digestion, air-drying, anaerobic digestion, composting, and lime stabilization.
3. Use of a process equivalent to one of the PSRPs, as determined by the permitting Authority.

In addition to pathogen reduction requirements, the Part 503 rule includes vector attraction reduction requirements (VAR) for biosolids that are applied to land. All Class A and B biosolids must meet one of the requirements for vector attraction reduction. Vectors are animals involved in the transmission of infectious diseases to humans. In the case of sludge disposal, there is particular concern with insect vectors that might be attracted to the disposal site as a result of management practices. The PFRPs, PRSPs and VAR requirements are listed in Appendices A, B, and C, respectively.

2.3.2 Stabilization Processes

Stabilization includes processes that primarily break down the organic fraction of the sludge in order to reduce the mass and produce an aesthetically acceptable (odorless) sludge. Pathogen reduction occurs as an important side effect, but rarely was monitored or documented in the past. However, reduction in pathogen and vector

attraction is paramount in biosolids that are to be land applied or surface applied under the current regulations. The major sludge stabilization processes include anaerobic digestion, aerobic digestion, composting, alkaline (lime) stabilization, and air-drying. The effectiveness of stabilization processes in reducing the densities of indicator bacteria and pathogenic organisms in municipal wastewater sludge is discussed below.

Anaerobic digestion

Anaerobic digestion has been used widely for the stabilization of wastewater sludge for more than a half of a century, both in the United States and abroad. Data on volatile solids reduction are readily available. One of the goals of this study is the evaluation of the reduction of indicator and pathogenic organisms during anaerobic digestion. Therefore, a detailed discussion of this process is presented in section 2.3.3.

Aerobic digestion

The objective of the aerobic digestion process is the destruction of volatile solids, i.e., the biodegradable solids in the sludge, resulting in a reduction in the volume of sludge that requires further processing. The aerobic digestion process is effective in reducing the volatile solids from 35 to 50%. The EPA Part 503 regulations require that a 38% volatile solids reduction be achieved to attain the vector attraction reduction requirements. Aerobic digestion is classified as a process to significantly reduce pathogens (PSRP).

Reduction of pathogens in the aerobic digestion process has been addressed by various investigators. Kuchenrither and Benefield (1983) reported that bacterial indicators (i.e., fecal coliforms and fecal streptococci) die off increasingly as

temperature in aerobic digestion is raised from 20°C to 40°C. Farrah and Bitton (1984) detected *Salmonella* sp. densities of 0.8 to 33 MPN/g in aerobically digested sludges from three wastewater treatment plants. Martin et al, 1990 (as cited by Bitton, 1994) suggested that the reduction in enteric bacteria and viruses during aerobic digestion of sludge depends on both temperature and detention time. However, the mesophilic aerobic digestion process appears to be ineffective in reducing helminth ova such as *Ascaris* (Pedersen, 1981).

The autothermal thermophilic aerobic digestion (ATAD) is becoming more popular because the treated biosolids contain lower pathogen densities and higher solids.

Autothermal Thermophilic Aerobic Digestion

The ATAD is an aerobic digestion process that operates at thermophilic temperatures (40 to 80°C) without supplemental heat. The oxygen concentration, volatile solids content, and mixing in the ATAD reactor result in the degradation of organics solids to carbon dioxide, water, and nitrogen by-products, as well as the release of heat. The process can be controlled at thermophilic temperatures to achieve greater than 38% volatile solids destruction and pathogen reduction sufficient to meet Class A biosolids requirements if sufficient insulation, hydraulic detention time (HDT), and adequate solids concentration are provided (WEF, 1995).

The results of investigations carried out in North America indicate that the ATAD process can reduce significantly the number of pathogenic organisms in sewage sludge. Kabrick and Jewell (1982) found that *Salmonella* sp. were reduced to below detection limits at 45°C and 24-hour detention time and viruses were completely inactivated at temperatures greater than or equal to 40°C and a pH greater

than or equal to 7.0 by the ATAD process. Parasitic ova were significantly reduced by the aerobic thermophilic system; however, at 49°C, ova were not inactivated completely.

In another study, fecal coliforms, fecal streptococci and *Salmonella* were determined as pathogen indicators. Their results showed that fecal coliforms and fecal streptococci densities were less than 100 MPN/g wet solids in the majority of the 12 samples tested and *Salmonella* was not detected in any sample at three sewage sludge treatment facilities (Kelly et al.1993). At least two reactors must be constructed in series and temperatures of at least 55°C must be consistently obtained in the second or final reactor (Kelly et al.1993).

Composting

Composting is a biochemical stabilization process that prepares wastewater residuals for beneficial use as a soil conditioner or fertilizer. The process is self-heating that destroys pathogens and produces a humus-like material. Approximately 20 to 30 percent of the volatile solids are converted to carbon dioxide, water vapor and heat. The three major types of composting systems in the United States are aerated static pile, windrow process, and in-vessel processes. A survey showed that 198 composting facilities were in operation in the U.S, in 1997 (BioCycle, 1998 as cited by U.S. EPA, 1999). The compost heats to temperatures in the pasteurization range of 50 to 70°C (120 to 160°F) as the organic material in the sludge decomposes and the enteric pathogenic organisms are destroyed (WEF, 1995).

Temperature is the key factor controlling survival of pathogens. Temperature/time relationships for pathogen inactivation in actual composting operations are summarized in Table 2-3.

Composting where temperatures reach the thermophilic range should eliminate practically all-viral, bacterial, and parasitic pathogens (Water Pollution Control Federation, 1984 as cited by WEF, 1995). However, some fungi, such as *Aspergillus fumigatus*, are thermotolerant and survive the composting process.

Table 2-3: Temperature and Time for Pathogen Destruction in Compost

Microorganisms	Exposure time for destruction at various temperature (hr)		
	40-55°C	60°C	65°C
Salmonella newport			25
Salmonella	168	116	
Poliovirus type 1		1.0	
Candida albicans		72	
Ascaris lumbricoides		4.0	1.0
Mycobacterium tuberculosis			336

From WEF, 1995

The results of laboratory studies demonstrate the effective inactivation of viruses during composting and curing (Bitton, 1994; Pedersen, 1981). Protozoan cysts and helminth eggs in composting sludge are inactivated at a temperature of 55°C or above readily (Brandon, 1978 as cited by Bitton, 1994). Theis and Storm (1978) reported that after 17 days in an experimental compost facility during which temperatures of 63°C (146°F) were measured, all the *Ascaris suum* ova recovered in the samples showed evidence of degeneration. A survey about the occurrence of pathogens in compost and compost products indicated that no significant health hazard was associated with parasitic helminth ova or enteric viruses (Yanko, 1988). The effectiveness of composting in inactivating pathogen bacteria, viruses, and helminths has been demonstrated; however, concerns about the regrowth of pathogenic bacteria have been raised. The regrowth of pathogenic bacteria such as

Salmonella sp., *Yersinia enterocolitica* and toxigenic *E. coli* in compost and compost products was observed regularly (Yanko, 1988). This author pointed out that composts modified with various materials to produce commercial soil amendments contained significantly higher concentrations of bacteria and fungi than the base compost material. It appears that the regrowth phenomenon is related to the nutrient level.

Air drying

Biosolids are dried on sand beds or on paved or unpaved basins. Drying beds originally were developed to dewater digested sludge; however, significant pathogen inactivation characteristics have been reported. The EPA Part 503 regulations consider air-drying as a process to significantly reduce pathogens (PSRP). When air-drying is to be used, the EPA Part 503 regulations require the biosolids dry for a minimum of 3 months. The ambient average daily temperature must be above 0°C during 2 of the 3 months

Sludge dewaters in conventional sand drying beds as water drains through the sludge mass and supporting sand and evaporates from the surface exposed to the air. Most of the water leaves the sludge by drainage, thus the provision of an adequate under drain system is essential. The dried sludge is removed from the sand. Recently, paved basins have been used as an alternate to sand drying beds. Paved basins may be of two types: 1) a drainage type, and 2) a decanting type. The drainage type paved basin functions similar to a conventional sand bed. Sludge removal is with a front-end loader. The decanting-type basin depends on the decanting of the supernatant and mixing of the drying sludge to enhance evaporation. Decanting may remove about 20 to 30 percent of the water with a well-settling sludge. Solids concentration may range

from 40 to 50 percent for a 30 to 40 days drying time in an arid climate for a 12 in (300 mm) sludge layer (Metcalf and Eddy, 1991).

Several authors have reported that sludge drying to 95% solids results in a reduction in bacterial pathogens ranging from 0.5 to almost 4 logs. However, viruses generally are detected in dried sludge; thus, sludge drying may not be a reliable process for complete inactivation of viruses (Bitton, 1994). Dewatered anaerobically digested and centrifuge cake sludges that were aged for a minimum of 1.5 years in lagoons and then dried in paved basins to a concentration of at least 60 percent solids, under controlled operating conditions, reached Class A sludge criteria with respect to fecal coliforms, *Salmonella*, viruses, and helminth ova (Tata et al.1997).

Alkaline Stabilization

The principal objectives of alkaline stabilization are to substantially reduce the number and prevent the regrowth of pathogenic and odor-producing organisms. Therefore, health hazard associated with the biosolids is minimum (WEF, 1995).

Alkaline chemicals are added to raise the pH of the sludge that is maintained at a sufficient length of time. At a pH 12 for at least 2 hours of contact time and homogeneous alkaline chemical/sludge mixing, pathogens and microorganisms are inactivated or destroyed.

Two methods of lime stabilization are (1) addition of lime to sludge prior to dewatering, termed “liquid lime or prelime stabilization”, and (2) addition of lime to sludge after dewatering, termed “ dry lime or postlime stabilization”. The traditional lime stabilization is classified by the U.S. EPA as a process to significantly reduce pathogens (PSRP), and meets the requirements for Class B biosolids. Many of the advanced alkaline stabilization technologies meet the U.S. EPA definition of

processes to further reduce pathogens (PFRPs) and the Class “A” sludge requirements (WEF, 1995).

Total coliform, fecal coliform, and fecal streptococci densities were reduced by more than 99.9% by using liquid lime stabilization at a pH ≥12.0 in full-scale studies in Lebanon, Ohio (U.S. EPA, 1979). *Salmonella* and *Pseudomonas aeruginosa* were reduced to below detectable levels; however, liming did not completely reduce the viable Ascaris parasites (Pedersen, 1983). Christensen, 1987 (as cited by WEF, 1995) reported that fecal coliform and streptococcus organisms were reduced by at least two orders of magnitude by using dry quicklime doses of 13 and 40% on a dry-weight basis as calcium hydroxide. Studies by Malina et al (1993) in anaerobically digested sludge have demonstrated that quicklime doses of 0.14 to 0.37 lb CaO/lb wet sludge at a pH of 12 and a temperature of approximately 70°C for 30 minutes were able to reduce fecal coliform and fecal streptococci densities up to 6.43 and 5.57 logs, respectively. *Salmonella* sp. also were reduced to below detectable limits. Therefore, dry lime stabilization pasteurization appears to meet PFRP criteria and the Class A sludge requirements. The reduction of bacterial organisms by different stabilization processes are summarized in Table 2-4.

Table 2-4: Log Reduction of Bacteria by Municipal Sludge Stabilization Processes

Process	Fecal Coliform	Fecal Streptococcus
Anaerobic digestion (35°C)		
Mean	1.84	1.48
Range	1.44 - 2.33	1.1 – 1.94
Aerobic digestion		
20°C [1]	1.0	1.0
30°C [2]	2.0	1.64
Composting	≥4	2.9
Liquid lime stabilization		
Raw primary	5.1	2.4
Waste activated	3.2	3.2
Mixed primary and trickling		
Filter humus, 4% solids	2.6	1.8
Storage		
10°C	-	1.0
20°C	-	1.5
30°C	-	2.0

WEF, 1995

[1] Laboratory study, 35-day detention time

[2] Laboratory study, 30-day detention time

Disinfection Processes

Sludge disinfection is important consideration to satisfy the strict regulations for the beneficial use of biosolids. Disinfection of liquid and dewatered sludges can be accomplished by pasteurization, long-term storage, heat drying, heat treatment, and irradiation.

Pasteurization

Sewage sludge pasteurization is achieved by raising and maintaining the temperature of the sludge to 70°C (158°F) for 30 minutes. This process is energy-intensive; however, pasteurization effectively destroys helminth eggs and most

bacterial and viral pathogens. Heat pasteurization is a proven technology in Europe that requires skills such as boiler operation and the understanding of high-temperature and pressure processes. The EPA Part 503 regulations consider pasteurization as a process to further reduce pathogens (PFRP).

Long-term storage of liquid and dewatered digested sludge

Liquid digested sludge normally is stored in earthen lagoons in land application systems. Storage is often necessary to store sludge during periods when weather or crop considerations preclude application. In this case, storage facilities can perform a dual function by providing disinfection as well as storage (Metcalf and Eddy, 1991).

Ahmed and Sorensen (1997) indicated that pathogenic organisms (i.e., *Salmonella typhimurium, Yersinia enterocolitica*, *Campylobacter jejuni*, and *Ascaris suum* eggs) in dewatered digested sludges can be reduced to below detection limits in storage piles, irrespective of mixing conditions and pile temperatures in five WWTPs in Utah. Average storage times for reduction of *S. typhimurium, Y. enterocolitica* and *Ascaris suum* eggs to below detection limits were 90 days, 75 days and 330 to 400 days, respectively. The most important factor for the destruction of *A. suum* was storage time.

Irradiation

Irradiation consists of subjecting sewage sludge to isotopes emitting gamma radiation (i.e., cobalt-60 or cesium-137) or to high-energy electron beams. The agents that disinfect sludge are fast electrons that originate from the interaction of gamma rays or in an electron-beam (EB) generator (White, 1984 as cited by Lewis-Jones et

al., 1991). This process essentially destroys the microbial genetic material. The level of the irradiation is relatively low and does not result in the production of radioactive sludge (Ahlstrom and Lessel, 1986 as cited by Bitton, 1994). Gamma and beta irradiation have been categorized by EPA as processes to further reduce pathogens (PFRP's). The sludge must absorb irradiation to a dose of 1 Mrad.

Ahlstrom and Lessel, 1986 (as cited by Bitton, 1994) compiled the D_{10} values (absorbed radiation dose required to reduce a population of microbes by one order of magnitude, i.e., one-log or 90%) for reduction of selected pathogenic organisms. Bacterial organisms including S. *thyphimurium* were efficiently reduced at D_{10} values ranging from 14 to 250 krad. Enteric viruses were reduced at the same D_{10} values from 165 to 350 krad. *Ascaris* sp., and *Aspergillus fumigatus* were inactivated at D_{10} values of less than 66 and 50 to 60 krad, respectively. Viruses are the pathogens most resistant to irradiation.

Heat Drying

Heat drying involves using active or passive dryers to destroy pathogens and remove water from sewage sludge. The EPA Part 503 regulations consider heat drying as a process to further reduce pathogens (PFRP). In this process, sewage sludge solids are dried with hot gases at temperatures greater than 80°C to reduce the moisture content to 10% or lower. Pathogenic bacteria, viruses, and helminth ova are reduced to below detectable levels in properly operated heat-drying systems.

Heat Treatment

This process involves heating sludge at temperatures up to 260°C (500°F) and a pressure up to 400 psig (2760 kN/m2) for approximately 30 min. Heat treatment is

used to break down the gel structure, and to improve the release of water from the sludge solids. The sludge is sterilized and dewaters readily (Metcalf and Eddy, 1991). The EPA Part 503 regulations consider heat treatment as a process to further reduce pathogens (PFRP). The liquid sewage sludge must be heated to a temperature of at least 180°C (356°F) for 30 minutes. Pathogenic bacteria, viruses, and helminth ova are reduced to below detectable levels in properly operated heat treatment systems.

2.3.3 Anaerobic Digestion

Biological stabilization of the sludge solids by anaerobic digestion is frequently an integral process prior to disposal and/or land application of biosolids. Anaerobic digestion was operated to stabilize sludge in 52% of 117 POTW facilities surveyed (Edgar et al. 1998). Anaerobic digestion typically produces a Class B biosolids (U.S. EPA, 1992).

Anaerobic digestion of municipal wastewater sludge is the transformation of complex organics solids in the absence of dissolved oxygen to gaseous end products such as methane and carbon dioxide and to an innocuous sludge. A net reduction in odors, pathogen concentration, volatile solids and volume of sludge requiring further processing also are accomplished.

The microbial stabilization of wastewater sludge is a sequential process in which volatile solids (organic materials) are hydrolyzed to simpler soluble organic compounds by facultative heterotrophic organisms. These soluble organic compounds are fermented by acid-producing facultative bacteria to volatile acids, carbon dioxide and some hydrogen gas. The volatile acids are converted primarily to methane gas by anaerobic-forming bacteria (Haug et al., 1998). The anaerobic digestion process is limited primarily by the growth rate of methane-producing bacteria, which in turn are limited by temperature, pH, volatile acid, and alkalinity concentrations. Detention time in the reactor affects the remaining conversion of volatile acids to methane (Pedersen, 1981).

Design and Control Considerations for Anaerobic Digestion

The design parameters for anaerobic digestion include hydraulic detention time, volatile solids loading, temperature, and mixing. Digestion performance usually is based on volatile solids destruction and gas or methane production. Malina (1992) reported optimum and extreme environmental conditions for maximum methane production in anaerobic digesters (Table 2-5). The parameters that would provide an insight into the condition of the environment early enough to avert digester failure are the volatile acids concentration, alkalinity, pH, and the carbon dioxide content of the gas. These parameters are interrelated and one variable may directly or indirectly affect the others. Therefore, close monitoring of these variables is essential to the control of the anaerobic system. The relationship between alkalinity, pH, and carbon dioxide content at 35°C are presented in Figure 2-1 (McCarty, 1964). The effect of temperature and detention time on the destruction of volatile solids and gas production has been widely documented; however, the relationship among detention time, temperature, and solids loading on the reduction of pathogens has not yet described in full detail. The design and operating variables during anaerobic digestion are discussed below.

Hydraulic Detention Time

The primary objective of anaerobic digestion is the destruction of volatile solids (organic matter). The key to efficient digestion is the development and maintenance of a large, stable, viable population of methane-forming bacteria. Therefore, the most important parameter for system design and operation is the solids detention time (SDT)[hydraulic detention time].

Table 2-5: Environmental and Operating Conditions for Maximum Methane Production during Anaerobic Digestion of Municipal Sludges

Variable	Optimum	Extreme
pH	6.8-7.4	6.4-7.8
Oxidation Reduction Potential (ORP)(mv)	-520 to -530	-490 to -550
Volatile Acids (mg/L as acetic acid)	50-500	>2,000
Alkalinity (mg/L as $CaCO_3$)	1500-3,000	1,000-5,000
Temperature		
Mesophilic	30-35°C (86-95 °F)	20-40 °C 68-104 °F
Thermophilic	50-56 °C 122-132 °F	45-60 °C 113-140 °F
Hydraulic Detention Time (days)	10-15	7-30
Gas Composition		
Methane (CH_4) (%v)	65-70	60-75
Carbon Dioxide (CO_2)(%v)	30-35	25-40

Malina (1992)

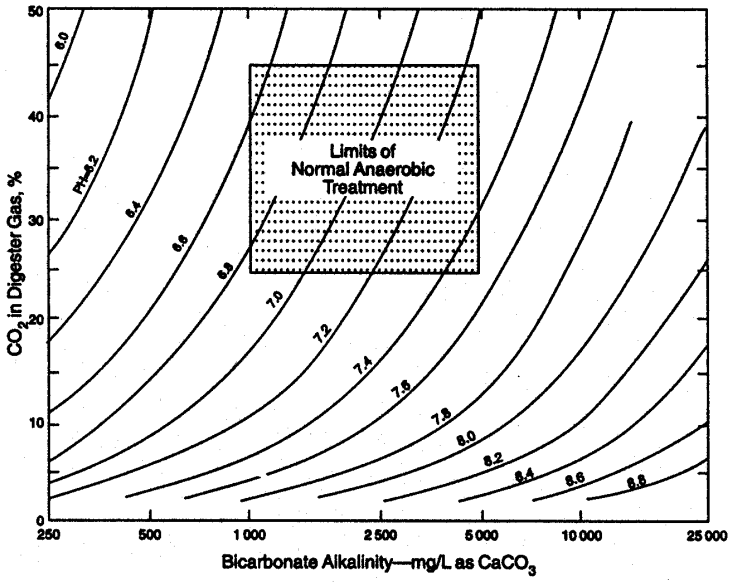

Figure 2-1: Relationship between Bicarbonate Alkalinity, pH, and Carbon Dioxide Percentage in Anaerobic Treatment (McCarty, 1964).

A minimum SDT is essential in the anaerobic digestion process to ensure that the necessary microorganisms are being produced at the same rate as methane organisms leave the system in the digested sludge. The system fails from washout of the microbial population of methanogens at SDT below a critical time. This critical SDT depends on the generation time of the methane-forming bacteria (i.e., the time required to double the microbial population of methane-forming bacteria). The generation times for methane-forming bacteria range from less than two days to 30 days at a temperature of 35°C (95 °F) depending on the organism. Anaerobic digesters are commonly operated on the no recycle basis; therefore, in this study, the terms hydraulic detention time (HDT) and solids retention time (SRT) will be used alternatively

The correct determination of the reactor volume to ensure sufficient stabilization of the sludge organics and methane gas production depends primarily on the HDT. Typical HDTs range from 15 to 20 days; however, the EPA Part 503 calls for minimum HDTs of 15 days at 35°C, or up to 60 days at 20°C to meet the Class "B" biosolids requirements.

Solids Loading

Standard rate digestion is characterized by intermittent feeding, no heating, no auxiliary mixing, detention times of 30 to 60 days, and organic loadings of 0.64 to 1.60 kg VS/m^3-d (40 to 100 lb VS/1000 ft^3-d). The functions of digestion, sludge thickening and supernate formation are carried out simultaneously in the digester High rate systems are characterized by heating, thickening, auxiliary mixing, detention times of 15 to 20 days, and organic loadings ranging from 1.6 to 3.2 kg VS/m^3-d (100 to 200 lb VS/1000 ft^3-d)(WEF, 1995). Some high rate systems are

carried out in a two-stage process, i.e., a heated mixed digester followed by a second digestion not heated nor mixed. The function of the second-stage digester is the gravity separation and concentration of the digested sludge solids; thus reducing the volume of sludge requiring disposal. A supernatant results in a two-stage system. The supernatant contains a considerable amount of volatile solids and organic constituents and cannot be disregarded in evaluating digester performance or estimating the load on the treatment plant. The supernatant requires treatment before disposal (Haug et all, 1998). The relationship between the concentration of solids in the feed sludge and the organic loading to the digestion tank for a given hydraulic detention time is illustrated in Figure 2-2.

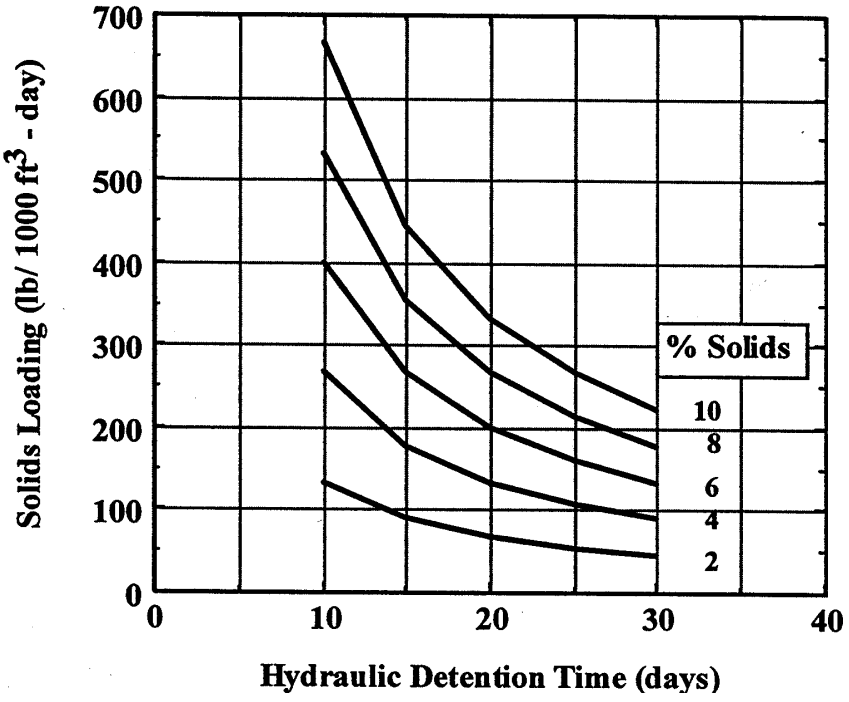

Figure 2-2: Effects of Volatile Solids Content in the Feed Sludge and Hydraulic Detention Time on the Organic Loading to Anaerobic Digestion Systems (Haug et al. 1998).

Temperature

Anaerobic digesters generally are operated in the mesophilic temperature range 30-38°C (86 to 100°F) or thermophilic range 50-60°C (122 to 140°F). Most of the digesters have been designed to operate in the mesophilic range, however, considerable research is being undertaken to evaluate the potential of the thermophilic range. Thermophilic microorganisms are considered to be more sensitive to temperature changes than mesophilic organisms. Several advantages have been reported for thermophilic digestion over mesophilic digestion, including increased degree of organic matter destruction, improved dewaterability characteristics, and increased destruction of pathogenic bacteria (Parkin and Owen, 1986). Limitations of the process include extreme sensitivities of the organisms to the defined temperature range, a higher net energy input (consequently higher operation costs) than for mesophilic digestion, and the production of biosolids with a comparatively more offensive odor (WEF, 1995).

Pathogen Reduction during Anaerobic Sludge Digestion

The main objectives of anaerobic digestion are the production of stabilized biosolids, reduction of pathogens, and reduction of the amount of biosolids by partial destruction of volatile solids. Pathogen reduction is required to reduce public health concerns related to beneficial use of biosolids. The U.S.EPA established strict regulations that dictate specific pathogen reduction performance requirements for the beneficial use of anaerobically digested biosolids.

Indicator and Pathogenic Bacteria

Berg and Berman (1980) found that temperature is the primary factor in the die-off of indicator bacteria at 20 days of HDT. The reduction of indicator organisms

at 49°C was greater than at 35°C. At mesophilic conditions, FC were reduced in more than 1.69 log units. Carrington and Harman (1984) estimated the decimal decay-rates for *Salmonella duesseldorf* using the equation (2-1) developed by Ginnivan et al. (1980).

$$Pw/Pf = R/(10^{R\theta kd} - 1 + R) \qquad \text{Eq. (2-1)}$$

where P_w and P_f are the pathogen content of the withdrawal and feed, respectively, R is the fraction of the total volume replaced at each feeding, θ is the mean sludge retention time (days), and k_d is the decimal decay rate (d^{-1}), i.e., the fraction of the original population surviving after unit time. The inactivation rates for *Salmonella* were greater in heated anaerobic digesters. Decay rates for S. duesseldorf were greater at 48°C (k_d = 3.4 d^{-1} at 20 days SRT) than at 35°C (k_d = 1.15 d^{-1} at 20 days SRT). There was no consistent tendency for decay rates for *Salmonella* to depend on the detention period.

Pike et al. (1988) reported that the inactivation rates for *Salmonella duesseldorf* were greater at 49°C than at 35°C. The results showed that at 35°C, *Salmonella duesseldorf* densities were reduced from 91% (10 days detention time) to 99.8% (20 days detention time). The decimal decay rates for *Salmonella* followed first order kinetics at mesophilic conditions.

Stukenberg et al. (1994) found that the effectiveness of the mesophilic anaerobic digestion process in reducing fecal coliform (FC) densities averaged 2 logs (base 10) in 54 wastewater treatment plants (WWTP). Approximately 47 out of 54 plants (87%) showed FC densities below the limit established by current regulations (40 CFR Part 503) for Class B biosolids (i.e., $2x10^6$ or 6.3 logs MPN/g TS).

Ponugoti et al (1997) reported that FC, FS, and *Salmonella* densities were reduced 2.70, 2.0, and 1.51 log units, respectively, during mesophilic anaerobic digestion. Performance data indicate that FC and FS reductions increased as the retention time increased, but decreased slightly as the VSS loading increased. Anaerobic digestion was superior to aerobic (mesophilic) digestion in reducing pathogen densities levels under the field conditions studied.

Farrel et al. (1988) observed that changing the feeding pattern from draw/fill (D/F) to fill/draw (F/D) affected on the reduction of fecal coliforms and virus counts, but did not affect volatile solids destruction. Reductions of both bacterial and virus counts were greater using the D/F feeding mode than the F/D pattern, but the effect was more greater for bacteria than for viruses. The experiments were carried out at 35°C and a mean hydraulic detention time of 14 days. Reductions of 2.85, 2.40, and 2.29 logs were reported for TC, FC and FS, respectively, during D/F operation. However, under the F/D operations, reductions of 1.28, 1.16, and 1.21 for TC, FC, and FS were reported. Virus inactivation was 0.97 and 0.74 logs, respectively, for D/F and F/D patterns. A material balance for the number of organisms and first order kinetics led to the development of equation 2-2. This expression may be used to calculate for F/D or D/F operation, either a rate constant from digester feed and product concentrations or digester performance knowing the rate constant.

$$\frac{v}{V}\frac{C_f}{C_d} = \left(1 + \frac{v}{V}\right)e^{k\theta} - 1 \qquad \text{Eq. (2-2)}$$

where v = volume withdrawn and added, V = digester volume before volume v is fed, k is the first order rate constant, θ is the time from addition to withdrawal, C_f is the microbial density in feed, and C_d is the microbial density in sludge withdrawn. The use of equation 2-2 at detention times other than the HDT at which the data were collected (i.e., 14 days) is not recommended unless data on effect of HDT (shorter or longer) on bacterial inactivation rates are determined.

Lee et al. (1989) reported lab-scale experiments that were conducted at two temperatures (35°C and 53°C) and two different HDTs, 10 and 20 days. Pathogens density reductions at 53°C were greater than those at 35°C. Thermophilic anaerobic digestion at 53°C and 10 days of SRT reduced indicator bacteria to essentially undetectable levels. Under mesophilic conditions and a SRT of 20 days, the two-phase anaerobic digestion achieved greater log bacterial density reduction than single-stage digestion by 0.91, 0.80, and 0.48 logs units for FC, EC, and FS, respectively. The bacterial density reductions under mesophilic conditions increased slightly as the HDT was increased from 10 to 20 days but the results were not statistically significant at a 95% confidence level.

Watanabe et al (1997) in their field study including 17 wastewater treatment facilities throughout Japan reported that FC densities were reduced by 2 log units during mesophilic anaerobic digestion. The densities of *Salmonella* were also reduced, but the digested sludge did not meet the EPA Part 503 requirements for Class A biosolids. Lab-scale anaerobic digesters that were operated at detention times of 10, 20, and 30 days at 35°C, and 10 and 20 days at 55°C indicate that temperature affected the inactivation of bacteria but the effect of detention time under mesophilic conditions was not conclusive. At 55° and 20 days (SRT), the densities of indicator

and pathogenic bacteria were reduced to levels that meet the EPA Part 503 requirements for Class A biosolids.

Viruses

Sanders, et al (1979) found that viral recovery was a direct function of temperature. As temperature increased from 34°C to 37°C to 50°C, the importance of heat in the loss of recoverable virus also increased. Detention times of 5, 10, and 15 days had a smaller but direct effect on viral recovery in the mesophilic range. The effect of detention time decreased as temperature increased. Detention time had the greatest effect of viral recovery at 34°C. The results indicate that the effects of volatile solids loading on viral removal were not conclusive.

Berg and Berman (1980) found that temperature is the primary factor in the die-off of viruses. At 49°C and 20 days of HDT, the reduction of viruses increased over reduction at 35°C. At mesophilic conditions, viruses were reduced in more than 1.0 log units whereas at thermophilic digestion, the numbers of viruses were reduced by 2 to 3 logs. The authors concluded that neither the TC, the FC, nor the FS are good indicators for the presence of viruses in mesophilic or thermophilic digested anaerobic sludges.

Farrel et al. (1988) reported that at 35°C and a mean hydraulic detention time of 14 days, draw/fill (D/F) to fill/draw (F/D) had a considerable effect on the reduction of fecal coliforms and virus counts, but little effect on volatile solids removal. Reductions of bacterial and virus counts were greater using the draw/fill-feeding mode than the fill/draw, yet the effect was more significant with bacteria than with viruses.

Lee et al. (1989) reported that in thermophilic anaerobic digestion at 53°C, enteroviruses were reduced to essentially undetectable levels; however, no difference in the viral density was observed between the conventional (single-stage) and the two-phase digesters in both 10 and 20-day SRTs under mesophilic conditions (35°C).

Helminths

Analyses of sewage sludge samples that were collected from a full-scale anaerobic digester operating at 35°C and 14-day SRT, and sludge retention basins (HDT not specified), showed that, significantly fewer ova from the lagooned sludge embryonated (17% of 100 ova) as compared to the ova from the fresh sludge that embryonated, i.e., 55% of 110 ova (Arther et al., 1981). Storage was suggested as the factor in the reduced embryonation of the parasite ova in lagooned sludge.

Laboratory experiments that were carried out at 35°C and 10, 16 and 20 days, and 48°C at 10 and 20 days, confirmed that the inactivation rates for the ova of *Ascaris suum* were greater in heated anaerobic digesters at 48°C than those at 35°C. No consistent tendency of the decay rates with detention period was observed for Ascaris ova (Carrington and Harman, 1984).

Pike et al. (1988) reported that the inactivation rate for *Ascaris suum* ova was greater at 49°C than at 35°C. Mesophilic anaerobic digestion (35°C) had little effect on viability of Ascaris ova. Reductions of the order of 99% or more occurred in thermophilic digestion or heating to at least 55°C for at least 15 minutes.

Lee et al. (1989) indicated that anaerobic digestion at 53°C and detention time of 10 days reduced the densities of Ascaris eggs to essentially undetectable levels.

Chapter 3: Experimental Materials and Methods

This research involved two phases. Phase I focused on field studies to determine the effectiveness of various conventional sludge treatment processes in reducing indicator and pathogenic organisms. Phase II involved lab-scale studies to evaluate the effect of several operating variables on the reduction of indicator and pathogenic bacteria during mesophilic anaerobic digestion of wastewater sludge.

3.1 PHASE I: FIELD STUDIES

The fate and survival of fecal coliforms, fecal streptococci, *Salmonella* sp, and helminth ova in various treatment processes at the Hornsby Bend Biosolids Management Plant (HBBMP) in Austin, Texas was evaluated in this phase of the project. Combined primary and waste activated sludge from three wastewater treatment plants (WWTPs): Govalle, Walnut Creek, and South Austin Regional Plant are pumped to the HBBMP for processing.

Approximately 2,082 cubic meters (550,000 gallons) of combined primary and waste activated sludge are treated daily at HBBMP. The process train includes gravity belt thickening, anaerobic digestion, air-drying, (or mechanical dewatering), and windrow composting (Figure 3-1). The raw sludge enters an equalization tank and is concentrated from 1.6 to 7.8 percent by belt thickening using a liquid polymer (Magnifloc1598 C™). The thickened sludge is pumped to the anaerobic digesters, which at the time of the study were operated with no sludge heating and a long hydraulic detention time (> 60 days). The digested sludge was air-dried in open paved rolled concrete basins to concentrate the solids to approximately 20 percent. About 45 percent of the dried solids are applied to adjacent land where hay is grown for animal

fodder. The remaining biosolids are composted aerobically. The side streams of the sludge treatment facility (i.e., filtrates from the thickening and dewatering processes as well as the decant from the air-drying basins) subsequently are treated in a facultative stabilization pond. The pond effluent is treated in a greenhouse-enclosed Duckweed (*Lemna giba*) pond. A portion of the effluent is used for irrigation and the rest is discharged to the Colorado River.

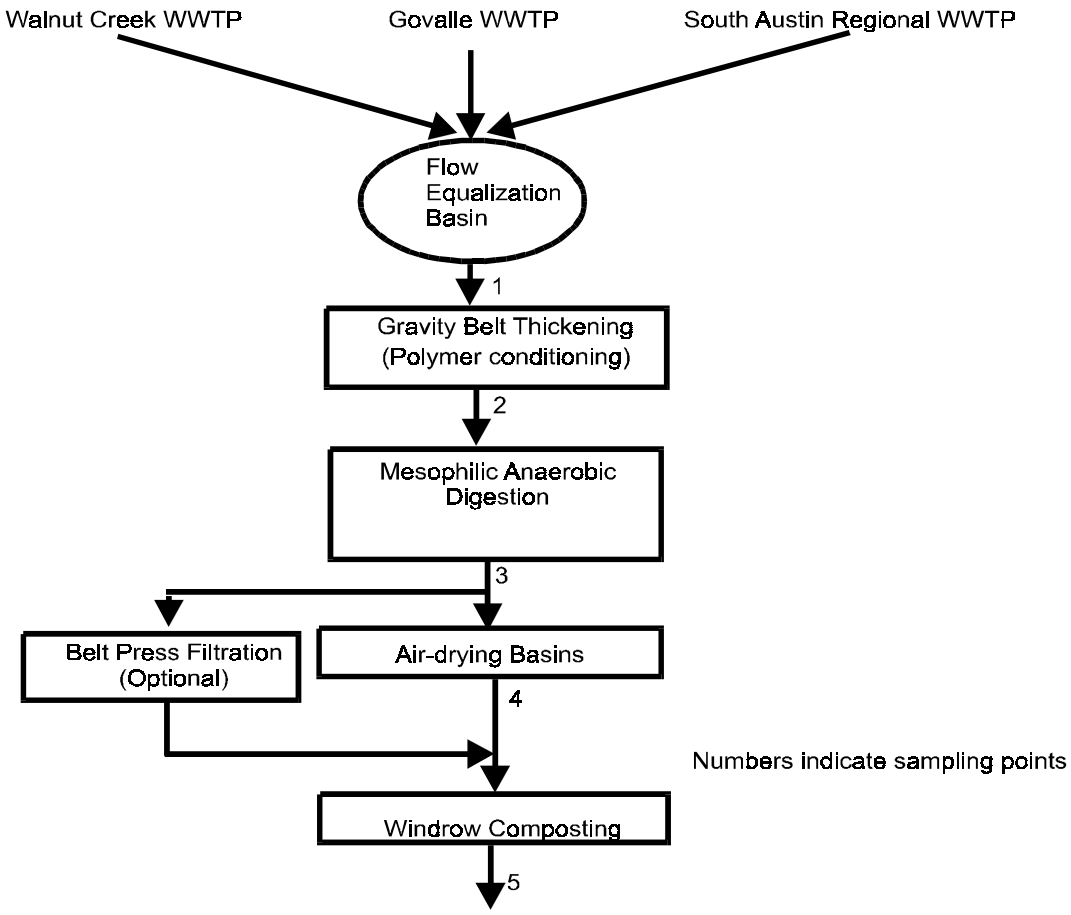

Figure 3-1: Hornsby Bend Biosolids Management Plant Diagram, Austin, Texas.

The air-drying process involves an area of approximately 11.1 ha (27.5 acre), which is divided into five basin cells of 2.2 ha (5.50 acre) each. The cells have the

following dimensions: 800-ft length, 300-ft width, and 3-ft depth. The system was operated at a solids application rate of approximately 450 dry metric tons/ha (199.8 ton/acre) and an average depth of 0.91 m (3-ft). The contents of the basins were turned three times per week using a Brown Bear™ or equipment such as a tractor with a horizontal auger. The biosolids were held in the basins for a period ranging from 7 months to 12 months or even longer to reach the 20 percent solids concentration required. Ambient temperature and precipitation controlled the holding time.

The composting process that includes windrow composting, curing and storage covers an area of about 5.7 ha (14 acres). The windrows are 16-ft wide, 6-ft high, and 600-ft long. Approximately 500 cubic yards of dewatered sewage solids and 1200 cubic yards of bulking agents (i.e., wood chips and/or yard trimmings) are mixed to form the windrow. The mixture of bulking agents and air–dried biosolids were turned 8 to 10 times, to maintain aerobic conditions, for a period of approximately 6 weeks with a Scarab™. This procedure satisfies the minimum criteria of 15 days at 55°C with 5 turnings established by the EPA for a Class A sludge. The composted biosolids were “cured” in stockpiles for a period ranging from 60 to 90 days. After curing, a 3/8-inch trommel screen was used to separate the wood chips from the finished compost. Most of the composted biosolids are sold to private vendors, and a small fraction is available to the public and for use by non-profit organizations. Approximately 3,632 metric tonnes per year (4,000 tons) of compost are produced from the 12,264 metric tonnes (13,507 tons) of dry solids processed at the Hornsby Bend Facility. Therefore, the composted sludge is approximately 30 percent by weight of the dry solids in the raw wastewater sludge.

The densities of indicator and pathogen organisms were determined by collecting and assaying a number of samples from various points along the sludge process train. Analytical procedures for assaying indicator organisms are presented in the Standard Methods for the Examination of Water and Wastewater (1995). Pathogenic organisms such as *Salmonella* species and helminth ova were determined by the analytical methods referenced by the U.S. EPA (1992).

3.1.1 Collection of Samples

Samples of raw sludge and wastewater solids after different treatment processes were collected at least two times per month from October 1996 to March 1998 at each of the following points (Figure 3-1):

1. Influent wastewater sludge,
2. Belt thickening sludge,
3. Anaerobic digested biosolids,
4. Air-dried sludge, and
5. Windrow composted biosolids.

Approximately 41 samples were collected at each sampling point. The analysis of samples was performed at the laboratories of the Center for Research in Water Resources (CRWR) of The University of Texas at Austin. All the sludge samples were collected by using 1-L presterilized high-density polyethylene (HDPE) bottles. The sampling procedure at each sampling point is presented below:

Influent wastewater solids

Four grab samples of raw wastewater solids were collected at the existing sampling points in the thickening building at intervals of fifteen minutes each. The

temperature and pH of each grab sample were recorded. A composite sample of approximately 1-L was prepared from the four grab samples. The composite sample was preserved on ice and transported to the laboratories of The University of Texas at Austin for analysis. Bacteriological analyses of all samples were performed within 24 hours after collection.

Gravity belt thickening sludge

Four grab samples were collected directly from the thickener troughs at intervals of 15 minutes each. Grab samples were taken by using 59-mL (2-oz) sterile sampler scoops. In general, only two out of the six existing thickeners were in operation at a time. A composite sample of approximately 200 mL was prepared from the four grab samples. Sludge samples were kept on ice and transported to the laboratory for analysis.

Anaerobic digested biosolids

The grab samples of sludge from the anaerobic digestion process were collected either from the bottom of the anaerobic digestion tanks or from the pipe that conveys the withdrawn digested sludge to the air-drying basins. When the pipe was utilized, four grab samples of approximately 250 mL each were collected at 30 minutes intervals. Alternatively, the samples that were taken from the digesters were collected from different tanks. The temperature and pH were recorded immediately after taking every grab A composite sample of approximately 1-L was prepared from the four grab samples, placed on ice and transported to the laboratory for analysis.

Air-dried biosolids

The grab samples were collected at four different points of the selected air-drying basin. The basin sampled usually contained the most concentrated sludge (and usually most aged biosolids). During the course of this study, samples were collected from each of the five paved drying basins at least once. The anaerobically digested biosolids usually are held in the air-drying cells until the solids concentration is approximately 20 percent. Uncontaminated subsamples were taken by digging a hole into the sludge bed with a sterile spatula to a depth of approximately eight inches. The sample was collected from the hole with another uncontaminated spatula or a 59-mL (2-oz) disposable sterile spoon and placed into a HDPE bottle. A 200 mL composite sample was then prepared from the four grab samples, placed on ice, and taken to the laboratory for analysis.

Composted biosolids

The composted biosolids grab samples of the final product were collected directly from the piles. Generally, eight subsamples were taken to prepare a composite sample of approximately 800 grams. The grab samples were obtained by clearing a path with a sterile spatula to a depth of approximately ten inches. The sample was collected from the hole with another uncontaminated spatula or a 59-mL (2-oz) sterile disposable spoon and placed into a 1-L HDPE bottle. The samples were put on ice and transported to the laboratories for analysis.

3.1.2 Physical and Chemical Analyses

The raw sludge and biosolids samples were thoroughly mixed prior to analysis. The following physical and chemical analysis were performed:

Temperature

The temperature of the raw sludge and anaerobically digested biosolids grab samples was determined immediately after collection. A 110°C mercury thermometer was used for the temperature determination.

pH

The pH of the raw sludge and anaerobically digested biosolids was determined immediately after sample collection. A portable pH meter (Corning Model Check-Mate 90 with a glass electrode) was used for the analysis.

Total and Volatile Solids.

The total solids and volatile solids concentrations of the sludge were determined by placing a known volume of each sludge type in an evaporating porcelain dish and following the procedures established in the Standard Methods (1995). Total solids are those remaining after the water is driven off by heating the sludge sample at 103°C overnight. After the total solids were determined, the dried residue was placed in a 550°C furnace for sixty minutes to drive off the volatile solids. The ash is cooled in a desiccator before weighing.

Alkalinity and Volatile Acids

The concentrations of alkalinity and volatile acids were determined by titration. A 50-mL portion of the supernatant that had been separated from the anaerobically digested biosolids, by centrifuging at 3,800 rpm for 15 minutes, was used for this analysis.

The alkalinity was estimated by titration of the liquid fraction of the sludge sample with 0.1 N sulfuric acid according to the Standard Methods (1995) and was

reported as the concentration in milligrams per liter (mg/L) as calcium carbonate ($CaCO_3$). An aliquot of the same sample was used for the analysis of volatile acids. A direct titration method using 0.1 N sodium hydroxide as described by DiLallo and Albertson (1961) was followed. The sample was acidified to a pH = 3.3 with sulfuric acid, boiled gently for three minutes on a hot plate, and cooled to room temperature; the pH of the sample was then raised to 4. The volume of 0.1 N sodium hydroxide required to raise the pH from 4 to 7 was recorded and used as a measure of the volatile acids concentration. The alkalinity and volatile acids were determined by multiplying the sulfuric acid and sodium hydroxide volumes required for the titration times 100 and 120, respectively. The volatile acids were reported as mg/L as acetic acid.

3.1.3 Bacteriological Analysis

Bacterial analyses performed in this phase of the study included total coliforms (TC), fecal coliforms (FC), and fecal streptococci (FS). *Salmonella* species also were determined. All the bacterial cultures were performed using the multiple-tube procedure. The results of the examination of replicate tubes and dilutions are reported in terms of the Most Probable Number (MPN) of organisms present. This number, based on certain probability formulas, is an estimate of the mean density of the organisms in the sample.

Glassware and Culture Media Preparation

Sampling bottles, pipettes, dilution water and all of the culture media were autoclaved at 121°C and 15 psi for 15 minutes. All petri dishes used in the bacteriological analyses were pre-sterilized, disposable plastic. The culture media

required for the indicator bacteria analysis were prepared according to the Difco Manual (1984). The enrichment medium for the *Salmonella* assays (i.e., dulcitol selenite broth, DSE) was prepared according to the procedure established by Kenner and Clark (1974).

Sample Dilutions

Preliminary tests were conducted to determine the dilution factors for each sample and to verify the test procedures. Dilution factors for fecal coliforms were estimated to be approximately 10^{-4} to 10^{-7} for raw and thickened sludge, 10^{-2} to 10^{-5} for anaerobically digested biosolids, and 10^{-1} to 10^{-4} for air dried, and composted biosolids. The following dilutions were selected for analysis of fecal streptococci: 10^{-4} to 10^{-7} for raw and thickened sludge, 10^{-2} to 10^{-5} for anaerobic digested biosolids, and 10^{-1} to 10^{-4} for air dried, and composted biosolids. Four series of five tubes were utilized for the assays of indicator bacteria, and the traditional three series of five tubes were used for the analysis of *Salmonella* (U.S. EPA, 1992). The dilution factors utilized for the assays of *Salmonella* in the raw sludge and all the four types of biosolids samples were 10, 1.0 and 10^{-1}, respectively.

Analytical procedures

Total coliform

The total coliform and fecal coliform tests were performed using the multiple-tube procedure (Standard Methods, 1995). The multiple-tube technique involves presumptive, confirmed, and completed steps. A series of fermentation tubes containing lauryl tryptose broth were inoculated with the appropriate amount of sample in the presumptive phase. The inoculated test tubes were incubated at 35 ±

0.5°C. After 24 ± 2 h, each tube was shaken gently and examined for growth, gas, and acidic production (i.e., shades of yellow color). The tubes were reincubated and reexamined at the end of 48 ± 3 h, if no gas or acidic reaction was evident. Production of an acidic reaction or gas in the tubes within 48 ± 3 h constitutes a positive presumptive reaction. A sterile metal loop (3 mm in diameter) was used to transfer a loop of culture from the positive tubes in the presumptive phase to tubes containing brilliant green bile broth (BGBB). The inoculated BGBB tubes were incubated at 35 ± 0.5°C. The formation of gas in the tubes within 48 ± 3 h constitutes a positive confirmed phase.

Fecal coliforms

The fecal coliforms tests were performed using the multiple-tube technique. The procedure consisted of inoculating all positive presumptive tubes (from the total coliform test) to fermentation tubes containing EC medium. The inoculated EC broth tubes were incubated in a water bath at 44 ± 0.2°C for 24 ±2 h. Gas production in a EC broth tube within 24 hours or less is considered a positive fecal coliform reaction.

Fecal streptococci

The multiple-tube test was utilized for assaying the fecal streptococci. Azide dextrose broth (ADB) was the culture medium specified by the Standard Methods (1995). The inoculated ADB tubes were incubated at 35 ± 0.5°C for 24 ± 2 h. Each tube was examined for turbidity at the end of the test period. If no turbidity was observed, the samples were reincubated and examined at the end of 48 ± 3 h.

The confirmation phase for the fecal streptococci test was performed using bile esculin agar (U.S EPA, 1978). The inoculated petri dishes containing

approximately 10 mL of the culture medium were incubated at 35 ± 0.5°C for 24 ± 2 h. Brownish-black colonies with brown halos confirm the presence of fecal streptococci.

Salmonella Species

The procedure developed by Kenner and Clark (1974) and referenced by the U.S EPA (1992), was utilized for determining the *Salmonella* species. This technique, which utilizes the multiple-tube method, involves basically four phases: enrichment, isolation, biochemical characterization, and verification or confirmation.

Inoculated test tubes containing dulcitol selenite broth (DSE) were incubated at 40 ± 0.2°C for one and two days. After primary incubation at 40°C, surface loopfuls were removed from each multiple-tube culture and streaked on petri dishes containing xylose lysine desoxycholate agar (XLD) to isolate colonial growth. The streaked petri dishes were inverted and incubated at 37°C for a period not to exceed 24 h. Positive incubated XLD plate cultures contain typical clear, pink-edged, black centered colonies. The *Salmonella* colonies are picked to triple sugar iron (TSI) agar slants for typical appearance, purification, and identity tests. Typically, *Salmonella* sp. slant cultures, incubated overnight at 37°C, give an unchanged or alkaline red-appearing slant; the butt is blackened by H_2S, is acid yellow, and has gas bubbles. Typical colonies are subjected to the urease test. Urease-negative tubes are retained for presumptive serological test.

3.1.4 Helminth Ova Analysis

A series of preliminary tests were performed using the two most common procedures for analyzing helminth ova in biosolids or biosolids products. These

analytical techniques have been reported by Yanko (as cited by U.S EPA, 1992) and Little (as cited by Berk et al, 1993).

After preliminary tests using the two protocols, it was found that both methods gave similar results. The Yanko technique was selected for two reasons: (1) this method has been chosen by the EPA for compliance with the Part 503 regulations, and (2) the laboratory analysis is faster than the alternate technique, yet the incubation time in both methods is the same. Helminth ova were analyzed in the following sludge samples: raw wastewater solids, anaerobically digested, air dried, and composted biosolids.

In this study, the method suggested by Yanko (EPA, 1992) was used for the identification of the most frequent helminth ova found in municipal wastewater, i.e., *Ascaris* sp, *Trichuris* sp, and *Toxocara* sp. The analytical procedure depends principally on the use of a saturated solution of zinc sulfate (specific gravity of 1.2), to separate the parasite eggs (specific gravity < 1.2) from the rest of the sludge solids, by flotation. A summary of the protocol is presented below:

Biosolids samples are processed by first blending with buffered water containing a surfactant (i.e., Tween-80). The blend is screened through a 48-size sieve to remove large particles. The solids in the screened portion are allowed to settle out and the supernatant decanted. The sediment is subjected to density gradient centrifugation using zinc sulfate (specific gravity 1.20). This flotation procedure yields a layer most likely to contain *Ascaris* and some other parasitic ova. The resulting concentrate is incubated at 26°C for 20 to 30 days before microscopic examination for parasite ova. The complete procedure has been included in Appendix D.

3.1.5 Analytical Quality Control Procedures

The microbiological analytical procedures were assessed by qualitative and quantitative controls:

Qualitative Controls

Each lot of culture medium was tested with known positive and negative control cultures for the bacteria under test. The control cultures utilized in the examinations of indicator bacteria are shown in Table 3-1 (Standard Methods, 1995). *Salmonella* typhi was used as the positive control culture during the examination of *Salmonella*. Pure control cultures were obtained from the School of Biological Sciences of the University of Texas at Austin.

Table 3-1: Control Cultures for Microbiological Tests

Group	Control Culture	
	Positive	Negative
Total coliforms	*Escherichia coli*	*Staphylococcus aureus*
Fecal coliforms	*E. coli*	*Streptococcus faecalis*
Fecal streptococci	*Streptococcus faecalis*	*E.coli*

Quantitative Controls

The precision of the analytical methods for enumeration of indicator bacteria was evaluated according to the following procedure:

- Duplicate analyses of indicator bacteria were performed in the beginning of the study using seven samples of anaerobically digested sludge. Duplicate analyses were recorded as D_1 and D_2.
- Bacterial densities (MPN/g) were transformed into logarithmic concentrations (log MPN/g).

- The range, R, for each pair of transformed duplicates was determined; then, the mean range, $\overline{R}$, was calculated as the average of these ranges.
- The precision criterion was calculated as the product 3.27 $\overline{R}$.
- At least 10% of the anaerobically digested sludge samples were run in duplicate throughout the study. After transforming the bacterial densities into logarithmic concentrations, their range, R, was calculated. If their range, R, was greater than the precision criterion (3.27 $\overline{R}$), then there was a 99% probability that the laboratory variability was excessive and corrective action was required before making further analyses.

The numerical calculation of the precision criteria for indicator bacteria is illustrated in Appendix E.

Duplicate samples for helminth ova were compared with the precision criterion reported by Yanko. The analytical technique for enumeration of Salmonella was assessed only by qualitative controls. In all cases the differences between sample duplicates were less than the precision criteria. The results of precision criteria are summarized in Table 3-2.

Table 3-2: Results of Precision Criteria

Test	Criterion Value
Total Coliform	0.7390
Fecal Coliform	0.9038
Fecal Streptococci	0.3603
*Salmonella**	1.1315
Total Parasites*	0.5702

* Yanko (1988)

3.2 PHASE II: LAB-SCALE STUDIES

3.2.1 Introduction

The second phase of this study involved the use of bench-scale anaerobic digesters to evaluate the effect of several operating variables on the densities of indicator and pathogenic bacteria. An experimental design was used to evaluate the statistical significance of temperature, detention time, and solids loading on the reduction of the densities of FC, FS, and *Salmonella* during mesophilic anaerobic digestion of wastewater sludge. Four bench-scale anaerobic digesters were operated from March 1998 to October 1999 at temperatures of 25°C and 35°C. Detention times of 7, 15, 30, and 45 days and solids loading rates of 0.9, 1.80 and 2.70 kg VS/m^3-day were other variables used. Sludge samples to feed the reactors were collected from the HBBMP.

3.2.2 Statistical Experimental Design

A three-factor factorial experimental design was used to evaluate the statistical significance of the effects of temperature, hydraulic retention time, and volatile solids loading on the concentration of indicator and pathogenic bacteria in the mesophilic anaerobic digestion process. Factorial designs allow a large number of variables (factors) to be investigated in few experimental runs. Two aspects related to any experimental problem include the design of the experiment and the statistical analysis of data (Montgomery, 1998). In this study, the design of the experiment involved (1) selection of the response variable, (2) choice of factors, levels, and ranges, and (3) selection of the experimental design. Once the experiment was performed, analysis of variance (ANOVA) was used for the statistical analysis of data. The methodology for the design and performance of the experiment is presented below. The statistical analysis of data is presented in the section 4.2.2.

Selection of the response variable

The EPA Part 503 regulations classified the anaerobically digested sludge as Class B biosolids (i.e., FC < $2x10^6$ MPN/g TS). This phase of the study addressed the inactivation of pathogens in the mesophilic anaerobic digestion process. The response variables included in this study were the densities of FC, FS, and *Salmonella*. The fecal coliform density was selected as the main response variable. Fecal streptococci, a pathogen indicator, and *Salmonella* sp., the most important pathogenic bacteria also were selected as response variables because of their public health significance.

Choice of Factors, Ranges and Levels

Factors

Several possible mechanisms for the inactivation of pathogens in the anaerobic digestion process have been reported. However, only the effects of temperature have been documented widely. Detention time and solids loading also have been suggested as factors that influence the fate and survival of pathogens in that process; however, information is still limited (i.e., detention time), or not yet available (i.e., solids loading). Thus, the selected factors for this study were hydraulic detention time (HDT), temperature, and volatile solids loading.

Ranges and Levels

The ranges and levels of these factors were selected based on reported typical and extreme values at which anaerobic digesters might be operated. Ranges were considered to include the whole "spectrum" of levels at which mesophilic anaerobic digesters have been operated. Levels, on the other hand, included the specific (discrete) values at which the bench-scale experiments were performed. The selected ranges for each factor were 7 to 45 days for hydraulic detention time, 25°C to 35°C for temperature, and 0.9 to 2.70 kgVS/m^3-d for organic loading. The levels chosen for each factor were 7, 15, 30, and 45 days for HDT, 25°C and 35°C for temperature, and 0.90, 1.80, and 2.70 kgVS/m^3-d for volatile solids loading. The factors and levels included in the experimental design are presented in Table 3-3.

Table 3-3: Factors and Levels Included in the Experimental Design

Factor	No. of levels	Levels
Hydraulic Detention Time	4	7, 15, 30, 45 days
Temperature of Digestion	2	25°C, 35°C
Organic Loading	3	0.90, 1.80, 2.70 kgVS/m^3-d (56.2, 112.4, and 168.6 lbVS/1000 ft^3-d)

Selection of the Experimental Design

Two basic principles of experimental designs are replication and randomization. Replication of the operating conditions would allow estimating the measurement error. Three replicates were selected for each factor-level combination. Furthermore, the sequence in which the experimental runs were carried out was determined randomly. Design parameters and operating criteria that were published in the literature indicated the selection of a three-factor factorial design with four levels for factor A (detention time), two levels for factor B (temperature), and three levels for factor C (solids loading) for this study. Therefore, one complete replicate of the factorial design would involve 24 experimental runs. The general arrangements of the three-factor factorial design and the sequence in which the experimental runs were performed are shown in Table 3-4.

Table 3-4: General Arrangement of the Three-Factor Factorial Design

HDT	Temperature = 25 Celsius			Temperature = 35 Celsius		
	Loading ($kgVS/m^3$-d)			Loading ($kgVS/m^3$-d)		
	0.90	1.80	2.70	0.90	1.80	2.70
7 days	9	13	17	10	14	18
15 days	1	5	21	2	6	22
30 days	3	7	19	4	8	20
45 days	11	15	23	12	16	24

Numbers in cells indicate the order in which the experimental runs were performed.

Performing the experiment

Four bench-scale anaerobic digesters were operated simultaneously throughout the research. Two digesters were maintained at room temperature (25°C) and two at 35°C in constant temperature incubators. The experimental apparatus is shown in Figure 3-2. A 4.5-L glass carboy (4-L effective sludge volume) was used as the digester; gas was collected over a saturated sodium chloride solution in a glass bottle. Mixing of the contents of the digester was maintained by using a magnetic stirrer. The digesters were started-up with digesting sludge collected from the single-stage anaerobic digesters at the HBBMP. The digesters were operated during two time periods for each factor-level combination, i.e., acclimation and monitoring performance at pseudo-steady state conditions. Pseudo-steady state was defined as the time when a relatively constant VS reduction was attained (Lee et al, 1989).

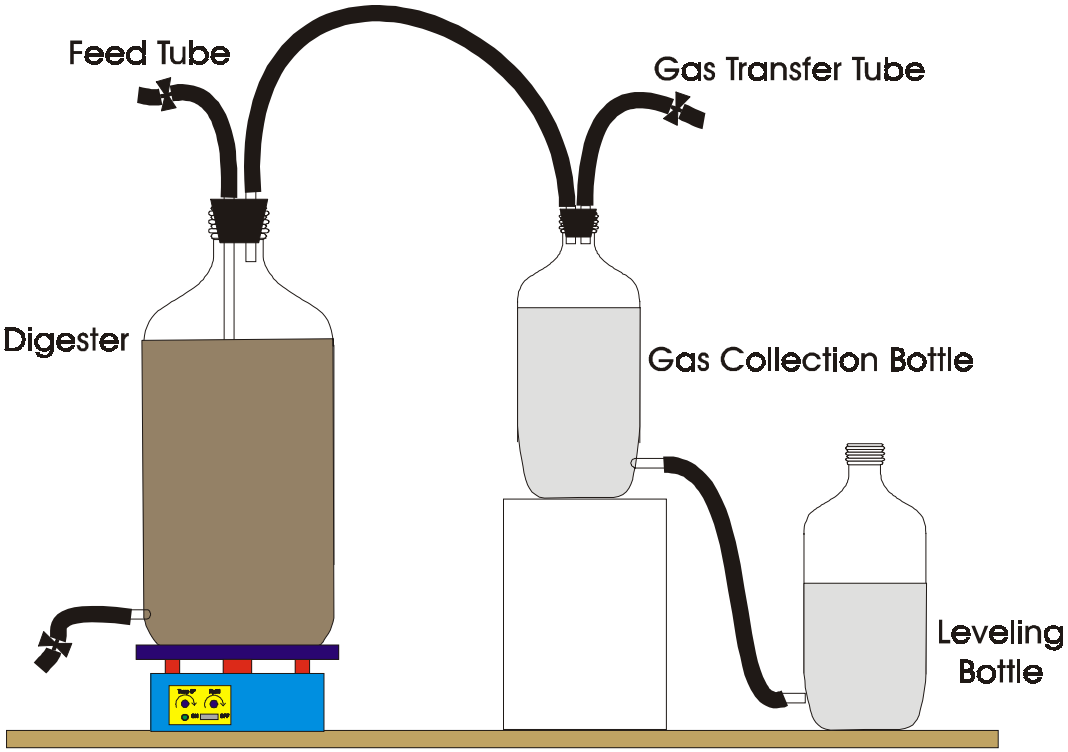

Figure 3-2: Bench-Scale Anaerobic Digesters and Gas Collection Apparatus

Sludge samples were collected daily during both the acclimation and monitoring periods and physical and chemical parameters were determined. Temperature and pH were recorded daily. Alkalinity, volatile acids, and total and volatile solids were determined at least once per week. Gas composition (carbon dioxide and methane content) was evaluated by gas chromatography at least twice per month. Samples for bacterial analysis were taken once per week during the pseudo-state period and analyzed for TC, FC, FS, and *Salmonella*. At least three samples were analyzed for each experimental test regardless the hydraulic detention time associated with each factor-level combination. A minimum of 66 samples were taken and analyzed for bacteria during this study. All the physical-chemical, and bacterial analyses were performed using the procedures discussed in sections 3.1.2 and 3.1.3,

respectively. An aliquot of stored sludge was allowed to room temperature before addition to the digesters. The digesters were fed once per day. A draw-fill mode of operation was adopted to eliminate contamination of the withdrawn sludge with fresh sludge.

Combined primary-secondary raw sludge was collected from the HBBMP once per week and stored in a refrigerator at 4°C. A sample of digested sludge was withdrawn from the bottom of the digester after recording the volume of gas produced. Prior to drawing a sample, each unit was vigorously shaken to insure the removal of a representative sample of the digesting sludge. As the sample of digesting sludge was withdrawn, the gas that was produced and collected in the brine bottle displaced the volume in the digester.

Feed sludge was added by gravity through the tube in top of the digester. Sludge thickening was almost always required to maintain the designed volatile solids loading rates. Make-up water was added to the digesters to maintain a constant volume in the digester and to compensate for any vapor that was carried into and trapped in the gas collection system. After the addition of the feed material, the gas in the collection bottle was vented using the leveling bottle.

Chapter 4: Results and Discussion

The results and discussion of the field-scale (Phase I) and lab-scale (Phase II) are presented below. This chapter is divided into three sections. Section 4-1 presents the results of the field-scale studies to evaluate the effectiveness of belt thickening, anaerobic digestion, air-drying, and composting in the reduction of FC, FS, *Salmonella*, and helminth ova. The results of the lab-scale studies to establish the effect of temperature, hydraulic detention time, and volatile solids on the densities of FC, FS and *Salmonella*, during conventional mesophilic anaerobic are included in Section 4-2. Finally, a summary of field-scale and lab-scale results for the anaerobic digestion process is presented in Section 4-3.

4.1 Results of Phase I - Field Studies

The effectiveness of various sludge treatment processes at the Hornsby Bend Biosolids Management Plant (HBBMP) in Austin, Texas, for the inactivation of indicator and pathogenic organisms in municipal wastewater sludge was investigated. The densities of fecal coliforms (FC), fecal streptococci (FS), *Salmonella*, and helminth ova in raw sludge and gravity belt thickened sludge were assayed. Similarly, these organisms as found in anaerobic digestion, air-drying, and composting processes were monitored. The basis for comparison were the requirements established in the U.S.EPA Part 503 “Standards for the Use or Disposal of Sewage Sludge” for achieving Class “A” and Class “B” biosolids. The reductions of indicator and pathogen organisms were determined by subtracting the densities of fecal coliforms (FC), fecal streptococci (FS), *Salmonella* and helminth ova in the effluent of each

process from those in the raw sludge. These results were compared with literature data.

4.1.1 Physical and Chemical Results

The physical and chemical characteristics of the sludge collected during the sampling period (October 1996 – March 1998) are summarized in Tables 4-1 and 4-2. The operating data for the anaerobic digesters reported by personnel of HBBMP during the period of study are presented in Table 4-3. The data presented in Table 4-1 indicate that the average total and volatile solids contents for the raw sludge, anaerobic digestion, air-drying, and composting processes are in agreement with those

Table 4-1: Total and Volatile Solids in Raw and Stabilized Sludge

Type of Biosolids	**n**	**Range**		**Average**	**Standard Deviation**
Total Solids (%)					
Raw Sludge	41	0.9	2.9	1.6	0.4
Gravity Belt Thickener	41	4.4	11.8	7.8	1.6
Anaerobic Digester	41	2.5	7.1	4.9	1.0
Air-Drying Basins	41	6.1	40.4	18.6	7.6
Composting	41	49.2	75.9	60.3	5.5
Volatile Solids (%)					
Raw Sludge	41	57.2	75.9	69.4	4.0
Gravity Belt Thickener	41	56.5	78.6	71.8	4.5
Anaerobic Digester	41	45.8	62.0	53.8	4.2
Air-Drying Basins	41	43.5	59.6	50.1	3.7
Composting	41	33.2	54.7	42.7	4.7

Table 4-2: Physical and Chemical Characteristics of Digested Sludge.

Parameter	**n**	**Range**		**Average**	**Standard Deviation**
pH	41	6.2	7.7		
Temperature, °C	41	15.9	29.9	24.2	3.4
Alkalinity, mg/L as $CaCO_3$	41	4,210	8,154	6,606	853
Volatile acids, mg/L as acetic acid	41	186	1,830	821	437

reported in the literature (Metcalf and Eddy, 1991). However, the volatile solids concentration in the gravity belt thickened sludge (71.8%) was slightly higher than the value observed in the raw sludge (69.4 %). Organic polymer used for thickening may be the cause of this difference. At the time of this study, a cationic polymer, Magnifloc 1598C™, was used at a dose of approximately 10 pounds per ton dry of sludge solids, were being used to enhance the thickening of raw sludge. A paired t test showed that the average of the differences in the volatile solids content was significant at the 95% confidence level. These results suggest that the increase in the VS content was caused by the effect of the thickening process rather than by random variation. The 95% confidence interval for mean VS differences ranged from -2.910 to -1.860. Since the confidence interval does not include a difference of zero, there is a 95% of confidence that the VS difference between the two types of biosolids is not zero. Therefore, these results indicate that there is 95% confidence that the VS content in the thickening sludge was higher than that in the raw sludge. We conjecture that the significant difference may be attributed to the presence of an organic polymer that was used during the thickening process.

Table 4-3: Characteristics of the Anaerobic Digesters at the Hornsby Bend Biosolids Management Plant, Austin, Texas (October 1996 - March 1998).

Parameter	**Units**	**Range**		**Average**
pH		7.2	7.6	
Temperature	°C (°F)	12.5 (54.5)	35.0 (95.0)	22.8 (73.1)
Alkalinity,	mg/L as $CaCO_3$	4,592	7,579	6,562
Volatile Acids	mg/L as Acetic Acid	76.7	220	121
Hydraulic Detention Time	days	ND	ND	75
Volatile Solids Loading	$kgVS/m^3$-d ($lbVS/ft^3$-d)	ND	ND	0.74 (0.045)
Methane	%	57.5	64.2	62.1
Carbon Dioxide	%	35.8	42.6	37.9

Calculated from operating records maintained at HBBMP.
ND: Not determined

The results of physical and chemical analyses for the digested sludge collected during the evaluation period (Table 4-2) compare favorably with the characteristics reported for the anaerobic digesters at HBBMP (Table 4-3). The operating conditions for the anaerobic digesters at the HBBMP during this study were within the optimum and extreme ranges reported by McCarty, 1964 (Figure 2-1) and Malina, 1992 (Table 2-5) for normal anaerobic digestion. The pH, volatile acids, and gas composition were in the optimum range of operation; however, the temperature, alkalinity, and detention time were within the extreme range of operation. The volatile solids loading and hydraulic detention time for the anaerobic digesters at the HBBMP during the period of study were 0.74 kg VS/m^3-d (0.045 lb/ft^3-d) and 75 days, respectively. The hydraulic detention time was longer than the range recommended by WEF (1995) for design of low-rate, unmixed, anaerobic digesters (i.e., 30-60 days).

4.1.2 Biological Results

The results of total coliforms (TC), fecal coliforms (FC), fecal streptococci (FS), *Salmonella* and helminth ova analyses are presented in Tables 4-4 through 4-6. The densities of bacteria and helminth ova throughout the sludge processing train are illustrated in Figures 4-1 and 4-2, respectively. Complete data for the biological results are presented in Appendices F and G. Summary bacterial data expressed as the most probable number of organisms per gram of total solids (MPN/g TS) for TC, FC, FS and *Salmonella* are presented in Table 4-4. Bacterial densities in wastewater are characterized by extreme values (high and low numbers) and a significant variation between them. A lognormal (geometric) distribution instead of the normal distribution describes the bacterial densities in wastewater sludge (U.S.EPA, 1992). Therefore, geometric means rather than arithmetic averages were used for comparison with EPA Part 503 regulations (the geometric mean is the antilog of the mean of the log densities). The densities of TC, FC, FS and *Salmonella* expressed as the $\log_{10}$MPN/g TS and geometric means are presented in Table 4-5. A summary of the helminth ova counts in the specific sludge treatment processes is presented in Table 4-6. The log reduction of bacterial densities between the feed sludge and the effluent of each process is presented in Table 4-7. The densities of *Salmonella* (MPN/4 g TS) and helminth eggs (ova/4 g TS) are shown in Tables 4-8 and 4-9, respectively. *Salmonella* and helminth ova densities per 4 grams TS make direct comparisons with the limits established for Class A biosolids. The results of FC, FS, *Salmonella*, and helminth ova and the reduction effectiveness at each sludge treatment process are discussed below.

Table 4-4: Bacterial Densities in Municipal Sludge Treatment Processes

Source	n	Range (MPN/g TS)		Average (MPN/g TS)	Standard Deviation (MPN/g TS)
Raw Sludge					
Total Coliforms	41	6.96E+07	1.00E+09	3.74E+08	2.91E+08
Fecal Coliforms	41	5.00E+06	2.90E+08	5.08E+07	5.68E+07
Fecal Streptococci	41	1.50E+06	1.58E+08	2.24E+07	2.87E+07
Salmonella	24	2.00E+00	6.00E+01	1.19E+01	1.23E+01
Gravity Belt Thickening					
Total Coliforms	41	4.41E+06	2.62E+08	1.19E+08	7.70E+07
Fecal Coliforms	41	1.90E+06	2.86E+08	2.37E+07	4.49E+07
Fecal Streptococci	41	2.14E+05	5.45E+07	8.04E+06	1.13E+07
Anaerobic Digestion					
Total Coliforms	41	3.24E+02	2.09E+06	3.20E+05	5.06E+05
Fecal Coliforms	41	2.80E+01	6.40E+05	1.10E+05	1.68E+05
Fecal Streptococci	41	3.24E+02	4.00E+06	7.13E+05	1.04E+06
Salmonella	24	3.00E-01	3.40E+00	1.27E+00	8.34E-01
Air-Drying					
Total Coliforms	41	1.70E+01	2.29E+04	1.56E+03	3.94E+03
Fecal Coliforms	41	1.00E+00	1.48E+04	8.34E+02	2.65E+03
Fecal Streptococci	41	1.36E+02	8.20E+05	3.98E+04	1.48E+05
Salmonella	24	8.00E-02	5.00E-01	1.97E-01	1.58E-01
Composting					
Total Coliforms	41	3.00E-01	4.98E+03	7.59E+02	1.17E+03
Fecal Coliforms	41	3.00E-01	4.39E+03	5.17E+02	9.57E+02
Fecal Streptococci	41	1.00E+00	1.45E+04	1.76E+03	2.91E+03
Salmonella	24	3.00E-02	4.00E-02	3.50E-02	7.07E-03

Table 4-5: Transformed Bacterial Densities in Municipal Sludge Treatment Processes.

Source	Range (log MPN/g TS)		Average (log MPN/g TS)	Standard Deviation (log MPN/g TS)	Geometric Mean MPN/g TS
Raw Sludge					
Total Coliforms	7.8	9.0	8.5	0.3	2.91E+08
Fecal Coliforms	6.7	8.5	7.5	0.4	3.28E+07
Fecal Streptococci	6.2	8.2	7.1	0.5	1.25E+07
Salmonella	0.3	1.8	0.9	0.4	8.47E+00
Gravity Belt Thickening					
Total Coliforms	6.6	8.5	7.9	0.4	8.70E+07
Fecal Coliforms	6.3	8.5	7.1	0.4	1.27E+07
Fecal Streptococci	5.3	7.7	6.6	0.5	4.06E+06
Anaerobic Digestion					
Total Coliforms	0.5	6.3	4.4	1.3	2.73E+04
Fecal Coliforms	0.5	5.8	4.1	1.2	1.29E+04
Fecal Streptococci	2.5	6.6	5.0	1.1	1.03E+05
Salmonella	-0.5	0.5	0.0	0.3	1.02E+00
Air-Drying					
Total Coliforms	1.2	4.4	2.7	0.6	5.45E+02
Fecal Coliforms	0.0	4.2	1.7	1.1	4.53E+01
Fecal Streptococci	2.1	5.9	3.4	0.8	2.75E+03
Salmonella	-1.1	-0.3	-0.8	0.3	1.58E-01
Composting					
Total Coliforms	-0.5	4.5	2.1	1.3	1.27E+02
Fecal Coliforms	-0.5	3.6	1.4	1.4	2.56E+01
Fecal Streptococci	-0.5	4.4	2.5	1.3	3.41E+02
Salmonella	-1.5	-1.4	-1.5	0.1	3.46E-02

41 sludge samples were analyzed for TC, FC, and FS, and 24 for *Salmonella*

Table 4-6: Total and Viable Helminth Ova in Municipal Sludge Treatment Processes

Type of Helminth Ova	Range (ova/g TS)		Average (ova/g TS)	Median (ova/g TS)	Standard Deviation (ova/g TS)
Total Helminth Ova					
Raw Sludge	1.00	7.50	2.77	2.64	1.29
Anaerobic Digestion	1.73	5.99	4.02	3.99	1.18
Air-Drying	1.15	6.44	2.98	2.83	1.28
Composting	0.11	0.53	0.25	0.22	0.11
Viable Helmith Ova					
Raw Sludge	0.09	3.53	0.92	0.68	0.77
Anaerobic Digestion	0.20	3.74	1.68	1.87	0.82
Air-Drying	0	3.28	0.54	0.14	0.93
Composting	0	0	0	0	0

22 sludge samples were analyzed for helminth ova

Raw Sludge

Fecal Coliforms in Raw Sludge

The fecal coliform (FC) densities in the raw sludge ranged from 5.00E+06 to 2.90E+08 MPN/g with an average of 5.08 E+07 MPN/g TS. The standard deviation (5.68E+07 MPN/g TS) was greater than the average, which illustrates the significant variation of bacterial measurements in wastewater sludge. Typical FC densities reported in the literature for unstabilized raw solids range from 3.69E+08 MPN/100 mL (Berg and Berman, 1980) to more than 1.4E+09/100mL (WEF, 1995). Direct comparison is not possible due to difference in units, but the results observed in this study are in agreement with those numbers. The log densities ranged from 6.7 to 8.5 MPN/g TS with an average of 7.5 MPN/g TS (geometric mean = 3.28E+07 MPN/g TS) .The use of transformed values (i.e., log MPN/g TS) instead of raw data (MPN/g

TS) is a common procedure in statistical analysis of data to stabilize the variance or standard deviation of bacterial counts. A constant variance of the response variable is a requisite for the use of ANOVA for comparison of bacterial log means.

Fecal Streptococci in Raw Sludge

The concentrations of fecal streptococci (FS) in the raw sludge ranged from 1.50E+06 to 1.58E+08 MPN/g TS and averaged 2.24E+07 MPN/g TS (Table 4-4). Berg and Berman (1980) and Williams (1991) reported average FS levels of 2.92E+07 MPN/100 mL and 7.6E+07 MPN/g TS in mixed raw sludge. The FS densities observed in this study compare favorably with the numbers reported in the literature. The FS log densities ranged from 6.2 to 8.2 MPN/g TS and averaged 7.1 MPN/g TS (geometric mean = 1.25E+07). In the raw sludge, the FS densities were lower than FC concentrations by less than one order of magnitude.

Salmonella in Raw Sludge

The *Salmonella* counts (Table 4-4) ranged from 2.0 to 60 MPN/g TS with an average of 11.9 MPN/g of TS. When expressed in logarithms (Table 4-5), the *Salmonella* densities ranged from 0.3 to 1.8 MPN/g TS and averaged 0.9 MPN/g TS (geometric mean = 8.47 MPN/g TS). The densities of *Salmonella* observed in this study are in agreement with the levels published by Sedita et al (1994) who reported *Salmonella* counts ranging from 1.9 to 126 MPN/g for seven Wastewater Treatment Plants (WWTP) in the Chicago area.

Helminth Ova in Raw Sludge

Helminth ova numbers in raw and stabilized sludge usually are lower than indicator bacterial densities; therefore, actual counts (ova/g TS) rather than

transformed densities (i.e., $\log_{10}$ ova/g TS) will be considered in the discussion of results. The densities of helminth ova in the sludge processing train are presented in Table 4-6. The number of total helminth ova in the raw sludge varied from 1.0 to 7.5 ova/g TS with an average of 2.77 ova/g TS (Table 4-6). The average density of viable helminth ova was 0.92 ova/g of TS. The data indicate that approximately 33.2 % of the total helminth ova in the raw sludge are infective.

Gravity Belt Thickening

Fecal Coliforms in Gravity Belt Thickening

The number of fecal coliforms in the thickened sludge ranged from 1.90 E+06 to 2.86E+08 MPN/g TS with an average of 2.37E+07/g TS. When expressed in logarithms (Table 4-5), the bacterial densities ranged from 6.3 to 8.5 MPN/g TS and averaged 7.1 MPN/g TS (geometric mean = 1.27E+07 MPN/g TS). The reduction of FC densities between the raw sludge and the thickened sludge was 0.4-logs (<90%) (Table 4-7).

Fecal Streptococci in Thickened Sludge

The FS concentration in the thickened sludge ranged from 2.14E+05 to 5.45E+07 MPN/g TS with an average of 8.04E+06 MPN/g TS (Table 4-4). The log densities ranged from 5.3 to 7.7 MPN/g TS and averaged 6.6 MPN/g TS (geometric mean = 4.06E+06 MPN/g TS). The average reduction of FS between the influent (raw sludge) and the thickened sludge was 0.5-logs (<0.90) (Table 4-7).

Table 4-7: Reduction of Bacterial Densities by Municipal Sludge Processing

Type of Bacteria	Log MPN/g TS	Log reduction
Total Coliforms		
Raw Sludge	8.5	
Gravity Belt Thickening	7.9	0.5
Aerobic Digestion	4.4	4.0
Air-Drying	2.7	5.7
Composting	2.1	6.4
Fecal Coliforms		
Raw Sludge	7.5	
Gravity Belt Thickening	7.1	0.4
Aerobic Digestion	4.1	3.4
Air-Drying	1.7	5.9
Composting	1.4	6.1
Fecal Streptococci		
Raw Sludge	7.1	
Gravity Belt Thickening	6.6	0.5
Aerobic Digestion	5.0	2.1
Air-Drying	3.4	3.7
Composting	2.5	4.6
Salmonella		
Raw Sludge	0.93	
Anaerobic Digestion	0.01	0.9
Air-Drying	-0.80	1.7
Composting	-1.46	2.4

Anaerobic Digestion

Fecal Coliforms in Anaerobically Digested Sludge

The FC densities in the effluent of the anaerobic digestion process ranged from 28 to 6.40E+05 MPN/g TS (average density of 1.10E+05 MPN/g TS). The FC densities observed in this study are lower than the reported by Berg and Berman, 1980 (6.04E+06 MPN/100mL) and Williams, 1991(9.5E+07 MPN/g TS). The FC densities expressed in logarithms ranged from 0.5 to 5.8 MPN/g TS with an average of 4.1 MPN/g TS. The reduction of FC in the anaerobic digestion process was 3.0-logs or 99.9% (3.4-logs or >99.9% when the thickening process was included). This value is greater than the reductions reported by Berg and Berman, 1980 (2.97-logs), WEF, 1995 (1.84-logs) and Ponugoti et al, 1997 (2.94-logs) for mesophilic anaerobic digesters. The geometric mean (1.29E+04 MPN/g TS) clearly meets the EPA Part 503 requirements for Class "B" biosolids (i.e., FC < 2 x 10^6 MPN/g TS). However, the FC density failed to meet the requirements for Class "A" biosolids (i.e., FC < 1,000 MPN/g TS).

Fecal Streptococci in Anaerobically Digested Sludge

The FS numbers in the effluent of the anaerobic digestion process ranged from 324 to 4.00E+06 MPN/g and averaged 7.13E+05 MPN/g TS. These results are lower than the reported by Berg and Berman, 1980 (2.27E+06 MPN/100mL) and Williams, 1991 (6E+07 MPN/g TS). The log densities of FS varied from 2.5 to 6.6 MPN/g and averaged 5.0 MPN/g TS (geometric mean = 1.03E+05 MPN/g TS). The average FS reduction in the anaerobic digesters was 1.6-logs (2.1-logs when the thickening process was included) (Table 4-7). The reduction of FS log densities observed in this

study is higher than that reported by Berg and Berman, 1980 (0.65-logs), Williams, 1991 (0.5-logs), and the WEF, 1995 (1.48-logs), but lower than the reported by Ponugoti et al, 1997 (2.0-logs). The data presented in Figure 4-1 indicate that FS were more resistant to inactivation than FC.

Salmonella in Anaerobically Digested Sludge

The *Salmonella* concentration in the anaerobically digested sludge ranged from 0.3 to 3.4 MPN/g TS and averaged 1.27 MPN/g TS. The density of *Salmonella* in the anaerobically digested sludge, when expressed in the same units than the current regulations was 4.1 MPN/4 g TS (Table 4-8). This density is above the limit established by the EPA Part 503 regulations for Class "A" biosolids (i.e., *Salmonella* $\leq$ 3 MPN/4 g TS). Therefore, the anaerobically digesting sludge did not meet the pathogen requirements for Class "A" biosolids. The reduction of *Salmonella* between the raw sludge and the anaerobic digestion process was 0.9-logs (~90%).

Helminth Ova in Anaerobically Digested Sludge

The total helminth ova densities in the anaerobically digested sludge ranged from 1.73 to 5.99 ova/g TS and averaged 4.02 ova/g TS (Table 4-6). Approximately 41.8 % of the total helminth ova were viable or infective (i.e., 1.68 ova/g TS). The density of total and viable helminth eggs in the anaerobically digested sludge was higher than the observed in the raw sludge. The median viable counts increased from 0.68 ova/g TS in raw sludge to 1.87 ova/g TS after anaerobic digestion (Figure 4-2). A factor that may account for such an increase is that the concentration of solids in the digested sludge is higher, while the volatile solids in the digested sludge is lower than in the raw sludge.

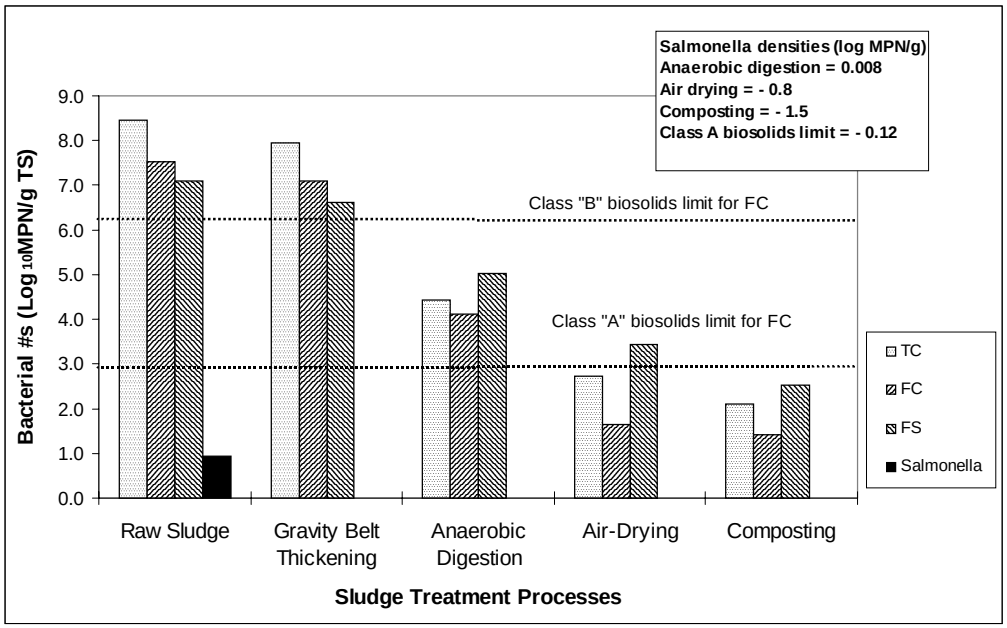

Figure 4-1: Densities of Total Coliform, Fecal Coliform, Fecal Streptococci, and *Salmonella* in Municipal Sludge Treatment.

Table 4-8: *Salmonella* Densities in Municipal Sludge Processing

Sludge Treatment Process	Geometric Means (MPN/4 g TS)
Raw Sludge	33.88
Anaerobic Digestion	4.08
Air-drying Basins	0.63
Composting	0.14

The destruction of volatile solids may release more ova from the sludge matrix. Reimers at al. (1981) observed a similar pattern for helminth ova after mesophilic anaerobic digestion. The EPA Part 503 regulations have established that the concentration of helminth ova in a Class "A" sludge must be less than 1 viable ovum/ 4 grams of dry total solids (< 0.25 ovum/g TS). When expressed in the same

units than current pathogen reduction regulations (Table 4-9), the viable helminth density in the digesting sludge (7.48 MPN/4 g TS) exceeds by far the limit established by the EPA Part 503 for Class "A" biosolids (< 1 ovum/4 g TS). Therefore, the mesophilic anaerobically digesting sludge cannot be considered as a Class "A" biosolids, in terms of helminth ova.

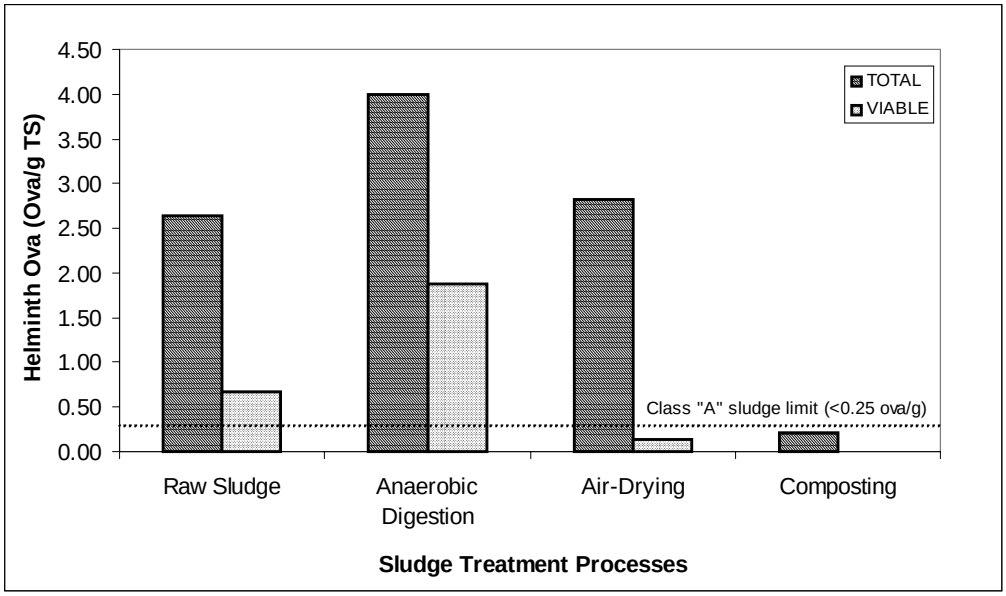

Figure 4-2: Densities of Helminth Ova in Municipal Sludge Treatment.

Table 4-9: Viable Helminth Ova in Municipal Sludge Processing

Sludge Treatment Process	Median (ova/4g TS)
Raw Sludge	2.72
Anaerobic Digestion	7.48
Air-Drying	0.56
Composting	0

Air-Drying

Fecal Coliforms in Air-Dried Sludge

The bacterial counts in this process varied from 1.0 to 1.48E+04 and averaged 834 MPN/g TS. The average log density was 1.7 MPN/g TS (geometric mean = 45.3 MPN/g TS). The geometric mean was higher than that reported by Tata et al, 1997 (3.6 MPN/g TS) for a digested sludge that was air-dried to 60 percent solids. In this study, the FC densities observed in 36 out of 41 monitored samples were lower than the limit established for Class "A" biosolids The reduction of FC densities between the raw sludge and the air-dried sludge was 5.9 logs (Table 4-7). This value compares favorably with the 5.36 log reduction reported by Tata et al. (1997).

Fecal Streptococci in Air-Dried Sludge

The FS numbers in the air-dried sludge ranged from 136 to 8.20E+05 MPN/g TS and averaged 3.98E+04 MPN/g TS (Table 4-4). Logarithmic densities ranged from 2.1 to 5.9 MPN/g TS an averaged 3.4 MPN/g (geometric mean = 2750 MPN/g TS). Williams (1991) reported FS values in the range from 45 to 1.4E+05 MPN/g TS with an average of 3.8E+04MPN/g TS for the same facility. In this study, the reduction of FS between the influent (raw sludge) and the air-dried sludge was 3.7-logs. Williams (1991) reported an average reduction of 2.8-logs for the same plant.

Salmonella in Air-Dried Sludge

The *Salmonella* assays for air-dried sludge samples, for the most part, were below detection limits (i.e., < 2 MPN/100 mL). The arithmetic and geometric means were 0.2 and 0.16 MPN/g TS, respectively The average total solids content in the air-dried sludge was 18.6 %. A direct comparison with the limits established by current

regulations for Class “A” biosolids required that the geometric means of *Salmonella* be expressed as MPN/4 TS instead of MPN/g TS (Table 4-8). The densities of *Salmonella* (0.63 MPN/4 g TS) were below the limit established by EPA Part 503 for Class “A” biosolids (i.e., < 3 MPN/4 g TS). The results of this study are in agreement with the *Salmonella* counts reported by Tata et al, 1997 (0.39 MPN/4 g TS) for a digested sludge that was air-dried to 60 percent solids.

Helminth Ova in Air-Dried Sludge

Total helminth eggs in the air-dried sludge ranged from 1.15 to 6.44 ova/g TS and averaged 2.98 ova/g TS. The average and median viable helminth counts were 0.54 and 0.14 ova/g, respectively. The median concentration of viable helminth eggs (0.56 ova/4g TS) was below the limit established for Class “A” biosolids (< 1 ovum/4 TS); however, only 16 out of 22 samples contained viable helminth ova counts less than the limit for Class A biosolids. The relationship between the solids content of the sludge and detention time is presented in Figure 4-3. The correlation coefficient (R^2) between these variables was 0.7797. The data in Figure 4-3 indicate that the solids content in the air-dried sludge increased as the storage time increased, since more water evaporates with time during periods of no rainfall. The results for viable helminth ova and detention time are plotted in Figure 4-4. The trend of the data in Figure 4-4 shows that the viable helminth ova density decreased consistently up to approximately 254 days. After 254 days, the viable helminth ova density remained constant until the end of the sampling period (570 days). The data in Figure 4-4 also indicate that after 254 days of storage, the viable helminth ova was reduced to a level below the limit established for Class “A” biosolids (i.e., < 0.25 ova/g TS).

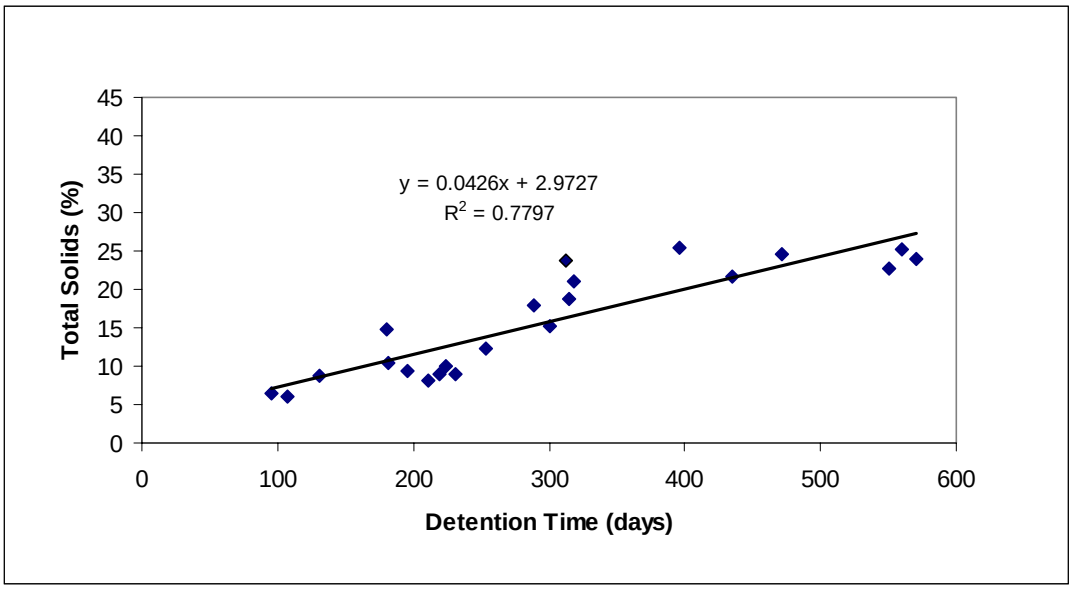

Figure 4-3: Relationship between Detention Time and Total Solids in Air-dried Sludge.

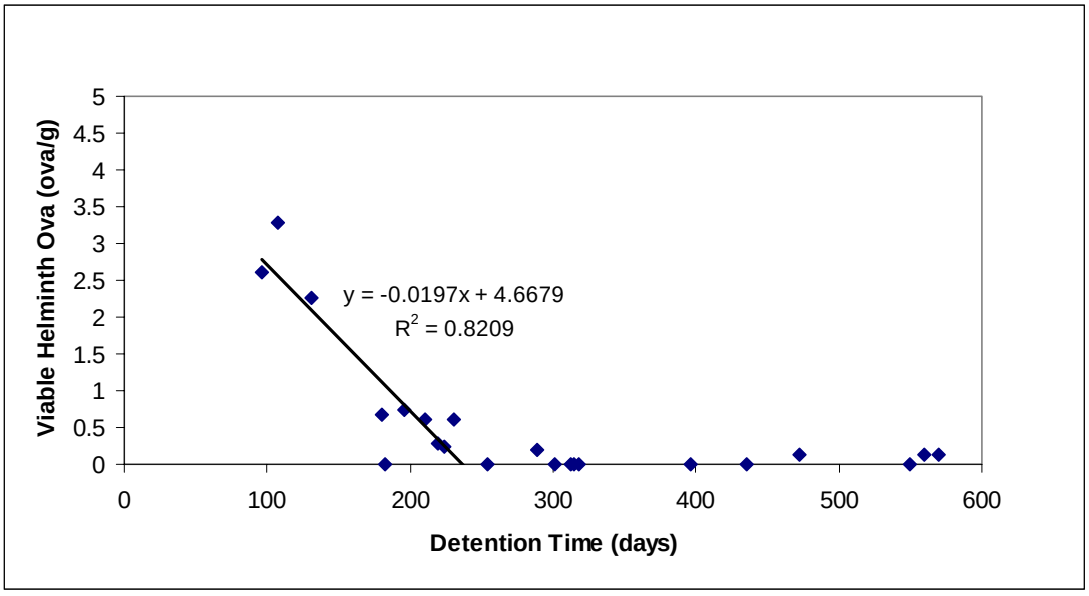

Figure 4-4: Effect of Detention Time on Viable Helminth Ova in Air-dried Sludge.

These data compare favorably with those reported by Ahmed et al. (1997), who stated that destruction of Ascaris eggs to below detention limits (BDL) was achieved after 330 days of storage in piles. The data in Figure 4-3 indicate that the solids content in the air-dried sludge was approximately 12.3 percent after 254 days. A multiple linear regression was performed to test the possible correlation between viable helminth ova and solids content and detention time up to 254 days.

The test indicated that only the detention time was linearly correlated with the viable helminth ova density. For instance, the t-test "p" value (0.001) indicated that there is significant evidence that the coefficient of the detention time variable is not zero. On the other hand, the t-test "p" value for the solids content was 0.168, which was greater than the significance level (0.05). Therefore, the solids content variable was not linearly correlated with the viable helminth ova density. Linear regression of the viable helminth ova on detention time values up to 254 days yielded the following equation:

$$y = -0.0197X + 4.6679 \qquad \text{(Eq. 4-1)}$$

where y is the viable helminth ova density (ova/g TS) and X is the storage time (days). At the time of the study, the operating practices in the air-drying process at HBBMP were focused primarily on dewatering the digested biosolids prior to composting rather than to reducing their pathogen content. The detention time values that were used in Figure 4-4 were calculated using records at HBBMP of the dates when the basins started to be filled with digested sludge. It should be noted, however, that fresh sludge was occasionally mixed with partially dried sludge. Therefore, the

sludge grab samples that were collected randomly in any basin may have had different storage times. Therefore, the detention times presented above should be considered as preliminary values. The data observed in this study indicate that air-drying of anaerobically digested sludge may have potential as an alternative low technology process to meet Class "A" sludge requirements for both bacteria and helminth ova. Further investigation is required to define the optimum detention time (design parameter) for air-dried basins.

Windrow Composting

Fecal Coliforms in Composted Sludge

The FC counts in the composted sludge ranged from 0.3 to 4,390 MPN/g TS with an average density of 517 MPN/g TS. The average log density was 1.4 MPN/g TS (geometric mean = 25.6 MPN/g TS). The reduction of FC between the raw sludge and the composted sludge was 6.1 logs (Table 4-7). This value is higher than the reported by Williams, 1991 (5.2-logs) for the same facility. The density of FC in the composted sludge was far below the limit established for Class "A" biosolids.

Fecal Streptococci in Composted Sludge

The fecal streptococci densities in the composted sludge varied from 1.0 to 14,500 MPN/g TS and averaged 1,760 MPN/g TS (Table 4-4). The geometric mean was 341 MPN/g TS (Table 4-5). The reduction of FS densities between the unstabilized sludge and the composted sludge was 4.6-logs (Table 4-7). This value is the same as that reported by Williams (1991) for the same facility and higher than that reported by the WEF, 1995 (2.9 logs) for composting processes. The data presented

by WEF (1995) do not specify the type of the composted solids. Similarly to the anaerobic digestion and air-drying processes, fecal streptococci were more resistant to inactivation than fecal coliforms (Figure 4-1).

Salmonella in Composted Sludge

The *Salmonella* densities in the composted sludge were below detection limits (BDL) for the most part. Both the arithmetic and geometric mean densities were 0.035 MPN/g TS. The densities of *Salmonella* in the composted sludge (0.14 MPN/ 4g TS) clearly met the limits established for Class "A" biosolids.

Helminth Ova in Composted Sludge

The densities of total helminth ova in the composted sludge ranged from 0.11 to 0.53 ova/g TS with an average of 0.25 ova/g TS (Table 4-6). However, no viable helminth ova were found in any of the 22 samples analyzed. Therefore, the windrow-composted sludge can be considered as a Class "A" biosolids in terms of the viable helminth eggs concentration.

4.1.3 Statistical Analysis

A statistical analysis was performed to assess the average differences in the densities of indicators and pathogens in paired samples of raw sludge and in the sludge from the mesophilic anaerobic digestion, air-drying, and composting processes. The standard procedure for evaluating such average differences is to construct a null hypothesis, which is tested statistically using a paired t-test. The classical null hypothesis states that the difference between two methods is zero. An alternate procedure to constructing a null hypothesis is to compute the difference and the confidence interval in which the difference is expected to fall (Berthouex and

Brown, 1994). Both procedures were utilized in this study to assess the significance of the average differences in the densities of indicators and pathogens. The null hypothesis procedure is presented first.

The null hypothesis states that the average densities of indicator or pathogen populations between the biosolids collected at two treatment processes are equal. In other words, it states that the average of the differences in the densities of indicators or pathogens in samples of biosolids collected at two treatment processes is equal to zero.

$$H_o: \mu_1 = \mu_2 \text{ (or } \mu_d = \mu_1 - \mu_2 = 0) \qquad \text{(Eq. 4-2)}$$

where μ is the average indicator or pathogen density and μ_d is the expected average of differences in the densities of indicator and pathogens.

The alternative hypothesis states that the average densities of indicator or pathogen populations between the biosolids collected at two treatment processes are not equal. In other words, it states that the average of the difference in the densities of indicators or pathogens in samples of biosolids collected at two treatment processes is not equal to zero.

$$H_1: \mu_1 \neq \mu_2 \text{ (or } \mu_d \neq 0) \qquad \text{(Eq. 4-3)}$$

The test statistic, *t* is defined as follows:

$$t = \frac{\overline{d} - \mu_d}{S_d / \sqrt{n}} \qquad \text{(Eq. 4-4)}$$

where t is the t-statistic

$\bar{d}$ is the average of paired differences in the densities of indicators or pathogens

μ_d is the hypothesized difference = 0

S_d is the standard deviation of the paired differences of indicator or pathogen densities, and

n is the number of pairs of values.

The standard deviation of the paired differences of indicator or pathogen densities is calculated using the following equation:

$$S_d = \sqrt{\frac{\sum \left(d_i - \bar{d}\right)^2}{n-1}} \qquad \text{(Eq. 4-5)}$$

where d_i is difference in the densities of indicators or pathogens in each pair of values

$\bar{d}$ is the average of paired differences in the densities of indicators or pathogens

n is the number of pairs of values

The null hypothesis would be rejected if $|t| > t_{\alpha/2,\, n-1}$

where $t_{\alpha/2}$ is the value from a t-distribution table

α is the significance level (i.e., 0.05), and

n-1 is the number of degrees of freedom.

Alternatively, the 95% confidence intervals were calculated as follows:

$$\bar{d} \pm t_{\alpha/2}\left(S_d / \sqrt{n}\right) \qquad \text{(Eq. 4-6)}$$

The results of the paired t-tests of the densities of FC, FS, *Salmonella*, and viable helminth ova in the raw sludge and in the biosolids collected after thickening, anaerobic digestion, air-drying, and composting are presented in Tables 4-10, 4-12, 4-14, and 4-16, respectively. The 95% confidence intervals for FC, FS, *Salmonella* and viable helminth ova are presented in Tables 4-11, 4-13, 4-15, and 4-17, respectively. The confidence intervals reported in Tables 4-11, 4-13, 4-15, and 4-17 are for the average differences between the column and row level densities.

The results presented in Table 4-10 indicated that the average of the differences in the densities of FC for paired samples of raw sludge and biosolids after treatment were statistically significant at the 95% confidence level, except for the difference between the FC densities in the paired samples of air-dried and composted sludge. Furthermore, the data in Table 4-11 showed that the 95% confidence intervals for the average of the differences in the densities of FC for all pairs did not include the value zero, which suggests that the FC densities for all pairs were statistically different, except for the air-dried and composted sludges. The results in Tables 4-10 and 4-11 indicate that there was no statistical evidence that the FC mean densities in air-dried and composted biosolids were different at the 95% confidence level. The failure to have a statistically significant value may be due to the high variability of the FC densities. The results presented in Table 4-12 indicated that the average of the differences in the densities of FS for all pairs of biosolids were statistically significant at the 95% confidence level. The data in Table 4-13 showed that the 95% confidence intervals for the average of the differences in the densities of FS for all pairs did not

include the value zero. Therefore, the results of the statistical analyses indicate that the FS densities for all pairs of biosolids were different.

The results in Tables 4-14 and 4-15 indicated that the average of the differences in the densities of *Salmonella* between raw sludge and anaerobically digested biosolids and between raw sludge and air-dried sludge were statistically different at the 95% confidence level. In contrast, the results of the null hypothesis and confidence intervals (Tables 4-14 and 4-15) showed that the average of the differences in densities of *Salmonella* between raw and composted sludge, between anaerobically digested and air-dried biosolids, and between anaerobically digested and composted sludges were not statistically different at the 95% confidence level.

These results are not conclusive because the densities of *Salmonella* in air-dried sludge, and composted biosolids were expected to be statistically different than those in raw sludge and anaerobically digested biosolids. A factor that may have accounted for failing to reject the null hypothesis was the reduced number of samples that were included in the statistical analysis.

Salmonella was detected in a small number of 21 samples of air-dried sludge and composted biosolids that were collected; therefore, statistical tests are relatively insensitive. For example, the comparison of raw biosolids and composted biosolids is based on only two samples, and it is of no surprise that the result was inconclusive.

The results presented in Table 4-16 indicate that the average of the differences in the counts of viable helminth ova for all pairs of biosolids were statistically significant at the 95% confidence level, except for the difference in the ova counts between raw sludge and air-dried biosolids. Furthermore, the data in Table 4-17 show that the 95% confidence intervals of the average of the differences in the counts of

Table 4-10: Results of Paired t-test of FC Differences for Sludge Treatment Processes

Treatment Processes	n	$t = \frac{\overline{d} - \mu_d}{S_d / \sqrt{n}}$	$t_{\alpha/2.\ n-1}$	Result
Raw Sludge and Thickened Biosolids	40	5.0	2.02	Reject Null Hypothesis
Raw Sludge and Digested Biosolids	40	15.83	2.02	Reject Null Hypothesis
Raw Sludge and Air-Dried Biosolids	40	32.7	2.02	Reject Null Hypothesis
Raw Sludge and Composted Biosolids	40	26.89	2.02	Reject Null Hypothesis
Thickened Sludge and Digested Biosolids	40	15.0	2.02	Reject Null Hypothesis
Thickened Sludge and Air-Dried Biosolids	40	26.93	2.02	Reject Null Hypothesis
Thickened Sludge and Composted Biosolids	40	24.74	2.02	Reject Null Hypothesis
Anaerobically Digested and Air-Dried Biosolids	40	9.75	2.02	Reject Null Hypothesis
Anaerobically Digested and Composted Biosolids	40	11.56	2.02	Reject Null Hypothesis
Air-Dried and Composted Biosolids	40	0.73	2.02	Accept Null Hypothesis

FC densities expressed in log MPN/g

Table 4-11: 95% Confidence Intervals for FC Mean Differences in Sludge Processing

Source	Raw Sludge	Thickened Sludge	Anaerobically Digested Sludge	Air-Dried Sludge
Thickened Sludge	(0.2470,0.5829)			
Anaerobically Digested Sludge	(2.986, 3.861)	(2.603, 3.415)		
Air-Dried Sludge	(5.536, 6.266)	(5.074, 5.898)	(1.963, 2.991)	
Composted Sludge	(5.621, 6.536)	(5.200, 6.127)	(2.190, 3.119)	(-0.316, 0.670)

Table 4-12: Results of Paired t-test of FS Differences for Sludge Treatment Processes.

Treatment Processes	n	$t = \frac{\overline{d} - \mu_d}{S_d / \sqrt{n}}$	$t_{\alpha/2.\ n-1}$	Result
Raw Sludge/ Thickened Biosolids	40	5.15	2.02	Reject Null Hypothesis
Raw Sludge /Digested Biosolids	40	11.45	2.02	Reject Null Hypothesis
Raw Sludge/Air-Dried Biosolids	40	25.08	2.02	Reject Null Hypothesis
Raw Sludge/Composted Biosolids	40	22.07	2.02	Reject Null Hypothesis
Thickened Sludge/Digested Biosolids	40	8.42	2.02	Reject Null Hypothesis
Thickened Sludge/Air-Dried Biosolids	40	22.46	2.02	Reject Null Hypothesis
Thickened Sludge/Composted Biosolids	40	23.40	2.02	Reject Null Hypothesis
Anaerobically Digested/ Air-Dried	40	9.64	2.02	Reject Null Hypothesis
Anaerobically Digested /Composted Biosolids	40	10.63	2.02	Reject Null Hypothesis
Air-Dried/ Composted Biosolids	40	3.88	2.02	Reject Null Hypothesis

FS densities expressed in log MPN/g

Table 4-13: 95% Confidence Intervals for FS Mean Differences in Sludge Processing

Source	Raw Sludge	Thickened Sludge	Anaerobically Digested Sludge	Air-Dried Sludge
Thickened Sludge	(0.300, 0.688)			
Anaerobically Digested Sludge	(1.716, 2.452)	(1.208, 1.972)		
Air-Dried Sludge	(3.356, 3.945)	(2.872, 3.440)	(1.238, 1.895)	
Composted Sludge	(4.077, 4.900)	(3.649, 4.340)	(1.947, 2.862)	(0.401, 1.275)

n = 40

Table 4-14: Results of Paired t-tests of *Salmonella* Differences for Sludge Treatment Processes.

Treatment Processes	*n*	$t = \frac{\bar{d} - \mu_d}{S_d / \sqrt{n}}$	$t_{\alpha/2.\ n-1}$	Result
Raw Biosolids – A. Digested Biosolids	17	3.74	2.12	Reject Null Hypothesis
Raw Sludge - Air-Dried Biosolids	6	2.68	2.57	Reject Null Hypothesis
Raw Sludge- Composted Biosolids	2	2.27	12.71	Accept Null Hypothesis
Anaerobically Digested- Air-Dried	3	2.61	4.3	Accept Null Hypothesis
Anaerobically Digested – Composted Biosolids	2	1.63	12.71	Accept Null Hypothesis
Air-Dried - Composted Biosolids	0	-	-	ND

Densities expressed in MPN/g TS
ND: Not determined

Table 4-15: 95% Confidence Intervals for *Salmonella* Mean Differences in Sludge Processing.

Source	Raw Biosolids	Anaerobically Digested Biosolids	Air-Dried Biosolids
Anaerobically Digested Biosolids	5.30, 19.13 (17)		
Air-Dried Biosolids	0.87, 42.50 (6)	-0.702, 2.875 (3)	
Composted Biosolids	-0.702, 2.875 (2)	-7.423, 9.603 (2)	ND

Densities expressed in MPN/g TS
ND: Not determined
Numbers in parenthesis indicate pairs of values that were analyzed.

Table 4-16: Results of Paired t-test of Viable Helminth Ova Differences for Sludge Treatment Processes.

Treatment Processes	n	$t = \frac{\overline{d} - \mu_d}{S_d / \sqrt{n}}$	$t_{\alpha/2.\ n-1}$	Result
Raw Sludge and Anaerobically Digested Biosolids	22	3.02	2.08	Reject Null Hypothesis
Raw Sludge and Air-Dried Sludge	22	1.43	2.08	Accept Null Hypothesis
Raw Sludge and Composted Biosolids	22	5.61	2.08	Reject Null Hypothesis
Anaerobically Digested and Air-Dried Biosolids	22	3.96	2.08	Reject Null Hypothesis
Anaerobically Digested and Composted Biosolids	22	9.62	2.08	Reject Null Hypothesis
Air-Dried Sludge and Composted Biosolids	22	2.73	2.08	Reject Null Hypothesis

Densities expressed in ova/g TS

Table 4-17: 95% Confidence Intervals for Viable Helminth Ova Mean Differences in Municipal Sludge Processing.

Source	Raw Sludge	Anaerobically Digested Sludge	Air-Dried Sludge
Anaerobically Digested Sludge	-1.277, -0.235 (22)		
Air-Dried Sludge	-0.171, 0.928 (22)	0.580, 1.262 (22)	
Composted Sludge	0.580, 1.262 (22)	1.314, 2.039 (22)	0.130, 0.955 (22)

Viable helminth ova densities expressed as ovum/g TS
Numbers in parenthesis indicate pairs of values included in the analysis.

viable helminth ova for all pairs of treatment processes did not include zero, which suggests that the counts of viable helminth ova between all pairs of biosolids were different, except for the difference in the ova counts between raw sludge and air-dried biosolids. The results in Tables 4-16 and 4-17 indicated that there is not convincing evidence that the average viable helminth ova in raw sludge and air-dried are statistically different at the 95% confidence level. This result is inconclusive because the viable helminth ova counts in the air-dried sludge were expected to be lower than those in the raw sludge. The failure to have a significant difference may be due to the high variability of the viable helminth ova counts.

4.1.4 Summary of Field-Scale Studies

The results of the analyses of indicator and pathogenic bacteria and helminth ova in the raw sludge and the biosolids after four treatment processes included in the study are presented in Figures 4-1 and 4-2, respectively. The data in Figure 4-1 indicate that the densities of fecal coliforms (FC), and fecal streptococci (FS) in the raw sludge decreased consistently through gravity thickening, mesophilic anaerobic digestion, air-drying and windrow composting processes. The densities of bacterial indicators decreased slightly during the thickening process, but the densities decreased to a greater extent after anaerobic digestion, air-drying and composting processes. Paired t-tests revealed that the mean densities of FC and FS in the thickened biosolids were statistically lower than those in the raw sludge, at the 95% confidence level. Moreover, the average densities of FC and FS in the air-dried biosolids were statistically lower than those in the anaerobically digested sludge at the 95% confidence level. However, the mean densities of FC in the composted biosolids were not statistically lower than those in the air-dried biosolids at the 95% confidence

level. The densities of FC in the digested sludge were well below the limit established by the EPA Part 503 for Class B biosolids (FC < $2x10^6$ MPN/g TS). However, the densities of FC in the digested sludge did not meet the FC limit for Class A biosolids (FC< 1000 MPN/g TS). It should be noted that the average densities of FC and *Salmonella* after the air-drying and composting process were below the limit established by the Part 503 for Class A biosolids (FC < 1000 MPN/g and *Salmonella* < 3 MPN/g TS). The data also indicated that the densities of FS in all the processes that were evaluated were consistently higher than those of FC. FS were more resistant to inactivation than FC; therefore, FS may be a better indicator for monitoring pathogen survival during conventional sludge treatment processes.

The densities of helminth ova throughout the sludge processing train at HBBMP are presented in Figure 4-2. The data in Figure 4-2 indicate that helminth ova densities increased after the mesophilic anaerobic digestion process, then decreased in the air-dried and composted biosolids. Paired t-tests showed that the viable helminth ova counts in the anaerobically digested biosolids were statistically greater than those in the raw sludge, at the 95% confidence level. A factor that may account for the increase of viable ova in the anaerobically digested sludge may be the increase in total solids content of the digested sludge and the release of more ova caused by the destruction of volatile solids from the sludge matrix. Paired t-tests indicated that the viable helminth ova counts in the air-drying beds were statistically lower than those after the anaerobic digestion process, at the 95% confidence level. Furthermore, the counts of viable helminth ova after composting were statistically lower than those in the air-dried sludge process, at the same 95% confidence level. The median viable helminth ova in the air-dried sludge was below the limit

established by the EPA for Class A biosolids (viable helminth ova < 1 ovum/4 g TS). Detention time rather than solids content appeared to be the factor that controls the reduction of viable helminth ova in air-drying in rolled concrete basins after to 254 days of storage. The data observed in this study suggest that air-drying of anaerobically digested sludge may be a potential alternative low technology process to meet Class A sludge requirements for helminth ova, FC and *Salmonella*. The data in Figures 4-1 and 4-2 also indicated that the windrow composting process produce a Class A biosolids in terms FC, *Salmonella*, and helminth ova.

Overall, the data in Figures 4-1 and 4-2 indicate that the use of conventional sludge treatment processes, in series namely mesophilic anaerobic digestion followed by air-drying, and windrow composting is an effective processing train to produce Class A biosolids in terms of bacteria, helminth ova and vector attraction reduction requirements (e.g., >38% destruction of volatile solids).

4.2 RESULTS OF PHASE II - LAB-SCALE ANAEROBIC DIGESTION

Land application of stabilized municipal wastewater sludge for beneficial use has drawn considerable attention. Approximately 2.1 million dry tons of Class B sludge or 41% of the 6.9 million tons of biosolids generated in the US, in 1998, were applied to land. Several technologies can be used to produce Class B biosolids; however, a survey by (Edgar et al, 1998) in 117 POTWs indicated that anaerobic digestion seems to be the process of choice. The destruction of organic matter during anaerobic digestion of municipal sludge is well documented. The effects of temperature on the inactivation of FC, FS, and *Salmonella* have been documented; however, limited information on the fate and survival of FC, FS, and *Salmonella* in anaerobic sludge digestion at different hydraulic detention times is available and almost no data on the effect of organic loading is available. Understanding the individual effects of temperature, detention time, and organic loading as well as their interactions on the densities of indicator and pathogenic bacteria could provide guidance for designers and (POTWs) operators in determining the optimum conditions at which the mesophilic anaerobic digesters can comply with the EPA Part 503 regulations in terms of pathogen and vector attraction reduction requirements.

The second phase of this study focused on an evaluation of the fate of indicator and pathogenic bacteria in bench-scale anaerobic digesters at different hydraulic detention times, temperatures, and organic loadings. A factorial experimental design was used to determine the number of experimental runs and the sequence of performing each factor-level combination. Analysis of variance (ANOVA) was used to determine the statistical significance of the effects of the three selected factors on the densities of FC, FS, and *Salmonella*. The results of the

performance of the mesophilic anaerobic digestion in stabilizing the raw sludge and the relationship among time, temperature and organic loading and the bacterial densities in the digested sludge at all factor-level combinations is discussed below. Complete data on operating characteristics for the anaerobic digesters are presented in Appendix H.

4.2.1 Anaerobic Digestion Performance

Four bench-scale anaerobic digesters were operated from March 1998 to October 1999. The results of performance indicators (i.e., destruction of volatile solids and gas production) and process control parameters (i.e., pH, alkalinity, and volatile acids) at all operating conditions are summarized in Table 4-18. The effects of hydraulic detention time and organic loading on the reduction of volatile solids at 25°C and 35°C are presented in Figures 4-5 and 4-6, respectively. These data indicate that increasing the detention time will increase the percent destruction of volatile solids to approximately 50 to 60%. Increasing the temperature from 25°C to 35°C increases the rate of destruction of volatile solids. These results are in agreement with those reported by WEF (1995). The analyses of variance of the results of anaerobic digestion indicate that the individual effects of the hydraulic detention time and temperature were statistically significant on the destruction of volatile solids at the 95% confidence level. The two-factors interactions between time and organic loading and between time and temperature also were statistically significant at the same confidence level, at all factor-levels combinations. However, the two-factor interaction between temperature and loading only was statistically significant when the organic loading changed from 0.90 1.80 kgVS/m^3-d rather than when varied from 1.80 to 2.70 kgVS/m^3-d.

Table 4-18: Average Operating Characteristics of the Anaerobic Digestion Process

HDT	Temp.	Organic Loading	Influent			Effluent			VS Red.	pH	Alkalinity as $CaCO_3$	Volatile Acids as acetic acid	Gas Production	Gas Composition	
			TS,	VS,	Volatile Fraction,	TS,	VS,	Volatile Fraction,						CH_4	CO_2
Days	°C	kg/m^3-d	g/L	g/L	%	g/L	g/L	%	%		mg/L	mg/l	m^3/kg VS-d	%	%
7	24.8	0.89	8.4	6.2	73.9	6.9	4.6	66.6	26.2	6.1 – 6.5	610	436	0.5	48.0	52.0
	25.4	1.79	19.5	12.6	64.6	15.6	9.4	60.1	26.6	6.6 – 6.9	1270	424	0.9	60.2	39.9
	25.4	2.69	28.9	18.9	65.3	26.8	15.7	58.8	16.5	6.7 – 6.7	1577	284	1.5	59.6	40.3
	35.4	0.89	8.4	6.2	74.0	6.1	3.7	61.2	40.3	6.7 – 6.8	917	104	1.1	60.0	40.0
	35.2	1.79	19.5	12.6	64.6	14.4	8.0	55.5	36.5	7.1 –7.1	1595	180	1.2	60.7	39.4
	34.8	2.69	28.9	18.9	65.3	23.2	12.8	55.1	32.3	6.9 – 7.2	1893	96	1.2	61.5	38.1
15	24.9	0.87	19.8	13.1	66.2	14.9	8.2	54.8	36.7	7.0 – 7.1	2013	180	1.0	66.1	33.9
	24.7	1.83	39.8	27.5	69.1	31.5	17.5	55.4	36.5	7.0 – 7.0	2580	276	1.0	61.3	38.7
	25.1	2.68	64.0	40.2	62.8	44.0	23.8	54.0	41.3	6.8 – 6.8	2507	732	0.6	56.5	43.5
	35.3	0.87	19.8	13.1	66.2	13.0	6.7	51.5	48.0	7.0 – 7.2	2135	99	1.0	63.0	37.0
	35.1	1.83	39.8	27.5	69.1	29.7	15.4	51.9	43.6	7.0 – 7.1	2798	117	1.2	58.5	41.5
	35.3	2.68	64.1	40.2	62.8	41.8	20.6	49.3	49.1	7.1 –7.2	3143	588	0.9	55.0	45.0
30	24.9	0.88	40.1	26.5	66.1	28.7	15.7	54.7	38.8	7.0 – 7.1	2983	195	1.2	57.3	42.7
	25.0	1.84	80.0	55.3	69.1	48.7	25.9	53.3	53.2	7.0 – 7.0	3628	351	0.9	52.8	47.2
	25.1	2.55	121.8	76.5	62.7	74.5	37.1	49.7	51.4	7.3 – 7.3	4203	404	0.7	56.5	43.5
	34.6	0.88	40.1	26.5	66.2	25.8	13.0	50.5	50.1	7.1 –7.2	3018	108	1.0	62.4	37.6
	35.3	1.82	79.1	54.7	69.2	49.2	25.0	50.9	54.1	7.0 – 7.0	3980	327	0.9	57.7	42.3
	34.8	2.55	121.8	76.5	62.7	70.7	33.7	47.6	55.8	7.6 – 7.6	5217	640	0.7	58.8	42.2
45	25.0	0.88	46.5	39.8	73.4	33.1	19.5	59.1	50.5	7.2 – 7.8	3508	153	0.9	50.8	49.2
	25.6	1.67	112.2	75.1	67.2	61.0	33.1	54.3	56.0	7.2 – 7.3	4523	474	0.8	61.6	38.4
	35.1	0.88	53.9	39.7	73.4	35.0	19.6	56.0	51.0	7.5 – 7.8	4243	279	1.0	59.8	40.2
	35.0	1.67	112.3	75.1	67.2	59.5	31.4	52.8	58.2	7.4 – 7.6	5718	1197	0.8	64.5	35.5

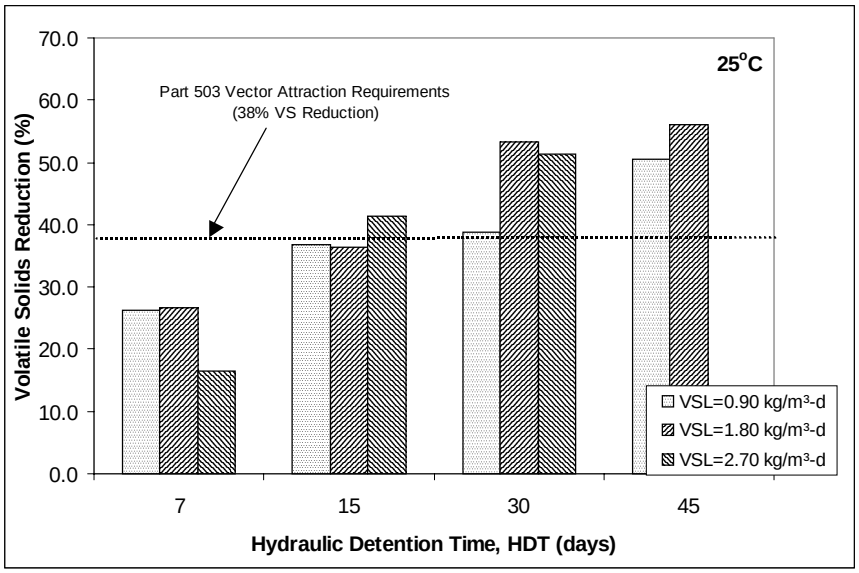

Figure 4-5: Effects of Hydraulic Detention Time and Organic Loading on the Reduction of Volatile Solids at 25°C

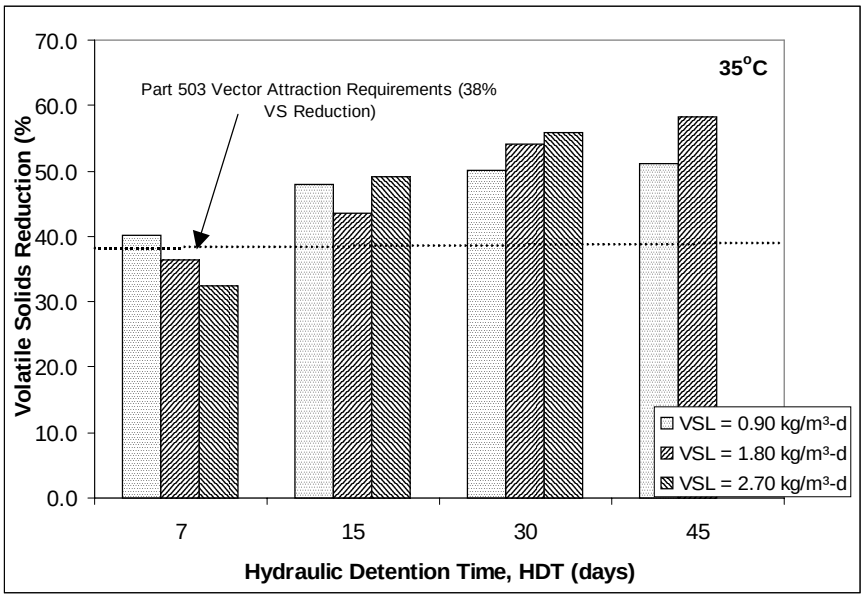

Figure 4-6: Effects of Hydraulic Detention Time and Organic Loading on the Reduction of Volatile Solids at 35°C.

The reduction in volatile solids at 35°C and 15 days for all the three loadings clearly was above the 38% established by the Part 503 Vector Attraction Reduction (VAR) Requirements, while at 25°C and 15 days the VS reduction at only one of the organic loadings was in compliance with the regulations. The maximum volatile solids reduction at both temperatures occurred at a detention time of 45 days and an organic loading of 1.80 kgVS/m^3-d.

The effects of hydraulic detention time and organic loading on pH, alkalinity, and volatile acids at 25°C and 35°C, are illustrated in Figures 4-7 and 4-8, respectively. The data in these figures indicate that pH, alkalinity, and volatile acids were for the most part within the limits reported in the literature by McCarty (1964); and Malina (1992) for normal anaerobic treatment. A good understanding of the interrelationships between pH, alkalinity, and carbon dioxide content of the gas are essential to the control of the anaerobic digestion system. At 25°C, the pH ranged from 6.2 to 7.5 at all organic loading and detention time conditions. The pH = 6.2 was slightly below the range for normal operation and occurred at the shortest detention time (7 days) and at the lowest VS loading (0.90 kgVS/m^3-d). The reduction of volatile solids at these conditions was below the limit established by VAR requirements (i.e., 38%). At 35°C, the pH ranged from 6.8 to 7.7 at all organic loading and detention time conditions. The pH = 7.7 was slightly above the range for normal operation and occurred at the longest detention time (45 days) and the lowest organic loading (0.90 kgVS/m^3-d). Overall, the pH values observed at both temperatures and at all detention times fell within the ranged established in the literature for normal operation.

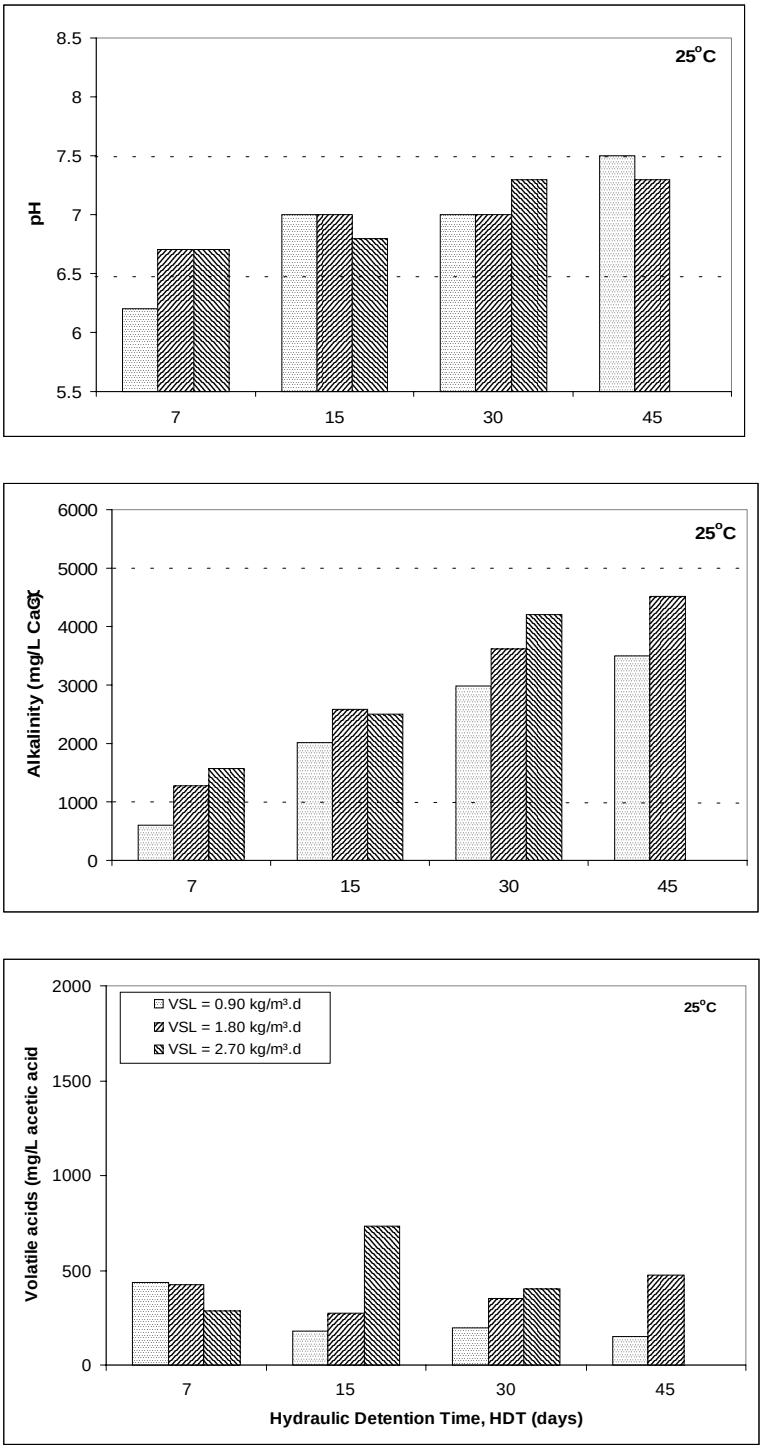

Figure 4-7: Effects of Time and Loading on pH, Alkalinity and Volatile Acids at 25°C.

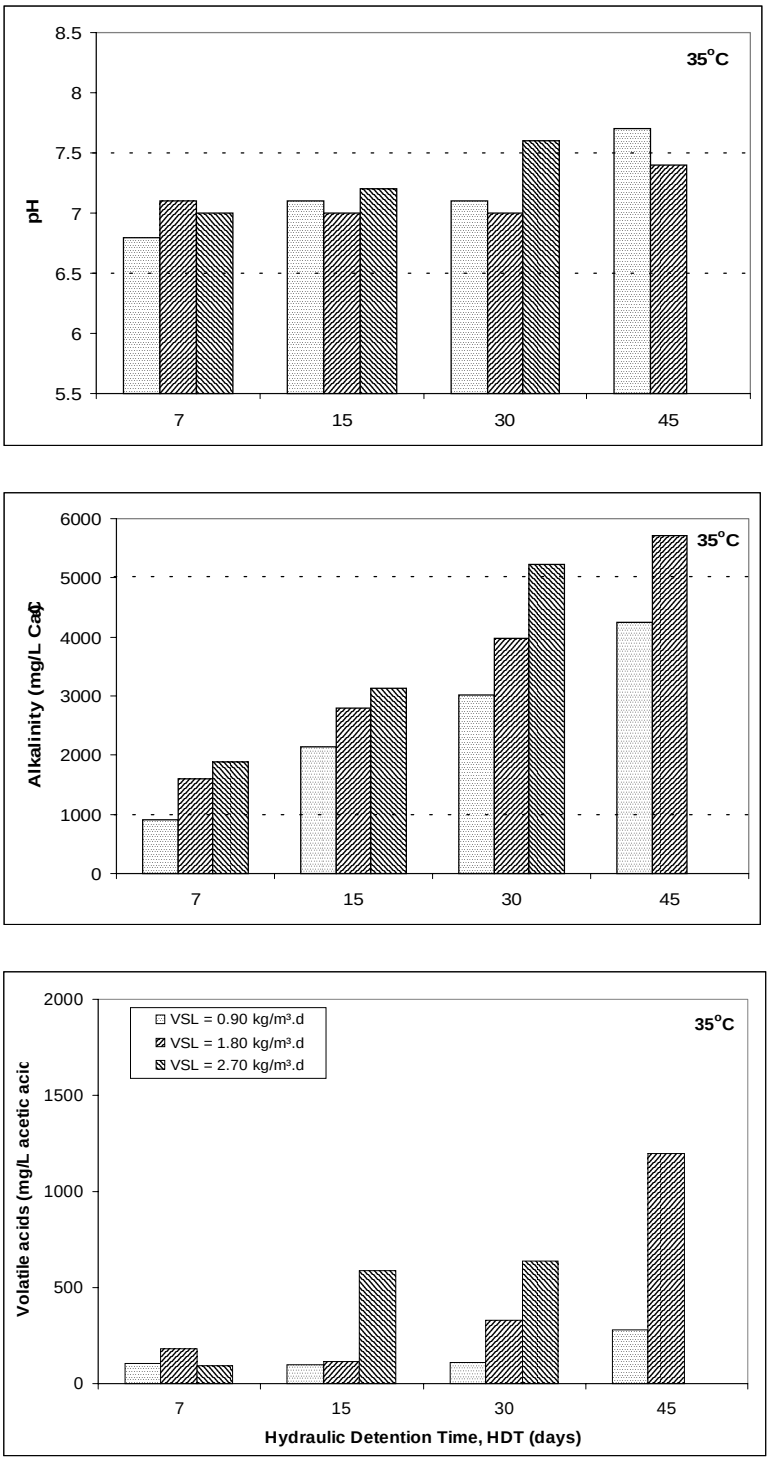

Figure 4-8: Effects of Time and Loading on pH, Alkalinity and Volatile Acids at 35°C

The alkalinity of the anaerobically digesting sludge is important because it indicates the buffer in the system; therefore, sufficient alkalinity is essential for proper pH control. The type of alkalinity is affected by pH and the partial pressure of the carbon dioxide content in the overhead gas.

The data presented in Figures 4-7 and 4-8 indicate that at both temperatures the alkalinity in the digesting sludge increased with an increasing detention time and an increase in VS loading. A factor that might account for this trend is the relation between alkalinity and the concentration of organic solids in the sludge, i.e., the higher the detention time and the volatile solids loading, the greater the concentration of organic solids in the reactor and, consequently, the higher the alkalinity concentrations.

The alkalinity ranged from 610 to 4523 mg/l as $CaCO_3$ at all the three organic loadings at 25°C. The alkalinity of 610 mg/l as $CaCO_3$ was below the range for normal operation (1000 to 5000 as $CaCO_3$) and occurred at the shortest detention time (7 days) and at the lowest VS loading (0.90 kgVS/m^3-d). At these operating conditions, the pH was 6.2 and the VS reduction was less than 38%. The alkalinity ranged from 917 to 5718 mg/L as $CaCO_3$ at all the three VS loadings at 35°C. The alkalinity of 917 as $CaCO_3$ occurred at the shortest detention time and the lowest VS loading was slightly below the range for normal operation. The alkalinity of 5718 mg/L as $CaCO_3$ occurred at the longest detention time and intermediate organic loading and was above the range for normal operation. However, the VS reduction at these conditions was 58.2%, which is far above the limit established by the EPA Part 503 for VAR.

The carbon dioxide content of the gas ranged from 33.9 to 52% at all the three organic loadings at 25°C. The CO_2 = 33.9% fell within the range for optimum operation (30 to 35%) and occurred at 15 days and at the lowest VS loading (0.90 kgVS/m^3-d). The CO_2 = 52% was above the range for extreme operation (25 to 40 %) and occurred at 7 days and at the lowest VS loading (0.90 kgVS/m^3-d). At these operating conditions, the pH ranged from 6.1 to 6.5 and the VS reduction was less than 38%. The CO_2 ranged from 35.5 to 45% at all the three VS loadings at 35°C. The lower limit (CO_2 = 35.5%) was slightly above optimum conditions and occurred at the longest detention time and the intermediate VS loading (1.80 kgVS/m^3-d). The CO_2 = 45% was slightly above the range for extreme operation and occurred at 15 days and at the highest VS loading (2.70 kgVS/m^3-d).

The data presented in Figures 4-8 and 4-9 indicate that, at both temperatures, the concentration of volatile acids in the digesting sludge tended to increase with an increase in the organic loading at detention times of 15, 30 and 45 days. This effect was more significant when the organic loading was increased from 1.80 kgVS/m^3-d to 2.70 kgVS/m^3-d than when changed from 0.90 kgVS/m^3-d to 1.80 kgVS/m^3-d. This pattern was more evident at 35°C than it is at 25°C.

4.2.2 Effects of Time, Temperature, and Organic Loading on Bacterial Densities

The statistical significance of the effects of detention time, temperature, and organic loading (factors or independent variables) on the densities of fecal coliforms, fecal streptococci, and *Salmonella* (response variables) during bench-scale anaerobic digestion was evaluated using a factorial experimental design and the analysis of variance (ANOVA) procedure.

The three selected factors were tested at the following levels: detention time (factor A) was investigated at four levels (7, 15, 30 and 45 days), temperature (factor B) was examined at two levels (25°C and 35°C), and organic loading (factor C) was tested at three levels (0.90, 1.80, and 2.70 kgVS/m^3-d). Therefore, one complete replicate of the factorial design would involve 24 experimental runs. In this study, however, 22 experimental runs were completed and two individual tests were missed. The two missing runs were planned at a detention time of 45 days and a VS loading of 2.70 kg/m^3-d, at both temperatures. The concentration of solids in the feed sludge required to produce the organic loading of 2.70 kg/m^3-d was much higher than could be obtained with the lab equipment. Three replicates were analyzed for each experimental run; thus, 66 observations were collected throughout the experiment.

One of the principal steps in factorial designs is the statistical analysis of data. Planning and selection of the design were discussed previously in Section 3.2.2. The standard procedure for testing the equality of several factors means is the analysis of variance (ANOVA). The analysis of variance is a procedure for testing two or more treatment processes to determine whether their sample means could

have obtained from populations with the same true mean. The classical null hypothesis states that there is no difference in treatment (or factors) means:

$$H_o: \mu_1 = \mu_2 = \ldots. \mu_a \quad \text{(Eq. 4-7)}$$

where μ_1, μ_2, and μ_a are the treatment or factor averages. The alternative hypothesis states that there are differences in at least one pair (i, j) of treatment averages.

$$H_1: \mu_i \neq \mu_j \quad \text{(Eq. 4-8)}$$

The test is made by estimating the amount of variation **within** treatments and comparing it to the variance **between** treatments. If the treatments are alike (that is, from populations with the same mean), the variation within each treatment will be about the same as the variation between treatments. The null hypothesis of no difference in treatment means is tested using the F statistic, which is defined as follows:

$$F_0 = \frac{SS_{Treatments}/(a-1)}{SS_E/(N-a)} = \frac{MS_{Treatments}}{MS_E} \quad \text{(Eq. 4-9)}$$

where $SS_{Treatments}$ is the sum of squares between treatments, a are the treatment averages, a *-1* are the degrees of freedom (between treatments), SS_E is the sum of squares due to error, N are the total number of observations, N - a are the degrees of freedom (within treatments). $MS_{Treatments}$ is the mean square of treatments and MS_E is the mean square of errors. Complete definitions for $SS_{Treatments}$ and SS_E are presented by Montgomery (1998).

The null hypothesis H_o would be rejected if $F_0 > F_{\alpha,a-1,N-a}$, where F_0 is computed from Eq. 4-9 and $F_{\alpha,a-1,N-a}$ denotes the upper α percentage point of the F distribution (i.e., 95% confidence level) with *a-1* and *N - a* degrees of freedom.

The computations of ANOVA were run in MINITAB®, a standard statistical software package. The use of the analysis of variance to test formally for no differences in treatment means requires that certain assumptions be satisfied. One such assumption is that the errors (or residuals) are normally and independently distributed variables with mean zero and constant but unknown variance σ^2. In this study, that assumption was met by transforming the levels of FC, FS, and *Salmonella* from MPN/g TS to log densities ($\log_{10}$ MPN/g TS). Probability plots of the data produced a straight line suggesting that the transformed errors were log normally distributed. The U.S. EPA (1992) has suggested that the bacterial densities in wastewater sludge are best described by a log normal distribution rather than by the normal distribution.

Effects of Time, Temperature and Organic Loading on the Fate (Concentration) of Fecal Coliforms.

Anaerobically digested sludge has been classified by the EPA Part 503 regulations as Class B biosolids (i.e., FC < $2x10^6$ MPN/g TS). Lee et al (1989) and Stukenberg et al (1994) reported that mesophilic anaerobic digestion yields an average of 2-log reduction in fecal coliforms. Several factors have been mentioned as possible mechanisms for the inactivation of FC in mesophilic anaerobic digestion. However, only temperature has been experimentally documented. In addition to temperature, hydraulic detention time and organic loading also might affect the

densities of this pathogen indicator. The results of ANOVA for the log FC densities during bench-scale anaerobic digestion are discussed below.

The actual and transformed densities of FC, expressed as MPN/g TS and log MPN/g TS, are presented in Tables 4-19 and 4-20, respectively. The effects of detention time and organic loading on the log-reduction of FC at 25°C and 35°C are illustrated in Figures 4-9 and 4-10, respectively. The densities of FC during anaerobic digestion at 25°C and 35°C at all operating conditions are presented in Figures 4-11 and 4-12, respectively.

The data in Figure 4-9 show that, at 25°C, the reduction of FC at all operating conditions was less than 2-logs, except at 30 days and loading of 0.90 kgVS/m^3-d, and at 45 days and the loadings of 0.90 and 1.80 kgVS/m^3-d. In contrast, at 35°, the FC densities were reduced in more than 2 logs at all operating conditions, except at 30 days and the highest loading (2.70 kgVS/m^3-d). The FC log reduction at 35° is in close agreement with the data reported by Stukenberg et al (1994) for well-operated mesophilic anaerobic digesters (i.e., 2-log units). The maximum FC log reductions, at both temperatures, occurred at the lowest organic loading (i.e., 0.90 kgVS/m^3-d) [56.2 lbVS/1000 ft^3-d], but at different detention times, e.g., at 25°C, the maximum FC reduction occurred at 45 days (2.43-logs), while at 35°C the maximum FC inactivation occurred at 30 days (2.87-logs).

The data presented in Figures 4-11 and 4-12 indicate that the FC densities at both temperatures and all operating conditions were below the limit established by the Part 503 regulations for Class “B” biosolids (FC< 6.3-log MPN/g), i.e., the digesting sludge at all operating conditions met the Class “B” biosolids requirements for FC. However, at some operating conditions, the 38% reduction of volatile solids

established by the VAR requirements was not met (Figures 4-5 and 4-6). It should be mentioned that the average FC density in the feed sludge throughout this study (7.05 log MPN/g TS) was slightly lower than that reported by the Berg and Berman (1980) for mixed primary and waste activated sludge (8.57 log MPN/100 mL)

Table 4-19: Raw Densities of Fecal Coliforms in Anaerobic Digestion

	25 Celsius			35 Celsius		
	0.9 kg VS/m^3-d	1.8 kg VS/m^3-d	2.7 kg VS/m^3-d	0.9 kg VS/m^3-d	1.8 kg VS/m^3-d	2.7 kg VS/m^3-d
7 days	4.00E+05	1.00E+06	2.59E+05	8.33E+04	2.00E+04	1.09E+05
	3.14E+06	1.50E+06	4.07E+05	1.33E+05	1.47E+05	5.42E+04
	1.14E+06	1.60E+06	3.46E+06	4.40E+04	3.57E+04	7.39E+04
15 days	2.27E+04	5.31E+05	1.78E+05	1.08E+04	7.50E+04	5.24E+04
	1.13E+05	2.67E+05	1.16E+05	8.46E+04	5.67E+04	7.86E+03
	3.33E+05	3.44E+05	6.36E+04	2.30E+04	1.79E+05	4.05E+04
30 days	2.76E+04	9.80E+04	4.54E+04	2.96E+03	9.80E+04	1.11E+05
	8.71E+03	1.04E+05	1.09E+05	9.00E+03	1.04E+05	7.14E+04
	8.28E+04	1.70E+05	1.89E+05	1.11E+04	6.12E+04	3.14E+04
45 days	3.78E+04	4.84E+04	ND	2.05E+05	4.06E+04	ND
	2.29E+05	3.61E+04	ND	3.51E+04	2.88E+04	ND
	9.38E+04	5.74E+04	ND	6.11E+04	3.61E+04	ND

FC densities in MPN/g TS
ND: Not determined

Table 4-20: Transformed Densities of Fecal Coliforms in Anaerobic Digestion

	25 Celsius			35 Celsius		
	0.9 kg VS/m^3-d	1.8 kg VS/m^3-d	2.7 kg VS/m^3-d	0.9 kg VS/m^3-d	1.8 kg VS/m^3-d	2.7 kg VS/m^3-d
7 days	5.60	6.00	5.41	4.92	4.30	5.04
	6.50	6.18	5.61	5.12	5.17	4.73
	6.06	6.20	6.54	4.64	4.55	4.87
15 days	4.36	5.73	5.25	4.03	4.88	4.72
	5.05	5.43	5.06	4.93	4.75	3.90
	5.52	5.54	4.80	4.36	5.25	4.61
30 days	4.44	4.99	4.66	3.47	4.99	5.05
	3.94	5.02	5.04	3.95	5.02	4.85
	4.92	5.23	5.28	4.05	4.79	4.50
45 days	4.58	4.68	ND	5.31	4.61	ND
	5.36	4.56	ND	4.55	4.46	ND
	4.97	4.76	ND	4.79	4.56	ND

FC densities in log MPN/g TS
ND: Not determined

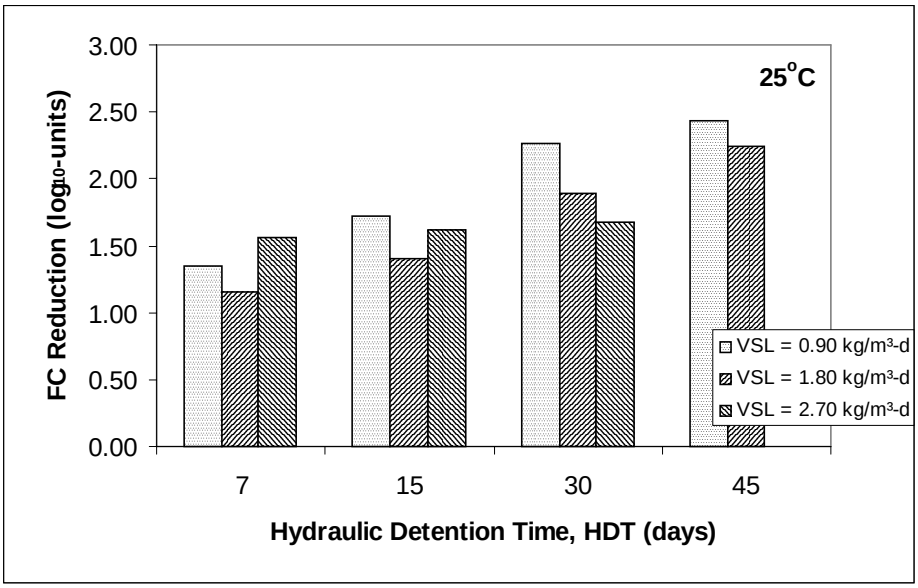

Figure 4-9: Effects of Hydraulic Detention Time and Organic Loading on Log-Reduction of Fecal Coliforms at 25°C.

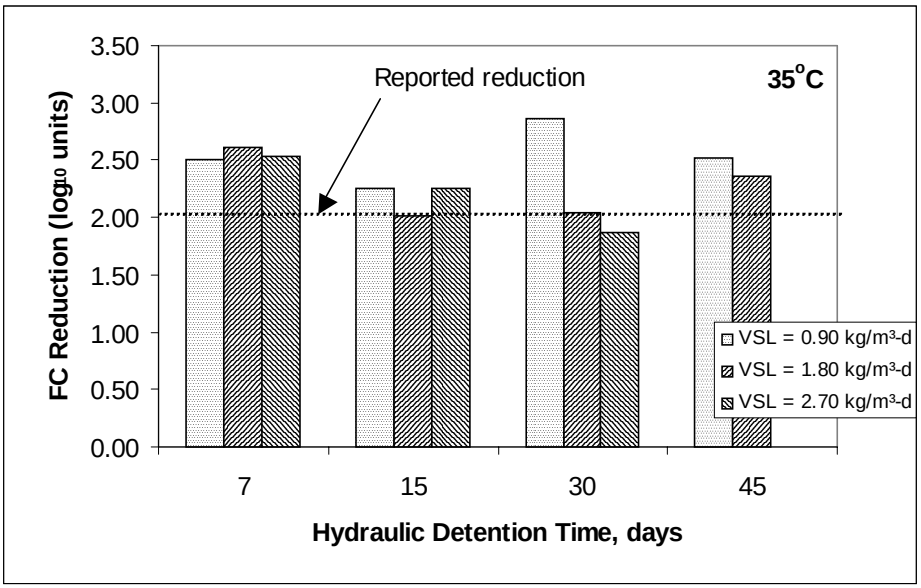

Figure 4-10: Effects of Hydraulic Detention Time and Organic Loading on Log-Reduction of Fecal Coliforms at 35°C.

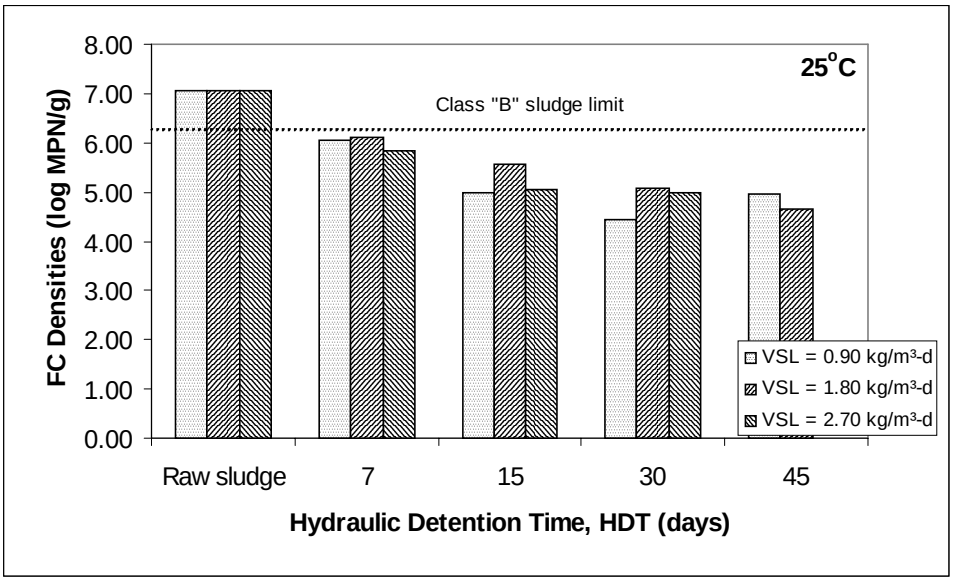

Figure 4-11: Densities of Fecal Coliforms in Anaerobic Digestion at 25°C.

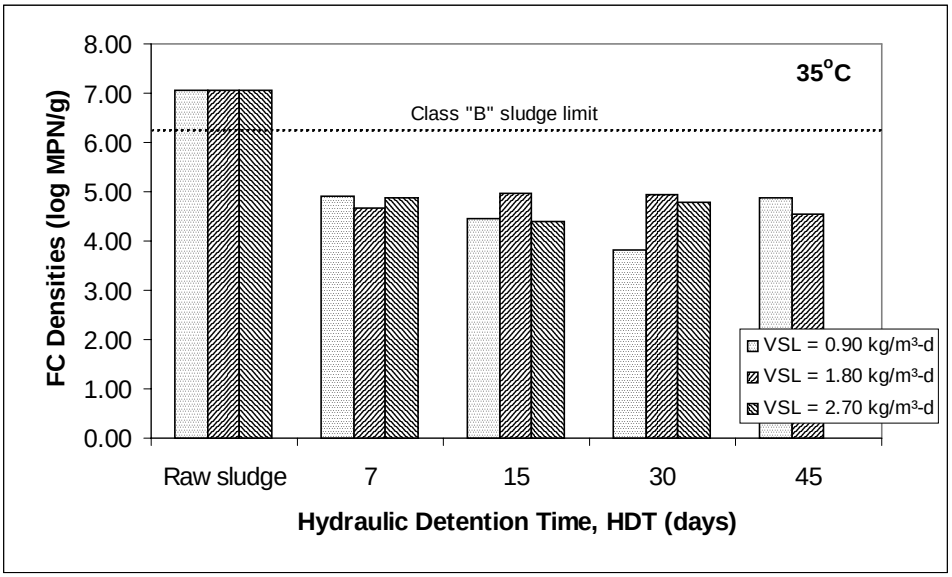

Figure 4-12: Densities of Fecal Coliforms in Anaerobic Digestion at 35°C.

This difference might have been a factor by which the digesting sludge always met the FC limit established for Class "B" biosolids. However, at 35°C and detention times greater than or equal to 15 days, the mesophilic anaerobic digestion process should be able to meet the pathogen and VAR requirements for Class B biosolids.

The bar graphs in Figures 4-9 and 4-10 indicate that the reduction of FC densities was greater at 35°C than it was at 25°C. The FC densities declined at an approximate constant rate from 7 through 30 days at 25°C. On the other hand, at 35°C the FC densities decreased rapidly at 7 days (> 2 logs), and then decreased at a much slower rate or even increased slightly as the detention time increased from 15 through 45 days. Two FC decay rates were observed at 35°C at 7, 15, and 30 days (i.e., a higher rate at 7 days, and a lower rate at 15 and 30 days), while the FC declined at an approximately constant rate at 25°C. Overall, the data in Figures 4-11 and 4-12 indicate that, at 25°C, the FC densities consistently decreased as the detention time increased from 7 through 30 days at all organic loadings. At 35°C, the tendency of the FC densities to decrease as the detention time increased from 7 through 30 days was observed only in the digesters with the lowest organic loading (0.90 kgVS/m^3-d); whereas at higher organic loadings (1.80 and 2.70 kgVS/m^3-d), the FC densities decreased at 7 days and then remained approximately constant at 15 and 30 days. At both temperatures, the minimum FC densities were not achieved at the longest detention time. This effect was more evident at 45 days and 0.90 kgVS/m^3-d.

Two ANOVA tests were carried out using the log FC densities as input data. All the three factors: temperature, detention time, and organic loading were evaluated in each ANOVA test. However, the number of factor levels included in each statistical test was different. The reason for using two ANOVA tests instead of one

was because the original factorial design (4-2-3) became unbalanced when two out of the 24 experimental runs were not carried out during the study. A requirement for using ANOVA for comparing treatment means is that factorial designs must be balanced. Therefore, the statistical analysis of the available factor-levels was split into two ANOVA tests. In the first analysis, hydraulic detention time was evaluated at 3 levels (7, 15 and 30 days), temperature was examined at two levels (25°C and 35°C), and organic loading was tested at 3 levels (0.90, 1.80 and 2.70 $kg/m^3.d$). This test was a 3-2-3 level-combination. The second test included all the four levels for detention time, two levels for temperature, and two levels for organic loading (i.e., 0.90 and 1.80 $kg/m^3.d$). This design was a 4-2-2 level-combination.

The results of ANOVA for the 3-2-3 and 4-2-2 tests are summarized in Tables 4-21 and 4-22, respectively. The data in these tables include F test and P-values (i.e., the smallest level of significance that would lead to rejection of the null hypothesis Ho) for all main effects and interactions. The plots for main effects and interactions for the 3-2-3 and 4-2-2 tests are presented in Figures 4-13 and 4-14, respectively. A nonsignificant main effect would plot as a nearly horizontal straight line, and nonsignificant interactions result as almost parallel plots.

The results of ANOVA presented in Tables 4-21 and 4-22 show similar results for both tests, e.g., the main effects of all three factors were statistically significant for both tests at the 95% confidence level. Furthermore, the interactions between time and temperature and between time and organic loading also were significant for both analyses. The P-values for main effects indicate that detention time and temperature were more significant than organic loading for both statistical tests.

Table 4-21: Results of ANOVA for Fecal Coliform Densities (3-2-3 factor-level test)

Factor	Type	Levels	Values	Units
Time	fixed	3	7 15 30	(days)
Temp	fixed	2	25 35	(C)
Load	fixed	3	0.9 1.8 2.7	()

Analysis of Variance for FC, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Time	2	5.1358	5.1358	2.5679	20.16	0.000
Temp	1	6.6290	6.6290	6.6290	52.05	0.000
Load	2	1.8496	1.8496	0.9248	7.26	0.002
Time*Temp	2	1.8208	1.8208	0.9104	7.15	0.002
Time*Load	4	2.1336	2.1336	0.5334	4.19	0.007
Temp*Load	2	0.0741	0.0741	0.0370	0.29	0.749
Time*Temp*Load	4	0.3035	0.3035	0.0759	0.60	0.668
Error	36	4.5852	4.5852	0.1274		
Total	53	22.5317				

Table 4-22: Results of ANOVA for Fecal Coliform Densities (4-2-2 factor-level test)

Factor	Type	Levels	Values	Units
Time	fixed	4	7 15 30 45	(days)
Temp	fixed	2	25 35	(C)
Load	fixed	2	0.9 1.8	()

Analysis of Variance for FC, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Time	3	5.0144	5.0144	1.6715	14.86	0.000
Temp	1	4.1831	4.1831	4.1831	37.19	0.000
Load	1	0.8086	0.8086	0.8086	7.19	0.011
Time*Temp	3	2.3819	2.3819	0.7940	7.06	0.001
Time*Load	3	2.7564	2.7564	0.9188	8.17	0.000
Temp*Load	1	0.0008	0.0008	0.0008	0.01	0.935
Time*Temp*Load	3	0.2295	0.2295	0.0765	0.68	0.571
Error	32	3.5990	3.5990	0.1125		
Total	47	18.9736				

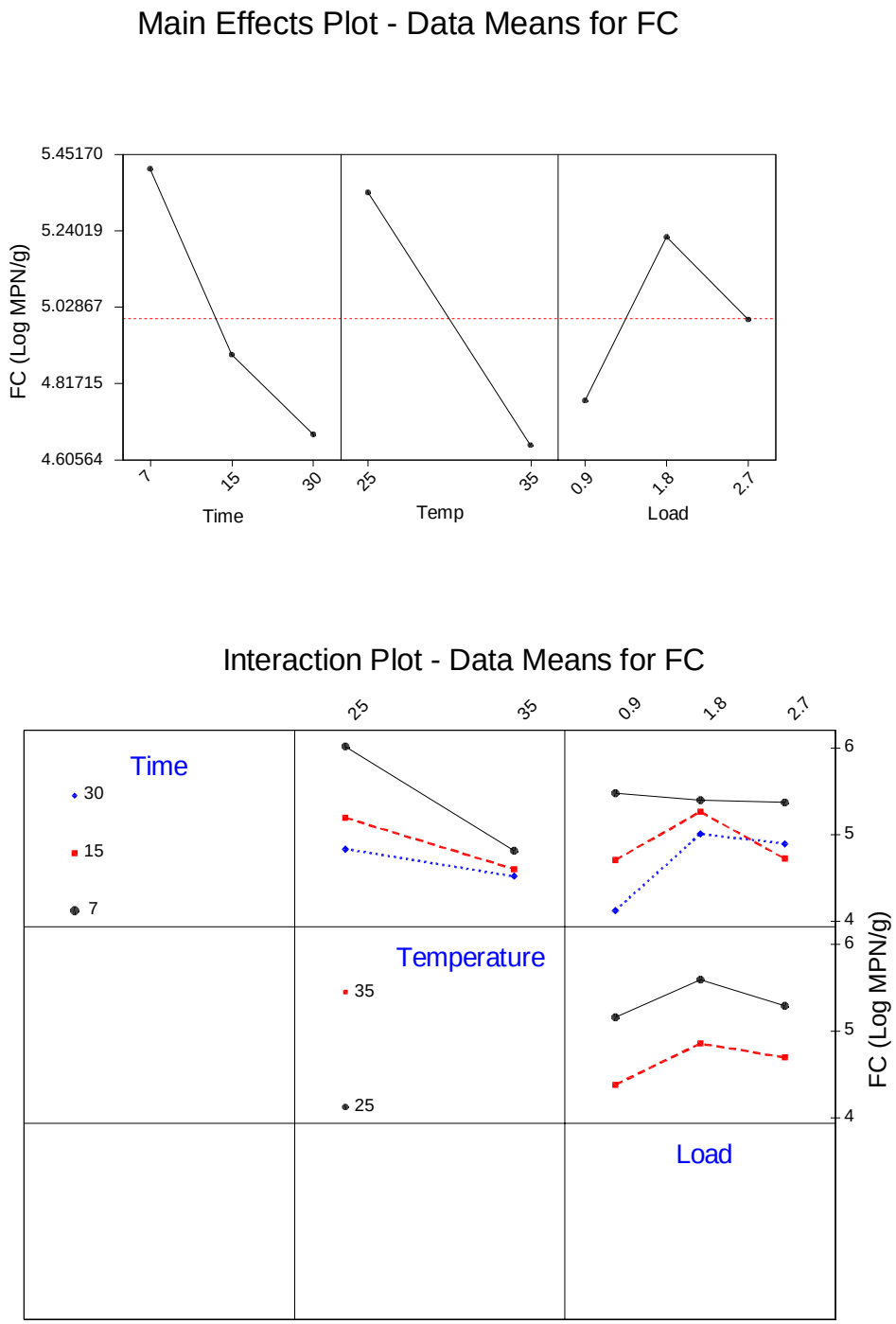

Figure 4-13: Effects and Interaction Plots for Fecal Coliforms (3-2-3 test)

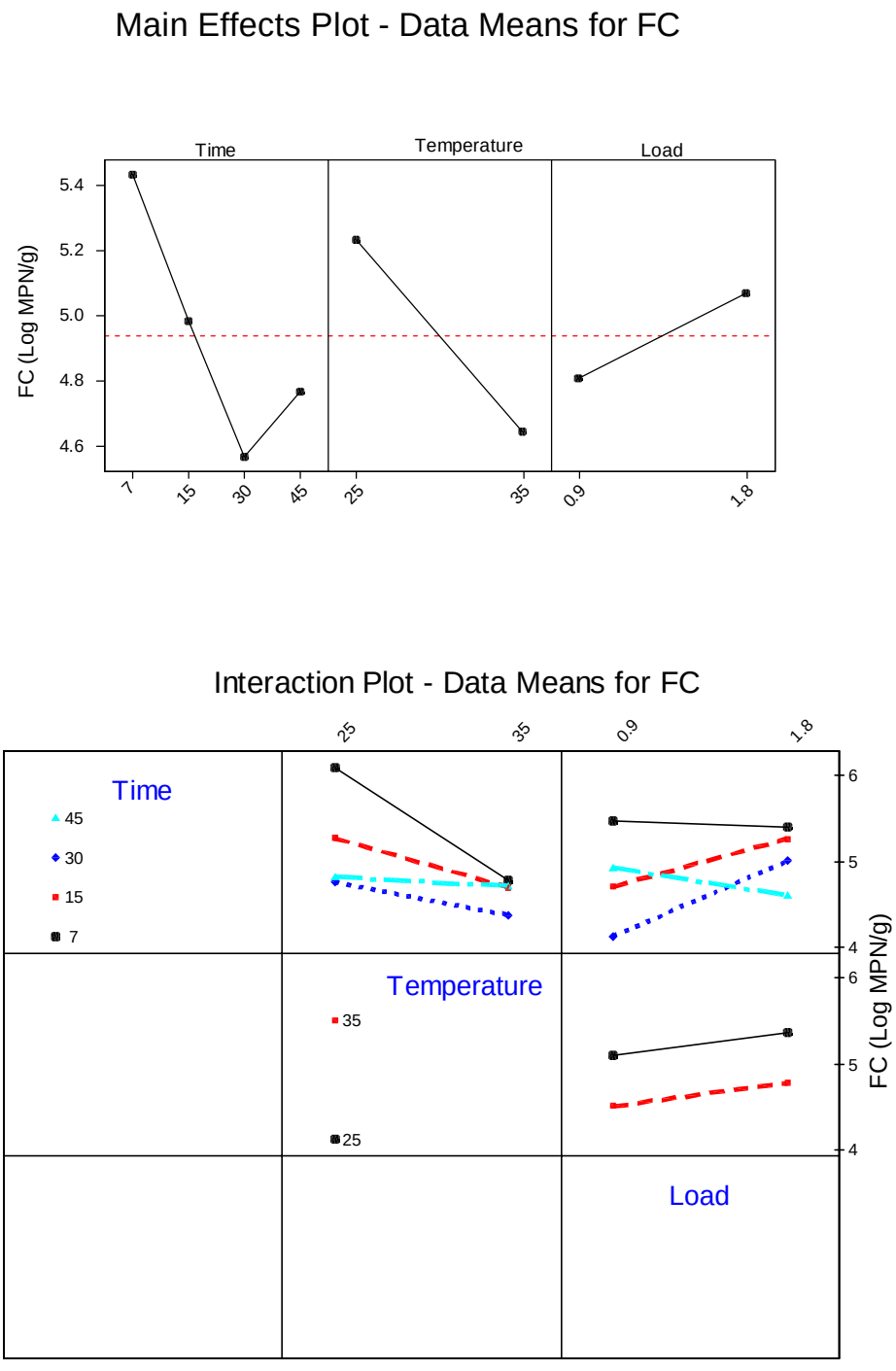

Figure 4.14: Effects and Interaction Plots for Fecal Coliforms (4-2-2 test)

In regard to two-factor interactions, the ANOVA data show that the interaction between time and temperature for the 3-2-3 test was more significant than the interaction between time and organic loading. In contrast, the data for the 4-2-2 design show that the interaction between time and organic loading was more significant than the interaction between time and temperature.

The plots for main effects for the 3-2-3 level-combination indicate that the effect of increasing the detention time on the reduction of FC was greater from 7 to 15 days than it was from 15 to 30 days. For instance, the FC densities declined at a higher rate from 7 to 15 days than from 15 to 30 days. The densities of FC decreased when temperature increased from 25°C to 35°C. The FC densities increased when the organic loading increased from 0.90 to 1.80 kgVS/m^3-d, then decreased when the organic loading increased to the highest loading. This pattern suggests that the organic loading is interacting with either time or temperature. The interaction between organic loading and time is statistically significant at the 95% confidence level. The plot for the interaction between time and temperature (Figure 4-13) suggests that the effect of increasing the temperature from 25°C to 35°C on the FC density was greater at 7 days than that at 15 and 30 days. The effect of increasing the temperature on the FC reduction was minimum at 30 days. The interaction between time and organic loading also is shown in Figure 4-13. The flat plot indicates that increasing the organic loading was non significant at 7 days. However, a significant interaction between detention time and organic loading was observed at 15 and 30 days at all organic loadings. This significant interaction is indicated by the crossing of plots for 15 and 30 days and the two highest organic loadings. Increasing the organic loading from 0.90 to 1.80 kgVS/m^3-d (56.2 to 112.4 lbVS/1000 ft^3-d) caused an increase in the

bacterial densities at 15 and 30 days. However, increasing the organic loading from 1.80 to 2.70 kgVS/m^3-d (112.4 to 168.6 lbVS/1000 ft^3-d) caused a slight decrease in the bacterial densities at both detention times. This effect was more significant at 15 days than it was at 30 days.

The data in Figure 4-14 show that for the 4-2-2 level test, both the main effects for all three factors and the two-factor interactions between time and temperature and between time and organic loading were statistically significant. The main effects plot shows that increasing the detention time from 7 through 30 days caused a consistent decrease in the FC densities. Increasing the temperature from 25°C to 35°C also caused a decrease in the FC densities. However, increasing the organic loading from 0.90 to 1.80 kgVS/m^3-d caused a slight increase in the concentration of FC. The interactions plot shows that both temperature and organic loading have an interactive effect with detention time on the FC density in the digesting sludge. The curve for interaction between time and temperature suggests that the effect of increasing the temperature from 25°C to 35°C was more significant at lower detention times than it was a higher detention times. The FC densities decreased at a higher rate at 7 days than they declined at 15 and 30 days. The effect of increasing the temperature on the FC concentration at 45 days was almost nonsignificant. The interaction between time and organic loading indicate that, at 7 days, increasing the organic loading from 0.90 to 1.80 kgVS/m^3-d caused a minimum effect on FC. However, at 15 and 30 days, increasing the organic loading caused an increase in the FC densities. Conversely, at 45 days, the effect of increasing the organic loading caused a slight decrease in the FC density.

Effects of Time, Temperature and Organic Loading on the Fate (Concentration) of Fecal Streptococci.

Fecal streptococci (FS) have been used to differentiate human fecal contamination from that of other warm-blooded animals. FS are more resistant to inactivation in conventional sludge treatment systems than FC. The results of ANOVA are used for examining the statistical significance of detention time, temperature, and organic loading, and their possible interaction, on the average log densities of FS during bench-scale mesophilic anaerobic digestion.

The observed data for actual and transformed FS densities are presented in Tables 4-23 and 4-24, respectively. The effects of detention time and organic loading on the log-reduction of FS counts at 25°C and 35°C are illustrated in Figures 4-15 and 4-16, respectively. The densities of FS in the digested sludge at different detention times, at 25°C and 35°C are presented in Figures 4-17 and 4-18, respectively.

The data presented in Figures 4-15 and 4-16 show that the log reduction of FS densities was greater at 35°C than at 25°C at all operating conditions. The FS log reduction increased rapidly at 7 days, at both temperatures and all the organic loadings. The FS reduction increased at a slower rate at 15 days and remained relatively constant at 30 days. However, when the detention time changed from 30 to 45 days, the FS log reduction increased at the lowest loading (0.90 kgVS/m^3-d) but decreased at the intermediate loading (1.80 kgVS/m^3-d). The maximum FS log-reduction occurred at 15 days and the highest organic loading (2.70 kgVS/m^3-d) at both temperatures. The maximum FS reductions were 1.45 logs at 25°C and 1.75 logs at 35°C. Overall, FS were more resistant to inactivation than FC during anaerobic digestion.

Table 4-23: Raw Densities of Fecal Streptococci in Anaerobic Digestion

	25 Celsius			35 Celsius		
	0.9 kg VS/m^3-d	1.8 kg VS/m^3-d	2.7 kg VS/m^3-d	0.9 kg VS/m^3-d	1.8 kg VS/m^3-d	2.7 kg VS/m^3-d
7 days	3.14E+06	1.29E+06	2.96E+05	5.05E+05	5.33E+05	1.36E+05
	1.86E+06	6.88E+05	4.81E+05	8.33E+05	8.67E+05	7.08E+04
	7.14E+06	2.00E+06	1.92E+06	1.60E+06	1.64E+05	5.65E+05
15 days	9.33E+05	5.31E+05	6.67E+05	6.66E+04	2.81E+05	1.67E+05
	7.33E+05	1.67E+06	6.51E+05	1.85E+05	2.67E+05	4.05E+05
	5.33E+05	8.75E+05	1.14E+06	3.85E+05	4.64E+05	7.14E+05
30 days	3.10E+06	5.88E+05	6.49E+05	1.11E+05	1.57E+05	1.25E+06
	7.74E+05	6.25E+05	2.19E+06	3.00E+05	5.00E+05	4.29E+05
	5.86E+05	5.11E+05	2.16E+06	2.96E+05	6.12E+05	4.29E+05
45 days	5.95E+06	8.06E+05	ND	2.05E+05	8.33E+05	ND
	4.57E+05	1.48E+06	ND	6.49E+05	8.47E+05	ND
	1.56E+06	1.48E+06	ND	4.72E+05	2.79E+05	ND

FS densities in MPN/g TS
ND: Not determined

Table 4-24: Transformed Densities of Fecal Streptococci in Anaerobic Digestion

	25 Celsius			35 Celsius		
	0.9 kg VS/m^3-d	1.8 kg VS/m^3-d	2.7 kg VS/m^3-d	0.9 kg VS/m^3-d	1.8 kg VS/m^3-d	2.7 kg VS/m^3-d
7 days	6.50	6.11	5.47	5.70	5.73	5.13
	6.27	5.84	5.68	5.92	5.94	4.85
	6.85	6.30	6.28	6.20	5.21	5.75
15 days	5.97	5.73	5.82	4.82	5.45	5.22
	5.87	6.22	5.81	5.27	5.43	5.61
	5.73	5.94	6.06	5.59	5.67	5.85
30 days	6.49	5.77	5.81	5.05	5.20	6.10
	5.89	5.80	6.34	5.48	5.70	5.63
	5.77	5.71	6.33	5.47	5.79	5.63
45 days	6.77	5.91	ND	5.31	5.92	ND
	5.66	6.17	ND	5.81	5.93	ND
	6.19	6.17	ND	5.67	5.45	ND

FS densities in log MPN/g TS
ND: Not determined

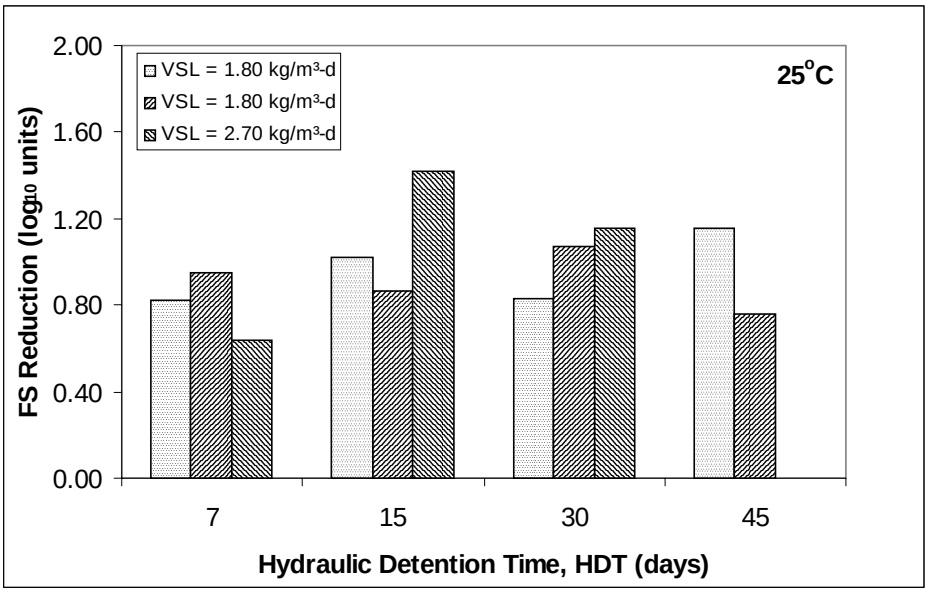

Figure 4-15: Effects of Hydraulic Detention Time and Organic Loading on Log Reduction of Fecal Streptococci at 25°C

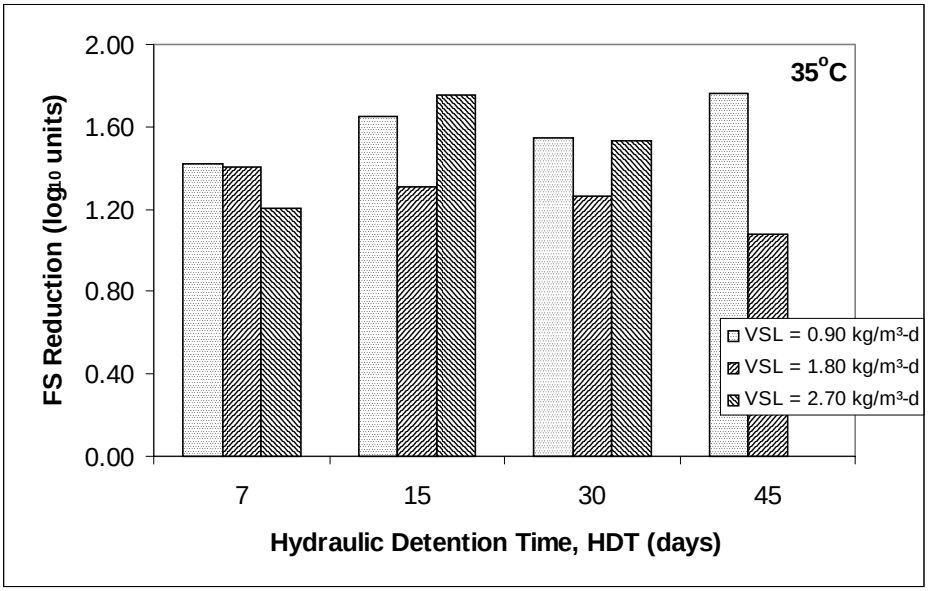

Figure 4-16: Effects of Hydraulic Detention Time and Organic Loading on Log Reduction of Fecal Streptococci at 35°C

The FS densities in the digesting sludge at all operating conditions and at 25°C and 35°C are presented in Figures 4-17 and 4-18, respectively. These data indicate that temperature was more significant than detention time in reducing the FS densities, yet the inactivation of FS was lower than FC at the same operating conditions. The data in Figures 4-17 and 4-18 indicate that the FS densities consistently decreased at 7 and 15 days, but increased at 30 and 45 days.

Two ANOVA tests were performed for the statistical analysis of the average log FS densities. All three factors were evaluated in each test. However, the number of factor levels in each test was different. The hydraulic detention time was evaluated at 3 levels (7, 15 and 30 days), temperature was examined at two levels (25°C and 35°C), and organic loading was tested at 3 levels (0.90, 1.80 and 2.70 $kg/m^3.d$) in the first analysis. This test was a 3-2-3 level-combination. The second test included all the four levels for detention time, two levels for temperature, and two levels for organic loading (i.e., 0.90 and 1.80 $kg/m^3.d$). This design was a 4-2-2 level-combination.

The results of ANOVA for the 3-2-3 and 4-2-2 designs are summarized in Tables 4-25 and 4-26, respectively. The data in these tables include F test and P-values for all the main effects and interactions. The plots for main effects and interactions for the 3-2-3 and 4-2-2 tests are included in Figures 4-19 and 4-20, respectively.

The results of ANOVA for the 3-2-3 test (Table 4-25) show that only the main effect of temperature and the interaction between time and organic loading were statistically significant at the 95% confidence level.

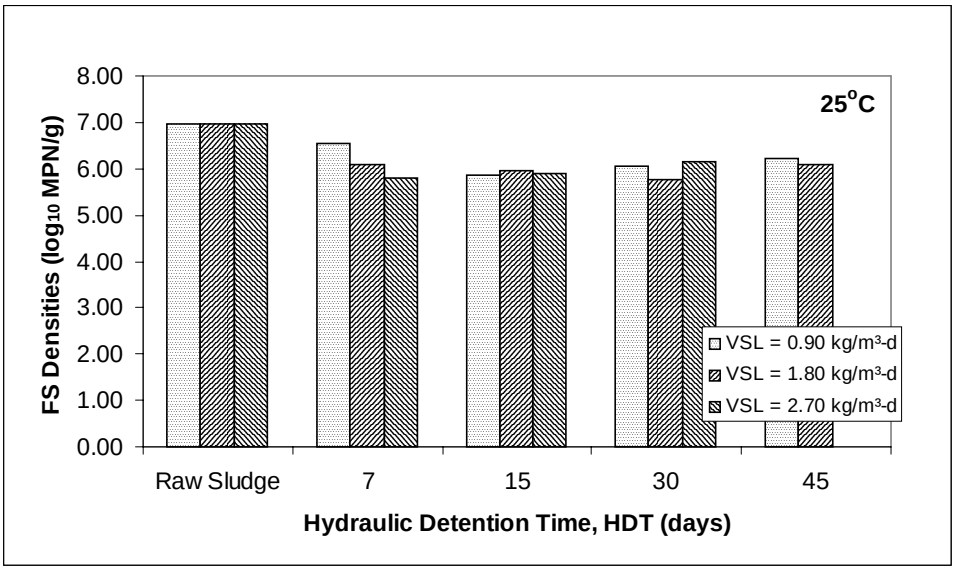

Figure 4-17: Densities of Fecal Streptococci in Anaerobic Digestion at 25°C

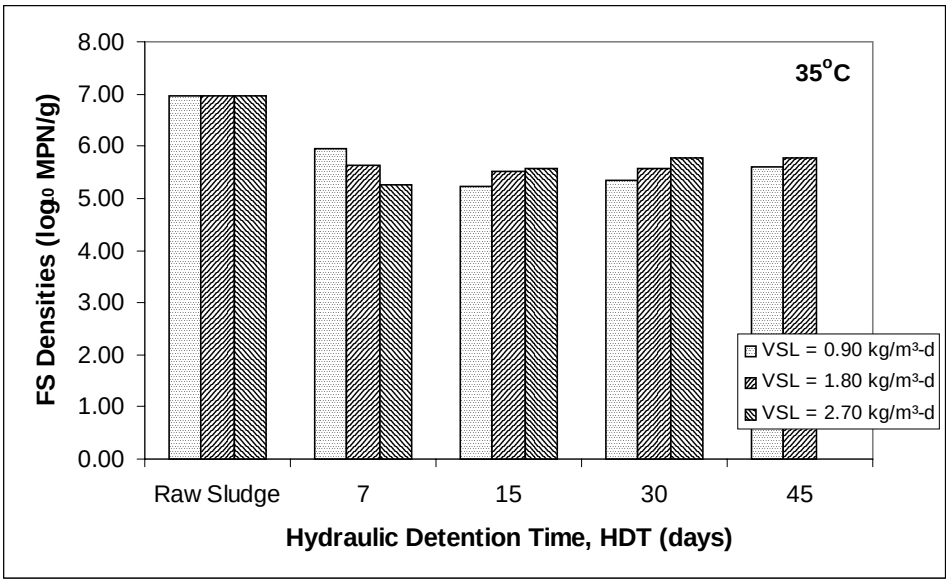

Figure 4-18: Densities of Fecal Streptococci in Anaerobic Digestion at 35°C

Table 4-25: Results of ANOVA for Fecal Streptococci Densities (3-2-3 factor-level test)

Factor	Type	Levels	Values	Units
Time	fixed	3	7 15 30	(days)
Temp	fixed	2	25 35	(C)
Load	fixed	3	0.9 1.8 2.7	(kgVS/m3-d)

Analysis of Variance for FS, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Time	2	0.37429	0.37429	0.18715	2.14	0.132
Temp	1	3.11520	3.11520	3.11520	35.65	0.000
Load	2	0.07185	0.07185	0.03592	0.41	0.666
Time*Temp	2	0.02891	0.02891	0.01446	0.17	0.848
Time*Load	4	1.96127	1.96127	0.49032	5.61	0.001
Temp*Load	2	0.19949	0.19949	0.09975	1.14	0.331
Time*Temp*Load	4	0.09301	0.09301	0.02325	0.27	0.898
Error	36	3.14553	3.14553	0.08738		
Total	53	8.98956				

Table 4-26: Results of ANOVA for Fecal Streptococci Densities (4-2-2 factor-level test)

Factor	Type	Levels	Values	Units
Time	fixed	4	7 15 30 45	(days)
Temp	fixed	2	25 35	(C)
Load	fixed	2	0.9 1.8	()

Analysis of Variance for FS, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Time	3	1.35734	1.35734	0.45245	5.28	0.004
Temp	1	2.96013	2.96013	2.96013	34.58	0.000
Load	1	0.02803	0.02803	0.02803	0.33	0.571
Time*Temp	3	0.01635	0.01635	0.00545	0.06	0.979
Time*Load	3	0.53898	0.53898	0.17966	2.10	0.120
Temp*Load	1	0.24367	0.24367	0.24367	2.85	0.101
Time*Temp*Load	3	0.06428	0.06428	0.02143	0.25	0.861
Error	32	2.73960	2.73960	0.08561		
Total	47	7.94839				

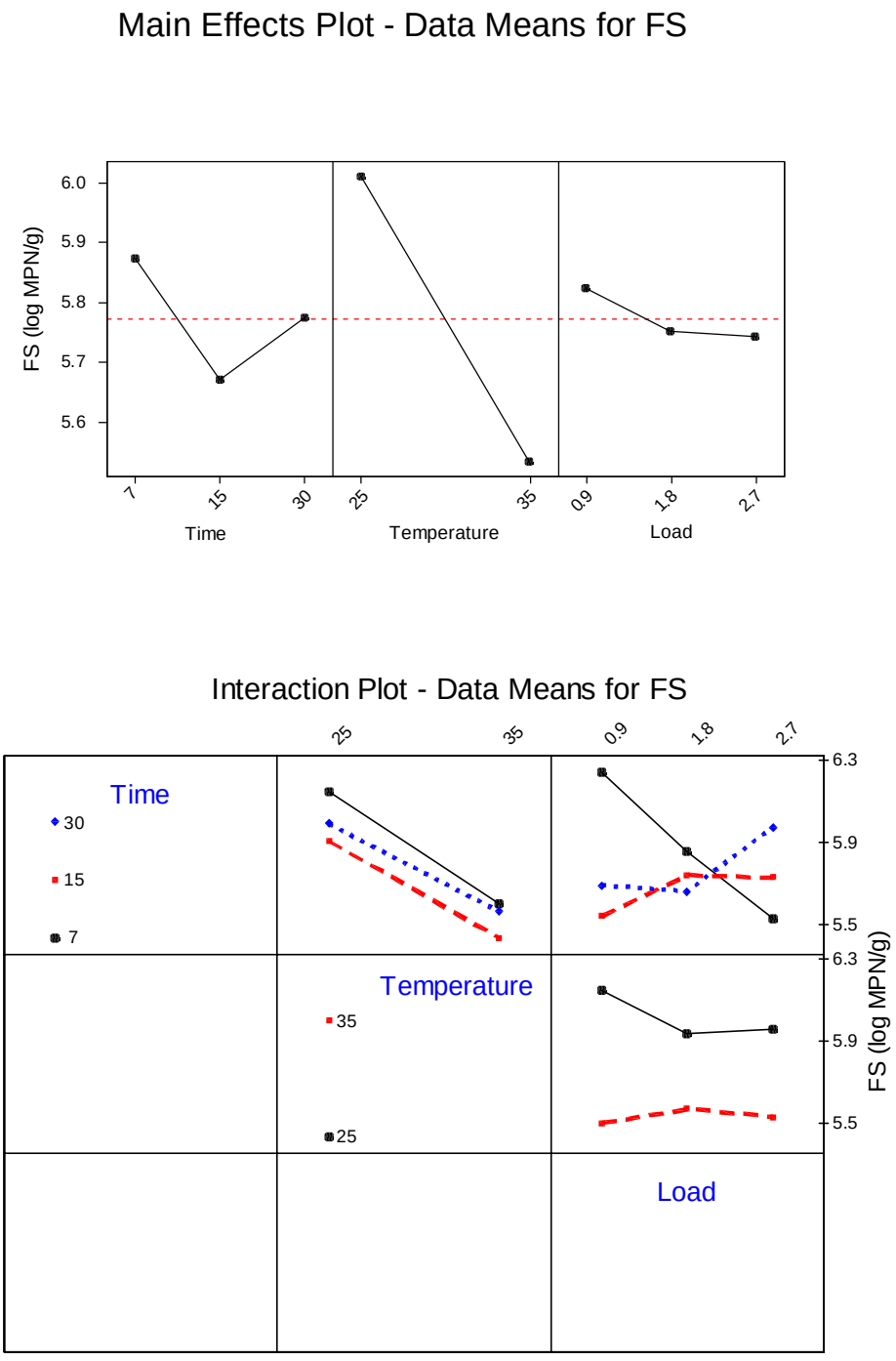

Figure 4-19: Effects and Interaction Plots for Fecal Streptococci (3-2-3 test)

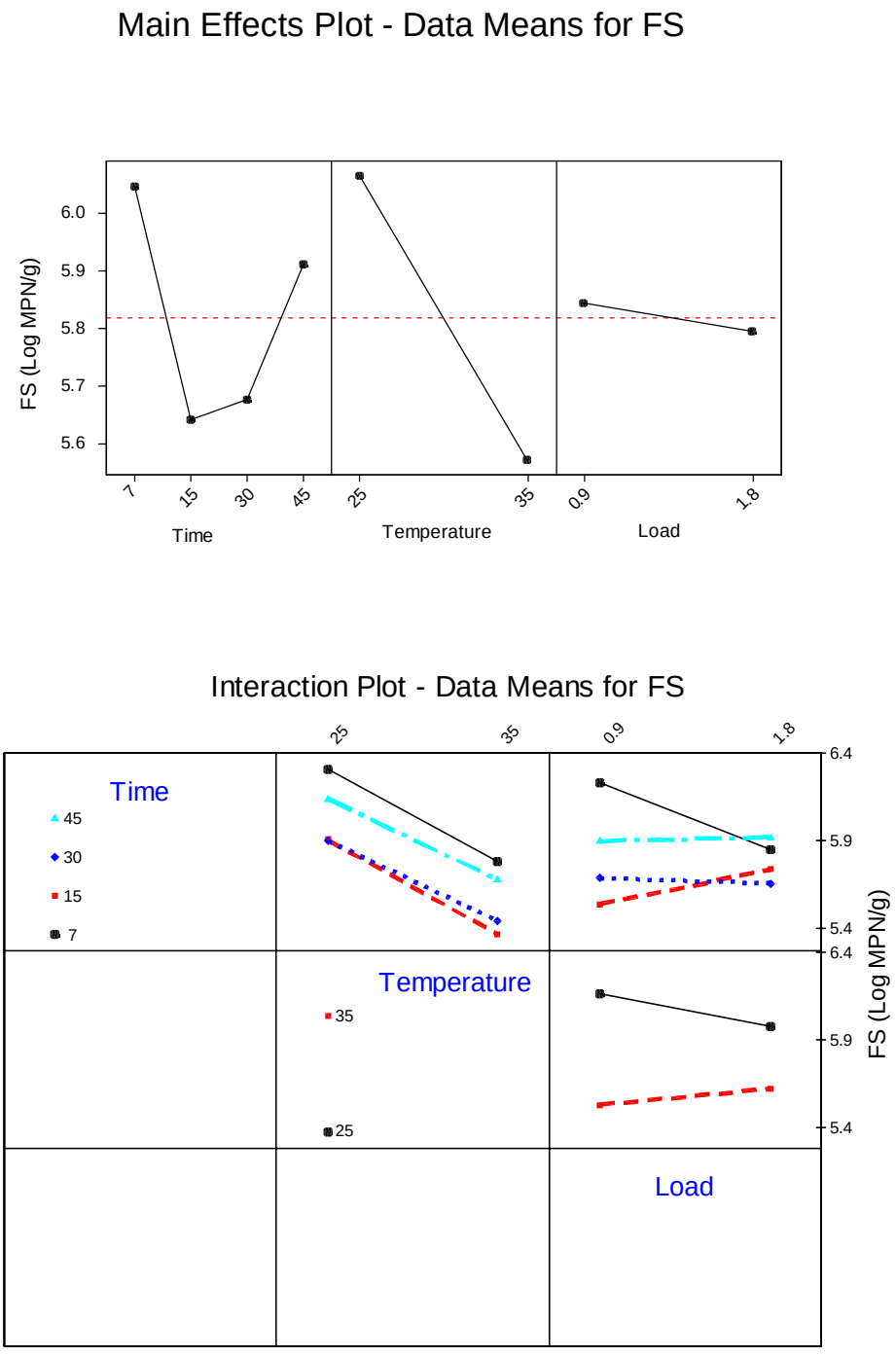

Figure 4-20: Effects and Interaction Plots for Fecal Streptococci (4-2-2 test)

The P-values show that temperature was more significant than the interaction between time and organic loading.

The plot for main effects for the 3-2-3 test (Figure 4-19) shows that the effect of temperature on the FS densities was greater than the effect of detention time and organic loading. The densities of FS decreased at a high rate when temperature varied from 25°C to 35°C regardless of hydraulic detention time and organic loading. The crossing of plots in Figure 4-19 indicates that time and organic loading have an interactive effect on the concentration of FS, e.g., the FS densities decreased consistently at 7 days as the organic loading increased from 0.90 to 1.80 kgVS/m^3-d and from 1.80 to 2.70 kgVS/m^3-d. However, at 15 and 30 days, increasing the organic loading from the lowest to the intermediate loading and from the intermediate to the highest loading caused a slight increase in the FS densities.

The results of ANOVA for the 4-2-2 combination are presented in Table 4-26. The P-values indicate that the main effects of detention time and temperature were significant. However, no interaction between factors was statistically significant at the 95% confidence level. The effect of temperature was more significant than detention time. The main effects plot (Figure 4-20) shows that the effect of temperature on the FS densities was greater than detention time and much greater than organic loading. The nearly horizontal line for the organic loading indicates a nonsignificant main effect for this factor. Increasing the detention time from 7 to 15 days caused a rapid decrease in the FS densities; however, the FS increased as the detention time increased from 15 to 30 days, and from 30 to 45 days. In general, only the main effect of temperature was statistically significant in both tests.

Effects on Time, Temperature, and Organic Loading on the Reduction of Salmonella.

The pathogenic bacteria of major concern in beneficial reuse of biosolids are *Salmonella*. The EPA Part 503 regulations have established a limit of 3 MPN per 4 grams of total solids (dry weight basis) for a stabilized sludge to be considered as a Class A biosolids to protect the public health and the environment. Anaerobically digesting sludge has been classified as Class B biosolids. However, evaluation of the fate and survival of *Salmonella* at different operating conditions of detention time, temperature, and organic loading would provide valuable insights into the limits of the anaerobic digestion in reducing this pathogenic bacteria.

The actual and transformed densities of *Salmonella* observed in this study are presented in Tables 4-27 and 4-28, respectively. The effects of hydraulic detention time and organic loading on the log reduction of *Salmonella* at 25°C and 35°C are illustrated in Figures 4-21 and 4-22, respectively. The densities of *Salmonella* during anaerobic digestion at 25°C and 35°C at all operating conditions are presented in Figures 4-23 and 4-24, respectively.

The data presented in Figures 4-21 and 4-22 indicate that the effects of time, temperature, and organic loading on *Salmonella* were more similar to the effects on FC than to FS. Therefore, using FC as an indicator of the presence of *Salmonella* is reasonable. The reduction of *Salmonella* densities was higher at 35°C than at 25°C at both temperatures and at the intermediate and highest organic loadings. The log reduction of *Salmonella* increased rapidly at 7 days and decreased slightly at 15 days. The log reduction of *Salmonella* increased at a lower rate at 30 and 45 days. This pattern was more evident for the bar graphs at 35°C than at 25°C.

Table 4-27: Raw Densities of *Salmonella* in Anaerobic Digestion

	25 Celsius			35 Celsius		
	0.9 kg VS/m^3-d	1.8 kg VS/m^3-d	2.7 kg VS/m^3-d	0.9 kg VS/m^3-d	1.8 kg VS/m^3-d	2.7 kg VS/m^3-d
7 days	12.86	1.20	11.10	11.67	<1.33(BDL)	1.82
	15.70	5.63	4.82	6.70	<1.33 (BDL)	0.83
	20.00	7.33	2.69	<4 (BDL)	<1.43(BDL)	1.74
15 days	4.00	4.40	2.00	1.00	0.63	0.48
	2.22	3.00	1.63	1.11	1.33	<0.48
	2.22	3.80	2.00	2.35	0.71	<0.48(BDL)
30 days	1.18	2.20	0.26		0.78	<0.28
	2.65	0.83	0.27	<0.61 BDL	<0.42(BDL)	0.29
	8.82	2.30	0.27	1.21	0.41	<0.29 (BDL)
				2.12		
45 days	1.10	<0.32(BDL)	ND	0.51	<0.33 (BDL)	ND
	3.14	1.80	ND	<0.54(BDL)	0.34	ND
	0.63	0.66	ND	<0.56 (BDL)	0.66	ND

Densities in MPN/g TS
ND: Not determined

Table 4-28: Transformed Densities of *Salmonella* in Anaerobic Digestion

	25 Celsius			35 Celsius		
	0.9 kg VS/m^3-d	1.8 kg VS/m^3-d	2.7 kg VS/m^3-d	0.9 kg VS/m^3-d	1.8 kg VS/m^3-d	2.7 kg VS/m^3-d
7 days	1.11	0.08	1.05	1.07	BDL	0.26
	1.20	0.75	0.68	0.83	BDL	-0.08
	1.30	0.87	0.43		BDL	0.24
15 days	0.60	0.64	0.30	0.00	-0.20	-0.32
	0.35	0.48	0.21	0.05	0.12	
	0.35	0.58	0.30	0.37	-0.15	
30 days	0.07	0.34	-0.59	0.08	-0.11	
	0.42	-0.08	-0.57	0.33		-0.54
	0.95	0.36	-0.57		-0.39	
45 days	0.04	0.26	ND	-0.29	-0.47	ND
	0.50	-0.18	ND		-0.18	ND
	-0.20		ND			ND

Densities in log MPN/g TS
ND: Not determined; BDL= Below detection limits

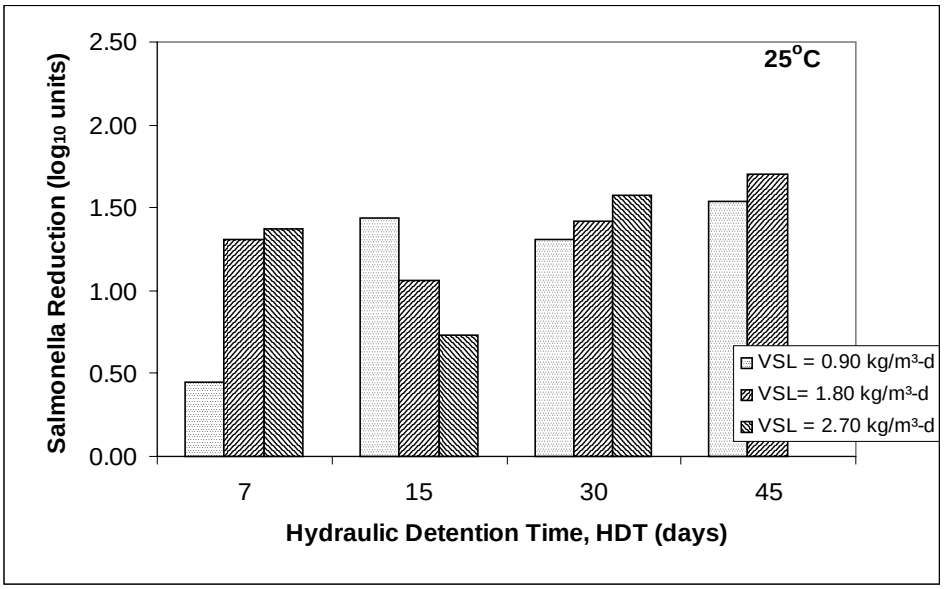

Figure 4-21: Effects of Hydraulic Detention Time and Organic Loading on Log-Reduction of *Salmonella* at 25°C.

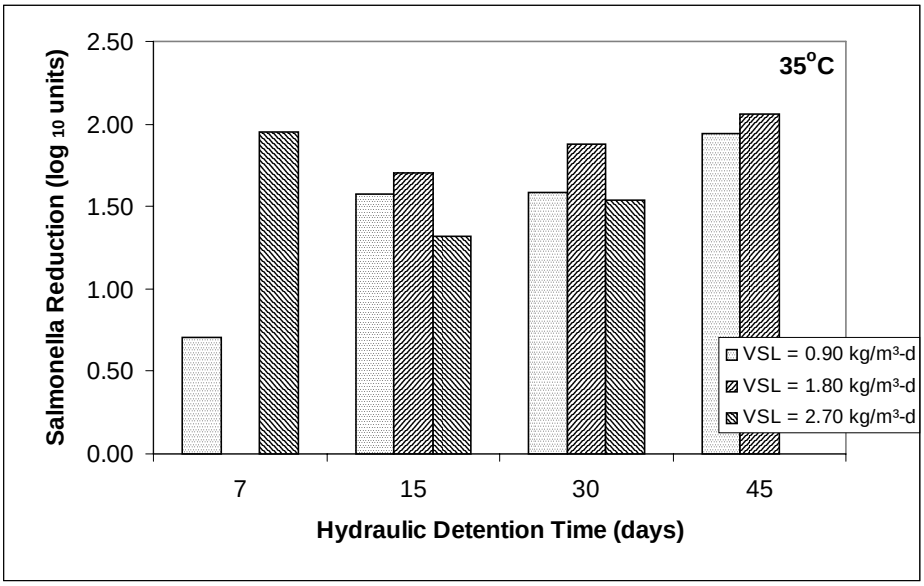

Figure 4-22: Effects of Hydraulic Detention Time and Organic Loading on Log-Reduction of *Salmonella* at 35°C.

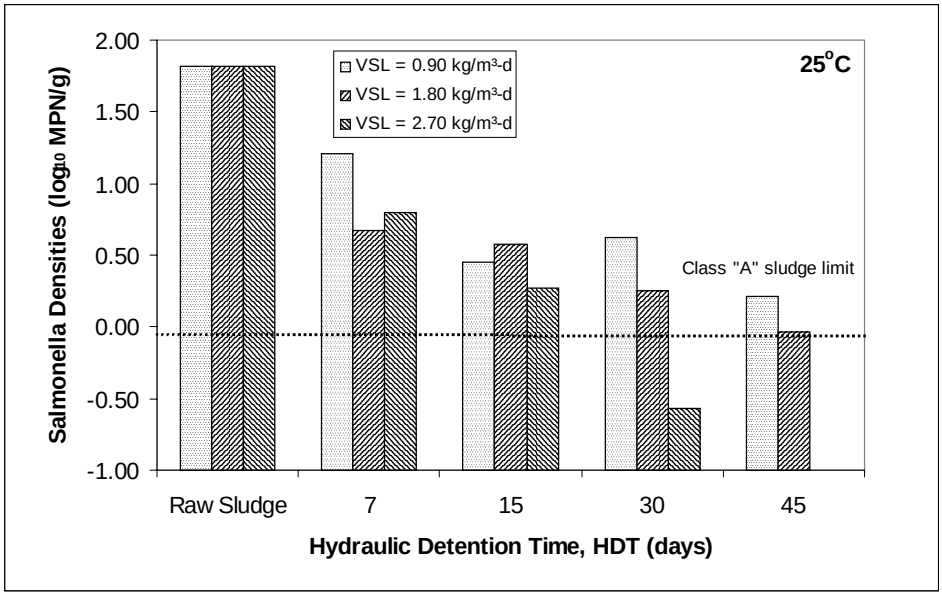

Figure 4-23: Densities of *Salmonella* in Anaerobic Digestion at 25 °C.

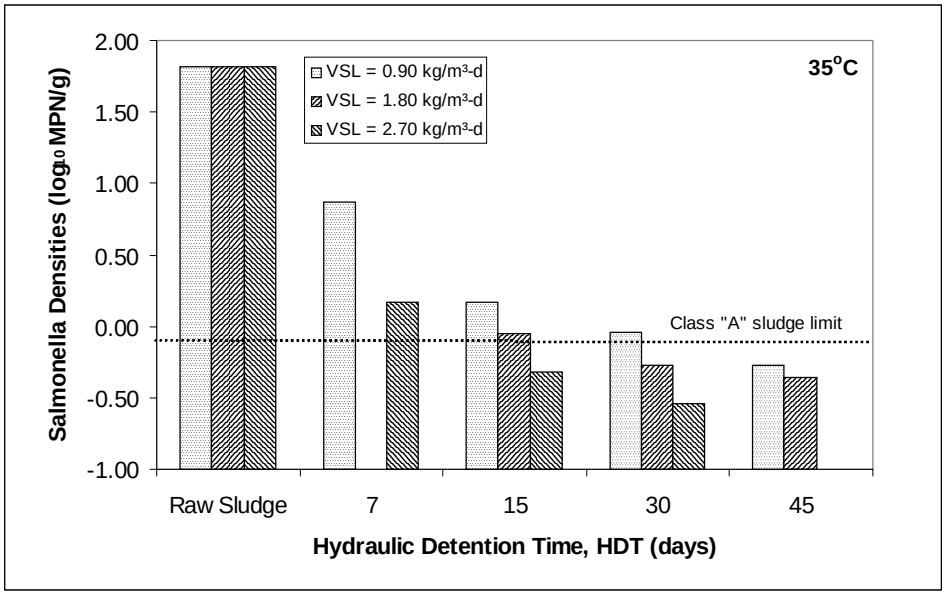

Figure 4-24: Densities of *Salmonella* in Anaerobic Digestion at 35 °C.

The maximum reduction in *Salmonella* densities occurred at 45 days, at both temperatures, and at the intermediate loading (1.80 kgVS/m^3-d), e.g., the maximum reduction was 1.70-logs at 25°C, while at 35°C the maximum reduction was 2.06-logs. The data in Figures 4-23 and 4-24 show that the densities of *Salmonella* decreased consistently as the detention time increased at all of the organic loadings, e.g., the *Salmonella* densities decreased rapidly at the shortest detention time (7 days), and declined at a lower rate at 15, 30, and 45 days. Interestingly, the trend of the data in Figure 4-24 shows that, at 35°C, the higher the organic loading the lower the concentration of *Salmonella*, at all detention times. This effect may be explained by the fact that at higher organic loadings, more antagonistic organisms may exist in the digester resulting in lower *Salmonella* densities.

The minimum *Salmonella* densities occurred at a detention time of 30 days at both temperatures and at the highest organic loading (2.70 kgVS/m^3-d). The minimum *Salmonella* densities were below the limit established by the Part 503 regulations for Class "A" biosolids (i.e., *Salmonell*a < 3 MPN/4g TS or –0.12 log MPN/g).

The densities of *Salmonella* in the feed sludge were low compared to concentrations reported in the literature (Ponugoti et al., 1997) yet the data compare favorably with the densities published by other authors who used the same analytical protocol (Tata et al, 1997). Nevertheless, when other analytical techniques were utilized, *Salmonell*a densities were much higher than the concentrations obtained in this study. A reliable and more accurate technique for the analysis of *Salmonella* in biosolids is still lacking. Therefore, the results of this study for *Salmonella* should be used with caution.

An ANOVA test was performed to the log densities of *Salmonella* for a 3-2-3 level-combination. The results of ANOVA are presented in Table 4-29. The F values and P-values indicate that the main effects of all three factors were statistically significant at the 95% confidence level. The interactions between time and temperature and between time and organic loading were also significant. From these two, the interaction between time and organic loading was more significant than the interaction between time and temperature.

Table 4-29: Results of ANOVA for *Salmonella* Densities (3-2-3 factor-level test)

Factor	Type	Levels	Values	Units
Time	fixed	3	7 15 30	(days)
Temp	fixed	2	25 35	(C)
Load	fixed	3	0.9 1.8 2.7	(kgVS/m3-d)

Analysis of Variance for Salmonel, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Time	2	255.171	255.171	127.586	29.62	0.000
Temp	1	117.189	117.189	117.189	27.21	0.000
Load	2	158.094	158.094	79.047	18.35	0.000
Time*Temp	2	48.374	48.374	24.187	5.62	0.008
Time*Load	4	148.578	148.578	37.145	8.62	0.000
Temp*Load	2	13.175	13.175	6.588	1.53	0.230
Time*Temp*Load	4	18.827	18.827	4.707	1.09	0.375
Error	36	155.064	155.064	4.307		
Total	53	914.473				

The plots for main effects and interactions are presented in Figure 4-25. The main effects plot show that increasing the temperature, detention time, and organic loading caused a decrease in the *Salmonella* densities, e.g., the effect of detention time on *Salmonella* was greater from 7 to 15 days than from 15 to 30 days. Similarly, the effect of organic loading was greater from 0.90 to 1.80 kgVS/m^3-d than from 1.80 to 2.70 kgVS/m^3-d.

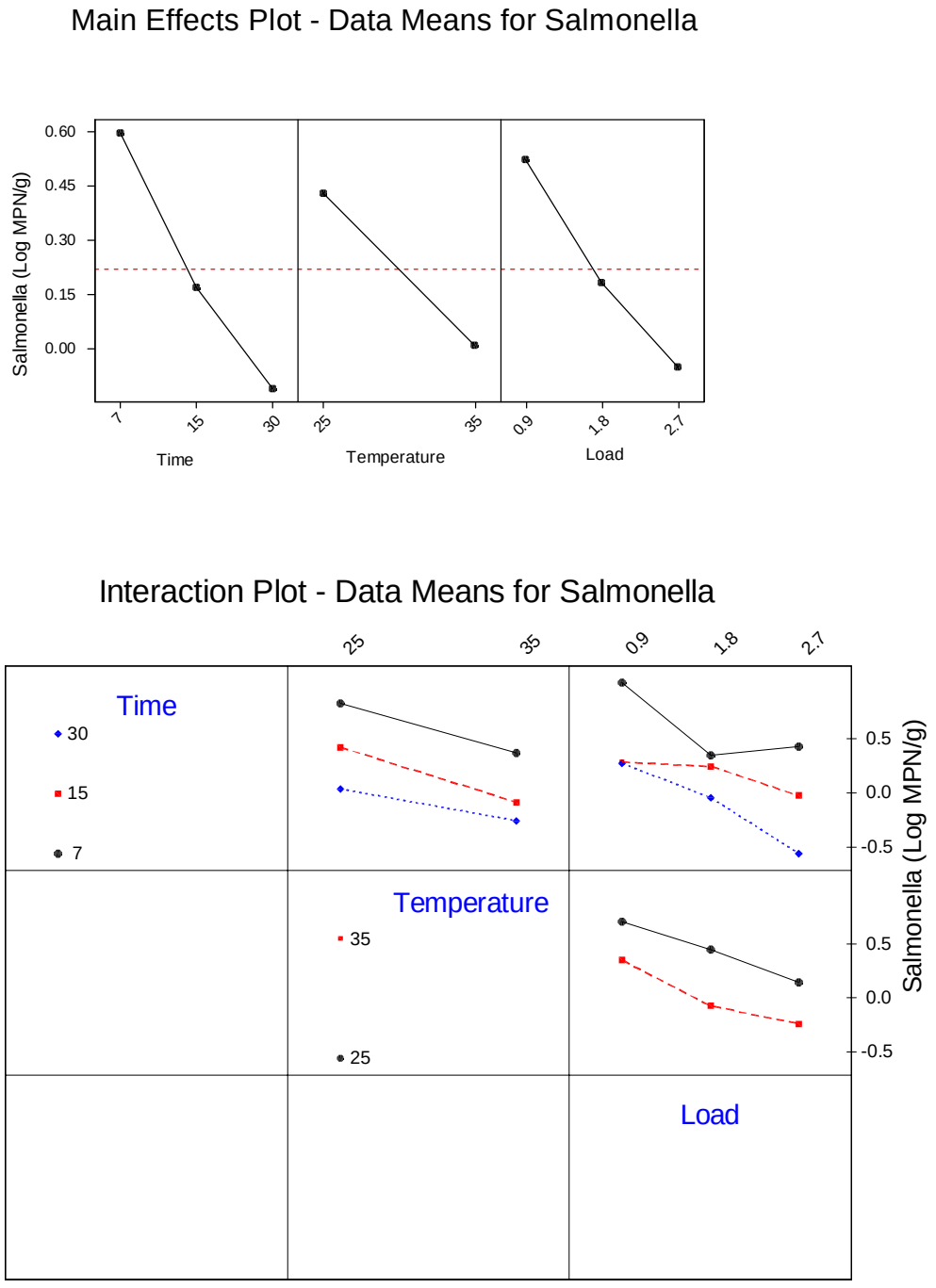

Figure 4-25: Effects and Interaction Plots for *Salmonella* (3-2-3 test)

The plot for interaction between time and temperature shows that the effect of increasing the temperature was greater at 7 days than it was at 15 days and 30 days. This pattern is similar to the observed for fecal coliforms. The plot for interaction between time and organic loading indicates that increasing the organic loading from 0.90 to 1.80 kgVS/m^3-d caused a higher decrease of *Salmonella* at 7 days than at 15 and 30 days. However, increasing the organic loading from 1.80 to 2.70 kgVS/m^3-d caused a slight increase in the *Salmonella* counts at 7 days but a higher decrease in the bacterial densities at 15 and 30 days.

4.3 SUMMARY OF ANAEROBIC DIGESTION RESULTS

The results of volatile solids and bacterial density analyses in the field-scale and lab-scale anaerobic digesters are summarized in Table 4-30. Since the full-scale anaerobic digesters were operated at extreme conditions of detention time (> 75 days) and organic loading (0.74 kg/m^3-d), direct comparison with the results for the laboratory digesters is not possible. However, the results of VS, FC, FS, and *Salmonella* for the full-scale digesters may be compared with the results from the lab-scale digesters that were operated at a detention time of 45 days and an organic loading of 0.90 kg/m^3-d. The results of destruction of volatile solids and reduction of indicator and pathogenic bacteria at full-scale and lab-scale anaerobic digesters, at heated (35°C) and unheated (~25°C) conditions are discussed below.

Volatile Solids Reduction

The destruction of the mass of volatile solids in full-scale unheated (24.2°C) anaerobic digesters at 75 days of HDT and organic loading of 0.74 kg/m^3-d was 51.5%, compared to 50.5% in lab-scale digesters at 45 days (HDT) and loading of 0.90 kg/m^3-d. These data compare favorably and are in agreement with those reported in the literature for unheated digesters that were operated at long detention times and low rate organic loadings (WEF, 1995). The VS fraction remaining in the digested sludge at full-scale and lab-scale conditions were 52.7 and 59.5% respectively.

Table 4-30: Results of Anaerobic Digesters at Full-Scale and Laboratory Conditions

Parameter	Unheated		Heated	
	Full-Scale Digester	**Laboratory Digester**	**Full-Scale Digester**	**Laboratory Digester**
Date	(Oct 1996 to Mar 1998)	(Mar 1998 to Oct 1999)	(May 2000 to Jun 2000)	(Mar 1998 to Oct 1999)
Temperature (^{O}C)	24.2	25	35	35
Detention Time (days)	75	45	50	45
VS Loading (kg/m^3-d)	0.74	0.90	0.74	0.90
VS Fraction (%)	52.7	59.1	55.7	56
VS Mass Reduction (%)	51.1	50.5		51
Fecal Coliforms				
Density (MPN/g TS)	1.29 x 10^4	9.33 x 10^4	1.39 x 10^4	7.59 x 10^4
EPA Required "Class B" biosolids Density (MPN/g TS)	2x10^6	2x10^6	2x10^6	2x10^6
Log reduction	3.0	2.43	2.6	2.5
Reported log Reduction	2	2	2	2
Fecal Streptococci				
Density (MPN/g TS)	1.03 x 10^5	1.62 x 10^6	7.07 x 10^4	3.98 x 10^5
Log reduction	1.6	1.16	1.2	1.77
Salmonella				
Density (MPN/4g TS)	4.1	6.4	ND	2.0
EPA Required "Class A" biosolids Density (MPN/4g TS)	3	3	3	3
Log reduction	0.9	1.55	ND	1.95

ND = not determined

Fecal Coliforms

The FC reduction in full-scale unheated anaerobic digesters was 3.0 logs, whereas at lab-scale conditions the FC inactivation was 2.43 logs. The data in Figure 4-9 indicate that, at lab-scale conditions and an organic loading of 0.90 kg/m^3-d, the log reduction of FC increased as the hydraulic detention time increased up to 45 days.

Therefore, it is expected that at 75 days, the FC reduction at lab-scale conditions approximate the reduction observed at full-scale conditions. The FC densities in the digested sludge at full-scale and lab-scale conditions were $1.29x10^4$ and $9.33x10^4$ MPN/g TS, respectively. These densities are below the limit established by the EPA Part 503 for Class B biosolids of $2x10^6$ MPN/g TS. The results for heated (35°C) anaerobic digesters indicate a FC reduction of 2.6 and 2.5 logs at full-scale (50 days and 0.74 kg/m^3-d) and lab-scale conditions (45 days and 0.90 kg/m3-d), respectively. These reductions are above the 2.0 logs reduction reported in the literature for anaerobic digesters by several authors (Lee et al, 1989; U.S. EPA, 1993; Stukenberg et al, 1994). The FC densities in the heated digesting sludge at full-scale and lab-scale conditions were $1.39x10^4$ and $7.59x10^4$ MPN/g, respectively. These values are below the limit established by the EPA for Class B biosolids. Therefore, the FC densities and FC inactivation observed in the lab-scale anaerobic digesters were similar to those observed in full-scale anaerobic digesters.

Fecal Streptococci

The FS reduction in unheated full-scale digesters at 75 days and 0.74 kg/m^3-d was 1.6 logs, whereas at lab-scale conditions at 45 days and 0.90 kg/m^3-d the FS reduction was 1.16 logs. These log reduction data are in good agreement and compare favorably with those reported by Williams (1991). The data in Figure 4-15 indicate that, at lab-scale conditions and the lowest organic loading (0.90 kg/m^3-d), the log-reduction of FS increased as the detention time increase from 30 to 45 days; therefore, it is expected that, at longer detention times (i.e., 75 days), the FS reduction in the lab digesters would approximate the value observed at the full-scale digesters. At 25°C, the FS densities in the digested sludge at full-scale and lab-scale conditions were

$1.03x10^5$ and $1.62x10^6$ MPN/g TS, respectively. The FS reduction for full-scale and lab-scale heated digesters were 1.2 logs and 1.77 logs, respectively. These data compare favorably with those reported by Berg and Berman (1980) and Ponugoti (1997). The geometric means of FS densities for full-scale and lab-scale heated (35°C) anaerobic digesters were $7.07x10^4$ and $3.98x10^5$ MPN/g TS, respectively.

Salmonella

In unheated (24.2°C) full-scale anaerobic digestion, the *Salmonella* reduction was 0.9 logs, whereas under lab-scale conditions the reduction was 1.55 logs. The geometric means of *Salmonella* densities at full-scale and lab-scale conditions were 4.1 and 6.4 MPN/4g TS, respectively. The EPA Part 503 *Salmonella* requirement for Class "A" biosolids is less than 3 MPN per 4 grams of TS (<0.75 MPN/g TS). Therefore, the densities of *Salmonella* in unheated full-scale digesters (24.2°C) and laboratory digesters (25°C) failed to meet the requirements for Class A biosolids. However, at 35°C and lab-scale conditions, the *Salmonella* density (2.0 MPN/4 g TS) was below the limit established by the EPA for Class A biosolids.

Chapter 5: Conclusions and Engineering Significance

The primary objective of this research was the development of a better understanding of the capabilities of several conventional sludge treatment processes including mesophilic anaerobic digestion, in reducing the densities of indicator and pathogenic organisms. This research was divided into two phases: Phase I: field-scale and Phase II: lab-scale studies.

5.1 CONCLUSIONS

5.1.1 Phase I – Field-scale Studies

The main objective of the field-scale studies was the evaluation of the effectiveness of belt thickening, anaerobic digestion, air-drying, and composting processes in the reduction of fecal coliforms, fecal streptococci, *Salmonella* and helminth ova in a biosolids management facility.

The conclusions that were generated from this phase of the research are the following:

- Observed reductions after anaerobic digestion followed by air-drying and composting in the fecal coliform, fecal streptococci, and S*almonella* densities were 6.1, 4.6, and 2.4 orders of magnitude (log_{10} units), respectively.
- Fecal streptococci were more resistant to inactivation than fecal coliforms during processing of the raw sludge by thickening, anaerobic digestion, air-drying, and windrow composting. Therefore, fecal streptococci may be a better indicator for monitoring pathogen survival during conventional sludge treatment processes.

The comparison of the removal efficiencies of indicator and pathogenic organisms in each sludge treatment process with the EPA Part 503 standards for the use and disposal of municipal sludge generated the following conclusions.

- Anaerobically digested sludge satisfied the EPA Part 503 requirements for Class B biosolids; however, the Class A biosolids requirements for fecal coliforms, *Salmonella*, and helminth ova densities in the digested sludge were exceeded.
- Air-dried biosolids in the majority of samples analyzed, satisfied the Class A biosolids criteria for bacteria and viable helminth ova. Air-drying of anaerobically digested sludge may be a potential alternative low-technology process for producing Class A biosolids.
- Aerobically composted sludge satisfied the Class A biosolids requirements for bacteria, and viable helminth ova.

5.1.2 Phase II – Lab-scale Studies

The objective of Phase II was an evaluation of the effects of temperature, hydraulic detention time, and volatile solids loading on the performance of mesophilic anaerobic digestion measured in terms of the densities of FC, FS, and *Salmonella*. The following conclusions are supported by the results of the lab-scale studies:

- The results of the ANOVA test for the 3-2-3 factor-level combination indicated that the individual effects of time, temperature, and organic loading on the FC densities were statistically significant at the 95% confidence level; however, the effects of time and temperature were more significant than organic loading. This results imply that increasing either the detention time from 7 to 15 to 30 days or the

temperature from 25°C to 35°C resulted in a decrease in the FC densities.

- The results of the 3-2-3 test indicated that the two-factor interactions on the FC densities between time and temperature and between time and organic loading were statistically significant at the 95% confidence level. The interaction between time and temperature had a larger F value than the interaction between time and organic loading. These results indicate that at detention times of 7, 15 and 30 days, increasing the temperature from 25°C to 35°C resulted in a decrease in the FC densities; however, the increase in temperature becomes less important as the detention time increases. The effect of increasing the organic loading was not conclusive. Increasing the organic loading from 0.9 to 1.80 kg/m^3-d at 15 and 30 days caused an increase in the FC densities; but increasing the organic loading from 1.80 to 2.70 kg/m^3-d, caused a decrease in the FC densities at the same detention times.
- The results of the ANOVA test for the 4-2-2 factor-level combination indicated that the two-factor interactions between time and organic loading and between time and temperature on the FC densities were statistically significant at the 95% confidence level. These results indicate that at detention times of 7,15, 30 and 45 days, increasing the temperature from 25°C to 35°C caused a decrease in the FC densities. However, increasing the organic loading from 0.90 to 1.80 kg/m^3-d caused an increase in the FC densities at 15 and 30 days, but a minimum decrease at 7 and 45 days.

- The results of the ANOVA test for the 3-2-3 factor-level combination indicated that only the effect of temperature on the FS densities was statistically significant at the 95% confidence level. The effect of the interaction between time and organic loading also was significant, but to a lesser degree than the effect of temperature alone.
- Only the individual effects of time and temperature on the FS densities were statistically significant at the 95% confidence level for the 4-2-2 test. However, temperature had a larger F value than that of detention time. These results indicate that increasing the temperature from 25°C to 35°C caused a greater decrease in the FS densities than the decrease caused by increasing the detention time from 15 through 45 days. Therefore, temperature is the principal mechanism that causes reduction of FS during mesophilic anaerobic digestion.
- The results of the 3-2-3 test indicated that the individual effects of detention time, temperature, and organic loading, and the two-factor interactions between time and temperature and between time and organic loading on the *Salmonella* densities in anaerobically digested sludge were all statistically significant at the 95% confidence level The statistical significance of the separate effects indicates that increasing either the detention time from 7 to 15 to 30 days, the temperature from 25 to 35°C, or the organic loading from 0.9 to 1.8 to 2.7 kg/m^3-d consistently resulted in a decrease in the *Salmonella* densities.
- The interaction between time and organic loading had a larger F value than the interaction between time and temperature on the *Salmonella*

densities. These results imply that the reduction in *Salmonella* as a result of the combined effect of time and organic loading was greater than the *Salmonella* reduction caused by the interaction between time and temperature. The statistical significance of the three factors and their two-factor interactions on *Salmonella* were similar to those observed for FC. However, assaying for *Salmonella* is essential to protecting public health.

The results of the lab-scale studies were compared with the EPA Part 503 regulations to generate the following conclusions:

- The fecal coliform density in the anaerobically digested sludge was below the limit established by the Part 503 requirements for Class B biosolids at detention times of 7, 15, 30, and 45 days, temperatures of 25 and 35°C, and organic loadings of 0.90, 1.80 and 2.70 kgVS/m^3-d.
- The destruction in volatile solids at 35°C during anaerobic digestion and all the organic loadings met the minimum 38 percent established by the Part 503 vector attraction reduction requirements (VAR) except at seven days of detention time and loadings of 1.80 and 2.70 kgVS/m^3-d.
- Minimum detention times for anaerobic digestion of 30 days at 25°C and of 15 days at 35°C are required to satisfy the pathogen and vector attraction reduction requirements for Class B biosolids in terms of FC densities and destruction of volatile solids.

- Anaerobic digestion at 35°C in lab-scale digesters at detention times of 30 and 45 days and organic loadings of 1.80 and 2.70 kgVS/m^3-d produced digested sludge that contained densities of *Salmonella* that were below the Part 503 Class A biosolids criteria.

5.2 ENGINEERING SIGNIFICANCE

The effects of conventional sludge treatment systems, including operating variables in mesophilic anaerobic digestion in reducing the densities of indicator and pathogenic organisms in wastewater sludge were investigated in this study. The results can be useful for operators, design engineers researchers, and regulatory officials to better understand the limits of various sludge treatment processes, individually or as part of a processing train, for producing biosolids that meet the requirements of the EPA Part 503 regulations and to protect the public health and the environment.

These biosolids treatment data provide guidance for designers and operators in optimizing anaerobic digestion for stabilizing volatile solids and disinfecting municipal sludge. Research has demonstrated that FC densities during mesophilic anaerobic digestion will be below the limit established by the EPA for Class B biosolids (FC < $2x10^6$ MPN/g TS) at detention times of 7, 15, 30, and 45 days at 25°C and 35°C. The minimum detention times at which the FC and VS reduction met the requirements for a Class B biosolids were at 25°C and detention time of 30 days (FC < $7x10^4$ MPN/g TS), and at 35°C and detention time of 15 days (FC < $4x10^4$ MPN/g TS). Currently, the Part 503 regulations for Processes to Significantly Reduce Pathogens (i.e., anaerobic digestion) require detention times between 15 days at 35°C and 60 days at 20°C to achieve Class B biosolids in terms of FC. The results observed in full-scale and lab-scale mesophilic anaerobic digestion clearly demonstrate that anaerobic digestion can reduce fecal coliform densities to well below those required for Class B biosolids (FC < $2x10^6$ MPN/g TS).

Appendix A: Processes to Further Reduce Pathogens

1. **Composting**
   Using either the within-vessel composting method or the static aerated pile composting method, the temperature of the biosolids is maintained at 55°C or higher for 3 days.
   Using the windrow composting method, the temperature of the biosolids is maintained at 55°C or higher for 15 days or longer. During the period when the compost is maintained at 55°C or higher, the windrow is turned a minimum of five times.

2. **Heat Drying**
   Biosolids are dried by direct or indirect contact with hot gases to reduce the moisture content of the biosolids to 10 percent or lower. Either the temperature of the biosolids particles exceeds 80°C or the wet bulb temperature of the gas in contact with the biosolids as the biosolids leave the dryer exceeds 80°C.

3. **Heat Treatment**
   Liquid biosolids are heated to a temperature of 180°C or higher for 30 minutes

4. **Thermophilic Aerobic Digestion**
   Liquid biosolids are agitated with air or oxygen to maintain aerobic conditions, and the mean cell residence time of the biosolids is 10 days at 55° to 60°C.

5. **Beta Ray Irradiation**
   Biosolids are irradiated with beta rays from an accelerator at dosages of at least 1.0 megrad at room temperature (i.e., 20°C).

6. **Gamma Ray Irradiation**
   Biosolids are irradiated with gamma rays from certain isotopes, such as Cobalt 60 and Cesium 137, at room temperature (i.e., 20°C).

7. **Pasteurization**
   The temperature of the biosolids is maintained at 70°C or higher for 30 minutes or longer.

U.S. EPA (1994) A Plain English Guide to the EPA Part 503 Biosolids Rule. EPA/832/R93/003

Appendix B: Processes to Significantly Reduce Pathogens

1. **Aerobic Digestion**
   Biosolids are agitated with air or oxygen to maintain aerobic conditions for a specific mean cell residence time at a specific temperature. Values for the mean cell residence time and temperature shall be between 40 days at 20°C and 60 days at 15°C.

2. **Air drying**
   Biosolids are dried on sand or on paved or unpaved basins. The biosolids dry for a minimum of 3 months. During 2 of the 3 months, the ambient average daily temperature is above 0°C.

3. **Anaerobic Digestion**
   Biosolids are treated in the absence of air for a specific mean cell residence time at a specific temperature. Values for the mean cell residence time and temperature shall be between 15 days at 35°C to 55°C and 60 days at 20°C.

4. **Composting**
   Using either the within-vessel, static aerated pile, or windrow-composting methods, the temperature of the biosolids is raised to 40°C or higher and maintained for 5 days. For 4 hours during the 5-day period, the temperature in the compost pile exceeds 55°C.

5. **Lime Stabilization**
   Sufficient lime is added to the biosolids to raise the pH of the biosolids to 12 after 2 hours of contact.

U.S.EPA (1994). A Plain English Guide to the EPA Part 503 Biosolids Rule. EPA/832/R93/003.

Appendix C: Vector Attraction Reduction Requirements

Option 1:	Meet 38% percent reduction in volatile solids content.
Option 2:	Demonstrate vector attraction reduction with additional anaerobic digestion in a bench-scale unit.
Option 3:	Demonstrate vector attraction reduction with additional aerobic digestion in a bench-scale unit.
Option 4:	Meet a specific oxygen uptake rate for aerobically digested biosolids.
Option 5:	Use aerobic processes at greater than 40°C for 14 days or longer
Option 6:	Alkali addition under specified conditions.
Option 7:	Dry biosolids with no unstabilized solids to at least 75 percent solids
Option 8:	Dry biosolids with unstabilized solids to at least 90 percent solids
Option 9:	Inject biosolids beneath the soil surface
Option 10:	Incorporate biosolids into the soil within 6 hours of application to or placement on land.
Option 11:	Cover biosolids placed on a surface disposal site with soil or other material at the end of each operating day. (Note: Only for surface disposal).
Option 12:	Alkaline treatment of domestic septage to pH 12 or above for 30 minutes without adding more alkaline material.

From U.S.EPA (1994) A Plain English Guide to the EPA Part 503 Biosolids Rule. EPA/832/R93/003.

Appendix D: Analytical Protocol for Helminth Ova

1. Introduction

This procedure has been adapted from Yanko's Analytical Method for Viable Helminth Ova (EPA, 1992). This technique identifies, quantifies and determines the presence of total and viable helminth ova (i.e., *Ascaris, Toxocara* and *Trichuris*) from wastewater sludge and sludge products. Solid samples are processed by blending with buffered water containing a surfactant (i.e., Tween-80). The blend is screened to remove large particles. The solids in the screened portion are allowed to settle out and the supernatant decanted off. The sediment is subjected to density gradient centrifugation using zinc sulfate (specific gravity 1.20). This flotation procedure yields a layer most likely to contain *Ascaris* and some other parasitic ova. After another centrifuging step, the sample is added 0.5% formalin and incubated at 26°C over three to four weeks for embryonation. The concentrate is then microscopically examined for parasite ova.

2. Sample Handling and Preservation.

- Solid samples are collected in either autoclaved screw cap bottles or sterile bags such as Whirl-Pak bags. Liquid sludge samples are collected in screw cap containers such as 1-L Nalgene bottles.
- Samples not analyzed promptly are stored at 0°C to 40°C.

3. Apparatus

- Standard light microscope
- 2-L Pyrex beakers
- 1-L Berzelius beakers.
- Table top centrifuge (A clinical centrifuge is optional for centrifuging 15 mL conical tubes)
- Rotor to hold eight 50 mL centrifuge tubes, preferably plastic.
- Rotor to hold eight 15 mL conical centrifuge tubes, preferably glass.

- 48 Tyler sieve.
- Large plastic funnel to support sieve.
- Test tube rack to accommodate 50 mL centrifuge tubes.
- Test tube rack to accommodate 15 mL conical centrifuge tubes.
- Number “0” rubber stoppers.
- Pasteur pipettes
- Incubator at 26°C
- Blender
- Vortex mixer

4. Reagents

- Phosphate-buffered water. Prepare stock phosphate buffer solution by dissolving 34.0 g potassium dihydrogen phosphate (KH_2PO_4) in 500 mL distilled water, adjusting to pH 7.2 ± 0.1 with 1 N NaOH.
- Add 1.25 mL stock phosphate buffer solution and 5.0 mL magnesium chloride solution (81.1 g $MgCl_2.6H_2O$/L distilled water) to 1 L distilled water.
- Prepare phosphate buffer working solution containing 0.1% (v/v) Tween 80. Adjust the pH to 7.2 ± 0.1 with 1 N NaOH.
- Tween 80.
- Zinc sulfate solution, sp. gr. 1.20. Weigh 454 g Zn SO_4 into 1 L deionized H_2O. Dissolve and check specific gravity with a hydrometer.
- Adjust specific gravity to 1.2 as necessary.
- 0.5% Formalin solution

5. Procedure

- Weigh 50 g (wet weight) of compost and blend at high speed for 1 min. with 450 mL phosphate buffered water (PBW) containing 0.1 percent of Tween 80 to achieve a ten percent suspension. If the sample is a liquid sludge (e.g., raw or digested sludge), pour it directly into a blender jar (300-400 mL) and add Tween-80 to 0.1percent v/v) prior to blending as above. Record volume tested.

- The % total solids of the sample is determined by the Standard Methods on a separate portion of the sample for use in the final calculation of ova/g dry weight.

- Pour the homogenized sample through a 48 mesh Tyler sieve held on a large funnel over a 2 L beaker.

- Wash the sample through the sieve with several rinses of warm tap water. Washings are caught in the beaker.

- Allow the screened and washed sample to settle overnight.

- Siphon off the supernatant to just above the settled layer of solids.

- Mix the settled material by swirling and then pour it into four 50 mL centrifuge tubes.

- Rinse the beaker two to three times and pour the rinsing into four 50 mL centrifuge tubes.

- Balance the tubes and centrifuge at 1250 RPM (400 x G) for 3 min.

- Pour off the supernatant and resuspend the pellet thoroughly in zinc sulfate solution, specific gravity 1.20.

- Centrifuge the zinc sulfate suspension at 1250 RPM for 3 min.

- Pour off the zinc sulfate supernatant into a 1-L Berzelius beaker, dilute to at least half the concentration with deionized water, cover and allow to settle 3 h or overnight.

- Aspirate the supernatant to just above the settled material.
- Resuspend the sediment by swirling and pipette into two to four 15 mL conical centrifuge tubes.
- Rinse the beaker two to three times with deionized water and pipette the rinse water into the tubes.
- Centrifuge the tubes at 1400 RPM (480 x G) for 3 min.
- Combine the pellets into one tube and centrifuge at 1400 RPM for 3 min.
- Resuspend the sediment with 4-5 mL of 0.5% formalin using a Vortex mixer. Add 0.5% formalin to fill the 15 mL conical tube.
- Centrifuge the tube at 1800 RPM for 3 min.
- Resuspend the pellet in 4 mL of 0.5% formalin and pour into a culture petri dish.
- Incubate the sample at 26°C for three to four weeks.
- Examine concentrates microscopically to enumerate detected ova
- Note viability based on the presence of embryonated ova whose larval forms can be induced to move when the light intensity is increased.
- Identify the ova and report as ova/g dry weight.

6. References

- U.S. EPA (1992). Analytical Method for Viable Helminth Ova. In Environmental Regulations and Technology: Control of Pathogens and Vector Attraction in Sewage Sludge. EPA/625/R-92/013.
- Berk, S. G., and Gunderson, J. H. (1993) Wastewater Organisms: A Color Atlas. Lewis Publishers
- Standards Methods for the Examination of Water and Wastewater (1995). 18th Edition, APHA, AWWA, WPCF, Washington, D.C.

Appendix E: Precision Criteria for Indicator Bacteria

Total Coliforms

Sample No	Duplicate Analyses		Logarithms of Counts		Range of Logarithms, (R_{log})
	D_1	D_2	L_1	L_2	$(L_1 - L_2)$
1	11400	18200	4.0569	4.2601	0.2032
2	38600	29500	4.5866	4.4698	0.1168
3	29500	5230	4.4698	3.7185	0.7513
4	1818	1818	3.2596	3.2596	0
5	182	182	2.2601	2.2601	0
6	295	91	2.4698	1.9590	0.5108
7	182	182	2.2601	2.2601	0

Calculations:

- $\sum$ of R_{log} = 1.5821
- $\bar{R} = \sum$ of R_{log}/No. samples = 1.5821/7 = 0.2260
- Precision Criterion = 3.27 $\bar{R}$ = 0.7390

Fecal Coliforms

Sample No	Duplicate Analyses		Logarithms of Counts		Range of Logarithms, (R_{log})
	D_1	D_2	L_1	L_2	$(L_1 - L_2)$
1	455	909	2.6580	2.9586	0.3006
2	5,320	6,820	3.7259	3.8338	0.1079
3	7,730	1,820	3.8882	3.2601	0.6281
4	909	909	2.9586	2.9586	0
5	46	91	1.6628	1.9590	0.2962
6	182	91	2.2601	1.9590	0.3011
7	182	91	2.2601	1.9590	0.3011

Appendix E (Continued)

Calculations:

- $\sum$ of R_{log} = 1.935
- $\bar{R}$ = $\sum$ of R_{log}/No. samples = 1.935/7 = 0.2764
- Precision Criterion = 3.27 $\bar{R}$ = 0.9038

FECAL STREPTOCOCCI

Sample No	Duplicate Analyses		Logarithms of Counts		Range of Logarithms, (R_{log})
	D_1	D_2	L_1	L_2	($L_1 - L_2$)
1	18200	11400	4.2601	4.0569	0.2032
2	36400	20500	4.5611	4.3118	0.2493
3	5454	6818	3.7367	3.8337	0.097
4	6818	6818	3.8337	3.8337	0
5	11364	11364	4.0555	4.0555	0
6	6818	11364	3.8337	4.0555	0.2218
7	6820	6820	3.8338	3.8338	0

Calculations:

- $\sum$ of R_{log} = 0.7713
- $\bar{R}$ = $\sum$ of R_{log}/No. samples = 0.7713/7 = 0.1102
- Precision Criterion = 3.27 $\bar{R}$ = 0.3603

Appendix F: Results of Bacterial Indicators Analyses at Field Conditions.

	10/10/96	10/16/96	10/22/96	10/29/96	11/5/96	11/12/96	11/19/96	11/26/96	12/3/96
TOTAL COLIFORMS (MPN/g)									
RAW SLUDGE	9.40E+08	9.40E+07	5.00E+08	5.60E+08	8.80E+07	7.50E+08	1.80E+08	2.50E+08	6.92E+08
GRAVITY BELT THICKENER	2.10E+08	4.80E+06	2.30E+08	1.30E+08	2.80E+07	1.90E+08	1.60E+08	1.90E+08	1.62E+08
ANAEROBIC DIGESTER	6.00E+03	1.35E+04	**3.83E+04**		722	3.09E+03	3.09E+03	618	4.40E+04
AIR DRYING BASINS	>=3.96E+0	383	2.32E+03	1.45E+03	6.28E+03	2.08E+03	898	172	375
COMPOSTING	217	<0.4 (BDL)	0.3	2	0.9	5	463	270	>=270
FECAL COLIFORMS (MPN/g)									
RAW SLUDGE	2.90E+08	2.80E+07	6.10E+07	5.00E+06	1.90E+07	1.20E+08	6.50E+07	2.00E+08	1.62E+07
GRAVITY BELT THICKENER	2.90E+06	3.80E+06	3.20E+07	2.40E+06	6.30E+06	5.90E+07	1.70E+07	1.50E+07	1.11E+07
ANAEROBIC DIGESTER	1.40E+03	5.20E+03	**667**		472	909	2.36E+03	491	2.80E+04
AIR DRYING BASINS	421	38	11	319	667	71	143	115	7
COMPOSTING	217	<0.4	< 0.3	<0.3	0.3	<0.3	370	80	59
FECAL STREPTOCOCCI, MPN/g									
RAW SLUDGE	2.06E+06	1.70E+07	8.90E+07	1.40E+07	8.10E+06	4.20E+07	5.30E+07	6.40E+07	1.62E+06
GRAVITY BELT THICKENER	4.60E+05	6.80E+06	4.30E+06	7.00E+06	1.10E+07	3.50E+06	3.00E+06	2.60E+06	1.31E+06
ANAEROBIC DIGESTER	3.20E+05	3.30E+04	**1.30E+04**		3.00E+04	1.60E+05	4.40E+04	5.50E+04	3.20E+04
AIR DRYING BASINS	[3]396	1.30E+03	1.11E+03	1.45E+03	941	3.73E+03	980	2.60E+03	667
COMPOSTING	[3]217	13	37	<0.3	408	12	46	[3]2.55E+03	1.52E+03

Appendix F (Continued)

	12/10/96	12/16/96	1/10/97	1/22/97	2/5/97	2/17/97	3/3/97	3/18/97
TOTAL COLIFORMS (MPN/g)								
RAW SLUDGE	3.85E+08	8.46E+08	2.31E+08	1.31E+08	3.57E+08	1.31E+08	1.29E+08	1.00E+09
GRAVITY BELT THICKENER	8.57E+07	1.90E+08	4.43E+07	2.40E+07	1.40E+08	>=2.86E+08	1.22E+08	2.54E+07
ANAEROBIC DIGESTER	2.71E+03	324	**725**	>=3.56E+03	>=4.71E+03	2.20E+05	<3	3.64E+04
AIR DRYING BASINS	1.35E+03	230	829	448	724	95	100	175
COMPOSTING	>=255	4.98E+03	228	2.73E+03	287	5	>=266	250
FECAL COLIFORMS (MPN/g)								
RAW SLUDGE	3.85E+07	3.85E+07	3.85E+07	3.85E+07	1.93E+07	1.31E+07	2.94E+07	8.75E+07
GRAVITY BELT THICKENER	7.62E+06	1.47E+07	1.39E+07	9.86E+06	2.19E+07	2.86E+08	1.89E+07	7.63E+07
ANAEROBIC DIGESTER	1.67E+03	2.80E+01	**435**	[3]3.56E+03	[3]4.71E+03	2.20E+05	<3	3.64E+04
AIR DRYING BASINS	49	14	54	35	14	<1.0	2	<1.0
COMPOSTING	[3]255	182	3	<3	<3	14	<0.3	<0.3
FECAL STREPTOCOCCI (MPN/g)								
RAW SLUDGE	2.54E+06	5.38E+06	2.54E+06	8.46E+06	1.57E+06	2.31E+07	1.00E+07	3.13E+07
GRAVITY BELT THICKENER	2.86E+06	4.31E+06	1.39E+07	1.83E+06	5.31E+05	2.14E+05	6.76E+06	1.36E+07
ANAEROBIC DIGESTER	1.04E+05	3.24E+02	**1.30E+04**	[3]3.56E+03	[3]4.71E+03	3.90E+04	364	[3]3.64E+03
AIR DRYING BASINS	7.20E+03	1.61E+02	683	2.49E+03	136	216	6.92E+03	1.12E+03
COMPOSTING	[3]2.55E+03	4.98E+03	[3]2.61E+03	3.86E+03	7	1	150	250

Appendix F (Continued)

	3/31/97	4/15/97	4/28/97	5/12/97	5/27/97	6/9/97	6/16/97	7/8/97
TOTAL COLIFORMS (MPN/g)								
RAW SLUDGE	2.67E+08	5.00E+08	2.00E+08	8.67E+07	3.33E+08	1.30E+08	1.67E+08	1.22E+08
GRAVITY BELT THICKENER	6.02E+07	2.54E+08	8.06E+07	7.14E+07	2.11E+08	1.50E+08	3.45E+07	1.07E+08
ANAEROBIC DIGESTER	4.07E+03	>=2.62E+03	>=2.76E+04	6.15E+05	1.22E+06	1.25E+06	**4.80E+04**	3.50E+05
AIR DRYING BASINS	17	233	865	957	293	297	337	407
COMPOSTING	1.42E+03	>=264	>=2.93E+04	1.71E+03	3.19E+02	1.83E+03	1.28E+02	5.20E+01
FECAL COLIFORMS (MPN/g)								
RAW SLUDGE	1.44E+08	2.78E+07	6.67E+07	2.00E+07	2.56E+07	1.04E+08	2.78E+07	4.44E+07
GRAVITY BELT THICKENER	2.05E+07	2.22E+07	2.74E+07	1.00E+07	1.05E+07	4.67E+07	5.80E+06	1.55E+07
ANAEROBIC DIGESTER	4.07E+03	>=2.62E+03	2.76E+04	6.15E+05	4.15E+05	2.00E+05	**2.60E+04**	3.56E+05
AIR DRYING BASINS	1	126	865	181	2	26	270	24
COMPOSTING	787	>=264	4.39E+03	9.49E+02	3.19E+02	1.02E+03	1.28E+02	6.00E+00
FECAL STREPTOCOCCI (MPN/g)								
RAW SLUDGE	2.44E+07	1.67E+07	2.50E+07	1.47E+07	3.00E+06	2.17E+07	1.50E+06	7.22E+06
GRAVITY BELT THICKENER	6.02E+06	3.81E+06	8.06E+06	4.29E+06	4.47E+05	5.00E+06	3.95E+05	5.95E+06
ANAEROBIC DIGESTER	>=2.71E+03	>=2.62E+03	>=2.76E+04	6.15E+05	7.32E+05	2.25E+06	**4.80E+05**	6.67E+05
AIR DRYING BASINS	3.70E+03	>=1.55E+03	>=1.54E+03	>=1.70E+04	>=1.95E+03	1.29E+04	8.99E+03	4.07E+03
COMPOSTING	>=252	>=264	>=2.93E+03	3.04E+03	1.69E+03	1.02E+03	4.04E+02	5.26E+02

Appendix F (Continued)

	7/21/97	8/5/97	8/25/97	9/9/97	9/29/97	10/15/97	10/28/97	11/21/97
TOTAL COLIFORMS (MPN/g)								
RAW SLUDGE	8.42E+08	4.50E+08	2.08E+08	4.55E+08	1.60E+08	3.33E+08	8.89E+07	6.96E+07
GRAVITY BELT THICKENER	3.57E+07	1.74E+08	3.29E+07	1.18E+08	4.41E+06	2.16E+07	3.90E+07	2.11E+08
ANAEROBIC DIGESTER	>=3.02E+05	6.00E+04	4.71E+04	5.77E+03	6.38E+03	1.91E+04	1.06E+04	**1.76E+04**
AIR DRYING BASINS	338	3.38E+03	329	8.51E+03	95	138	238	650
COMPOSTING	1.20E+00	0.4	415	9	8	164	1	873
FECAL COLIFORMS (MPN/g)								
RAW SLUDGE	3.68E+07	4.50E+07	9.17E+06	2.73E+07	3.00E+07	1.60E+07	2.78E+07	7.39E+06
GRAVITY BELT THICKENER	9.52E+06	3.04E+06	3.01E+06	1.18E+07	1.90E+06	1.22E+07	3.64E+06	2.11E+07
ANAEROBIC DIGESTER	3.21E+04	6.00E+04	4.70E+04	3.27E+03	4.68E+03	1.91E+04	1.06E+04	**5.49E+03**
AIR DRYING BASINS	56	3.38E+03	5	2.66E+03	45	<0.9 (BDL)	14	89
COMPOSTING	1.00E+00	0.4	277	4	8	1.4	<0.3(BDL)	279
FECAL STREPTOCOCCI (MPN/g)								
RAW SLUDGE	6.84E+06	1.10E+07	9.17E+06	2.73E+07	3.00E+07	1.47E+07	1.67E+07	5.65E+06
GRAVITY BELT THICKENER	9.52E+05	3.26E+06	2.33E+06	2.11E+07	7.35E+05	6.76E+06	3.90E+06	1.18E+07
ANAEROBIC DIGESTER	1.24E+06	1.40E+05	2.55E+05	4.62E+04	1.91E+05	1.91E+05	1.06E+06	**3.14E+05**
AIR DRYING BASINS	1.24E+04	>=1.08E+04	1.97E+05	1.60E+03	168	3.69E+03	1.43E+04	203
COMPOSTING	1.40E+01	4	225	>=282	126	2.92E+03	<0.3(BDL)	2.79E+03

Appendix F (Continued)

	11/28/97	12/8/97	12/15/97	1/14/98	1/27/98	2/17/98	2/28/98	3/24/98
TOTAL COLIFORMS (MPN/g)								
RAW SLUDGE	2.94E+08	>=8.42E+08	2.31E+08	9.41E+08	2.50E+08	>=5.52E+08	1.00E+09	1.83E+08
GRAVITY BELT THICKENER	1.50E+08	2.05E+08	2.62E+08	1.29E+08	7.35E+07	5.00E+07	1.15E+08	2.32E+08
ANAEROBIC DIGESTER	3.64E+05	1.14E+06	1.19E+05	2.09E+06	7.00E+05	7.32E+05	7.50E+05	9.60E+05
AIR DRYING BASINS	40	119	210	>=6.3E+03	544	>=2.5E+03	2.29E+04	568
COMPOSTING	2.46E+03	2.58E+03	112	>=2.55E+03	512	2.78E+03	>=2.81E+03	239
FECAL COLIFORMS (MPN/g)								
RAW SLUDGE	6.47E+06	1.26E+08	3.85E+07	7.65E+06	2.00E+07	**2.41E+07**	**8.13E+07**	1.17E+07
GRAVITY BELT THICKENER	3.67E+06	1.41E+07	4.92E+07	7.14E+06	2.50E+07	1.82E+07	2.18E+07	7.25E+06
ANAEROBIC DIGESTER	1.14E+05	2.04E+05	1.19E+05	3.02E+05	2.75E+05	1.95E+05	8.75E+04	6.40E+05
AIR DRYING BASINS	2	4	14	6.30E+03	7	>=2.5E+03	1.48E+04	45
COMPOSTING	46	1.45E+03	2	2.55E+03	154	1.56E+03	>=2.81E+03	147
FECAL STREPTOCOCCI , MPN/g								
RAW SLUDGE	4.12E+06	1.58E+08	1.85E+07	1.29E+07	1.42E+07	5.87E+07	1.50E+07	2.50E+07
GRAVITY BELT THICKENER	1.50E+07	2.82E+06	4.92E+07	7.14E+06	7.35E+06	5.45E+07	2.05E+07	4.35E+06
ANAEROBIC DIGESTER	5.45E+05	1.14E+06	1.19E+05	2.09E+06	2.25E+06	2.20E+06	4.00E+06	3.60E+06
AIR DRYING BASINS	969	1.38E+03	6.72E+03	197	>=6.70E+03	>=2.5E+04	8.20E+05	1.93E+05
COMPOSTING	2.46E+03	1.45E+04	545	>=2.55E+04	2.90E+03	5.21E+03	>=2.81E+04	1.47E+03

Bold numbers indicate average of duplicates

Appendix G: Results of *Salmonella* and Helminth Ova Analyses at Field Conditions.

	3/31/97	4/15/97	4/28/97	5/12/97	5/27/97	6/9/97	6/16/97	7/8/97
SALMONELLA (MPN/g)								
RAW SLUDGE	7	2	28	60	19	7.4	7	9.4
ANAEROBIC DIGESTER	<0.3 (BDL)	0.3	**1.6**	2	3.4	1.8	**0.8**	0.9
AIR DRYING BASINS	<0.1 (BDL)	<0.2 (BDL)	<0.2 (BDL)	0.2	<0.2 (BDL)	<0.2 (BDL)	<0.2 (BDL)	<0.2 (BDL)
COMPOSTING	<0.03 (BDL)	<0.03 (BDL)	<0.04 (BDL)	<0.04 (BDL)	<0.04 (BDL)	0.04	<0.04 (BDL)	<0.04 (BDL)
TOTAL HELMINTH OVA (Ova/g)								
RAW SLUDGE	2.5		2.9	1	2.5	2.6	1.9	3.33
ANAEROBIC DIGESTER	1.1		**1.9**	2.2	1.73	5.3	**3.7**	4.36
AIR DRYING BEDS	1.3	3.9	1.2	4.5	3.96	2.23	2.9	3.86
COMPOSTING	0.12	0.45	0.2	0.19	0.23	0.36	0.2	0.53
VIABLE HELMINTH OVA (Ova/g)								
RAW SLUDGE	0.8		0.6	0.67	0.56	0.6	0.7	1.39
ANAEROBIC DIGESTER	0		**0.2**	0.72	0.58	1.9	**1.5**	2.54
AIR DRYING BEDS	0	0.24	0	0.75	0.61	0.25	0.6	0
COMPOSTING	0	0	0	0	0	0	0	0

Appendix G (Continued)

	7/21/97	8/5/97	8/25/97	9/9/97	9/29/97	10/15/97	10/28/97	11/21/97
SALMONELLA (MPN/g)								
RAW SLUDGE	8.9	2	14	24	9	6	5	3.9
ANAEROBIC DIGESTER	0.4	<0.4 (BDL)	1.6	<0.4(BDL)	<0.29(BDL)	0.4	<0.4(BDL)	**<0.4(BDL)**
AIR DRYING BASINS	<0.2 (BDL)	<0.1 (BDL	0.5	0.2	0.11	<0.09(BDL)	<0.1(BDL)	<0.08(BDL)
COMPOSTING	<0.03 (BDL)	<0.04 (BDL)	<0.03(BDL)	<0.04(BDL)	<0.03(BDL)	<0.04(BDL)	<0.03(BDL)	<0.03(BDL)
TOTAL HELMINTH OVA (Ova/g)								
RAW SLUDGE	1.32	3.75	3.33	2.72	7.5	2.67	3.05	1.55
ANAEROBIC DIGESTER	2.94	4.6	4.03	3.96	5.69	3.89	3.4	**3.44**
AIR DRYING BEDS	2.8	3.72	1.15	2.26	2.61	3.23	1.75	2.85
COMPOSTING	0.25	0.41	0.26	0.35	0.26	0.12	0.11	0.17
VIABLE HELMINTH OVA (Ova/g)								
RAW SLUDGE	1.05	1.75	1.25	0.46	3.53	1	0.27	0.09
ANAEROBIC DIGESTER	2.04	1.97	1.86	2.46	0.71	2.49	1.36	**1.88**
AIR DRYING BEDS	0.28	0.68	0	0	0.19	0	0	0.14
COMPOSTING	0	0	0	0	0	0	0	0

Appendix G (Continued)

	11/28/97	12/8/97	12/15/97	1/14/98	1/27/98	2/17/98	2/28/98	3/28/98
SALMONELLA (MPN/g)								
RAW SLUDGE	19	4.7	13	5.3	5	4.1	7.5	14.2
ANAEROBIC DIGESTER	0.45	0.91	**0.48**	<0.46(BDL)	1	1.7	2.25	1.6
AIR DRYING BASINS	0.09	<0.08(BDL)	<0.08(BDL)	0.08	<0.08(BDL)	<0.3(BDL)	<0.3(BDL)	<0.22 (BDL)
COMPOSTING	0.03	<0.03(BDL)	<0.03(BDL)	<0.03(BDL)	<0.03(BDL)	<0.03(BDL)	<0.03(BDL)	<0.04 (BDL)
TOTAL HELMINTH OVA Ova/g								
RAW SLUDGE	2.35	2.1	2.22	3.06	3.67	2.24	1.75	3.35
ANAEROBIC DIGESTER	4.56	5.99	**4.86**	3.9	5.64	4.02	3.25	5.06
AIR DRYING BEDS	2.79	1.98	3.36	2.1	1.39	3.65	4.92	6.44
COMPOSTING	0.2	0.32	0.16	0.21	0.17	0.17	0.41	0.31
VIABLE HELMINTH OVA, Ova/g								
RAW SLUDGE	1.18	0.52	0.68	0.94	0.33	0.34	0.25	2.1
ANAEROBIC DIGESTER	1.9	3.74	**1.46**	0.78	2.26	1.83	0.81	1.9
AIR DRYING BEDS	0	0.14	0	0	0.14	2.6	3.28	2.27
COMPOSTING	0	0	0	0	0	0	0	0

Bold numbers indicate average of duplicate

Appendix H: Operating Characteristics of Anaerobic Digesters at Lab-Scale Conditions

Appendix H (Operating Characteristics at Temperature = 25 °C, HDT = 7 days, and Organic Loading = 0.90 kg/m^3-d)

Date	HRT	Temp.	VS Load	TS in	VS in	VS in	TS out	VS out	VS out	VS red	pH	Alkalinity	Vol. Acids	Gas Prod.	Methane	Carbon
	days	Celsius	kg/ m^3-d	g/L	g/L	%	g/L	g/L	%	%		mg/L	mg/L	m^3/kg VS		Dioxide
												as $CaCO_3$	as acetic acid	destroyed-d	%	%
2/14/1999	7	26	0.91	8.5	6.4	74.8										
2/15/1999	7	23.9	0.91	8.5	6.4	74.8										
2/16/1999	7	24.1	0.91	8.5	6.4	74.8					6.3					
2/17/1999	7	24	0.91	8.5	6.4	74.8					6.2					
2/18/1999	7	24	0.91	8.5	6.4	74.8					6.2					
2/19/1999	7	24	0.91	8.5	6.4	74.8					6.1					
2/20/1999	7	24	0.91	8.5	6.4	74.8					6.1					
2/21/1999	7	27	0.91	8.5	6.4	74.8					6.1				50.4	49.6
2/22/1999	7	24.8	0.91	8.5	6.4	74.8					6.1					
2/23/1999	7	24	0.91	8.5	6.4	74.8					6.1					
2/24/1999	7		0.91	8.5	6.4	74.8	7.2	4.9	67.6	23.5	6.1	480	504	0.5	49.6	50.4
2/25/1999	7	23	0.87	8.1	6.1	75.0										
2/26/1999	7	25	0.87	8.1	6.1	75.0					6.1					
2/27/1999	7	26	0.87	8.1	6.1	75.0					6.1					
2/28/1999	7	23.5	0.87	8.1	6.1	75.0					6.2					
3/1/1999	7	24.8	0.87	8.1	6.1	75.0					6.2					
3/2/1999	7	25	0.87	8.1	6.1	75.0					6.2					
3/3/1999	7	24.6	0.87	8.1	6.1	75.0					6.3					
3/4/1999	7	24.7	0.87	8.1	6.1	75.0					6.3					
3/5/1999	7		0.87	8.1	6.1	75.0	6.8	4.6	67.7	24.3	6.3	550	384	0.6		
3/6/1999	7	25.6	0.87	8.1	6.1	75.0					6.2					
3/7/1999	7	25	0.87	8.1	6.1	75.0					6.5					
3/8/1999	7	25	0.89	8.8	6.2	71.2										
3/9/1999	7	25.2	0.89	8.8	6.2	71.2					6.3					
3/10/1999	7	26	0.89	8.8	6.2	71.2					6.4					
3/11/1999	7	25.2	0.89	8.8	6.2	71.2					6.4					
3/12/1999	7	25.4	0.89	8.8	6.2	71.2					6.4					
3/13/1999	7		0.89	8.8	6.2	71.2	6.7	4.3	64.5	30.9		800	420	0.4	44	56
3/14/1999	7	25	0.89	8.8	6.2	71.2					6.4					
3/15/1999	7	25	0.89	8.8	6.2	71.2					6.5					
AVG	**7**	**24.8**	**0.89**	**8.4**	**6.2**	**73.9**	**6.9**	**4.6**	**66.6**	**26.2**		**610**	**436**	**0.5**	**48**	**52**

Appendix H (Operating Characteristics at Temperature = 25 °C, HDT = 7 days, and Organic Loading = 1.80 kg/m^3-d)

Date	HRT	Temp.	VS Load	TS in	VS in	VS in	TS out	VS out	VS out	VS red	pH	Alkalinity	Vol. Acids	Gas Prod.	Methane	Carbon
	days	Celsius	kg m^3/-d	g/L	g/L	%	g/L	g/L	%	%		mg/L	mg/L	m^3/kg VS		Dioxide
												as $CaCO_3$	as acetic acid	destroyed-d	%	%
5/12/1999	7	25.0	1.7	17.3	12.0	69.2					6.6					
5/13/1999	7	28.0	1.7	17.3	12.0	69.2					6.6					
5/14/1999	7	26.0	1.7	17.3	12.0	69.2					6.6					
5/15/1999	7		1.7	17.3	12.0	69.2										
5/16/1999	7	23.0	1.7	17.3	12.0	69.2					6.6					
5/17/1999	7		1.7	17.3	12.0	69.2	14.4	9.5	66.0	20.7		1250		0.8		
5/18/1999	7	25.0	1.9	21.2	13.0	61.2					6.6					
5/19/1999	7	28.0	1.9	21.2	13.0	61.2										
5/20/1999	7		1.9	21.2	13.0	61.2										
5/21/1999	7	24.0	1.9	21.2	13.0	61.2					6.6					
5/22/1999	7		1.9	21.2	13.0	61.2					6.6					
5/23/1999	7	24.0	1.9	21.2	13.0	61.2					6.6					
5/24/1999	7	27.0	1.9	21.2	13.0	61.2					6.6					
5/25/1999	7		1.9	21.2	13.0	61.2	17.1	10.0	58.5	23.0		1170	468	0.9	62	38
5/26/1999	7		1.8	19.4	12.6	64.6										
5/27/1999	7		1.8	19.4	12.6	64.6					6.7					
5/28/1999	7		1.8	19.4	12.6	64.6					6.7					
5/29/1999	7	26.0	1.8	19.4	12.6	64.6					6.7					
5/30/1999	7	25.0	1.8	19.4	12.6	64.6					6.7					
5/31/1999	7	25.5	1.8	19.4	12.6	64.6					6.8					
6/1/1999	7		1.8	19.4	12.6	64.6	15.7	9.1	58.1	27.3		1150	444	0.9	58	42
6/2/1999	7	25.4	1.8	19.4	12.6	64.6					6.8					
6/3/1999	7	25.0	1.8	19.4	12.6	64.6					6.9					
6/4/1999	7	25.4	1.8	19.4	12.6	64.6					6.9					
6/5/1999	7	24.5	1.8	19.4	12.6	64.6					6.9					
6/6/1999	7	24.2	1.8	19.4	12.6	64.6					6.9					
6/7/1999	7		1.8	19.4	12.6	64.6	15.3	8.9	57.8	29.5	6.7	1510	360	0.8		
AVG		**25.4**	**1.8**	**19.5**	**12.6**	**64.6**	**15.6**	**9.4**	**60.1**	**25.1**		**1270**	**424**	**0.9**	**60**	**40**

Appendix H (Operating Characteristics at Temperature = 25 °C, HDT = 7 days, and Organic Loading = 2.70 kg/m^3-d)

Date	HRT	Temp.	VS Load	TS in	VS in	VS in	TS out	VS out	VS out	VS red	pH	Alkalinity	Vol. Acids	Gas Prod.	Methane	Carbon
	days	Celsius	kg m^3/-d	g/L	g/L	%	g/L	g/L	%	%		mg/L	mg/L	m^3/Kg VS		Dioxide
												as $CaCO_3$	as acetic acid	destroyed-d	%	%
7/17/1999	7	25.3	2.7	28.2	18.8	66.6										
7/18/1999	7		2.7	28.2	18.8	66.6										
7/19/1999	7	25	2.7	28.2	18.8	66.6					6.7					
7/20/1999	7	25.5	2.7	28.2	18.8	66.6					6.7					
7/21/1999	7	25	2.7	28.2	18.8	66.6					6.7					
7/22/1999	7		2.7	28.2	18.8	66.6	27.5	16.3	59.4	13.1	6.7	1360	300	1.8		
7/23/1999	7	25	2.7	28.2	18.8	66.6										
7/24/1999	7	25	2.7	28.2	18.8	66.6					6.7					
7/25/1999	7	27	2.7	28.2	18.8	66.6										
7/26/1999	7	25.4	2.7	28.2	18.8	66.6										
7/27/1999	7	25.5	2.7	28.2	18.8	66.6					6.7					
7/28/1999	7	26.5	2.7	28.2	18.8	66.6										
7/29/1999	7	24.5	2.7	28.2	18.8	66.6										
7/30/1999	7		2.7	28.2	18.8	66.6	26.5	15.8	59.7	15.7	6.7	1690	264	1.5	65	35
7/31/1999	7	24	2.7	30.3	19.0	62.8										
8/1/1999	7	28	2.7	30.3	19.0	62.8										
8/2/1999	7	25	2.7	30.3	19.0	62.8										
8/3/1999	7	25.2	2.7	30.3	19.0	62.8					6.7					
8/4/1999	7	25.1	2.7	30.3	19.0	62.8										
8/5/1999	7		2.7	30.3	19.0	62.8										
8/6/1999	7		2.7	30.3	19.0	62.8	26.4	15.1	57.3	20.7	6.7	1680	288	1.2	54.2	45.6
AVG	**7**	**25.4**	**2.7**	**28.9**	**18.9**	**65.3**	**26.8**	**15.7**	**58.8**	**16.5**		**1577**	**284**	**1.5**	**59.6**	**40.3**

Appendix H (Operating Characteristics at Temperature = 25 °C, HDT = 15days, and Organic Loading = 0.90 kg/m^3-d)

Date	HRT	Temp.	VS Load	TS in	VS in	VS in	TS out	VS out	VS out	VS red	pH	Alkalinity	Vol. Acids	Gas Prod.	Methane	CO_2
	days	Celsius	kg/ m^3-d	g/L	g/L	%	g/L	g/L	%	%		mg/L	mg/L	m^3/kg VS	%	%
8/12/1998	15		0.88	20.0	13.2	65.9	14.3	7.8	54.9	40.5	7.1	1960	108	1.0	68.5	31.5
8/13/1998	15	26	0.87	21.2	13.0	61.3					7					
8/14/1998	15	25.2	0.87	21.2	13.0	61.3										
8/15/1998	15	26	0.87	21.2	13.0	61.3					7					
8/16/1998	15	25.5	0.87	21.2	13.0	61.3					7					
8/17/1998	15	29.5	0.87	21.2	13.0	61.3					7					
8/18/1998	15	26	0.87	21.2	13.0	61.3										
8/19/1998	15	24	0.87	21.2	13.0	61.3					7					
8/20/1998	15		0.87	21.2	13.0	61.3	15.4	8.3	54.1	35.9	7	1960	192	1.0	64.5	35.5
8/21/1998	15	24	0.87	19.2	13.0	67.8					7					
8/22/1998	15	23	0.87	19.2	13.0	67.8					7					
8/23/1998	15		0.87	19.2	13.0	67.8					7					
8/24/1998	15		0.87	19.2	13.0	67.8					7					
8/25/1998	15		0.87	19.2	13.0	67.8										
8/26/1998	15		0.87	19.2	13.0	67.8										
8/27/1998	15		0.87	19.2	13.0	67.8										
8/28/1998	15		0.87	19.2	13.0	67.8										
8/29/1998	15		0.87	19.2	13.0	67.8	14.9	8.1	54.1	38.3	7			0.7		
8/30/1998	15	22	0.87	19.2	13.0	67.8					7					
8/31/1998	15	25	0.87	19.2	13.0	67.8										
9/1/1998	15	24.8	0.87	19.2	13.0	67.8					7					
9/2/1998	15	24.8	0.87	19.2	13.0	67.8					7					
9/3/1998	15	24.3	0.87	19.2	13.0	67.8					7					
9/4/1998	15		0.87	19.2	13.0	67.8	15.2	8.6	56.5	33.9		2010	336	1.0	64.9	35.1
9/5/1998	15	22	0.88	19.5	13.3	68.1					7					
9/6/1998	15	22.2	0.88	19.5	13.3	68.1					7					
9/7/1998	15	24.1	0.88	19.5	13.3	68.1										
9/8/1998	15	27.5	0.88	19.5	13.3	68.1										
9/9/1998	15	25.3	0.88	19.5	13.3	68.1					7					
9/10/1998	15	25.8	0.88	19.5	13.3	68.1										
9/11/1998	15	25.2	0.88	19.5	13.3	68.1					7					
AVG	**15**	**24.9**	**0.87**	**19.8**	**13.1**	**66.2**	**14.9**	**8.2**	**54.8**	**37.8**		**2012.5**	**180**	**1.0**	**66.1**	**33.9**

Appendix H (Operating Characteristics at Temperature = 25 °C, HDT = 15days, and Organic Loading = 1.80 kg/m^3-d)

Date	HRT	Temp.	VS Load	TS in	VS in	VS in	TS out	VS out	VS out	VS red	pH	Alkalinity	Vol. Acids	Gas Prod.	Methane	CO_2
	days	Celsius	kg/ m^3-d	g/L	g/L	%	g/L	g/L	%	%		mg/L	mg/L	m^3/kg VS	%	%
11/18/1998	15		1.83	41.2	27.5	66.6										
11/19/1998	15	25	1.83	41.2	27.5	66.6										
11/20/1998	15	25	1.83	41.2	27.5	66.6										
11/21/1998	15		1.83	41.2	27.5	66.6	31.7	16.6	52.3	39.5		2050	108	0.7	63.1	36.9
11/22/1998	15	25	1.85	38.8	27.8	71.8										
11/23/1998	15	24.9	1.85	38.8	27.8	71.8										
11/24/1998	15	25.5	1.85	38.8	27.8	71.8										
11/25/1998	15	25	1.85	38.8	27.8	71.8										
11/26/1998	15	24.9	1.85	38.8	27.8	71.8										
11/27/1998	15	24.5	1.85	38.8	27.8	71.8										
11/28/1998	15	25	1.85	38.8	27.8	71.8										
11/29/1998	15	25	1.85	38.8	27.8	71.8										
11/30/1998	15		1.85	38.8	27.8	71.8										
12/1/1998	15	22	1.85	38.8	27.8	71.8										
12/2/1998	15		1.85	38.8	27.8	71.8	30.2	17.6	58.2	36.9		2540	324	1.0	56.8	43.2
12/3/1998	15	23	1.81	41.3	27.2	66.0					7					
12/4/1998	15	24	1.81	41.3	27.2	66.0										
12/5/1998	15	24	1.81	41.3	27.2	66.0										
12/6/1998	15	22	1.81	41.3	27.2	66.0										
12/7/1998	15	25.5	1.81	41.3	27.2	66.0										
12/8/1998	15		1.81	41.3	27.2	66.0	32.3	18.2	56.4	33.0		2710	444	1.2	63.9	36.1
12/9/1998	15	25.4	1.80	39.2	27.1	69.2					7					
12/10/1998	15	26.5	1.80	39.2	27.1	69.2										
12/11/1998	15	24	1.80	39.2	27.1	69.2										
12/12/1998	15	26	1.80	39.2	27.1	69.2										
12/13/1998	15	26	1.80	39.2	27.1	69.2										
12/14/1998	15		1.80	39.2	27.1	69.2	31.9	17.5	54.7	35.6		3020	228			
12/15/1998	15		1.80	39.2	27.1	69.2										
AVG	**15**	**24.7**	**1.83**	**39.8**	**27.5**	**69.1**	**31.5**	**17.5**	**55.4**	**36.3**		**2580**	**276**	**1.0**	**61.3**	**38.7**

Appendix H (Operating Characteristics at Temperature = 25 °C, HDT = 15days, and Organic Loading = 2.70 kg/m^3-d)

Date	HRT	Temp.	VS Load	TS in	VS in	VS in	TS out	VS out	VS out	VS red	pH	Alkalinity	Vol. Acids	Gas Prod.	Methane	Carbon
	days	Celsius	kg/ m^3-d	g/L	g/L	%	g/L	g/L	%	%		mg/L	mg/L	m^3/kg VS		Dioxide
												as $CaCO_3$	as acetic acid	destroyed-d	%	%
9/23/1999	15	25	2.79	67.4	41.8	62.0										
9/24/1999	15	24.6	2.79	67.4	41.8	62.0										
9/25/1999	15		2.79	67.4	41.8	62.0	45.1	24.8	55.0	40.6	6.8	2280	564			
9/26/1999	15	26.5	2.66	63.3	39.8	63.0										
9/27/1999	15		2.66	63.3	39.8	63.0										
9/28/1999	15	25	2.66	63.3	39.8	63.0										
9/29/1999	15	25	2.66	63.3	39.8	63.0										
9/30/1999	15	24.8	2.66	63.3	39.8	63.0										
10/1/1999	15	25	2.66	63.3	39.8	63.0										
10/2/1999	15		2.66	63.3	39.8	63.0	43.4	23.3	53.6	41.6	6.8	2700	708	0.6	56.5	43.5
10/3/1999	15		2.66	63.3	39.8	63.0										
10/4/1999	15	25	2.66	63.3	39.8	63.0										
10/5/1999	15	25	2.66	63.3	39.8	63.0										
10/6/1999	15		2.66	63.3	39.8	63.0										
10/7/1999	15		2.66	63.3	39.8	63.0										
10/8/1999	15		2.66	63.3	39.8	63.0	43.6	23.3	53.4	41.6		2540	924	0.5		
AVG	**15**	**25.1**	**2.68**	**64.0**	**40.2**	**62.8**	**44.0**	**23.8**	**54.0**	**41.3**		**2507**	**732**	**0.6**	**56.5**	**43.5**

Appendix H (Operating Characteristics at Temperature = 25 °C, HDT = 30 days, and Organic Loading = 0.90 kg/m^3-d)

Date	HRT	Temp.	VS Load	TS in	VS in	VS in	TS out	VS out	VS out	VS red	pH	Alkalinity	Vol. Acids	Gas Prod.	Methane	CO_2
	days	Celsius	kg/ m^3-d	g/L	g/L	%	g/L	g/L	%	%		mg/L	mg/L	m^3/kg VS	%	%
8/12/1998	30		0.88	40.1	26.4	65.9	25.1	14.0	55.8	47.1	7.1	3020	144	1.1	61.7	38.3
8/13/1998	30	26.5	0.87	42.8	26.2	61.3					7					
8/14/1998	30	24.9	0.87	42.8	26.2	61.3					7					
8/15/1998	30	26	0.87	42.8	26.2	61.3					7					
8/16/1998	30	25.1	0.87	42.8	26.2	61.3					7					
8/17/1998	30	28.5	0.87	42.8	26.2	61.3					7					
8/18/1998	30	26.2	0.87	42.8	26.2	61.3					7					
8/19/1998	30		0.87	42.8	26.2	61.3					7					
8/20/1998	30		0.87	42.8	26.2	61.3	28.6	15.8	55.4	39.7	7	2820	396	1.1	58.8	41.2
8/21/1998	30	23.5	0.86	38.1	25.8	67.8					7					
8/22/1998	30	23	0.86	38.1	25.8	67.8					7					
8/23/1998	30		0.86	38.1	25.8	67.8					7					
8/24/1998	30		0.86	38.1	25.8	67.8					7					
8/25/1998	30		0.86	38.1	25.8	67.8										
8/26/1998	30		0.86	38.1	25.8	67.8										
8/27/1998	30		0.86	38.1	25.8	67.8										
8/28/1998	30		0.86	38.1	25.8	67.8										
8/29/1998	30	20	0.86	38.1	25.8	67.8	29.3	15.6	53.2	39.6	7			1.1		
8/30/1998	30	22	0.86	38.1	25.8	67.8					7					
8/31/1998	30	26	0.86	38.1	25.8	67.8										
9/1/1998	30	25.4	0.86	38.1	25.8	67.8					7					
9/2/1998	30	26.1	0.86	38.1	25.8	67.8					7					
9/3/1998	30	24.8	0.86	38.1	25.8	67.8					7					
9/4/1998	30		0.86	38.1	25.8	67.8	31.3	17.2	54.9	33.4		3280	108	1.5		
9/5/1998	30	22	0.93	41.3	28.0	67.8					7					
9/6/1998	30	24.2	0.93	41.3	28.0	67.8					7					
9/7/1998	30	24.7	0.93	41.3	28.0	67.8										
9/8/1998	30	27.5	0.93	41.3	28.0	67.8										
9/9/1998	30	26.5	0.93	41.3	28.0	67.8					7					
9/10/1998	30	25	0.93	41.3	28.0	67.8										
9/11/1998	30	25	0.93	41.3	28.0	67.8					7					
AVG	**30**	**24.9**	**0.88**	**40.1**	**26.5**	**66.1**	**28.7**	**15.7**	**54.7**	**40.6**		**2983**	**195**	**1.2**	**57.3**	**42.7**

Appendix H (Operating Characteristics at Temperature = 25 °C, HDT = 30 days, and Organic Loading = 1.80 kg/m^3-d)

Date	HRT	Temp.	VS Load	TS in	VS in	VS in	TS out	VS out	VS out	VS red	pH	Alkalinity	Vol. Acids	Gas Prod.	Methane	CO_2
	days	Celsius	kg/ m^3-d	g/L	g/L	%	g/L	g/L	%	%		mg/L	mg/L	m^3/kg VS	%	%
11/18/1998	30	25.4	1.99	89.8	59.8	66.6										
11/19/1998	30		1.99	89.8	59.8	66.6										
11/20/1998	30		1.99	89.8	59.8	66.6										
11/21/1998	30		1.99	89.8	59.8	66.6	50.9	25.1	49.4	58.0	7	2870	120		49.7	50.3
11/22/1998	30		1.85	77.2	55.4	71.8										
11/23/1998	30	25.5	1.85	77.2	55.4	71.8										
11/24/1998	30	25.5	1.85	77.2	55.4	71.8										
11/25/1998	30	25	1.85	77.2	55.4	71.8										
11/26/1998	30	25	1.85	77.2	55.4	71.8										
11/27/1998	30	24.5	1.85	77.2	55.4	71.8										
11/28/1998	30		1.85	77.2	55.4	71.8										
11/29/1998	30		1.85	77.2	55.4	71.8										
11/30/1998	30	23	1.85	77.2	55.4	71.8										
12/1/1998	30	23	1.85	77.2	55.4	71.8										
12/2/1998	30		1.85	77.2	55.4	71.8	47.5	26.0	54.8	53.0		3750	240	0.7	54.6	45.4
12/3/1998	30	26.5	1.68	76.3	50.3	66.0					7					
12/4/1998	30	25	1.68	76.3	50.3	66.0										
12/5/1998	30	25	1.68	76.3	50.3	66.0										
12/6/1998	30	23.4	1.68	76.3	50.3	66.0										
12/7/1998	30	26	1.68	76.3	50.3	66.0										
12/8/1998	30	25.4	1.68	76.3	50.3	66.0	47.4	25.8	54.4	48.7		3780	864	1.1	54.0	46.0
12/9/1998	30		1.90	82.3	56.9	69.2										
12/10/1998	30	26.2	1.90	82.3	56.9	69.2										
12/11/1998	30	24	1.90	82.3	56.9	69.2										
12/12/1998	30	25.4	1.90	82.3	56.9	69.2										
12/13/1998	30	25.3	1.90	82.3	56.9	69.2										
12/14/1998	30		1.90	82.3	56.9	69.2	48.8	26.7	54.7	53.1		4110	180			
12/15/1998	30		1.90	82.3	56.9	69.2										
AVG	**30**	**25.0**	**1.84**	**80.1**	**55.3**	**69.2**	**48.7**	**25.9**	**53.3**	**53.2**		**3627.5**	**351**	**0.9**	**52.8**	**47.2**

Appendix H (Operating Characteristics at Temperature = 25 °C, HDT = 30 days, and Organic Loading = 2.70 kg/m^3-d)

Date	HRT	Temp.	VS Load	TS in	VS in	VS in	TS out	VS out	VS out	VS red	pH	Alkalinity	Vol. Acids	Gas Prod.	Methane	CO_2
	days	Celsius	kg/m^3-d	g/L	g/L	%	g/L	g/L	%	%		mg/L	mg/L	m^3/kg VS	%	%
9/20/1999	30	25.1	2.4	117.5	72.8	62.0										
9/21/1999	30	25.2	2.4	117.5	72.8	62.0										
9/22/1999	30		2.4	117.5	72.8	62.0										
9/23/1999	30		2.4	117.5	72.8	62.0										
9/24/1999	30	25.4	2.4	117.5	72.8	62.0										
9/25/1999	30		2.4	117.5	72.8	62.0	76.8	39.2	51.1	46.1		3960	240	0.8		
9/26/1999	30		2.6	124.2	78.2	63.0										
9/27/1999	30	25	2.6	124.2	78.2	63.0										
9/28/1999	30	24.8	2.6	124.2	78.2	63.0										
9/29/1999	30	25.2	2.6	124.2	78.2	63.0										
9/30/1999	30	24.7	2.6	124.2	78.2	63.0										
10/1/1999	30	25.4	2.6	124.2	78.2	63.0										
10/2/1999	30		2.6	124.2	78.2	63.0	72.5	35.7	49.2	54.4	7.3	4120	348	0.6	56.5	43.5
10/3/1999	30		2.6	124.2	78.2	63.0										
10/4/1999	30		2.6	124.2	78.2	63.0										
10/5/1999	30		2.6	124.2	78.2	63.0										
10/6/1999	30		2.6	124.2	78.2	63.0										
10/7/1999	30		2.6	124.2	78.2	63.0										
10/8/1999	30		2.6	124.2	78.2	63.0	74.2	36.3	48.9	53.6		4530	624	0.7		
AVG		**25.1**	**2.6**	**122.1**	**76.5**	**62.7**	**74.5**	**37.1**	**49.7**	**51.4**		**4203**	**404**	**0.7**	**56.5**	**43.5**

Appendix H (Operating Characteristics at Temperature = 25 °C, HDT = 45 days, and Organic Loading = 0.90 kg/m^3-d)

Date	HRT	Temp.	VS Load	TS in	VS in	VS in	TS out	VS out	VS out	VS red	pH	Alkalinity	Vol. Acids	Gas Prod.	Methane	CO_2
	days	Celsius	kg/ m^3-d	g/L	g/L	%	g/L	g/L	%	%		mg/L	mg/L	m^3/kg VS		
												as $CaCO_3$	as acetic acid	destroyed-d	%	%
2/26/1999	45	25.4	0.86	51.8	38.8	75.0										
2/27/1999	45	26	0.86	51.8	38.8	75.0										
2/28/1999	45	24	0.86	51.8	38.8	75.0										
3/1/1999	45	25	0.86	51.8	38.8	75.0										
3/2/1999	45	25.2	0.86	51.8	38.8	75.0										
3/3/1999	45	24.6	0.86	51.8	38.8	75.0										
3/4/1999	45	24.8	0.86	51.8	38.8	75.0										
3/5/1999	45		0.86	51.8	38.8	75.0	35.5	20.8	58.7	46.4	7.2	3860	144	1.0		
3/6/1999	45	25.4	0.86	51.8	38.8	75.0										
3/7/1999	45	25	0.86	51.8	38.8	75.0										
3/8/1999	45		0.88	55.6	39.6	71.2										
3/9/1999	45		0.88	55.6	39.6	71.2										
3/10/1999	45	25.5	0.88	55.6	39.6	71.2										
3/11/1999	45		0.88	55.6	39.6	71.2										
3/12/1999	45		0.88	55.6	39.6	71.2										
3/13/1999	45		0.88	55.6	39.6	71.2	32.0	18.8	58.8	52.4	7.4	3870	180	0.8	49	51
3/14/1999	45	25	0.88	55.6	39.6	71.2										
3/15/1999	45	23	0.88	55.6	39.6	71.2										
3/16/1999	45	25.2	0.90	54.4	40.5	74.4										
3/17/1999	45		0.90	54.4	40.5	74.4										
3/18/1999	45		0.90	54.4	40.5	74.4										
3/19/1999	45		0.90	54.4	40.5	74.4										
3/20/1999	45	25	0.90	54.4	40.5	74.4										
3/21/1999	45		0.90	54.4	40.5	74.4										
3/22/1999	45		0.90	54.4	40.5	74.4										
3/23/1999	45		0.90	54.4	40.5	74.4										
3/24/1999	45	25	0.90	54.4	40.5	74.4										
3/25/1999	45		0.90	54.4	40.5	74.4										
3/26/1999	45	25.4	0.90	54.4	40.5	74.4										
3/27/1999	45		0.90	54.4	40.5	74.4					7.7	3400	132			
3/28/1999	45		0.89	55.4	40.0	72.2										
3/29/1999	45	25	0.89	55.4	40.0	72.2										

3/30/1999	45	25.6	0.89	55.4	40.0	72.2										
3/31/1999	45	25.5	0.89	55.4	40.0	72.2										
4/1/1999	45	25.4	0.89	55.4	40.0	72.2										
4/2/1999	45	25.2	0.89	55.4	40.0	72.2										
4/3/1999	45		0.89	55.4	40.0	72.2										
4/4/1999	45		0.89	55.4	40.0	72.2										
4/5/1999	45	24	0.89	55.4	40.0	72.2	31.7	18.9	59.8	52.6	7.8	2900	156	0.7	52.6	47.4
AVG	**45**	**25.0**	**0.88**	**54.2**	**39.8**	**73.4**	**33.1**	**19.5**	**59.1**	**50.5**		**3508**	**153**	**0.9**	**50.8**	**49.2**

Appendix H (Operating Characteristics at Temperature = 25 °C, HDT = 45 days, and Organic Loading = 1.80 kg/m^3-d)

Date	HRT	Temp.	VS Load	TS in	VS in	VS in	TS out	VS out	VS out	VS red	pH	Alkalinity	Vol. Acids	Gas Prod.	Methane	CO_2
	days	Celsius	kg m^3/ -d	g/L	g/L	%	g/L	g/L	%	%		mg/L	mg/L	m^3/kg VS	%	%
6/16/1999	45	25	1.63	106.5	73.4	69.0					7.3					
6/17/1999	45		1.63	106.5	73.4	69.0										
6/18/1999	45		1.63	106.5	73.4	69.0										
6/19/1999	45		1.63	106.5	73.4	69.0										
6/20/1999	45		1.63	106.5	73.4	69.0										
6/21/1999	45		1.63	106.5	73.4	69.0										
6/22/1999	45	26	1.63	106.5	73.4	69.0										
6/23/1999	45		1.63	106.5	73.4	69.0	59.5	32.4	54.4	55.9		4510	636	1.0	56.7	43.3
6/24/1999	45	25.2	1.65	106.4	74.4	69.9					7.3					
6/25/1999	45	25.4	1.65	106.4	74.4	69.9										
6/26/1999	45	26	1.65	106.4	74.4	69.9										
6/27/1999	45	25.4	1.65	106.4	74.4	69.9										
6/28/1999	45		1.65	106.4	74.4	69.9										
6/29/1999	45	25.4	1.65	106.4	74.4	69.9										
6/30/1999	45		1.65	106.4	74.4	69.9	61.9	34.1	55.0	54.2		4240	444	0.8	66.8	33.2
7/1/1999	45		1.69	114.0	76.0	66.7										
7/2/1999	45	25.5	1.69	114.0	76.0	66.7					7.2					
7/3/1999	45		1.69	114.0	76.0	66.7										
7/4/1999	45	25.4	1.69	114.0	76.0	66.7										
7/5/1999	45	25	1.69	114.0	76.0	66.7										
7/6/1999	45		1.69	114.0	76.0	66.7	61.5	33.6	54.6	55.9		4610	396	0.8	62.3	37.7
7/7/1999	45		1.72	120.7	77.2	63.9										
7/8/1999	45		1.72	120.7	77.2	63.9										
7/9/1999	45		1.72	120.7	77.2	63.9										
7/10/1999	45	27	1.72	120.7	77.2	63.9										
7/11/1999	45		1.72	120.7	77.2	63.9										
7/12/1999	45	26.5	1.72	120.7	77.2	63.9										
7/13/1999	45	25.4	1.72	120.7	77.2	63.9										
7/14/1999	45	25.4	1.72	120.7	77.2	63.9										
7/15/1999	45		1.72	120.7	77.2	63.9										
AVG	**45**	**25.6**	**1.67**	**112.3**	**75.3**	**67.2**	**61.0**	**33.1**	**54.3**	**56.0**		**4523**	**474**	**0.8**	**61.6**	**38.4**

Appendix H (Operating Characteristics at Temperature = 35 °C, HDT = 7 days, and Organic Loading = 0.90 kg/m^3-d)

Date	HRT	Temp.	VS Load	TS in	VS in	VS in	TS out	VS out	VS out	VS red	pH	Alkalinity	Vol. Acids	Gas Prod.	Methane	CO_2
	days	Celsius	kg/ m^3-d	g/L	g/L	%	g/L	g/L	%	%		mg/L	mg/L	m^3/kg VS		
												as $CaCO_3$	as acetic acid	destroyed-d	%	%
2/14/1999	7	36	0.91	8.5	6.4	74.8										
2/15/1999	7	35.4	0.91	8.5	6.4	74.8										
2/16/1999	7	35.2	0.91	8.5	6.4	74.8										
2/17/1999	7	35.5	0.91	8.5	6.4	74.8										
2/18/1999	7	34	0.91	8.5	6.4	74.8										
2/19/1999	7	35.1	0.91	8.5	6.4	74.8										
2/20/1999	7	36	0.91	8.5	6.4	74.8										
2/21/1999	7	35.2	0.91	8.5	6.4	74.8									60.3	39.7
2/22/1999	7	36	0.91	8.5	6.4	74.8										
2/23/1999	7		0.91	8.5	6.4	74.8										
2/24/1999	7		0.91	8.5	6.4	74.8	6.5	4.0	61.5	37.5	6.7	740	84	1.1	60.8	39.2
2/25/1999	7		0.87	8.1	6.1	75.0										
2/26/1999	7	33	0.87	8.1	6.1	75.0										
2/27/1999	7	35.4	0.87	8.1	6.1	75.0										
2/28/1999	7	36.5	0.87	8.1	6.1	75.0										
3/1/1999	7	36.5	0.87	8.1	6.1	75.0										
3/2/1999	7	35	0.87	8.1	6.1	75.0										
3/3/1999	7	35	0.87	8.1	6.1	75.0										
3/4/1999	7	36	0.87	8.1	6.1	75.0										
3/5/1999	7		0.87	8.1	6.1	75.0	5.7	3.5	62.7	41.6	6.8	910	84	1.1		
3/6/1999	7	35.4	0.87	8.1	6.1	75.0										
3/7/1999	7		0.87	8.1	6.1	75.0										
3/8/1999	7	35	0.89	8.8	6.2	71.2										
3/9/1999	7	36	0.89	8.8	6.2	71.2										
3/10/1999	7	35.5	0.89	8.8	6.2	71.2										
3/11/1999	7	35.4	0.89	8.8	6.2	71.2										
3/12/1999	7		0.89	8.8	6.2	71.2										
3/13/1999	7		0.89	8.8	6.2	71.2	6.1	3.6	59.4	41.7	6.8	1100	144	1.0	58.8	41.2
3/14/1999	7	35.4	0.89	8.8	6.2	71.2										
3/15/1999	7		0.89	8.8	6.2	71.2										
AVG	**7**	**35.4**	**0.89**	**8.4**	**6.2**	**73.9**	**6.1**	**3.7**	**61.2**	**40.3**		**917**	**104**	**1.1**	**60.0**	**40.0**

Appendix H (Operating Characteristics at Temperature = 35 °C, HDT = 7 days, and Organic Loading = 1.80 kg/m^3-d)

Date	HRT	Temp.	VS Load	TS in	VS in	VS in	TS out	VS out	VS out	VS red	pH	Alkalinity	Vol. Acids	Gas Prod.	Methane	CO_2
	days	Celsius	kg m^3/-d	g/L	g/L	%	g/L	g/L	%	%		mg/L	mg/L	m^3/kg VS		
												as $CaCO_3$	as acetic acid	destroyed-d	%	%
5/12/1999	7	35	1.71	17.3	12.0	69.2										
5/13/1999	7	35.4	1.71	17.3	12.0	69.2										
5/14/1999	7		1.71	17.3	12.0	69.2										
5/15/1999	7	36	1.71	17.3	12.0	69.2										
5/16/1999	7	35.8	1.71	17.3	12.0	69.2										
5/17/1999	7		1.71	17.3	12.0	69.2	12.6	7.7	60.9	36.1		1550	156	1.0		
5/18/1999	7	35.9	1.85	21.2	13.0	61.2										
5/19/1999	7		1.85	21.2	13.0	61.2										
5/20/1999	7	35.6	1.85	21.2	13.0	61.2										
5/21/1999	7	35.5	1.85	21.2	13.0	61.2										
5/22/1999	7	35	1.85	21.2	13.0	61.2										
5/23/1999	7	35	1.85	21.2	13.0	61.2										
5/24/1999	7	35	1.85	21.2	13.0	61.2										
5/25/1999	7		1.85	21.2	13.0	61.2	15.3	8.2	53.7	36.6		1150	84	1.1	60.4	39.6
5/26/1999	7	35	1.79	19.4	12.6	64.6										
5/27/1999	7	35	1.79	19.4	12.6	64.6										
5/28/1999	7	35	1.79	19.4	12.6	64.6										
5/29/1999	7	35	1.79	19.4	12.6	64.6										
5/30/1999	7		1.79	19.4	12.6	64.6										
5/31/1999	7	35.2	1.79	19.4	12.6	64.6										
6/1/1999	7		1.79	19.4	12.6	64.6	15.3	8.3	54.1	34.3	7.1	1840	180	1.3	60.9	39.1
6/2/1999	7		1.79	19.4	12.6	64.6										
6/3/1999	7		1.79	19.4	12.6	64.6										
6/4/1999	7		1.79	19.4	12.6	64.6										
6/5/1999	7	34.5	1.79	19.4	12.6	64.6										
6/6/1999	7	35	1.79	19.4	12.6	64.6										
6/7/1999	7		1.79	19.4	12.6	64.6	14.5	7.7	53.4	38.5		1840	300	1.2		
AVG	**7**	**35.2**	**1.79**	**19.5**	**12.6**	**64.6**	**14.4**	**8.0**	**55.5**	**36.4**		**1595**	**180**	**1.2**	**60.7**	**39.4**

Appendix H (Operating Characteristics at Temperature = 35 °C, HDT = 7 days, and Organic Loading = 2.70 kg/m^3-d)

Date	HRT	Temp.	VS Load	TS in	VS in	VS in	TS out	VS out	VS out	VS red	pH	Alkalinity	Vol. Acids	Gas Prod.	Methane	CO_2
	days	Celsius	kg/ m^3-d	g/L	g/L	%	g/L	g/L	%	%		mg/L	mg/L	m^3/kg VS		
												as CaCO3	as acetic acid	destroyed-d	%	%
7/17/1999	7	35	2.68	28.2	18.8	66.6										
7/18/1999	7		2.68	28.2	18.8	66.6										
7/19/1999	7	35	2.68	28.2	18.8	66.6										
7/20/1999	7	35	2.68	28.2	18.8	66.6										
7/21/1999	7	34.8	2.68	28.2	18.8	66.6										
7/22/1999	7		2.68	28.2	18.8	66.6	22.2	12.2	55.3	34.7	6.9	1400	96	1.1		
7/23/1999	7	34.6	2.68	28.2	18.8	66.6										
7/24/1999	7	34.6	2.68	28.2	18.8	66.6										
7/25/1999	7	34.5	2.68	28.2	18.8	66.6										
7/26/1999	7	34.5	2.68	28.2	18.8	66.6										
7/27/1999	7	35	2.68	28.2	18.8	66.6										
7/28/1999	7	35	2.68	28.2	18.8	66.6										
7/29/1999	7	35	2.68	28.2	18.8	66.6										
7/30/1999	7		2.68	28.2	18.8	66.6	23.9	13.6	56.9	27.5	7	2020	96	1.4	60.8	39.2
7/31/1999	7	34.8	2.72	30.3	19.0	62.8										
8/1/1999	7	34.6	2.72	30.3	19.0	62.8										
8/2/1999	7	34.5	2.72	30.3	19.0	62.8										
8/3/1999	7	34.6	2.72	30.3	19.0	62.8										
8/4/1999	7		2.72	30.3	19.0	62.8										
8/5/1999	7		2.72	30.3	19.0	62.8										
8/6/1999	7	34.5	2.72	30.3	19.0	62.8	23.4	12.4	53.1	34.8	7.2	2260	96	1.2	62.1	37.0
AVG	**7**	**34.8**	**2.69**	**28.9**	**18.9**	**65.3**	**23.1**	**12.8**	**55.1**	**32.4**		**1893**	**96**	**1.2**	**61.5**	**38.1**

Appendix H (Operating Characteristics at Temperature = 35 °C, HDT = 15 days, and Organic Loading = 0.90 kg/m^3-d)

Date	HRT	Temp.	VS Load	TS in	VS in	VS in	TS out	VS out	VS out	VS red	pH	Alkalinity	Vol. Acids	Gas Prod.	Methane	CO_2
	days	Celsius	kg m^3/-d	g/L	g/L	%	g/L	g/L	%	%		mg/L	mg/L	m^3/kg VS	%	%
8/12/1998	15			20.0	13.2	65.9			52.1	47.6	7.1	2040	108	0.9	61.7	38.3
8/13/1998	15	36	0.87	21.2	13.0	61.3					7.1					
8/14/1998	15	35.1	0.87	21.2	13.0	61.3					7.1					
8/15/1998	15		0.87	21.2	13.0	61.3					7.1					
8/16/1998	15	35	0.87	21.2	13.0	61.3					7.1					
8/17/1998	15		0.87	21.2	13.0	61.3					7.1					
8/18/1998	15	35.6	0.87	21.2	13.0	61.3					7.1					
8/19/1998	15	35.6	0.87	21.2	13.0	61.3					7.1					
8/20/1998	15		0.87	21.2	13.0	61.3	14.1	7.3	51.4	44.3	7.1	2000	96	1.2	64.6	35.4
8/21/1998	15	35	0.87	19.2	13.0	67.8					7.1					
8/22/1998	15	35	0.87	19.2	13.0	67.8					7.1					
8/23/1998	15	36.5	0.87	19.2	13.0	67.8					7.2					
8/24/1998	15	36	0.87	19.2	13.0	67.8					7.1					
8/25/1998	15		0.87	19.2	13.0	67.8										
8/26/1998	15		0.87	19.2	13.0	67.8										
8/27/1998	15		0.87	19.2	13.0	67.8										
8/28/1998	15		0.87	19.2	13.0	67.8										
8/29/1998	15	30	0.87	19.2	13.0	67.8	12.3	6.2	50.4	52.5	7.1			0.9		
8/30/1998	15	35	0.87	19.2	13.0	67.8					7.1					
8/31/1998	15	36	0.87	19.2	13.0	67.8										
9/1/1998	15	35.2	0.87	19.2	13.0	67.8					7.1					
9/2/1998	15	35.3	0.87	19.2	13.0	67.8					7.1					
9/3/1998	15	35.8	0.87	19.2	13.0	67.8					7					
9/4/1998	15		0.87	19.2	13.0	67.8	12.6	6.6	52.1	49.7		2300	96	1.2	62.6	37.4
9/5/1998	15	36.1	0.88	19.5	13.3	68.1					7.1					
9/6/1998	15	35.1	0.88	19.5	13.3	68.1					7.1					
9/7/1998	15	35.1	0.88	19.5	13.3	68.1										
9/8/1998	15		0.88	19.5	13.3	68.1										
9/9/1998	15	36.1	0.88	19.5	13.3	68.1					7.1					
9/10/1998	15	36.5	0.88	19.5	13.3	68.1										
911/1998	15	36.2	0.88	19.5	13.3	68.1										
9/12/1998	15		0.88	19.5	13.3	68.1	12.9	6.6	51.6	50.0		2200	96	1.0		
AVG	**15**	**35.3**	**0.87**	**19.8**	**13.1**	**66.2**	**13.0**	**6.7**	**51.5**	**48.8**		**2135**	**99**	**1.0**	**63.0**	**37.0**

Appendix H (Operating Characteristics at Temperature = 35 °C, HDT = 15 days, and Organic Loading =1.80 kg/m^3-d)

Date	HRT	Temp.	VS Load	TS in	VS in	VS in	TS out	VS out	VS out	VS red	pH	Alkalinity	Vol. Acids	Gas Prod.	Methane	CO_2
	days	Celsius	kg/ m^3-d	g/L	g/L	%	g/L	g/L	%	%		mg/L	mg/L	m^3/kg VS	%	%
11/18/1998	15	35	1.83	41.2	27.5	66.6										
11/19/1998	15	35.4	1.83	41.2	27.5	66.6										
11/20/1998	15	35.1	1.83	41.2	27.5	66.6										
11/21/1998	15		1.83	41.2	27.5	66.6	31.8	15.3	48.2	44.2	7	2430	120	1.1	60.6	39.4
11/22/1998	15	35	1.85	38.8	27.8	71.8										
11/23/1998	15	35	1.85	38.8	27.8	71.8										
11/24/1998	15	35	1.85	38.8	27.8	71.8										
11/25/1998	15		1.85	38.8	27.8	71.8										
11/26/1998	15	34.6	1.85	38.8	27.8	71.8										
11/27/1998	15	35.2	1.85	38.8	27.8	71.8										
11/28/1998	15	34.5	1.85	38.8	27.8	71.8										
11/29/1998	15	34.7	1.85	38.8	27.8	71.8										
11/30/1998	15	34.8	1.85	38.8	27.8	71.8										
12/1/1998	15	35	1.85	38.8	27.8	71.8										
12/2/1998	15		1.85	38.8	27.8	71.8	30.2	16.1	53.5	42.0		2900	108	1.2	59.2	40.8
12/3/1998	15	35	1.81	41.3	27.2	66.0					7					
12/4/1998	15	35	1.81	41.3	27.2	66.0										
12/5/1998	15	35	1.81	41.3	27.2	66.0										
12/6/1998	15	34.9	1.81	41.3	27.2	66.0										
12/7/1998	15	35.4	1.81	41.3	27.2	66.0										
12/8/1998	15		1.81	41.3	27.2	66.0	28.4	15.1	53.2	44.6		2490	108	1.2	55.8	44.2
12/9/1998	15	35.4	1.80	39.2	27.1	69.2					7.1					
12/10/1998	15	36	1.80	39.2	27.1	69.2										
12/11/1998	15	36	1.80	39.2	27.1	69.2										
12/12/1998	15	35	1.80	39.2	27.1	69.2										
12/13/1998	15	34.7	1.80	39.2	27.1	69.2										
12/14/1998	15		1.80	39.2	27.1	69.2	28.5	15.1	52.8	44.5		3370	132			
12/15/1998	15		1.80	39.2	27.1	69.2										
AVG	**15**	**35.1**	**1.83**	**39.8**	**27.5**	**69.1**	**29.7**	**15.4**	**51.9**	**43.8**		**2797.5**	**117**	**1.2**	**58.5**	**41.5**

Appendix H (Operating Characteristics at Temperature = 35 °C, HDT = 15 days, and Organic Loading =2.70 kg/m^3-d)

Date	HRT	Temp.	VS Load	TS in	VS in	VS in	TS out	VS out	VS out	VS red	pH	Alkalinity	Vol. Acids	Gas Prod.	Methane	CO_2
	days	Celsius	kg m^3/-d	g/L	g/L	%	g/L	g/L	%	%		mg/L	mg/L	m^3/kg VS	%	%
9/23/1999	15	35.4	2.79	67.4	41.8	62.0										
9/24/1999	15	36	2.79	67.4	41.8	62.0										
9/25/1999	15		2.79	67.4	41.8	62.0	41.8	21.1	50.5	49.5	7.1	2750	180	0.8		
9/26/1999	15	35.8	2.66	63.3	39.8	63.0										
9/27/1999	15	36	2.66	63.3	39.8	63.0										
9/28/1999	15	35	2.66	63.3	39.8	63.0										
9/29/1999	15	35.4	2.66	63.3	39.8	63.0										
9/30/1999	15		2.66	63.3	39.8	63.0										
10/1/1999	15	34.6	2.66	63.3	39.8	63.0										
10/2/1999	15		2.66	63.3	39.8	63.0	41.8	20.4	48.9	48.7	7.2	3350	600	0.9	55	45
10/3/1999	15	34.8	2.66	63.3	39.8	63.0										
10/4/1999	15	35.5	2.66	63.3	39.8	63.0										
10/5/1999	15	34.5	2.66	63.3	39.8	63.0										
10/6/1999	15		2.66	63.3	39.8	63.0										
10/7/1999	15		2.66	63.3	39.8	63.0										
10/8/1999	15		2.66	63.3	39.8	63.0	41.7	20.3	48.6	49.1		3330	984	1.0		
AVG	**15**	**35.3**	**2.68**	**64.0**	**40.2**	**62.8**	**41.8**	**20.6**	**49.3**	**49.1**		**3143**	**588**	**0.9**	**55**	**45**

Appendix H (Operating Characteristics at Temperature = 35 °C, HDT = 30 days, and Organic Loading =0.90 kg/m^3-d)

Date	HRT	Temp.	VS Load	TS in	VS in	VS in	TS out	VS out	VS out	VS red	pH	Alkalinity	Vol. Acids	Gas Prod.	Methane	CO_2
	days	Celsius	kg/ m^3-d	g/L	g/L	%	g/L	g/L	%	%		mg/L	mg/L	m^3/kg VS	%	%
8/12/1998	30		0.88	40.1	26.4	65.9	24.1	12.2	50.4	54.0	7.1	3030	120	1.0	63.4	36.5
8/13/1998	30	36	0.87	42.8	26.2	61.3					7.1					
8/14/1998	30	35.5	0.87	42.8	26.2	61.3					7.1					
8/15/1998	30	35.4	0.87	42.8	26.2	61.3					7.1					
8/16/1998	30	35.7	0.87	42.8	26.2	61.3					7.1					
8/17/1998	30	36.5	0.87	42.8	26.2	61.3					7.1					
8/18/1998	30	32	0.87	42.8	26.2	61.3					7.1					
8/19/1998	30	33	0.87	42.8	26.2	61.3					7.1					
8/20/1998	30		0.87	42.8	26.2	61.3	25.7	12.7	49.4	51.7	7	2970	96	1.0	61.4	38.6
8/21/1998	30	34.5	0.86	38.1	25.8	67.8					7.1					
8/22/1998	30	34	0.86	38.1	25.8	67.8					7.1					
8/23/1998	30	32	0.86	38.1	25.8	67.8					7.1					
8/24/1998	30	35.7	0.86	38.1	25.8	67.8					7.1					
8/25/1998	30		0.86	38.1	25.8	67.8										
8/26/1998	30		0.86	38.1	25.8	67.8										
8/27/1998	30		0.86	38.1	25.8	67.8										
8/28/1998	30		0.86	38.1	25.8	67.8										
8/29/1998	30	30	0.86	38.1	25.8	67.8	27.0	13.6	50.3	47.4	7.1			1.0		
8/30/1998	30	34	0.86	38.1	25.8	67.8					7.2					
8/31/1998	30	34	0.86	38.1	25.8	67.8										
9/1/1998	30	35.4	0.86	38.1	25.8	67.8					7.1					
9/2/1998	30	34.5	0.86	38.1	25.8	67.8					7.1					
9/3/1998	30	34	0.86	38.1	25.8	67.8					7.1					
9/4/1998	30		0.86	38.1	25.8	67.8						3130	108			
9/5/1998	30	35	0.94	41.3	28.1	68.1					7.1					
9/6/1998	30	34.8	0.94	41.3	28.1	68.1					7.2					
9/7/1998	30	35.6	0.94	41.3	28.1	68.1										
9/8/1998	30	35	0.94	41.3	28.1	68.1										
9/9/1998	30	35.8	0.94	41.3	28.1	68.1					7.1					
9/10/1998	30	35.8	0.94	41.3	28.1	68.1										
9/11/1998	30	35	0.94	41.3	28.1	68.1					7.1					
9/12/1998	30		0.94	41.3	28.1	68.1	26.5	13.7	51.8	51.1		2940	108	1.1		
AVG	**30**	**34.6**	**0.88**	**40.1**	**26.5**	**66.2**	**25.8**	**13.0**	**50.5**	**51.1**		**3018**	**108**	**1.0**	**62.4**	**37.6**

Appendix H (Operating Characteristics at Temperature = 35 °C, HDT = 30 days, and Organic Loading =1.80 kg/m^3-d)

Date	HRT	Temp.	VS Load	TS in	VS in	VS in	TS out	VS out	VS out	VS red	pH	Alkalinity	Vol. Acids	Gas Prod.	Methane	CO_2
	days	Celsius	kg m^3/-d	g/L	g/L	%	g/L	g/L	%	%		mg/L	mg/L	m^3/kg VS		
												as $CaCO_3$	as acetic acid	destroyed-d	%	%
11/18/1998	30	35.2	1.99	89.8	59.8	66.6										
11/19/1998	30	35.2	1.99	89.8	59.8	66.6										
11/20/1998	30	35	1.99	89.8	59.8	66.6										
11/21/1998	30		1.99	89.8	59.8	66.6	51.2	25.0	48.8	58.2	7	3460	204	0.7	58.9	41.1
11/22/1998	30		1.85	77.2	55.4	71.8										
11/23/1998	30	35	1.85	77.2	55.4	71.8										
11/24/1998	30	35.4	1.85	77.2	55.4	71.8										
11/25/1998	30	35	1.85	77.2	55.4	71.8										
11/26/1998	30	35	1.85	77.2	55.4	71.8										
11/27/1998	30	34.6	1.85	77.2	55.4	71.8										
11/28/1998	30	35	1.85	77.2	55.4	71.8										
11/29/1998	30	37	1.85	77.2	55.4	71.8										
11/30/1998	30	35	1.85	77.2	55.4	71.8										
12/1/1998	30	35	1.85	77.2	55.4	71.8										
12/2/1998	30		1.85	77.2	55.4	71.8	47.9	25.0	52.2	54.9		4050	288	1.0	58.8	41.2
12/3/1998	30	35	1.70	77.2	50.9	66.0					7					
12/4/1998	30	34.7	1.70	77.2	50.9	66.0										
12/5/1998	30	36.5	1.70	77.2	50.9	66.0										
12/6/1998	30	34.8	1.70	77.2	50.9	66.0										
12/7/1998	30	35	1.70	77.2	50.9	66.0										
12/8/1998	30		1.70	77.2	50.9	66.0	49.3	25.8	52.4	49.2		3750	552	1.1	55.5	44.5
12/9/1998	30	35.4	1.79	77.7	53.7	69.2										
12/10/1998	30	36	1.79	77.7	53.7	69.2										
12/11/1998	30		1.79	77.7	53.7	69.2										
12/12/1998	30		1.79	77.7	53.7	69.2										
12/13/1998	30		1.79	77.7	53.7	69.2										
12/14/1998	30		1.79	77.7	53.7	69.2	48.2	24.2	50.2	55.0		4660	264			
12/15/1998	30		1.79	77.7	53.7	69.2										
AVG	**30**	**35.3**	**1.82**	**79.1**	**54.7**	**69.2**	**49.2**	**25.0**	**50.9**	**54.3**		**3980**	**327**	**0.9**	**57.7**	**42.3**

Appendix H (Operating Characteristics at Temperature = 35 °C, HDT = 30 days, and Organic Loading =2.70 kg/m^3-d)

Date	HRT	Temp.	VS Load	TS in	VS in	VS in	TS out	VS out	VS out	VS red	pH	Alkalinity	Vol. Acids	Gas Prod.	Methane	CO_2
	days	Celsius	kg m^3/-d	g/L	g/L	%	g/L	g/L	%	%		mg/L	mg/L	m^3/kg VS		
												as $CaCO_3$	as acetic acid	destroyed-d	%	%
9/20/1999	30	35	2.4	117.5	72.8	62.0										
9/21/1999	30	34.8	2.4	117.5	72.8	62.0										
9/22/1999	30	34.5	2.4	117.5	72.8	62.0										
9/23/1999	30		2.4	117.5	72.8	62.0										
9/24/1999	30		2.4	117.5	72.8	62.0										
9/25/1999	30		2.4	117.5	72.8	62.0	71.9	35.1	48.9	51.8		5410	576	0.8		
9/26/1999	30		2.6	124.2	78.2	63.0										
9/27/1999	30	34.8	2.6	124.2	78.2	63.0										
9/28/1999	30	34.7	2.6	124.2	78.2	63.0										
9/29/1999	30		2.6	124.2	78.2	63.0										
9/30/1999	30	34.8	2.6	124.2	78.2	63.0										
10/1/1999	30		2.6	124.2	78.2	63.0										
10/2/1999	30		2.6	124.2	78.2	63.0	70.1	33.0	47.0	57.8	7.6	5100	384	0.7	58.8	42.2
10/3/1999	30		2.6	124.2	78.2	63.0										
10/4/1999	30	35	2.6	124.2	78.2	63.0										
10/5/1999	30	35	2.6	124.2	78.2	63.0										
10/6/1999	30	34.8	2.6	124.2	78.2	63.0										
10/7/1999	30	34.7	2.6	124.2	78.2	63.0										
10/8/1999	30		2.6	124.2	78.2	63.0	70.1	32.9	47.0	57.9		5140	960	0.8		
AVG	**30**	**34.8**	**2.6**	**122.1**	**76.5**	**62.7**	**70.7**	**33.7**	**47.6**	**55.8**		**5217**	**640**	**0.7**	**58.8**	**42.2**

Appendix H (Operating Characteristics at Temperature = 35 °C, HDT = 45 days, and Organic Loading = 0.90 kg/m^3-d

Date	HRT	Temp.	VS Load	TS in	VS in	VS in	TS out	VS out	VS out	VS red	pH	Alkalinity	Vol. Acids	Gas Prod.	Methane	CO_2
	days	Celsius	kg m^3/-d	g/L	g/L	%	g/L	g/L	%	%		mg/L	mg/L	m^3/kg VS	%	%
2/25/1999	45		0.86	51.8	38.8	75.0										
2/26/1999	45	34.5	0.86	51.8	38.8	75.0										
2/27/1999	45	35.4	0.86	51.8	38.8	75.0										
2/28/1999	45	35.2	0.86	51.8	38.8	75.0										
3/1/1999	45	35	0.86	51.8	38.8	75.0										
3/2/1999	45	35	0.86	51.8	38.8	75.0										
3/3/1999	45	36	0.86	51.8	38.8	75.0										
3/4/1999	45	34.8	0.86	51.8	38.8	75.0										
3/5/1999	45		0.86	51.8	38.8	75.0					7.5	4430	216			
3/6/1999	45	35.8	0.86	51.8	38.8	75.0										
3/7/1999	45		0.86	51.8	38.8	75.0										
3/8/1999	45	36	0.88	55.6	39.6	71.2										
3/9/1999	45		0.88	55.6	39.6	71.2										
3/10/1999	45	35	0.88	55.6	39.6	71.2										
3/11/1999	45		0.88	55.6	39.6	71.2										
3/12/1999	45	34.8	0.88	55.6	39.6	71.2										
3/13/1999	45		0.88	55.6	39.6	71.2	35.6	19.7	55.3	50.3	7.6	4860	276	1.0	60.2	39.7
3/14/1999	45	35	0.88	55.6	39.6	71.2										
3/15/1999	45	35.4	0.88	55.6	39.6	71.2										
3/16/1999	45	34.9	0.90	54.4	40.5	74.4										
3/17/1999	45		0.90	54.4	40.5	74.4										
3/18/1999	45		0.90	54.4	40.5	74.4										
3/19/1999	45		0.90	54.4	40.5	74.4										
3/20/1999	45	35	0.90	54.4	40.5	74.4										
3/21/1999	45	35	0.90	54.4	40.5	74.4										
3/22/1999	45	35	0.90	54.4	40.5	74.4										
3/23/1999	45	34.6	0.90	54.4	40.5	74.4										
3/24/1999	45	35.4	0.90	54.4	40.5	74.4										
3/25/1999	45	34.9	0.90	54.4	40.5	74.4										
3/26/1999	45	35.4	0.90	54.4	40.5	74.4										
3/27/1999	45		0.90	54.4	40.5	74.4	34.4	19.2	55.8	52.5	7.8	3500	228	1.1		
3/28/1999	45		0.89	55.4	40.0	72.2										
3/29/1999	45		0.89	55.4	40.0	72.2										
3/30/1999	45		0.89	55.4	40.0	72.2										
3/31/1999	45	34.5	0.89	55.4	40.0	72.2										

4/1/1999	45	35	0.89	55.4	40.0	72.2										
4/2/1999	45		0.89	55.4	40.0	72.2										
4/3/1999	45	35	0.89	55.4	40.0	72.2										
4/4/1999	45	34.7	0.89	55.4	40.0	72.2										
4/5/1999	45		0.89	55.4	40.0	72.2	34.9	19.9	57.0	50.3	7.7	4180	396	1.0	59.4	40.6
AVG	**45**	**35.1**	**0.88**	**54.2**	**39.7**	**73.4**	**35.0**	**19.6**	**56.0**	**51.0**		**4243**	**279**	**1.0**	**59.8**	**40.2**

Appendix H (Operating Characteristics at Temperature = 35 °C, HDT = 45 days, and Organic Loading = 1.80 kg/m^3-d

Date	HRT	Temp.	VS Load	TS in	VS in	VS in	TS out	VS out	VS out	VS red	pH	Alkalinity	Vol. Acids	Gas Prod.	Methane	CO_2
	days	Celsius	kg m^3/-d	g/L	g/L	%	g/L	g/L	%	%		mg/L	mg/L	m^3/kg VS	%	%
6/15/1999	45		1.63	106.5	73.4	69.0										
6/16/1999	45	35.0	1.63	106.5	73.4	69.0					7.4					
6/17/1999	45		1.63	106.5	73.4	69.0										
6/18/1999	45		1.63	106.5	73.4	69.0										
6/19/1999	45		1.63	106.5	73.4	69.0										
6/20/1999	45		1.63	106.5	73.4	69.0										
6/21/1999	45		1.63	106.5	73.4	69.0										
6/22/1999	45	35.0	1.63	106.5	73.4	69.0										
6/23/1999	45		1.63	106.5	73.4	69.0	57.9	30.6	52.9	58.3		5640	1764	1.0	63.3	36.7
6/24/1999	45	35.0	1.65	106.4	74.4	69.9					7.4					
6/25/1999	45		1.65	106.4	74.4	69.9										
6/26/1999	45	35.0	1.65	106.4	74.4	69.9										
6/27/1999	45		1.65	106.4	74.4	69.9										
6/28/1999	45	35.0	1.65	106.4	74.4	69.9										
6/29/1999	45		1.65	106.4	74.4	69.9										
6/30/1999	45		1.65	106.4	74.4	69.9	60.4	32.7	54.1	56.1		5960	1260	0.9	64.2	35.8
7/1/1999	45		1.69	114.0	76.0	66.7										
7/2/1999	45	35.0	1.69	114.0	76.0	66.7					7.4					
7/3/1999	45		1.69	114.0	76.0	66.7										
7/4/1999	45	35.0	1.69	114.0	76.0	66.7										
7/5/1999	45		1.69	114.0	76.0	66.7										
7/6/1999	45		1.69	114.0	76.0	66.7	58.6	30.8	52.5	59.5		5360	936	0.7	66	34
7/7/1999	45	35.0	1.72	120.7	77.2	63.9										
7/8/1999	45		1.72	120.7	77.2	63.9										
7/9/1999	45	35.0	1.72	120.7	77.2	63.9					7.6					
7/10/1999	45		1.72	120.7	77.2	63.9										
7/11/1999	45		1.72	120.7	77.2	63.9										
7/12/1999	45		1.72	120.7	77.2	63.9										
7/13/1999	45	35.0	1.72	120.7	77.2	63.9										
7/14/1999	45	35.0	1.72	120.7	77.2	63.9										
7/15/1999	45		1.72	120.7	77.2	63.9										
7/16/1999	45	35.0	1.72	120.7	77.2	63.9	61.0	31.6	51.8	59.0	7.4	5910	828	0.7	64.4	35.6
AVG	**45**	**35.0**	**1.67**	**112.3**	**75.3**	**67.2**	**59.5**	**31.4**	**52.8**	**58.2**		**5718**	**1197**	**0.8**	**64.5**	**35.5**

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