uOttawa

L'Université canadienne
Canada's university

FACULTÉ DES ÉTUDES SUPÉRIEURES ET POSTOCTORALES

uOttawa

L'Université canadienne
Canada's university

FACULTY OF GRADUATE AND POSDOCTORAL STUDIES

Ranya Sherif

AUTEUR DE LA THÈSE / AUTHOR OF THESIS

M.A.Sc. (Environmental Engineering)

GRADE / DEGREE

Program of Environmental Engineering

FACULTÉ, ÉCOLE, DÉPARTEMENT / FACULTY, SCHOOL, DEPARTMENT

Microaerobic Pretreatment of Extended Air Sewage Sludge for the Enhanced Destruction of Pathogenic Bacteria in Aerobic Digestion

TITRE DE LA THÈSE / TITLE OF THESIS

Dr. Kevin Kennedy

DIRECTEUR (DIRECTRICE) DE LA THÈSE / THESIS SUPERVISOR

Dr. Wayne Parker

CO-DIRECTEUR (CO-DIRECTRICE) DE LA THÈSE / THESIS CO-SUPERVISOR

EXAMINATEURS (EXAMINATRICES) DE LA THÈSE / THESIS EXAMINERS

Dr. Jason Zhang

Dr. R. Narbaitz

Dr. Ornita Basu

Gary W. Slater

Le Doyen de la Faculté des études supérieures et postdoctorales / Dean of the Faculty of Graduate and Postdoctoral Studies

MICROAEROBIC PRETREATMENT OF EXTENDED AIR SEWAGE SLUDGE FOR THE ENHANCED DESTRUCTION OF PATHOGENIC BACTERIA IN AEROBIC DIGESTION

Prepared By:

Ranya Sherif

Supervised By:

Wayne J. Parker and Kevin J. Kennedy

Thesis submitted to the
Faculty of Graduate and Postdoctoral Studies
In partial fulfillment of the requirements for the degree of

Master of Applied Science in Environmental Engineering

Ottawa-Carleton Institute of Environmental Engineering
Faculty of Graduate and Post-Doctoral Studies
University of Ottawa

Your file Votre référence
ISBN: 978-0-494-49278-9
Our file Notre référence
ISBN: 978-0-494-49278-9

Research Supervisor		**Civil Engineering** **University of Waterloo**
	Dr. Wayne Parker	
Co-Supervisor		**Civil/Chemical Engineering** **University of Ottawa**
	Dr. Kevin Kennedy	
Thesis Examiner		**Civil Engineering** **University of Ottawa**
	Dr. Roberto Narbaitz	
Thesis Examiner		**Chemical Engineering** **University of Ottawa**
	Dr. Jason Zheng	
Thesis Examiner		**Civil and Environmental Engineering** **Carleton University**
	Dr. Basu Ormeci	

Acknowledgements

The work presented here would not have been possible without the continued encouragement of my parents Aly Sherif and Fatma Maged. The opportunity to work on this research was made possible by my extremely knowledgeable and patient research supervisor Dr. Wayne Parker (University of Waterloo) who secured funding from both Environment Canada and the Ontario Ministry of Environment. Special thanks are accorded to Peter Seto from Environment Canada for his support of this project and also Mark Baker for assisting in coordinating transportation of frozen samples from Ottawa to various labs around Ontario. Dr. Kevin Kennedy (University of Ottawa) helped immensely in his co-supervision of the project by providing guidance in the scope of analysis and approach to writing the thesis.

The lab work would not have been possible without the assistance of Carleton University's Graduate Environmental Engineering Lab Technician, Marie-José Tudoret who not only helped in procurement of supplies but endured the high traffic of sludge that moved through the lab for over a year. All digesters and reactors used in the experiments were masterfully crafted by Pierre Trudel a valued and talented member of the support staff at Carleton's department of Civil and Environmental Engineering. The lab training and insights provided by (Dr.) Hamid Salsali who ran anaerobic digesters in the same lab during his doctorate research was invaluable in startup and understanding early results. I would also like to thank my colleague Laura Seaman for her assistance in procuring sludge weekly from the Rockland Treatment Plant and for feeding my reactors when I was ill or away.

This thesis is dedicated to the memory of my loving feline companion Missy who passed away while it was being written.

Abbreviations

AD	Anaerobic digestion
ATAD	Autothermal aerobic digestion
ATP	Aerobic thermophilic pretreatment
EPDM	Eethylene propylene diene monomer
CFU	Colony forming units
COD	Chemical oxygen demand
DO	Dissolved oxygen
HRT	Hydraulic residence time
LSD	Least squares difference
ORP	Oxidative-reductive potential
MPN	Most probable number
SBR	Sequencing batch reactor
SRT	Solids residence time
T_{90}	Time to kill 90% of population
TKN	Total kjeldahl nitrogen
TS	Total solids
TSS	Total suspended solids
VFA	Volatile fatty acid
VS	Volatile solids
VSS	Volatile suspended solids

Units of Measure

^{o}C	degrees Celsius (temperature)
CFU/mL	colony forming units/gram solids (microbial concentration)
d	days (time)
ft	feet (length)
h	hours (time)
mg/L	milligrams per litre (mass per volume concentration)
mV	millivolts (electric potential)
vvm	volume per volume per minute (flowrate)

Abstract

The inactivation of pathogens in sewage sludge reduces the risks of infection through contaminant pathways associated with handling and disposal. Domestic sludge sourced from a rural treatment plant was found to contain high levels of the indicator microoganisms *E. coli* and fecal coliforms and pathogenic bacteria *Salmonella* spp., *Shigella* spp., and *C. perfringens*. An effective and simple approach to enhance pathogen removal in a rural treatment setting was desired. Existing literature suggested that draw/fill staged configurations tended to yield better inactivation rates. Other literature suggested that the build-up of inhibitory compounds such as VFAs were detrimental to pathogens and that VFAs could be accumulated in higher temperature microaerobic conditions. The investigation of microaerobic pretreatment was trialed as a novel approach to staged digestion for improved pathogen removal. Microaerobic pretreatment of aerobically digested sludge improved inactivation of aerobic bacteria but the inactivation of persistent spores of *C. perfringens* were inconclusive.

Microaerobic pretreatment alone was investigated in three phases of the experiments and did not result in inactivation greater than one log reduction for any bacteria monitored. In Phase I where feed solids concentration was varied across four reactors, the lowest solids loading of 1.1% TS showed the best removal rates of pathogens. In Phase II, contact time was evaluated in terms of feeding frequency and residence time. It was found that less frequent feeding and longer residence times were more effective in removing pathogens as expected from the reactor kinetics and suggested by the literature.

The impact after digestion was found to be significant in Phase III for fecal coliforms, *E.coli, Salmonella* spp. and *Shigella* spp. It appeared that changes to the sludge matrix in microaerobic pretreatment improved digester performance in terms of pathogen removal.

Operating variables were monitored to gain an understanding of the factors impacting performance. Statistical analyses were performed at the 90% confidence interval to determine which if any factors differed significantly between systems and stages. The major findings were that mesophilic pretreatment (35°C) with air supplied at 0.06vvm yielded significantly higher ammonia levels after pretreatment than did pretreatment at ambient temperatures. This was hypothesized to account for the significantly greater extent of nitrification observed in downstream aerobic digestion over the effluents from digestion without pretreatment and those that were pretreated under ambient microaerobic conditions. Accordingly, the pH in those digesters was significantly lower than after digestion without pretreatment and after digestion with ambient microaerobic pretreatment. This enhanced depression in pH was hypothesized to account for the significantly enhanced inactivation in pathogens.

Table of Contents

List of Tables

List of Figures

1. Introduction

1.0 Problem Definition

The adequate treatment and disposal of sewage sludge is of great concern to public health and safety, especially in limiting the introduction of pathogenic organisms into the environment. Infected individuals within the community, animal waste, and also washings of food-borne pathogens introduce these organisms into the waste stream. There are several treatment technologies used to achieve sludge stabilization that involve both sanitation and adequate solids destruction to avoid vector attraction.

In urban centres, advanced treatment technologies such as anaerobic digestion (AD) and autothermal aerobic digestion (ATAD), are often used to effectively stabilize sludge. These technologies are economically feasible due to the availability of greater funding associated with the larger tax base. Both of these treatments operate at elevated temperatures, a factor that contributes to the reduction of pathogens in the sludge. These biological treatment methods require regular monitoring to remain operational given the sensitivity of the processes to reactor conditions. They are also demanding in terms of both capital and other operating costs.

In rural settings, however, less dense populations result in limited resources to fund public works including wastewater treatment. One major difference between an urban and rural wastewater treatment facility is the availability of operations staff to monitor and maintain the facilities. The challenge in rural facilities then becomes to design an effective system that can operate under minimal supervision. This makes the conventional yet effective sludge stabilization approach of AD or ATAD impractical due to their inherent sensitivity to process variables. It is for this reason that aerobic digestion

is often used in rural facilities since it is a more stable biological treatment method requiring less supervision, maintenance of equipment, and capital investment.

Aerobic digestion facilitates the endogenous decay of biomass under fully aerated conditions. It operates at solids retention times (SRT) of up to 40 days under continuous or semi-batch conditions with mixing typically provided by the air supplied. However, even under such long retention times, significant inactivation of pathogens is not necessarily achieved. This research proposes a method for the enhanced destruction of pathogens in rural domestic sewage sludge by means of microaerobic pretreatment. This approach was deemed promising since it requires minimal changes to existing systems and would not necessarily require excessive operator attention.

1.1 Research Objectives and Scope

The primary objective of this research was to assess the effectiveness of microaerobic pretreatment of rural domestic sludge for the enhanced destruction of pathogenic organisms at lab-scale. Monitoring indicator bacteria, such as fecal coliforms and *Escherichia Coli*, as well as human pathogens *Salmonella* spp., *Shigella* spp., and *Clostridium Perfringens*, assessed performance of this approach. Although other pathogenic organisms such as protozoans and helminthes exist, only bacteria were monitored in this study.

The pretreatment method was examined under various process conditions including feed concentration, feeding frequency, solids residence times, feed shear, and over the course of the seasons to assess both optimum conditions and system stability over time. To this end lab-scale reactors were run in semi-batch mode and monitored

over several months once they attained a pseudo-steady-state. Typical process variables such as solids content, oxidative-reductive potential (ORP), dissolved oxygen (DO), pH, temperature, alkalinity, nitrogen species, and fatty acids were monitored to gain an understanding of the abiotic factors and biological mechanisms that were operative within the systems. In this study, microaerobic conditions were defined as the intermediary zone between anaerobic and aerobic with measured values of ORP between -300mV and 100mV and DO values of less than 2mg/L.

The feed sludge was obtained on a weekly basis from a single source at the Rockland (Ontario) Wastewater Treatment Plant that employs extended air sequencing batch reactors for wastewater treatment. This source was assumed to be representative of extended air plants in general.

1.2 Thesis Organization

This study investigated the use of microaerobic pretreatment of aerobically digested sludge for enhanced pathogen destruction.

Chapter 2 provides a brief review of the literature focussed on the mechanisms that kill pathogens and staged sludge digestion research that focussed on pathogen destruction.

Chapter 3 presents the experimental design, constructed apparatus and methods employed for testing, monitoring, and evaluating the proposed treatment train. The results of the lab-scale examination that spanned over 12 months in three phases are presented and discussed in Chapters 4, 5, and 6 respectively.

Based on the results, in Chapter 7 conclusions are drawn and recommendations made for future process validation and implementation with a brief discussion of technical issues and risks. Finally, all raw data and output from statistical analyses are available electronically in the Appendices.

2. Literature Review

2.0 Introduction

The safe disposal or reuse of biosolids is imperative to public health and safety. There are many contaminants in wastewater sludge including pathogenic microorganisms, heavy metals, and endocrine disrupters that arise naturally or from anthropogenic sources such as personal care products. Land application of biosolids introduces contaminant fate pathways to groundwater by seepage, surface water by runoff, local food chains by vector attraction and directly into any food crops grown on the receiving soil. Many authors have identified health risks associated with applying both human sewage and animal waste on land in terms of the occurrence, survival, and infectivity of pathogens in the applied materials (Dumontet et *al.*, 1999).

Sewage sludge is in large part derived of fecal matter and hence may contain enteric pathogens that are excreted in feces and are infectious orally. Pathogens arise from human, pet and farm animal excreta; they can also arise from vegetable washings both domestic and commercial (EC, 2001).

In sewage, the type and quantity of pathogens varies depending on the health and size of the population in a given catchment area. In large communities, it is expected that a small percentage of people are host to endemic pathogens whose presence is expected at low levels in the sewage at any given time. However, in small communities, it takes fewer infected individuals to impact the quality of the sewage and therefore higher concentrations are expected. At the same time, in a healthy small community certain endemic pathogens may not appear in the sewage at all (EC, 2001). The presence of

hospitals, meat processing plants, and abbatoirs in the catchment area also affects the type and quantity of pathogens in sewage (EC, 2001).

Pathogenic microorganisms include certain bacteria, viruses, protozoa, yeasts, fungi, and helminthes. They are characterized by having adverse effects on human health. Typically, pathogens use products from their host's metabolic systems as nutrients and excrete wastes that are toxic (EC, 2001). Several pathogenic microorganisms that arise in sludge are listed in Table 2.1. The main endemic pathogens of concern include *Salmonella* and *Campylobacter* due to their predominance in cases of infection and enterohaemorragic *E. coli* (especially *E. coli* 0157:H7) which is potentially fatal although its presence is uncommon (Jones and Martin, 2003).

Adequate treatment technologies are required to attain pathogen reduction targets in sludge stabilization. Depending on the type of pathogen, destruction rates vary under different treatment conditions. For example, bacteria tend to live independently and multiply under favourable conditions but become compromised in sub-optimal growth conditions. However, certain types of bacteria such as *Clostridium* spp. form highly resistant spores that enhance their survival under harsh environmental conditions and thus are more difficult to remove (Bujoczek et *al*, 2001). Similarly, worms and protozoa produce eggs and cysts, respectively, that have increased resistance to stresses in their environment. However, these microorganisms cannot reproduce outside a host and so are found in low concentrations in sludge. Viruses also require a host in which to multiply but have been found to survive in harsh conditions. Given these variations, it is difficult to make statements on the overall pathogenicity of a sample based on enumeration of a single species (Dumontet et *al.*, 1999).

Table 2.1 Pathogenic microorganisms that may be found in sludge derived from fecal material (Adapted from EC, 2001)

Bacteria	**Protozoa**
Salmonella spp.	*Entamoeba histolytica*
Shigella spp.	*Giardia lambia*
Escherichia coli (enteropathogenic strains)	*Toxoplasma gondii*
Pseudomonas aeruginosa	*Sarcocystis*
Yersinia enterolitica	
Clostridium perfringens	
Clostridium botulinum	**Helminths**
Bacillus anthracis	*Taenia saginata*
Listeria monocytogenes	*Taenia solium*
Vibrio cholera	*Diphyllobothrium latum*
Mycobacterium spp.	*Echinococcus gramulosus*
Leptospira spp.	*Ascaris lumbricoides*
Campylobacter spp.	*Ancylostoma duodenale*
Staphylococcus	*Toxocara canis*
Streptococcus	*Toxocara cati*
	Trichuris trichura
Viruses	
Poliovirus	**Yeast**
Coxsackievirus	*Candida albicans*
Echovirus	*Candida krusi*
'New' enterovirus	*Candida tropicalis*
Adenovirus	*Candida guillermondii*
Reovirus	*Cryptococcus neoformans*
Hepatitis A-virus	*Trichosporon*
Rotavirus	
Astrovirus	**Fungi**
Calicivirus	*Aspergillus* spp.
Coronavirus	*Aspergillus fumigatus*
Norwalk-like calicivirus	*Phialophora richardsii*
Small round viruses	*Geotrichum candidum*
Parvovirus	*Trichophton* spp.
Adenoassociated viruses	*Epidermophyton* spp.
Influenza virus	

A comprehensive identification and enumeration of all pathogens in a diverse sludge matrix is extremely time and resource intensive and possibly risky. To overcome this, indicator organisms are often enumerated and assumed to represent the pathogenic populations. Indicator organisms should be present in high enough concentration to

ensure precise measurement, represent classes of microorganisms associated with contamination, be typically non-pathogenic and more robust than the most resistant pathogens present, and have means of simple, reliable, and precise quantification (Dumontet et *al.*, 1999). In conjunction with indicator microorganisms, human pathogens are also directly enumerated; typically by membrane filtration plate counts on selective media. Some indicator bacteria include fecal coliforms, fecal streptococci, and *E. coli*, (where certain strains such as 0157:H7 can also be pathogenic). Some human bacterial pathogens that are often enumerated are *Salmonella* spp., *Shigella* spp., and *C. perfringens* bacteria.

There exists controversy in the selection of appropriate microorganisms to monitor and regulate in order to assure sanitation of sludge. Specifically, Dumontet et *al.* (1999), cite several authors that view indicators such as fecal coliforms and fecal streptococci as inadequate for the assessment of sludge hygienicity. Several authors cited suggest the use of bacteriophage f2 as an indicator microorganism due to its high heat resistance (Dumontet et *al.*, 1999). However, mention of this indicator was not found in other literature. Another issue discussed in quantifying adequate sludge treatment is that of regulations that are based on maximum allowable concentrations of indicators versus those that are based on minimum concentration reductions. Presumably, an observed reduction indicates some action induced by treatment that would cause pathogen destruction. However, an observed low effluent concentration of indicator or pathogen taken on its own may be caused by the fact that it was not present in influent sludge due to catchment population variables. This could result in a multitude of other pathogens left untreated and unaccounted for. In general, measuring only one or two species as an

overall assessment of pathogenicity, as is often done, may be misleading since different types of pathogens can behave quite differently under the same conditions. Another shortcoming of many monitoring techniques is the apparent lack of replication both within sampling events and over time that would be required to have reasonable quantification given the high variability in biological systems and monitoring techniques.

The challenge in small communities is to meet pathogen inactivation targets with limited resources. This literature review examines the options available for pathogen reduction in sludge with the aim of identifying the technologies and the underlying mechanisms that kill pathogens.

2.1 Factors Affecting Pathogen Inactivation

There are several factors that affect the survival of pathogenic microorganisms in sewage sludge. In general, the destruction and inactivation of pathogens is caused by competition, depletion of nutrients, inhibition, and increased temperature (Ward et *al.*, 1999). EC (2001) reviewed four key parameters that affect the inactivation of pathogens including temperature, moisture, pH, and residence time.

2.1.1 Temperature

2.1.1.1 *Thermophilic Temperatures*

The most common approach to the inactivation of pathogens is to increase the process temperature to greater than optimal growth temperature for a certain period of time (EC, 2001). Metcalf and Eddy (2002) states that the thermophilic range spans 35-75°C with the optimum range between 55-65°C. The length of exposure required to

achieve inactivation depends both on the temperature and organism in question. Inactivation occurs once the temperature has increased beyond the thermal death point of the organism (Jones and Martin, 2003). The thermal death rates of certain common pathogens and parasites are listed in Table 2.2. EC (2001) reported that Strauch (1991 and 1998) developed a time-temperature graph (shown in Figure 2.1) for the complete inactivation of several enteric pathogens in sludge based on the work of Feacham et *al.*, (1983).

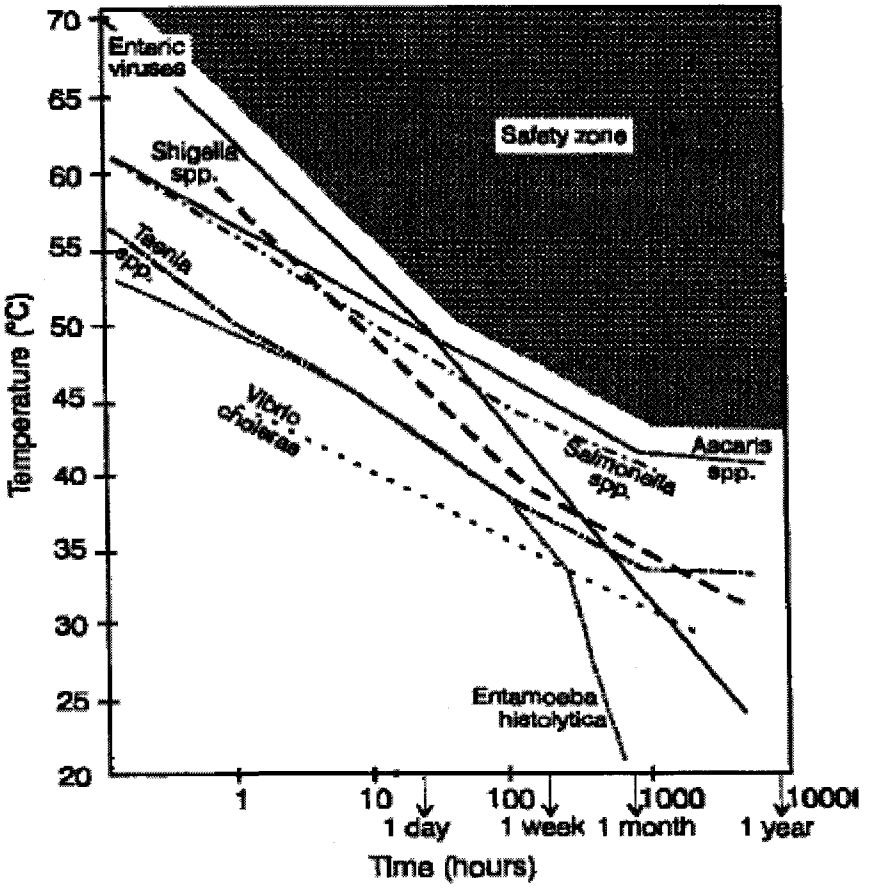

Figure 2.1: Time-temperature for the inactivation of various enteric pathogens in sludge (EC (2001) adapted from Strauch (1991 and 1998))

Dumontet et *al.* (1999) reported that at 50°C virus inactivation can reach 99.99% however the time required to attain this removal is not specified except for the poliovirus type I (0.13d). However, later in their report they state that thermophilic anaerobic sludge digestion can produce highly variable destruction rates of viruses ranging from 50 to 99%. For vegetative pathogenic bacteria it is reported that thermophilic AD residence times of 12-24h should result in adequately disinfected sludge; where the time to 90% destruction (T_{90}) values for *S. typhimurium, S. dublin, E. coli, S. aureus*, and *Erysipelotrix rhusiopathiae* are reported to be between 0.3 to 1.2h at 53°C.

In thermophilic aerobic digestion, it is reported that sludge can be sanitized when held 50°C for 23h, 55°Cfor 10h, or 60°C for 4h and virus populations can be reduced by 95% when held at 45°C for 5 days (Dumontet et *al.*, 1999).

Composting, which is applied to high suspended solids wastes, is a highly exothermic process that was reported to generate temperatures up to 82°C if left uncontrolled (Dumontet et *al.*, 1999) and can be effective for pathogen removal. However, this treatment technology is left outside of the scope of this review. The paper presented by Dumontet et *al.* (1999) provides a concise review of conclusions drawn by various authors regarding pathogen reduction on various treatment conditions. However, there was no critical analysis presented of methods used by the authors cited.

The minimum time-temperature relationships under USEPA regulation for Class A sludge are dependent on solids concentration where if the solids concentration of the sludge is 7% or greater,

$$D = (131.7 \times 10^6)/10^{0.14t} \qquad [2.1]$$

where D is time in days and t is temperature in °C (USEPA, 1999).

In this case the contact time should be at least 30 minutes and the temperature at least 50°C. Also, if sludge particle size is small and is heated by hot gas or immiscible fluid the heating time must be at least 15 seconds.

For cases where the solids concentrations of the sludge is less than 7% the the contact time should be at least 30 minutes and the temperature should be at least 50°C. If contact time is less than 30 minutes then equation 2.2 applies for sludges with solids content less than 7%.

$$D = (50.07 \times 10^{6})/10^{0.14t} \qquad [2.2]$$

EC (2001) reported that although the formulae agree with Strauch's graph at high temperatures the two diverge at low temperatures. This divergence is attributed to the fact that the USEPA developed a linear model, and used a logarithmic scale for time and incorporated a margin of safety. Figure 2.1 shows that the safety zone is bounded by an irregular curve that is neither directly proportional to time or temperature but rather related to the sensitivities of the microorganisms studied.

It should be noted that several of the minimum time requirements reported to meet USEPA regulation at various temperatures by EC (2001) do not actually agree with the calculated values.

Table 2.2: Temperature-time relationships at thermophilic temperatures for the inactivation of pathogens in sludge

Temperature	Time
70	7min[1], 30min[2], 1.3h (solids >7%)[3], 30min (solids <7%)[3]
65	30min[1], 6.3h (solids >7%)[3], 2.4h (solids <7%)[3]
60	2h[1], 31.5h (solids >7%)[3], 12.0h (solids <7%)[3]
55	15h[1], 4h [2], 2.6d (solids >7%)[3], 1d (solids <7%)[3]
50	3d[1], 13d (solids >7%)[3], 5d (solids <7%)[3]

[1] Feacham et *al.*, (1983) – inactivation of enteric pathogens (pure cultures)
[2] Carrington et *al.*, (1998) – inactivation of all pathogens (in sludge)
[3] USEPA (1999) requirements for Class A Biosolids (calculated)

High temperature alternatives such as thermophilic AD, autothermal aerobic digestion (ATAD), and composting have been found to successfully remove most pathogens from sludge (Dumontet et *al.*, 1999). However, resistant organisms such as the spores of *Clostridia* spp. and the cysts and eggs of protozoa and helminthes may persist. The above treatment technologies are not necessarily compatible with the available resources in small communities.

2.1.1.2 *Mesophilic Temperatures*

At mesophilic temperatures (30-35°C) a moderate amount of pathogen reduction is expected according to Strauch's graph (Figure 2.1). However, it is more likely that other effects such as acid formation, the presence of antagonistic compounds such as ammonia, and predation will have a greater effect on inactivation during mesophilic (anaerobic) digestion. In this temperature range the differences in pathogen survival time, sludge characteristics, and treatment types make it impossible to set out consistent

time-temperature relationships for complete pathogen inactivation. Other factors tend to affect pathogen inactivation at mesophilic temperatures and are discussed later.

According to the review by Dumontet et *al.* (1999), mesophilic AD can inactivate 50-99% of viruses in treated sludge. Batch AD was reported to perform better in reducing several bacterial pathogens than semi-batch processes but *C. jejuni* was unaffected by mesophilic AD in either case. They reported that other bacteria such as *Salmonella* spp., *C. perfringens*, and *Bacillus cereus* were commonly found in anaerobically digested effluents. To achieve similar pathogen destruction as thermophilic treatment much longer residence times are required.

Ponugoti et al., (1997) studied 12 facilities in the US over the course of a year and found that mesophilic AD (2.7, 1.87, and 1.51 log reductions) was superior to aerobic digestion (0.68, 0.89, and 0.68 log reductions) for removal fecal coliform, fecal streptococci, and *Salmonella* spp. respectively. It was reported that other authors found aerobic digestion tended to result in one log reductions of total enteric bacteria and that total coliforms were the most easily removed bacteria while fecal streptococci were resistant to destruction. In AD, fecal coliforms and fecal streptococci removal increased with solids loading rate within 0.64 to 1.60 $kg/m^3/d$ for standard-rate AD and 1.60 to 3.20 $kg/m^3/d$ for high-rate AD. However, *Salmonella* spp. did not show this trend. Fecal coliform removal was typically one log reduction higher than fecal streptococci.

2.1.2.3 *Psycrophilic Temperatures*

Similarly to the mesophilic range, under ambient conditions, temperature is not expected to play a major role in the inactivation of pathogens in sludge. In fact, colder temperatures may lead to the prolonged presence of pathogens in sludge. Jones (1976) reported that *Salmonella* spp. survival was greatest at 10°C in cattle slurries containing more than 5% solids.

At low temperatures, it is more likely that the production of fatty acids during stabilization and storage, and predation by other micro-flora such as protozoa would contribute more significantly to the destruction of pathogens. Few authors have focused on the destruction of pathogens at low temperatures. Singh et *al.*, (1994) assessed the degree of pathogen destruction in rabbit waste dosed with *E. coli, S. typhi, and S. aureus* under anaerobic and aerobic conditions at low temperatures (at 10, 20, and 37°C and at 5, 10, and 20°C respectively). Batch tests were performed where the aerobic samples were inoculated with *Arthrobacter* sp. isolated from Antarctica that showed growth between 0-25°C, optimum at 20°C. The T_{90} values for the pathogens monitored were reported and found to decrease with increased temperature (5-20°C) from 5.7 +/- 0.4d to 4.6 +/- 0.3d for *E. coli*, 10.2 +/- 0.3d to 7.8 +/- 1.0d for *S. typhi*, and 10.0 +/- 0.3d to 7.0 +/- 0.2d for *S. aureus* where the mean was reported about standard error for n = 4.

Under USEPA regulation there is only one process approved for the production of Class B sludge at ambient temperature – air drying (USEPA, 1999). This process is subject to several constraints. Essentially partially digested sludge would be applied to underdrained beds to a depth of at most 23 cm for a minimum drying period of 90 days

where the average daily temperature was required to be above freezing for at least 60 days during which they could not be covered in snow. This treatment at ambient temperature is estimated to yield a single log reduction of pathogens.

It is expected that at ambient temperatures, pathogenic bacteria adapted for growth in host tissues, such as *Salmonella* spp. and E. *coli*, will not multiply but rather will tend to decline (Jones and Martin, 2003). However, under certain conditions the growth of both *E. coli* and *Salmonella* spp. has been found to occur in previously sanitized compost (Jones and Martin, 2003). This draws attention to the importance of efficient and effective stabilization and sanitation of sludge prior to reuse to avoid pathogen regrowth as much as possible.

At ambient temperatures other factors such as extended residence time and inhibition by pH and volatile fatty acids are expected to play a more important role. This presents an opportunity for the adequate destruction of pathogens at small treatment plants where resources are limited. Conventional low-cost options that are operated at ambient temperature include extended aeration and oxidation ditch plants.

2.1.2 pH

The pH conditions in sludge treatment tend to negatively affect pathogens as they move to more extreme levels from neutral. The practice of liming or raising the pH level of sludge above 12 using lime has been found to inactivate pathogens in sludge by at least 2 log reductions. In lime sanitation pathogens are assumed to be inactivated when maintained for at least 2 hours at a pH of 12 (Dumontet et *al.*, 1999). This is in part caused by the heat generated in the lime-sludge mixture (EC, 2001). The combined

effects of high temperature (60-70°C) and pH (12) for 24h could also reduce resistant *Ascaris* ova (Strauch, 1998).

However, Parmar et *al.* (2001) found in sludge samples held for 24h at an ambient temperature of 23°C that *Salmonella* spp. were still present in a concentration of $4x10^5$CFU/100mL after 24h at pH 12 with lime though fecal coliforms were not detected; and both fecal coliforms and *Salmonella* spp. were present in concentrations of $2x10^5$ and $2x10^6$CFU/100mL respectively when the pH was raised with sodium hydroxide (initial concentrations were $4x10^8$ and $1.5x10^9$CFU/100mL respectively). Similar results to those of liming at 23°C were observed when the temperature was held at 50°C and pH at 12 for 3 hours. At 60°C, neither coliforms nor *Salmonella* spp. were detectable when held for 3h at pH 10 or at pH 12. The detection limit was based on an unusually high (10^7) dilution in this study, however the volumes used in the membrane filtration technique were not reported so it is difficult to determine the minimum reportable concentration. These results were presented as control data for the examination of enhancement of pathogen reduction by using alkaline protease enzymes at high pH. In one phase of the study the impact of 0.03% alcalase dosing at different pH levels (7, 10 and 12) held for 4 days at 40°C was assessed. Pathogen concentrations for "MPN", coliforms (assumed total based on media used), and *Salmonella* spp. were reported. It is assumed that "MPN" referred to the most probable number of heterotrophs however this was not defined by the author and is left out of this discussion. The authors state that alkaline enzyme "accelerates removal of pathogens from sewage sludge" which overstates the less than one log reduction reported for *Salmonella* spp. at pH 10 and the growth observed with alcalase at pH 12 over the control under these conditions.

However, at neutral pH a four log reduction was observed for alcalase-dosed sample which was more than the sample held at pH 10 under the same conditions without enzyme but less than that held at pH 12 without enzyme. The coliform concentrations for both the controls and enzyme treated samples were all below the detection limit except for the neutral alcalase dosed sample which resulted in a one log reduction below the original concentration.

Strauch (1998) reported that sludge raised to a pH of 12.6 should be stored for at least 3 months for complete pathogen removal. Bujoczek et *al.*, (2001) examined the effects of lime dosing, temperature, and storage time on the survival of fecal coliform bacteria, *Salmonella* spp. bacteria, and *C. perfringens* spores. They reported that the inactivation of *C. perfringens* spores was found elsewhere at the University of Tulane to behave similarly to *Ascaris* eggs and as such is a good candidate for modeling *Ascaris* die-off. It was also reported that the containment of sludge in long-term storage was important to prevent the volatilization of free ammonia evolved from lime thereby maintaining high pH throughout storage. Under contained conditions, fecal coliforms and *Salmonella* spp. were completely inactivated after one day below the detection limit when dosed with 20g CaO/kg TS and did not grow back in either long-term storage (6 to 9 months) at 20-22°C or 4-6°C. However, higher doses of 120g CaO/kg TS at 20-22°C and 80g CaO/kg TS at 4-6°C were required to inactivate more persistant *C. perfringens* below detection limits using standard media. Parallel testing with lyzozyme enriched media indicated that injured spores were never completely removed under the same conditions.

Pathogens have been found to decline at depressed pH associated with VFA production. Jones (1982) reported a 90% decline in *Salmonella* population in cattle slurry during the first 2 to 4 weeks of storage corresponding to a period of VFA production and pH decline from 7.5 to 6.5.

Fukushi et *al.*, (2003) investigated the survival of *Salmonella* spp. in acid-phase AD. They reported in the review portion of their research that the optimum pH for *Salmonella* spp. growth ranges from 6.2-7.2 and that significant inhibition occurs for *S. typhimurium* occurs below pH 4. The study aimed to separate the impact of pH and organic acid content in acid-phase digestion. Batch reactors at 37°C were fed with secondary sludge with an initial concentration of *Salmonella* spp. on the order of 10^5 MPN/100mL and volatile solids concentration of 5500mg/L. Samples were either spiked with various amounts from 0-9306mg/L of organic acids (acetic, propionic, and butyric acids) or pH adjusted to levels between 3.5 and 5.5 with sodium hydroxide and hydrochloric acid with a fixed amount of organics acids added (9306mg/L).

It was found that when pH was initially adjusted to pH 5.5 and organic acids added that the pH tended to increase slightly over time likely due to consumption of organic acids. *Salmonella* spp. tended to decrease with time but no obvious correlation between acid concentration and inactivation was observed. It took approximately 10 days to completely inactivate *Salmonella* spp. below detection limits. In fact the control where no acid was added achieved levels below detection limit on the eighth day.

In the second part of the study where pH levels were below 5.0 with initial organic acid concentrations of 9306 mg/L, *Salmonella* spp. were inactivated below

detection limits in 2d. However, at pH 5.5 it took 5d to inactivate *Salmonella* spp. This time did not agree with similar conditions tested in the first part of the study where it took 10d to inactivate *Salmonella* spp. at the same pH and organic acid concentration. However, no discussion was provided on this point.

Salsali et *al.*, (2006) found in a well-controlled experiment that the survival of *Salmonella* spp. in anaerobic digesters depended on pH, temperature, and chain length of volatile fatty acids. Digester effluents from reactors run at different temperatures (35, 42, and 49°C) were pH adjusted to 5.5 and dosed with mixed acids, and individual acids (acetic, propionic, and butyric) in the range of 0-6000mg/L, respectively. The samples were incubated for 24h and sampled over time to investigate the effect of temperature and fatty acid concentration on the inhibition of *Salmonella* spp. It was found that mixed acids had the highest inhibition at all temperatures but more so at the highest temperature. In general acetic acid showed the next highest inhibition followed by propionic, then butyric acid within each temperature group and higher temperatures increased inhibition for each acid tested. This effect was explained by the weakening of the cell membrane at higher temperatures and the potential for shorter chain toxic fatty acids to be more readily transported across cell membranes leading to the inihibition of *Salmonella* spp. It was stated in the paper that because the background concentration of organic acids in the highest temperature effluent was higher than the lowest acid dose level (750mg/L) that no additional acid was added to these samples leaving the reader to conclude that it should behave similarly to the control for that temperature. However, whereas the control declined moderately, the data presented showed that the *Salmonella* spp. concentration

decreased below the method detection limit for the 750mg/L samples for both the mixed and acetic acid groups. This anomaly was not discussed in the paper.

The effect of pH was examined in the second part of the study by Salsali et *al.*, (2006), where AD effluents from the same three temperatures were dosed with a fixed amount of mixed acids (750 mg/L) and pH adjusted to 4.5, 5.5, 6.5, and 7.5, respectively. In the mesophilic group (35°C), pH levels of 4.5 and 5.5 led to a decline of *Salmonella* spp. but growth was observed for pH levels of 6.5 and 7.5. No control (without added fatty acid) was presented so it is unclear if this effect was caused by pH or the added fatty acids. However, the control for pH 5.5 from the previous phase of the study did not show any significant decline in the mesophilic range. Also, the values for the mixed acid dosing 750 mg/L agreed for the mesophilic temperature range.

In the slightly thermophilic group (42°C), pH levels of 4.5, 5.5, and 7.5 had a significant negative impact on *Salmonella* spp. concentrations where the latter pH decreased concentrations below the detection limit; growth was observed after 24h at pH 6.5. It was hypothesized that at pH 7.5 and 42°C, conditions favourable to the growth of *Klebsiella* spp., fermentation of longer chain fatty acids into more toxic shorter chain fatty acids by that species may have occurred leading to greater inhibition of *Salmonella* spp. However, further study would be required to confirm this hypothesis.

Similarly to the mesophilic data, it was unclear whether the effect was caused by the added fatty acids or simply as a result of pH. However, the control data presented earlier in the paper for the pH 5.5 samples at the same temperature indicated a lesser decline in *Salmonella* spp. compared to the sample dosed with 750mg/L of mixed acid in

the first phase of the study (which agreed well with the data presented in the pH study for those conditions).

In the thermophilic group (49°C), *Salmonella* spp. concentrations declined for all pH levels tested. However, the data for pH 5.5 at 49°C with 750mg/L of mixed acids presented in this phase indicate a decline from approximately 5000CFU/100mL to approximately 3700CFU/100mL as read from the graph presented while the same conditions presented in the earlier phase of the study show a decline to below the detection limit (1000CFU/100mL). The only apparent difference in the two scenarios is that in the first phase, the high temperature mixed acid sample was not dosed with organic acids at the 750mg/L level, whereas according to the method described it was in the subsequent pH study. However, this would imply higher acid concentrations in the second part of the study and therefore increases rather than reported decreases in *Salmonella* spp. inactivation were expected. The inactivation observed in the second part of the study are similar to the control data presented for pH 5.5 at 49°C making it difficult to distinguish between the effect of temperature and the background concentration of fatty acids (reported as 853mg/L).

Overall, the second part of the study showed conclusively that *Salmonella* spp. were negatively effected by pH levels less than neutral, more so at higher temperatures.

In a similar study Singh et *al.*, (1994) found that whereas acetate had no inhibitory effect on *E. coli, S. typhi*, and *S. aureus*, propionate, butyrate, valerate, and mixed acids showed similar inhibition to approximately 50, 70, and 50% of the control populations of the respective bacteria when dosed at 4000mg/L and held at 20°C for 72h. The effect of

sulphide was determined to have the greatest inhibitory effect at the same concentration and conditions reducing *E. coli*, *S. typhi*, and *S. aureus* to 20, 10 and 7% of the concentrations measured in the control. In this study, pure cultures of pathogens in sterile nutrient broth were dosed with different fatty acids and sulphide and incubated for 72h at 20°C. The pH of the samples was not reported. The study aimed to identify the abiotic factors that contribute to pathogen inactivation in AD.

Surampalli et *al.*, (1994) found that the pH of sludge decreased during aerobic treatment (and in storage). These decreases were attributed to nitrification and carbon dioxide production. The endogenous decay of microorganisms in aerobic conditions results in the oxidation of cell tissue to carbon dioxide, water, and ammonia. It was reported that with sufficient oxygen and alkalinity, nitrifiers oxidize the available ammonia to nitrite and nitrate causing a drop in pH when there is insufficient alkalinity to buffer the sludge.

2.1.3 Residence Time

In both aerobic and anaerobic thermophilic processes where complete inactivation of pathogens occurs within hours and sludge stabilization takes several days, under these conditions the effect of residence time on pathogen inactivation becomes secondary to the required stabilization criteria. However, in mesophilic and ambient temperature treatment the effect of residence time on pathogen destruction becomes important. As previously noted, in these temperature ranges other effects such as the presence of fatty acids and other compounds that promote pathogen inactivation are the mechanisms of interest. When dealing with batch or plug-flow regimes, the afore described time-

temperature relations hold. However, for semi-batch operation where new material is introduced with a corresponding volume removed often daily, the possibility of removing sludge that has only been in treatment since the last feeding time is more probable. Operationally this means that it is important to remove treated sludge prior to feeding to avoid short-circuiting.

Farrell et *al.*, (1988) quantified the importance of a "draw and fill" approach as opposed to a "fill and draw" approach to maximize anaerobic digester effluent quality in terms of indicator bacteria such as fecal streptococci and total and fecal coliforms. They found that although feeding procedure in a semi-batch operation fed daily had little impact on variables such as solids and COD destruction, the draw/fill approach increased removal by 1-2 log reductions for the indicators measured.

For semi-batch treatments, the fraction of surviving pathogens in the effluent from treatment can be predicted as follows,

$$C/C_0 = R / (10^{DR\theta} - 1 + R) \qquad [2.3]$$

C/C_0 is the fraction surviving,
R is the fraction of the reactor content replaced each day,
$R\theta$ is the interval between feeds (expressed as days) and
D is the decimal decay rate in d^{-1}

EC (2001) gives an example of how this would effect the log reductions of a hypothetical pathogen during a range of mesophilic anaerobic digestion scenarios system with a decimal decay rate of 1 d^{-1} as shown in Table 2.3.

Table 2.3: Examples of log10 reduction of numbers of a hypothetical pathogen during a range of mesophilic anaerobic digestion situations (Adapted from EC, 2001)

Interval Between Feeds (h)	log_{10} reductions with 12 day HRT	Log_{10} reductions with 15 day HRT
2.4	0.61	0.69
24	2.04	2.10
72	4.01	4.18

This example illustrates that less frequent feeding for similar retention times leads to greater inactivation of pathogens.

Ponugoti et al., (1997) looked at the impact of residence time on fecal coliforms, fecal streptococci and *Salmonella* spp. in anaerobic and aerobic digestion by comparing measured concentrations from several full-scale facilities operating under different conditions. It was found that in AD, residence time had a positive effect on fecal coliforms where log reductions increased from 2.3 logs at 22 days up to 2.9 logs at 32 days HRT; similarly, fecal streptococci reductions increased from 1.45 logs at 22 days to 2.2 logs at 32 days. However, *Salmonella* spp., only improved slightly with residence time and was about 0.9 log reductions in AD. A similar analysis was performed for aerobic systems however, only fecal streptococci improved from 1.0 logs at 10 days to 1.4 logs at 90 days. For the same range of HRTs, fecal coliform removal was approximately 0.7 logs and *Salmonella* spp. removal was 0.8 to 0.9 logs.

2.2 Staged Treatment Options

Several staging approaches have been trialed mainly for the improvement of AD performance in terms of biogas production, solids, and pathogen destruction. With the exception of staging ATADs little work has been performed on introducing a

microaerobic stage in aerobic digestion. Some authors have investigated the staging from anaerobic to aerobic conditions which may show some similarities to microaerobic pretreatment of aerobic sludge given the transition from low negative to positive ORPs.

In recent years staged digestion has become a strategy increasingly researched and used to improve digestion performance and biogas production. Various configurations are used that stage oxidation states and/or temperatures to achieve desired results. Although many papers explore the impact of staging on effects such as biogas and dewaterability, there are fewer that focus on pathogen destruction.

In the case of AD, staging typically involves a short residence time, thermophilic, acid-phase digester followed by a longer residence time mesophilic methanogenic phase digester. This strategy has been found to decrease the total residence time required to meet VS destruction requirements (Schafer and Farrell, 2000).

Lee et *al.*, (1989) evaluated the impact of two-stage AD on enteric pathogen and virus destruction versus single-stage digestion at two different temperatures (35 and 53°C) and residence times for a total of four different conditions. The first stage was an acid phase digester that promoted the formation of fatty acids with a 1d residence time. The longer second stage (9 or 19d) served to destroy volatile solids and produce biogas. Feed sludge was a 2:1 mixture of waste activated to primary sludge from a municipal wastewater treatment facility of a large American city (Cincinatti, Ohio). Reactors were fed with a semi-batch draw-fill approach to minimize short-circuiting. Pathogen concentrations were determined in 8 sampling events over 8 weeks for each set of conditions for fecal coliforms, *E. coli*, fecal streptococcus, and enterovirus using

membrane filtration techniques. *Ascaris* ova die-off was also measured for thermophilic samples where the anaerobic digesters were spiked once with *Ascaris* eggs to obtain high enough concentrations for meaningful results. This study had a well-formed experimental plan with sufficient data to draw statistically meaningful results.

It was found that VS destruction was similar in staged versus conventional anaerobic digestion at the respective temperatures and residence times which did not agree with previous research cited. However, there was no detail provided on the feed sludge composition in the previous studies cited which is known to affect VS destruction. Slightly higher VS destruction was observed in thermophilic AD versus conventional for the conventional conditions and for the 20d residence time two-phase system. Interestingly, the VS destruction jumped from 3% to 10-12% in the acid phase digester when switching from mesophilic to thermophilic temperatures. The author concluded that acid-forming bacteria were activated in the higher temperature acid-phase digester versus the lower temperature acid-phase digester to account for the difference in VS destruction. However, there was no significant difference found between the acid concentrations at either temperature for the respective residence times. Based on the data presented, it is more likely that the higher temperature affected the kinetics of the general bacterial population resulting in an acceleration of the rate of destruction of easily degradeable solids.

In the mesophilic anaerobic conventional and staged digesters no significant difference at the 95% confidence level was found between the 10 and 20d SRT reactors for removal of FC, EC, and FS respectively. However, the log reductions after staged digestion was significantly higher than for conventional (increased by 0.5 for FC and EC,

and 0.9 for FS at 20d SRT). This was explained by the expected difference in change of concentration between completely mixed reactors in series versus a single stage completely mixed reactor.

An interesting approach was used by Lee et *al.*, (1989) to compare conventional and staged operation. First-order decay coefficients were calculated for the conventional mesophilic anaerobic conditions and used to calculate the expected reduction at the SRTs of the acid phase and methanogenic phase digesters. They were found in general to underestimate reduction in the acid phase digester and overestimate reduction in the methanogenic digester indicating that the acid phase accelerated inactivation of the more susceptible fraction of the populations measured leaving the hardier fraction to be less effectively removed downstream.

A similar comparison at thermophilic temperatures was not performed because many averages included values less than the detection limits, which would have skewed the data. It was found that acid-phase digestion resulted in log reductions greater than 4 for FC and EC and 3.6 and 3.9 log reductions for FS for 10d and 20d SRTs respectively. However, similar results were observed for conventional thermophilic AD. The log reductions observed for FC under thermophilic anaerobic conditions were similar to those observed by Zabranska et *al.*, (2003) at full scale where they found 4-5 log reductions for thermotolerant coliforms. However, in that study the SRT of the first stage (55°C) averaged 6.7d and the total residence time averaged 18.6d.

Another approach to AD enhancement involves an initial thermophilic aerobic stage (Pagilla et *al.*, 1996; Ward et *al.*, 1998; Hasegawa et *al.*, 2000). In a pilot-scale

study Pagilla et *al.*, (1996) examined the impact of aerobic thermophilic pretreatment (ATP) at 42°C for 1 day prior to AD (14 days) on pathogen destruction and *Norcadia* control; the destruction of fecal coliforms and *Salmonella* spp. was compared to full-scale anaerobically digested sludge from the same source. ATP was found to improve pathogen removal over AD alone by up to 3 log reductions of fecal coliforms from 10^6MPN/g TS to less than 10^3MPN/g TS where the initial feed concentration was 10^7 to 10^8MPN/g TS. The measured *Salmonella* spp. concentrations showed reductions from 3MPN/4g TS to less than 1MPN/g TS for both ATP and AD (alone) treated samples. In this study, VFA concentrations increased threefold from approximately 1000mg/L in the feed sludge up to 3000 mg/L. Unfortunately, details on process variables such as aeration rate, DO, or ORP in the ATP system tested were not provided in this study.

In a similar study Ward et *al.* (1998) examined the effect of ATP on AD by feeding two lab-scale semi-batch anaerobic digesters with feed sludge from before and after a full-scale ATP reactor with a 1d HRT operated at 65°C. They found that ATP improved fecal coliform removal by up to 4 log reductions after AD. The AD retention times tested ranged from 4-14d however the retention time for which the fecal coliform data were presented was not stated.

Hasegawa et *al.* (2000), investigated the impact of aerobic (0.4 vvm air and 1 to 3mg DO/L) and microaerobic (0.08vvm and 0-0.1mg DO/L) with thermophilic bacteria on AD with a focus on the attainable improvements to solids destruction and biogas production. A thermophilic bacteria was isolated and injected into feed sludge that was pretreated for 24h at temperatures ranging from 50°C to 80°C. It was found that VFAs could be accumulated under microaerobic conditions from 500mg/L after 1 day and up to

2200mg/L after five days – it was not clear from the paper at what temperature these observations were made. VFAs were not appreciably accumulated under aerobic conditions.

Several authors have researched autothermal aerobic digestion (ATAD) for improved digestion performance (Kelly et *al.*, 1993; Chu et *al.*, 1994; Mavinic et *al.*, 2001; Zhou and Mavinic, 2003). Kelly et al., (1993) found that ATAD is a viable technology to produce Class A (USEPA, 2003) biosolids for communities of 2 000 to 10000 people. Three demonstration-scale facilities were monitored where each system comprised of 2 reactors in series. The process operating variables were well defined in this study. Although not explicitly stated, these reactors appeared to be operating under microaerobic conditions with ORPs ranging from –350mV to 100mV, however the DO in these reactors was not reported. No literature was found on the impact of microaerobic pretreatment on conventional aerobic digestion. However, Arunachalam et *al.*, (2004) explored the impact of low DO conditions on pH, solids digestion, and fecal coliform removal on aerobic digestion. Several experiments were run and data presented in such a way that it was difficult to associate the results with the methodology used. The conclusions drawn did not necessarily compare fecal coliform removal between systems as much as they stated that low DO concentrations provided adequate conditions to meet Class B (USEPA, 2003) sludge requirements for pathogens (2000000 MPN/g TS).

2.3 Summary

In general there is a good deal of literature that describes the factors that affect pathogen removal in sludge digestion such as temperature, pH, residence time, and

feeding techniques. In general there is more literature available on the topic of AD likely due to the opportunity for economic benefits that can be gained from biogas evolution. The expected pathogen inactivation performance is well defined in conventional systems such as AD, aerobic digestion, and ATAD. However, the impact of various other staging configurations is a topic of increased exploration. Currently, there is a lack of research that specifically focuses on the impact of microaerobic pretreatment prior to conventional aerobic digestion. From the literature, it is expected that inhibitory conditions such as elevated VFA production under microaerobic conditions may enhance pathogen removal.

3. Experimental Methods

3.0 Purpose

The purpose of the experiments run was to determine the impact of sludge solids concentration, feeding frequency, hydraulic residence time (HRT), pretreatment temperature, and feed shearing on the performance of microaerobic pretreatment prior to aerobic digestion on pathogen destruction. The experiments were run in 3 phases. Phase I and II tested pretreatment only and Phase III tested the full treatment train including pretreatment and subsequent aerobic digestion.

3.1 Apparatus

Four pretreatment columns were constructed and run in semi-batch completely mixed mode, under microaerobic conditions. In Phase III, five fully aerobic completely mixed digesters were constructed, four of which received pretreated sludge while the fifth served as a control and received SBR sludge that was not pretreated. The overall treatment train is depicted in Figure 3.1. The design, construction, and operation of these reactors are further described below.

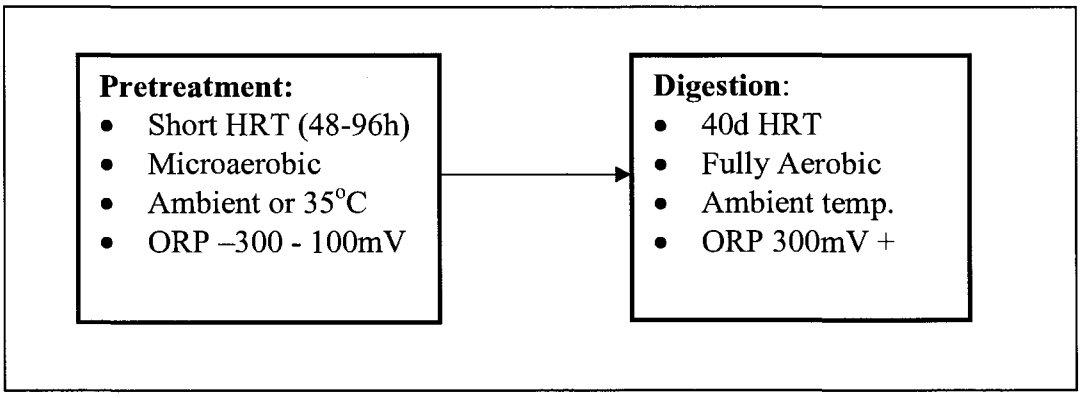

Figure 3.1: Microaerobic pretreatment to aerobic digestion treatment train

3.1.1 Microaerobic Pretreatment Columns

3.1.1.1 *Design Considerations*

The pretreatment reactors were designed to optimize the transfer of oxygen into lab-scale semi-batch completely mixed sludge digesters. It was also desired that the temperature, air supply, and short HRT be controlled variables in the experiments.

Oxygen transfer is most efficient with fine bubbles (as opposed to coarse bubbles with lower surface area to volume ratios) in deep columns of water that provide increased surface area and contact time for mass transfer. Therefore, tall and thin columns were employed to minimize the volume required and maximize the depth of the reactors. In this case, smaller reactor volumes made the study of short HRT practical by decreasing the amounts of feed sludge required. Aquarium air stone diffusers were found to deliver small volumes of fine air bubbles in water though they required regular replacement due to solids build-up on the stones when used in sludge as observed during regular inspections.

To allow semi-batch operation, inlet and outlet ports were provided. As the airflow into the reactors was intentionally small and thus insufficient for complete mixing, external mixing was provided. Also, as odour emissions in the lab were of concern, an off-gas capture system was designed to allow venting to the exhaust pipes in the lab.

3.1.1.2 *Reactor Design Summary*

The pretreatment columns were made from 10.2 cm (4" nominal) diameter SDR35 sewer grade PVC pipe and were 1m in height with 6L working volumes. The columns were heat traced with 6ft of variable wattage heating cable and insulated with 2 layers of reflective insulation. The temperature in each column was regulated with Precision Microcontroller Digital Temperature Controllers that were connected to Type K

thermocouples. Temperature probes were sealed in place with silicone at the mid-height of the columns.

The columns were aerated with air that was provided by a TetraTek 96 deep water pump and was filtered with Penn Plax check-valve air filters and regulated with rotameter style flowmeters. The airline entry was from the top of the column where it was secured in place with four 130 mm (½"nominal) diameter, 2.5cm long PVC tubes glued along the inside of the column to avoid interference with the mixing shaft and paddles. A single cylindrical aquarium type stone diffuser (2.5cm long and 1.25cm diameter) located at the bottom of each column provided fine-bubble aeration. To avoid stripping of water by dry air, bubbling through a large carboy upstream of the reactors humidified the air.

Each column was capped at the top with a standard 10.2cm (4" nominal) diameter SDR35 PVC caps with a #4 removable rubber stopper for feeding, a 12mm (5/16") diameter mixing shaft port, an off-gas port with a ¼" barbed fitting for the exhaust airline, and an inlet airline port.

The bottom of each column was equipped with a threaded cleanout plug to allow for air stone replacement. Outlet valves were located near the base of each column for removal of pretreated sludge as shown in Figures 3.2a and 3.2b.

Each column was completely mixed with a mixer equipped with 3-blade turbine impellors located 10cm from the bottom and another set at mid-height of the columns. The columns, mixers, and rotameters were mounted on a stainless steel frame. Every two weeks the reactors were temporarily emptied in order to clean and replace the diffusers where necessary and check the levels.

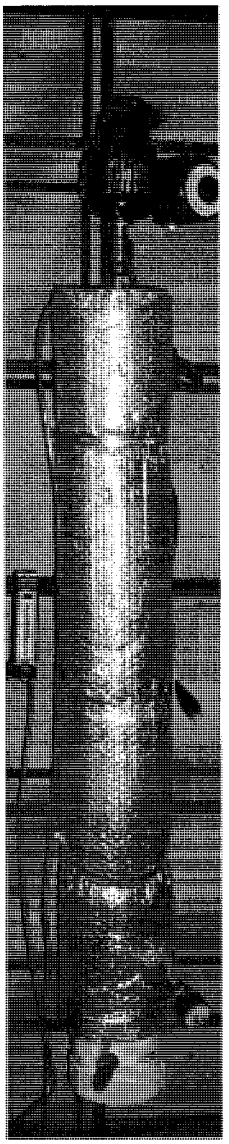

Figure 3.2a

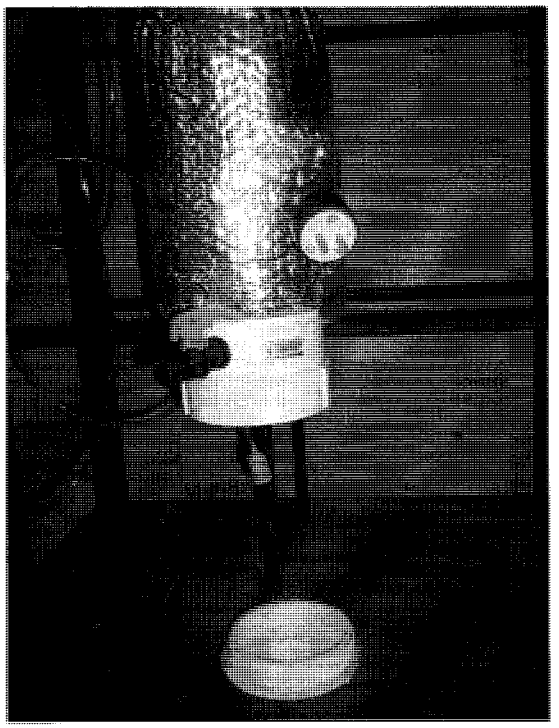

Figure 3.2b

Figure 3.2a: Microaerobic pretreatment column equipped with mixer, heat tracing, insulation, regulated airflow, and cleanout plug

Figure 3.2b: Close-up photo of pretreatment reactor, outlet, air stone and cleanout plug

3.1.2 Aerobic Digesters

3.1.2.1 *Design Considerations*

The aerobic digesters were designed to optimize the transfer of large amounts of oxygen into lab-scale semi-batch completely mixed sludge digesters. The digesters were also designed to have long residence times and temperature was not a controlled variable in the experiment. The reactors were set up to be fully aerobic, though the exact airflow was not controlled.

To deliver large amounts of air to the reactors, fine bubble ethylene propylene diene monomer (EPDM) rubber membrane diffusers were employed on the basis of their high capacity and resistance to plugging. The large airflow provided was adequate to provide complete mixing in the digesters whose circular geometry minimized dead space.

Circular tanks made of sewer grade piping were used for both their geometric and economic benefits. The aerobic digesters had minimal dead space given that the air supplied was sparged from the entire base of the columns.

These reactors had larger volumes to accommodate longer HRT. To allow semi-batch operation, outlet ports were provided with feeding provided directly into the top of the digesters. Odour emissions were mitigated by off-gas capture that was vented to exhaust pipes in the lab.

3.1.2.2 *Reactor Design Summary*

The aerobic digesters were made from 30.5cm (12" nominal) diameter SDR35 sewer grade PVC pipe. They were 80cm in height having working volumes of 40L each. Fine bubble aeration was provided with 22.9cm (9"nominal) diameter EPDM disc diffusers at the base of the columns. The diffusers were sealed in place with O-rings. The air supply was

provided by lab air that was filtered through a 1m column of fiberglass. The digesters were capped with circular 30mm (1/8") thick PVC sheet with 60mm (¼" nominal) barbed fitting off-gas ports for odour control. These lids were removed for feeding and digested effluent was removed from a valve at the base of the reactor.

3.2 Feed Sludge

Feed sludge was obtained from the Rockland, Ontario sequencing batch reactor (SBR) wastewater treatment facility on a weekly basis. The Rockland SBR facility treats about 500 m^3/day of rural domestic wastewater. It is designed to operate at an SRT of approximately 12 days. There are three SBRs which cycle through fill, react, settle, and decant modes. The decanted supernatant is sent to a polishing tank where it overflows to the Ottawa River. The waste sludge is aerobically digested with a residence time of 40 days and then sent to landfill. A single SBR basin at the facility is shown in Figure 3.3.

Figure 3.3: Contact basin at the Rockland, Ontario SBR facility

Operators at the plant would fill rain barrels with waste activated sludge (about 0.3% solids) during the settling cycle of the SBR as shown in Figure 3.4a and 3.4b. The sludge was allowed to settle for several hours until the supernatant was pumped off and the thickened sludge (approximately 1.5% w/v total solids (TS)) was retrieved and transported to Carleton University where it was stored at 4°C until needed.

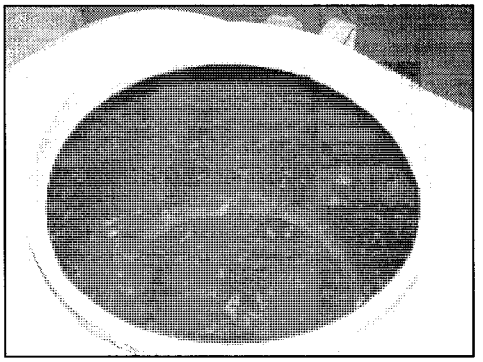

Figure 3.4a: Rain barrel full of waste activated sludge prior to settling and decanting

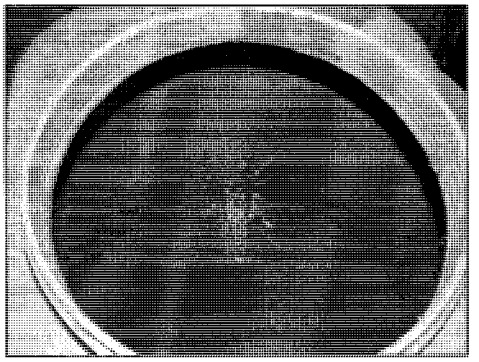

Figure 3.4b: Thickened waste activated sludge after settling and decanting

In phase I where solids concentration was varied, sludge was stored in the fridge at 4°C for one day to allow it to further settle (up to 2.5% w/v TS). The supernatant was then removed with a peristaltic pump and the concentrated sludge was mixed together in a large container and then measured into four buckets such that four feeds were prepared in the following ratios: 100% sludge; 80% sludge - 20% supernatant; 60% sludge - 40% supernatant; and 40% sludge - 60% supernatant;

Typically, a sufficient quantity was prepared to provide feed for a week. The sludge was stored in the fridge until feeding time when it was mixed and the required volumes were

heated to room temperature in glass beakers in a hot water bath then fed to the appropriate reactors.

In phase III two types of feed were used: fresh and mechanically sheared sludge. The mechanically sheared feed was prepared in an Ostler household blender at the highest setting for ten minutes. The extent of particle size reduction was determined at Accutest Labs using serial filtration and Standard Method 1040D (APHA, 1998).

3.3 Experimental Phases

3.3.1 Phase I

In phase I the effect of feed solids concentration in microaerobic pretreatment of SBR sludge was investigated in four pretreatment reactors. The columns were fed daily at approximately the same time with 3L of sludge to maintain a 48h HRT with 2.5%, 2.0%, 1.5%, and 1.0% feed solids concentrations respectively. The reactors were run at ambient temperatures and aerated at a rate of 350mL/min of humid air or 0.06vvm as determined during startup to achieve microaerobic conditions with low DO (<2mg/L) and low ORP (-300mV to 100mV). Feeding for all phases conducted while reactors were mixing using the draw/fill method to prevent short-circuiting.

3.3.2 Phase II

Phase II looked at the effect of contact time on pathogen destruction after microaerobic pretreatment only. A two by two factorial experiment compared feeding frequency by manipulating feed volume (50% and 25% reactor volume replacements) and HRT (48h and 96h) and was performed as shown in Table 3.1.

Table 3.1: Phase II controlled pretreatment reactor conditions

	HRT = 96h	**HRT = 48h**
50% Volume Replacement	*Reactor Name*: R1 *Airflow*: 0.06vvm *Temperature*: Ambient *Feed solids*: 1.5% *Feed frequency*: Every 2nd day *Feed Volume*: 3L	*Reactor Name*: R3 *Airflow*: 0.06vvm *Temperature*: Ambient *Feed solids*: 1.5% *Feed frequency*: Daily *Feed volume*: 3L
25% Volume Replacement	*Reactor Name*: R2 *Airflow*: 0.06vvm *Temperature*: Ambient *Feed solids*: 1.5% *Feed frequency*: Daily *Feed volume*: 1.5L	*Reactor Name*: R4 *Airflow*: 0.06vvm *Temperature*: Ambient *Feed solids*: 1.5% *Feed frequency*: Every 2nd day *Feed Volume*: 1.5L

3.3.3 Phase III

Phase III examined the effect of temperature and particle size on the performance of microaerobic pretreatment and subsequent digestion in a two by two factorial experimental design. The staged digestion systems were compared against a control digester. The semi-batch pretreatment reactors were operated with 96h HRTs, aerated at 0.06vvm and fed 50% of their volumes every other day with sludge of 1.5% solids concentrations. The four pretreatment reactors were operated under the conditions shown in Table 3.2.

Table 3.2: Phase III pretreatment reactor conditions

	T = ambient	**T = 35°C**
Sheared Feed	*Reactor Name*: R1 *Airflow*: 0.06vvm *Temperature*: Ambient *Feed solids*: 1.5% *Feed frequency*: Every 2nd day *Feed Volume*: 3L *Feed Type*: Sheared	*Reactor Name*: R3 *Airflow*: 0.06vvm *Temperature*: **35°C** *Feed solids*: 1.5% *Feed frequency*: Every 2nd day *Feed Volume*: 3L *Feed Type*: Sheared
Not Sheared Feed	*Reactor Name*: R2 *Airflow*:0.06 vvm *Temperature*: Ambient *Feed solids*: 1.5% *Feed frequency*: Every 2nd day *Feed Volume*: 3L *Feed Type*: Not Sheared	*Reactor Name*: R4 *Airflow*: 0.06vvm *Temperature*: **35°C** *Feed solids*: 1.5% *Feed frequency*: Every 2nd day *Feed Volume*: 3L *Feed Type*: Not Sheared

The effluents of the pretreatment reactors were used to feed 2L every other day to the downstream aerobic digesters with 40d HRTs. A fifth digester was fed in a similar manner with sludge that was not pretreated.

3.4 Sampling Protocol

For each phase of the study, the pretreatment reactors were allowed to stabilize for at least 3 HRTs prior to monitoring. The aerobic digesters (Phase III) were run for 3 months prior to monitoring. It was assumed that the reactors and digesters had attained a pseudo-steady state and that variations in measured variables could be attributed mainly to the variations of the feed sludge.

3.5 Analytical Methods

Several operating variables were measured to characterize the conditions of the feed as well as pretreatment and digester effluents. These included temperature, pH, oxidative-reductive potential (ORP), dissolved oxygen (DO), ammonia, total kjeldahl nitrogen (TKN), soluble TKN, combined nitrate and nitrite, total volatile fatty acids (VFAs), alkalinity, soluble chemical oxygen demand (COD), as well as total and suspended solids and volatile solids. Five microbial indicator and pathogenic organisms were monitored to assess the degree of pathogen destruction including fecal coliforms, *E.coli*, *Salmonella* spp., *Shigella* spp. and *C. perfringens*. Each variable was measured at least 7 times over the course of at least 2 months with the exception of TKN, which was measured 3 times in Phases II and III as external lab costs were prohibitive.

3.5.1 ORP

The ORP was measured directly in the reactors and digesters using a double-junction ORP probe and Orion 420p millivolt meter. The probe was allowed to stabilize for 10 minutes with 3 readings taken every two minutes thereafter. The probe was calibrated using Zobell solution on a weekly basis and after each time the battery in the meter was replaced.

3.5.2 DO

The DO content was measured in the reactors using a Martek DO probe suitable for low DO levels. According to the manual a flow of 100-250mL/min across the probe was required for proper readings. A flowtube was constructed to achieve this. However,

readings that were taken directly in the reactors appeared to be more stable. The membrane was changed and probe calibrated monthly.

The readings for DO were much less stable than the ORP readings. Hence, the probe was allowed to stabilize for 30 minutes and 3 readings were taken every two minutes thereafter.

3.5.3 TKN and Soluble TKN

Samples of feeds, reactors, and digesters were acidified with sulphuric acid for preservation and sent to Caduceon Environmental labs for analysis of TKN. Samples were also filtered using 934-AH Whatman glass fiber filter paper from which the filtrate was sent for analysis of soluble TKN.

3.5.4 Ammonia

An Orion ion selective probe was used to measure the ammonia concentrations in the reactors and feeds in duplicate at each sampling event. 50mL samples were drawn and 1.5mL of 10N NaOH were added to raise the pH above 12. The probe was inserted in the sample and allowed to stabilize with stirring. Measurements were obtained in mV and cross referenced with a calibration curve prepared using ammonia standards.

3.5.5 Total VFAs and Alkalinity

Samples from the feeds, reactors, and digesters were centrifuged for 15 minutes at 6000 rpm. The supernatant was titrated first to a pH of 5.1 then to 3.5 with a 0.1M H_2SO_4 solution using the method described by Andersen and Yang (1992) in duplicate. The

method detection limit was reported to be 0.1mM/L for bicarbonate concentrations (alkalinity) and 0.3mM/L for volatile fatty acids.

3.5.6 Soluble COD

Soluble COD was measured for feeds, reactors, and digesters at each sampling event. The COD closed reflux colorimetric assay was used (Standard Methods, 1998). Samples were centrifuged for 15min at 6000rpm, filtered through 934-AH Whatman filter, then acidified with sulphuric acid for future analysis. It was found that samples did not need to be diluted to be within range of the calibration curve.

3.5.7 pH

The pH was measured in at least duplicate at each sampling event of the feeds, reactors, and digesters where applicable (Phase III). 50mL samples were drawn and the pH was measured immediately with a pH electrode at the temperature of the reactors. The samples were stirred with a magnetic stir bar during measurement.

3.5.8 Microbial Analysis

Five indicator and pathogenic bacteria were monitored. Thermotolerant fecal coliforms, *Escherichia Coli, Salmonell*a spp., and *Shigella* spp. concentrations, were enumerated using membrane filtration on selective media as described in APHA (1998) and *Clostridium perfringens* was measured using a pour plate technique. All plates were incubated for 24 +/-2h with the media and incubation temperatures for each listed in Table 3.3. Helminth ova such as *Ascaris* eggs were not evaluated as they were assumed to be

represented by C.*perfringens* as reported by Bujoczek et *al.*,(2003) which was more economically feasible to measure.

Table 3.3: Selective media, incubation temperature, and techniques used in microbial enumerations

Bacteria	Media	Incubation Temperature	Technique
Fecal coliforms	Difco mFC Agar	45°C	Membrane Filtration
E. Coli	Difco mFC Basal with BCIG	37°C	Membrane Filtration
Salmonella and *Shigella* spp.	Difco SS Agar	37°C	Membrane Filtration
C. Perfringens	Difco SPS Agar	37°C (anaerobic)	Pour Plate

All samples were serially diluted in a phosphate buffer with intermittent vortexing between dilutions. At the outset of each steady state several dilutions were run to establish the appropriate dilution to test for a given sample. For samples with low pH and bacterial concentration, the pH was adjusted with a 1N solution of NaOH prior to testing as this was shown to improve recovery. Analysis on all samples began within 4 hours of being collected from the reactors.

4. Results: Phase I

4.0 Results and Discussion: Phase I

In the first phase of experimentation, a fixed airflow of 0.06vvm (350mL/min) was supplied while feed solids concentration was varied from 1.0% - 2.5% across four pretreatment reactors at ambient temperature. These reactors had hydraulic residence times (HRT) of 2 days and were fed daily in semi-batch mode (with draw/fill of 50% of the reactor volume used per feed). The total solids measured in the feed and effluent samples showed that the actual feed solids were quite similar to the target concentrations and that little solids destruction was observed (Figure 4.1). The significant increase in effluent versus feed solids in the first pretreatment column was associated with a faulty mixer and assumed settling.

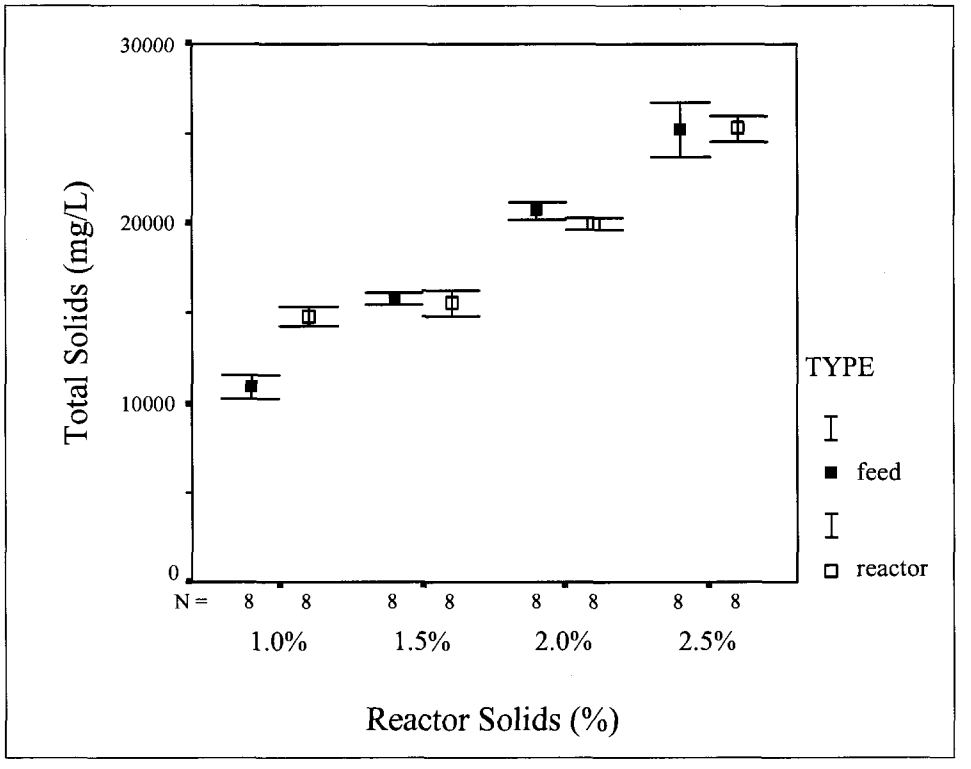

Figure 4.1: Error plot for 90% confidence interval around mean total solids (mg/L) by pretreatment reactor feed solids in phase I for feed and reactor effluent samples

A step down in ORP from approximately –100mV to –250mV was found between the 1.0% and 1.5% reactors as seen in Figure 4.2. The ORP in the 1.5% - 2.5% reactors were similar. The mean ORP measured in the reactors in this phase of the experiment confirmed that the selected airflow, reactor volume, residence time, and feed solids concentrations were appropriate to produce microaerobic conditions. The difference in ORP affected several other measured and observed variables. Qualitatively, it was observed that the samples from the least reducing reactor were light brown in colour and got progressively darker to black-brown in the most reducing reactor (highest solids). This darkening was explained by the increased reduction of ferric iron to ferrous iron with decreased ORP (Metcalf and Eddy, 2002). The dissolved oxygen (DO) concentrations were deemed unreliable in this phase since the probe was unable to stabilize within a reasonable range and timeframe and were therefore left out of the discussion.

The total TKN increased linearly with feed solids concentration and remained similar in the respective feed and effluent of the reactors indicating no significant nitrogen losses through nitrification/denitrification processes (Figure 4.3). A two to threefold increase in soluble total Kjeldahl nitrogen (TKN) and ammonia was observed in the three highest solids and most reducing reactors indicating that hydrolysis was more prevalent there than under the more oxidizing conditions of the low solids reactor (Figures 4.4 and 4.5).

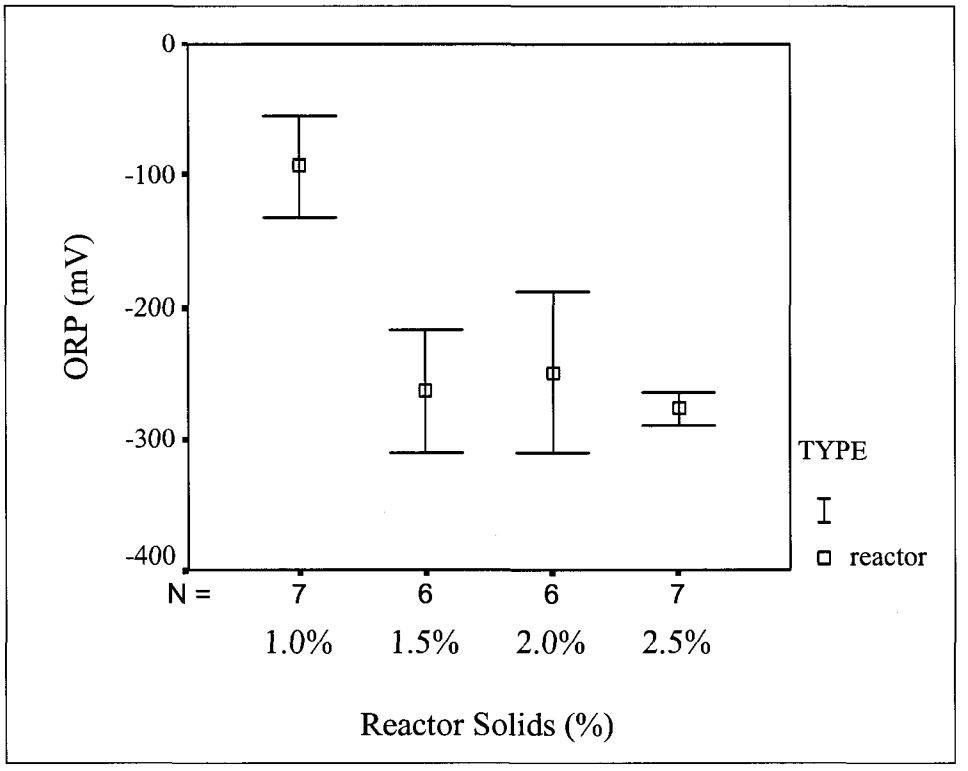

Figure 4.2: Error plot for 90% confidence interval around mean ORP by pretreatment reactor feed solids in phase I for reactor effluent samples

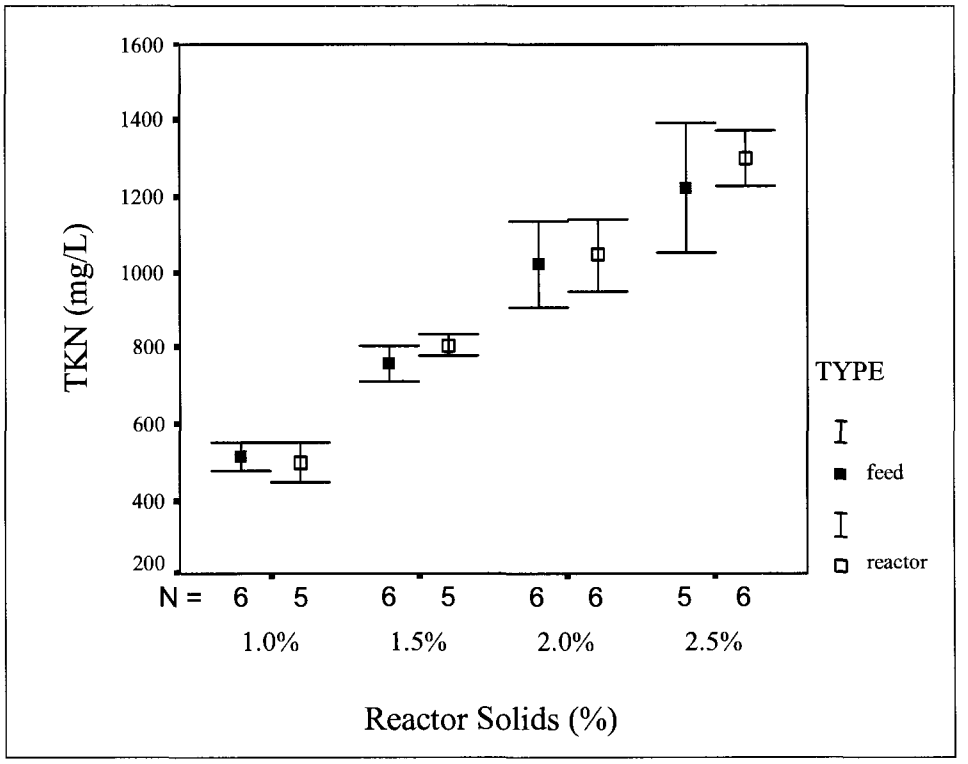

Figure 4.3: Error plot for 90% confidence interval around mean TKN (mg/L) by pretreatment reactor feed solids in phase I for feed and reactor effluent samples

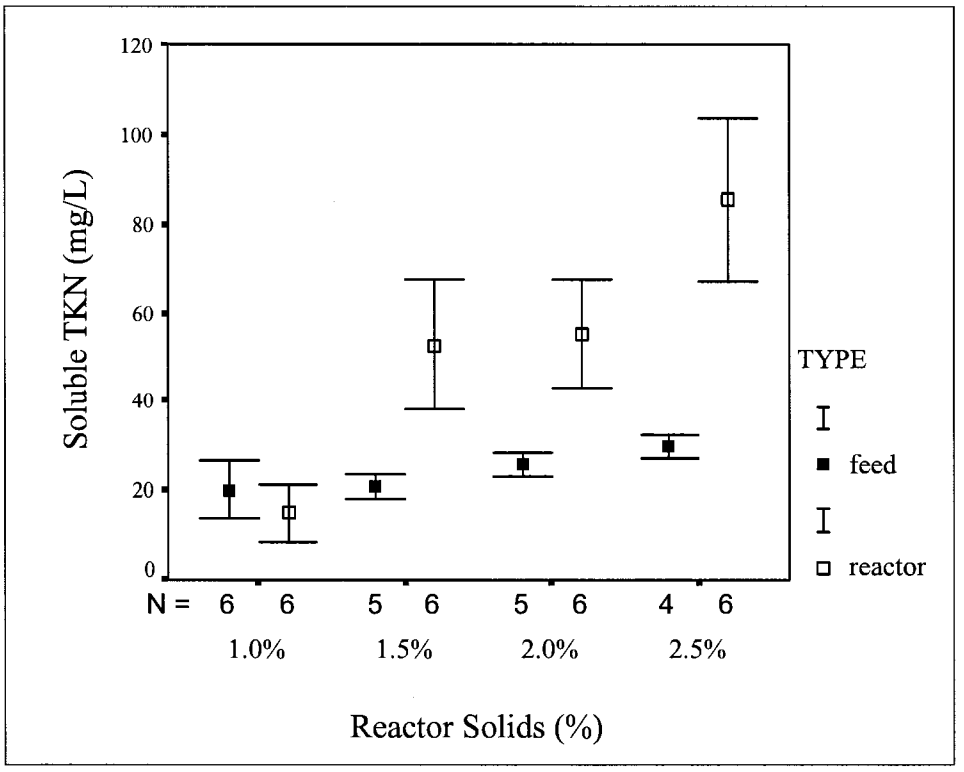

Figure 4.4: Error plot for 90% confidence interval around mean soluble TKN (mg/L) by pretreatment reactor feed solids in phase I for feed and reactor effluent samples

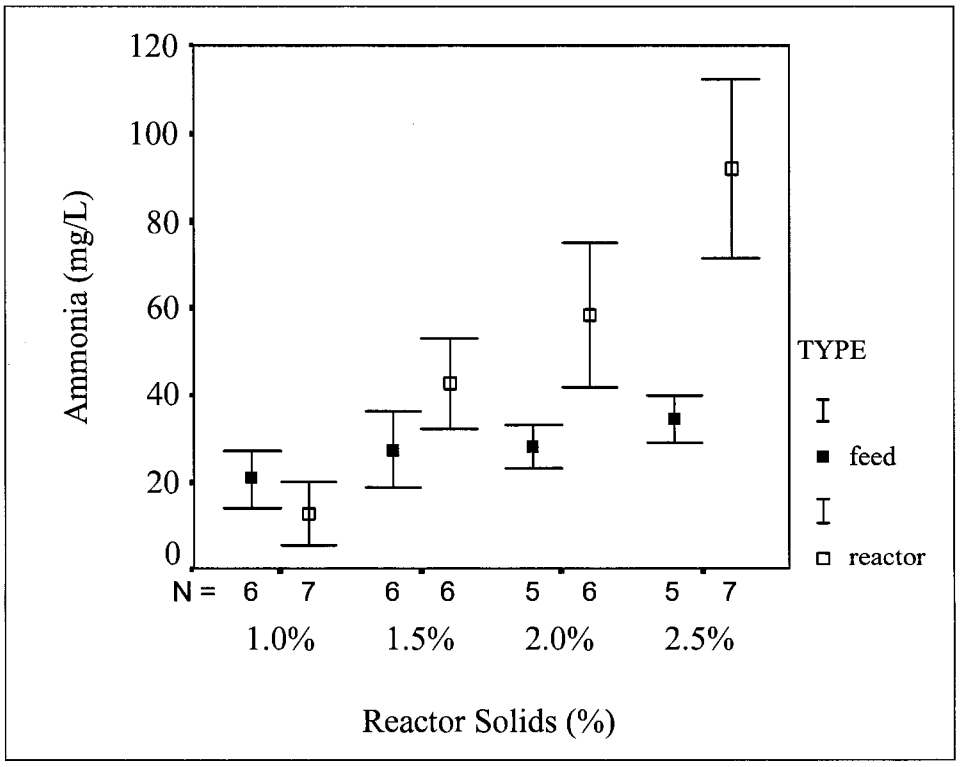

Figure 4.5: Error plot for 90% confidence interval around mean ammonia (mg NH_4^+/L) by pretreatment reactor feed solids in phase I for feed and reactor effluent samples

The total volatile fatty acid (VFA) concentrations from fermentation increased under more reducing conditions reaching 600mg acetate/L in the reactor fed with 2.5% total solids feed (Figure 4.6). These results suggest that facultative aerobes and anaerobes were more active with decreased oxygen availability as they were able to use alternative electron donors. The VFA concentrations were associated with the alkalinity and pH in the reactor effluents, an effect that is further discussed below.

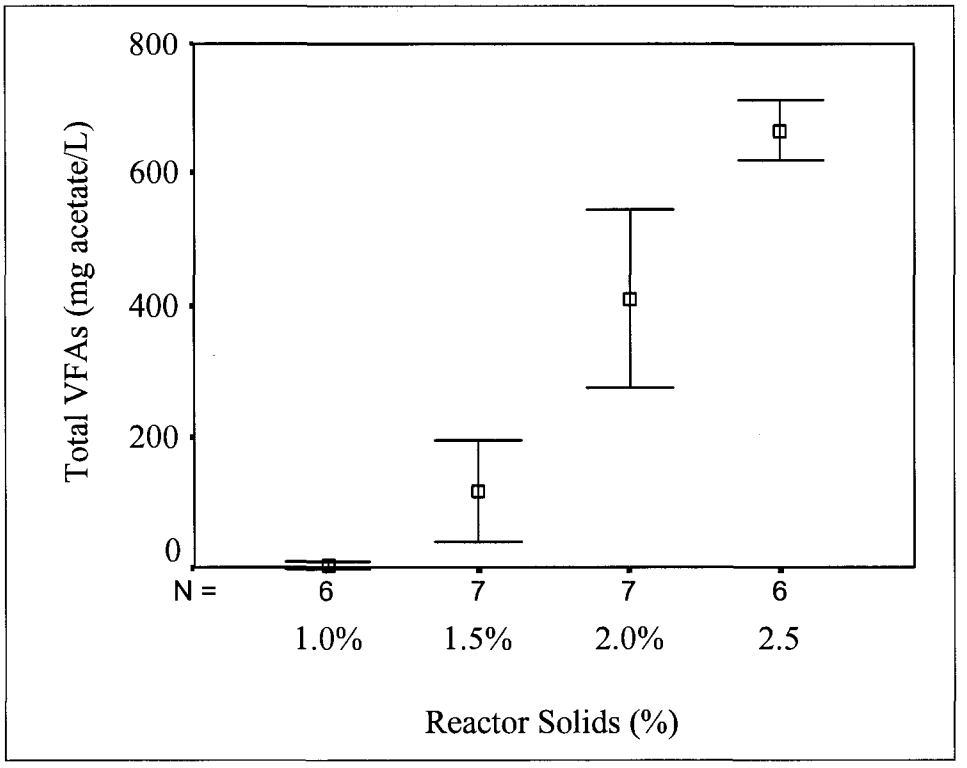

Figure 4.6: Error plot for 90% confidence interval around mean total VFAs (mg acetate/L) by pretreatment reactor feed solids in phase I for reactor effluent samples

The soluble chemical oxygen demand (COD) was seen to increase in the reactor feeds as expected with increased solids concentration. The low solids reactor achieved

some net soluble COD removal. However, the higher solids reactors remained insignificantly different between the respective feed and effluent samples. It was likely that the contribution of VFA production to soluble COD in these reactors masked the COD removed and kept the net soluble COD balance fairly even. However, in the 1.0% reactor where there was negligible production of VFAs, the soluble COD consumption became apparent as seen in Figure 4.7.

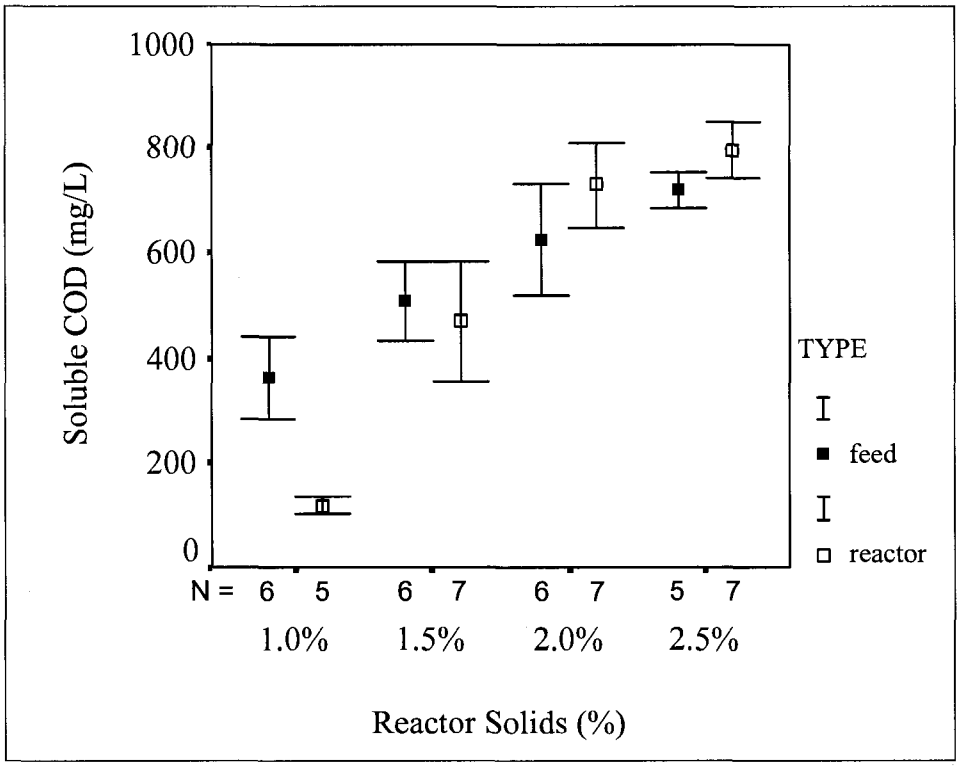

Figure 4.7: Error plot for 90% confidence interval around mean soluble COD (mg/L) by pretreatment reactor feed solids in phase I for feed and reactor effluent samples

The alkalinity in the feed samples remained fairly constant across all reactors. Some variation was observed in the effluent samples with the maximum found in the pretreatment reactor fed with 1.5% solids (Figure 4.8). The alkalinity levels were likely affected negatively by increased VFA concentrations (which dominated in the 2.0% and

2.5% feed solids reactors) and positively by increased ammonia concentrations (which dominated in the 1.5% feed solids reactor).

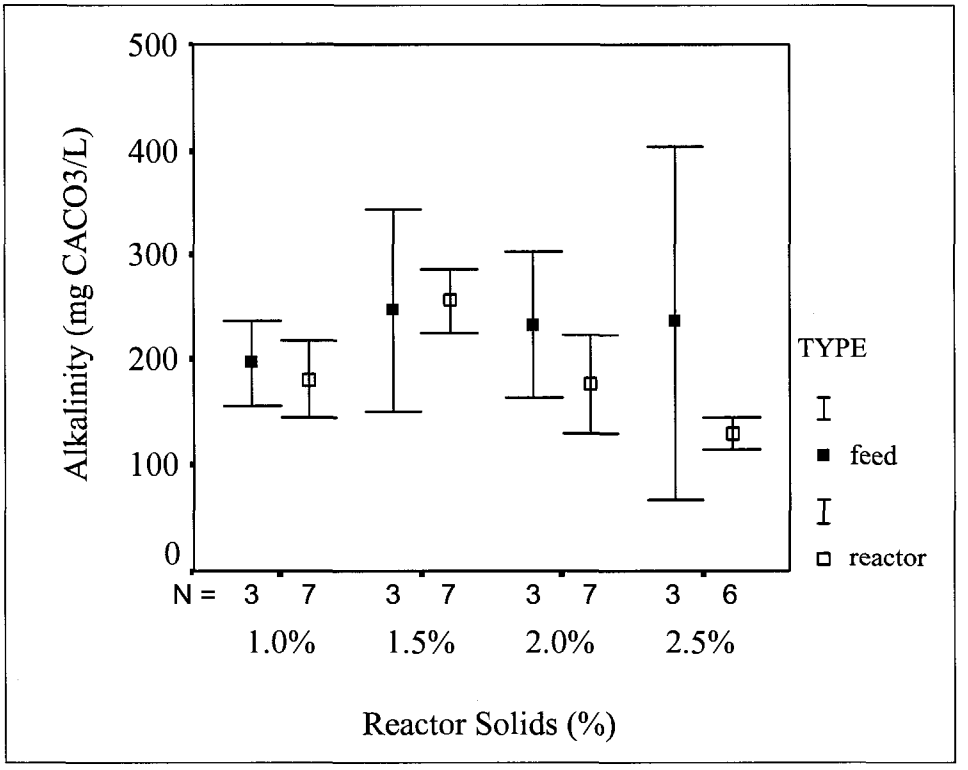

Figure 4.8: Error plot for 90% confidence interval around mean alkalinity (mg $CaCO_3$/L) by pretreatment reactor feed solids in phase I for feed and reactor effluent samples

The pH measured in the feed samples was fairly constant as seen in Figure 4.9. In the reactor effluent samples, the pH tended to increase significantly relative to the feed pH and followed a similar profile to the alkalinity with the highest mean observed in the 1.5% feed solids reactor. The factors affecting pH were likely the ammonia release, VFA production, and alkalinity. The variation in feed pH and alkalinity, which may have been caused by VFA production in feed storage, tended to stabilize after pretreatment.

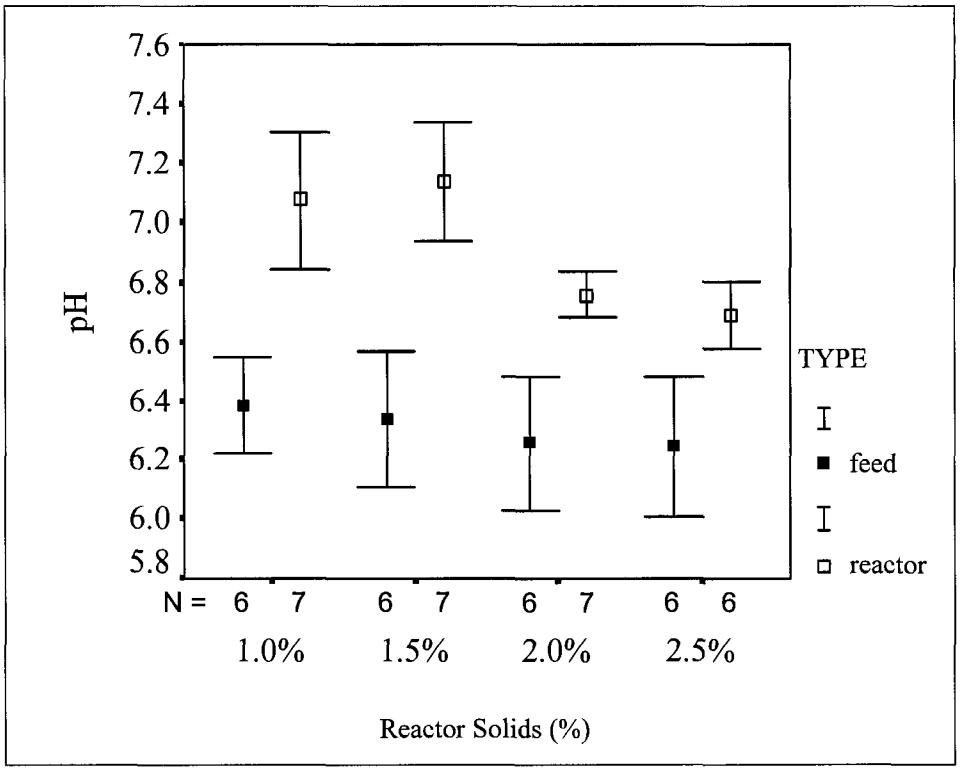

Figure 4.9: Error plot for 90% confidence interval around mean pH by pretreatment reactor feed solids in phase I for feed and reactor effluent samples

The optimum feed concentration for *E. Coli*, fecal coliforms, and *C. perfringens* mean log removal was 1.5% solids as shown in Figures 4.10, 4.11, and 4.14. However, there was a greater variability in the effluent fecal coliforms concentrations in these reactors than in the feeds and in the effluents of the 2.0% and 2.5% feed solids reactors. None of the fecal coliform or *C. perfringens* destructions observed after pretreatment were significant at the 90% confidence interval. However, the *E. coli* did show a significant decrease at the 90% confidence interval in the 1.0% and 1.5% feed solids reactors.

Similarly, the highest destructions observed for *Shigella* spp. and *Salmonella* spp. based on mean log reductions of CFU/gTS were found in the 1.0% and 1.5% feed solids reactors though the *Salmonella* spp destruction was not significant at the 90% confidence

interval in the 1.5% feed reactor as shown in Figure 4.12 and Figure 4.13. A significant decrease was also observed at the 90% confidence interval for *Shigella* spp. only in the 2.5% feed solids reactor. The 1.5% feed solids concentration was selected based on mean destructions prior to statistical analysis at the 90% confidence interval for further analysis in the subsequent phases of the experiments given its higher potential for *E. coli* and fecal coliform destruction. Statistical analysis of means at the 90% confidence interval later revealed that the 1.0% feed solids system would have also been a good candidate for further investigation based on pathogen removal. In general the log reductions of pathogens were small or negligible after pretreatment only especially for C. *perfringens* which was expected to be resistant to removal. The resistance of C. *perfringens* indicator in microaerobic pretreatment of sludge suggests that *Ascaris* ova if present would also be persistent under these conditions as reported by Bujoczek et al., (2001).

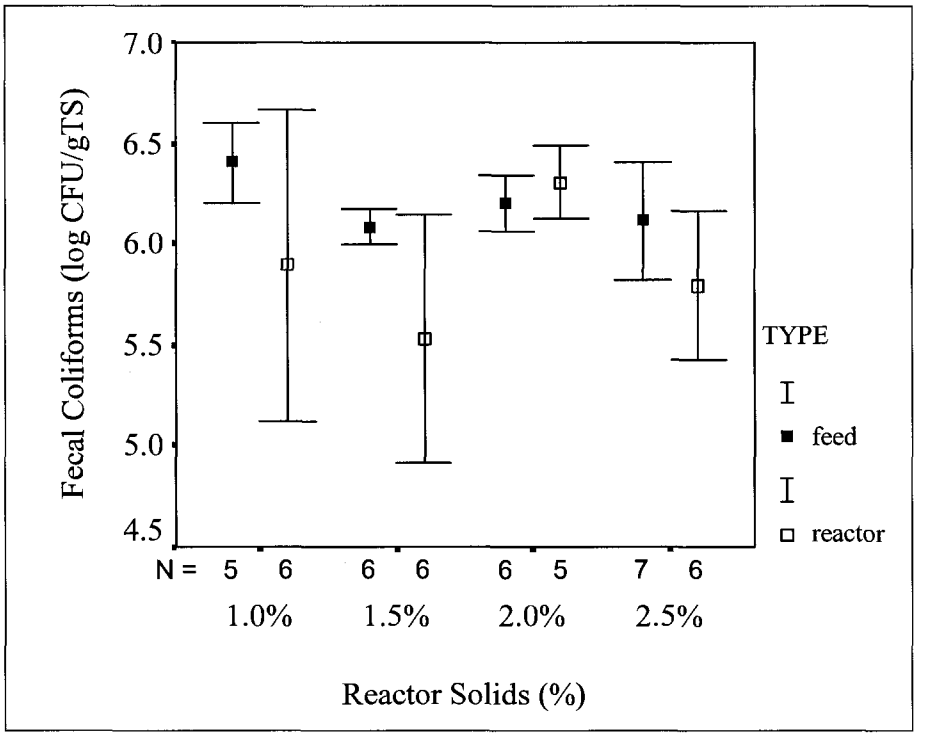

Figure 4.10: Error plot for 90% confidence interval around mean fecal coliform concentrations (log CFU/g TS) by pretreatment reactor feed solids in phase I for feed and reactor effluent samples

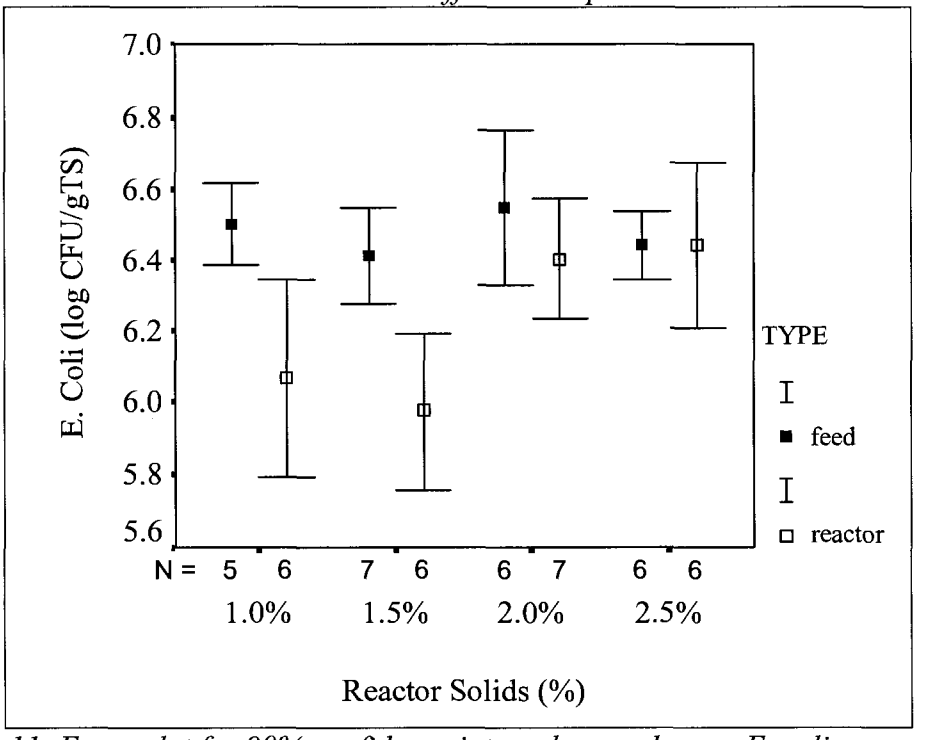

Figure 4.11: Error plot for 90% confidence interval around mean E. coli concentrations (log CFU/g TS) by pretreatment reactor feed solids in phase I for feed and reactor effluent samples

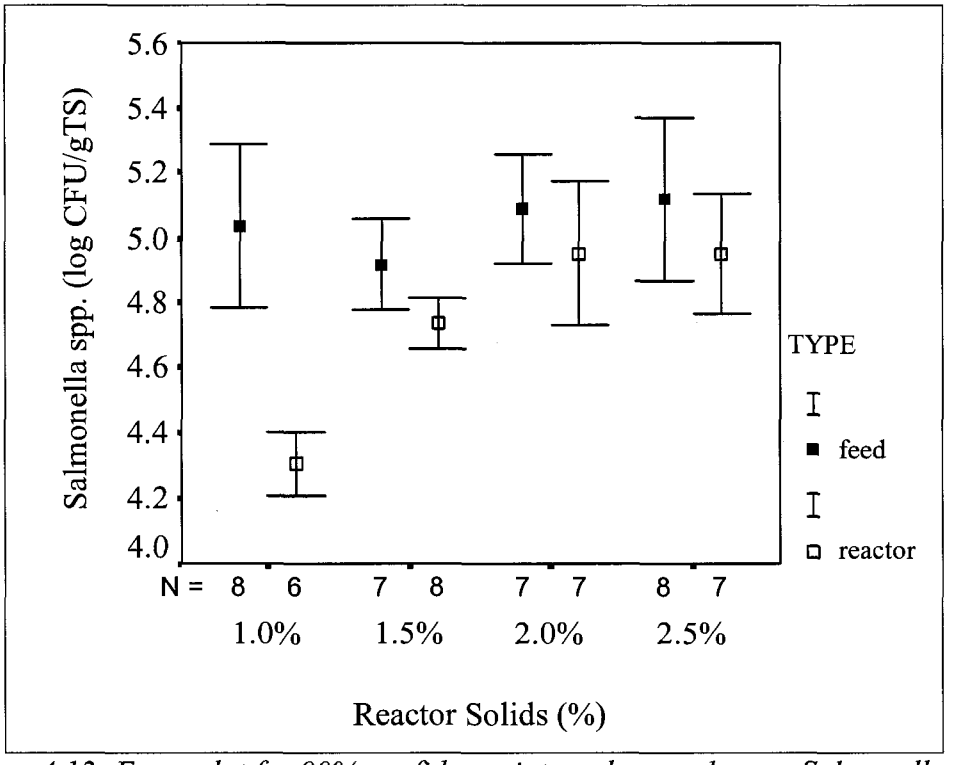

Figure 4.12: Error plot for 90% confidence interval around mean Salmonella spp. concentrations (log CFU/g TS) by pretreatment reactor feed solids in phase I for feed and reactor effluent samples

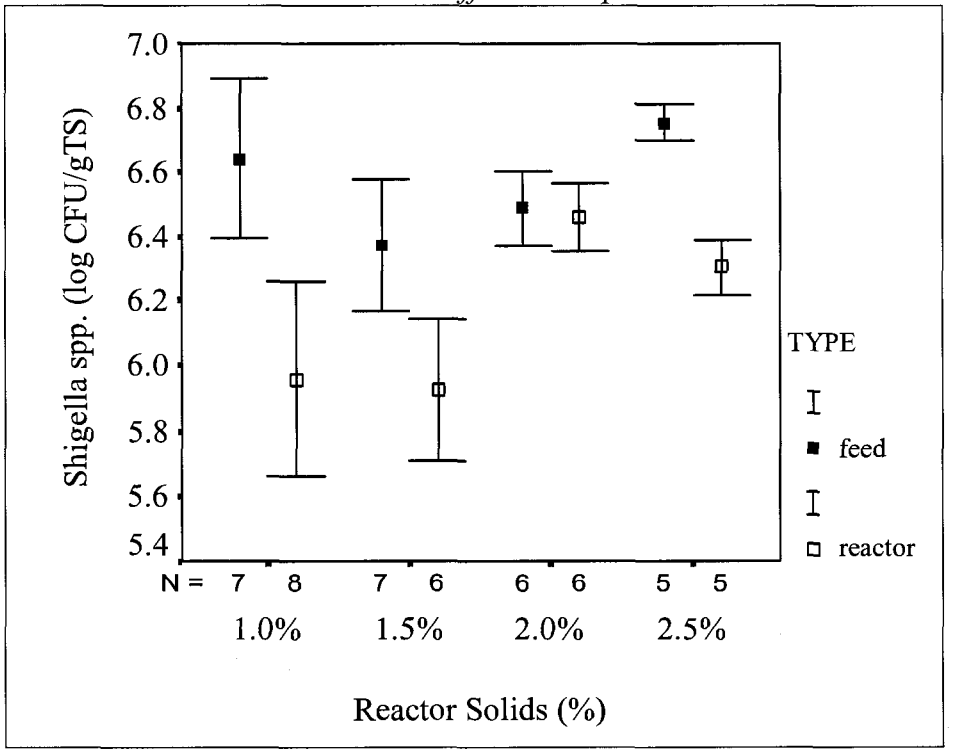

Figure 4.13: Error plot for 90% confidence interval around mean Shigella spp. concentrations (log CFU/g TS) by pretreatment reactor feed solids in phase I for feed and reactor effluent samples

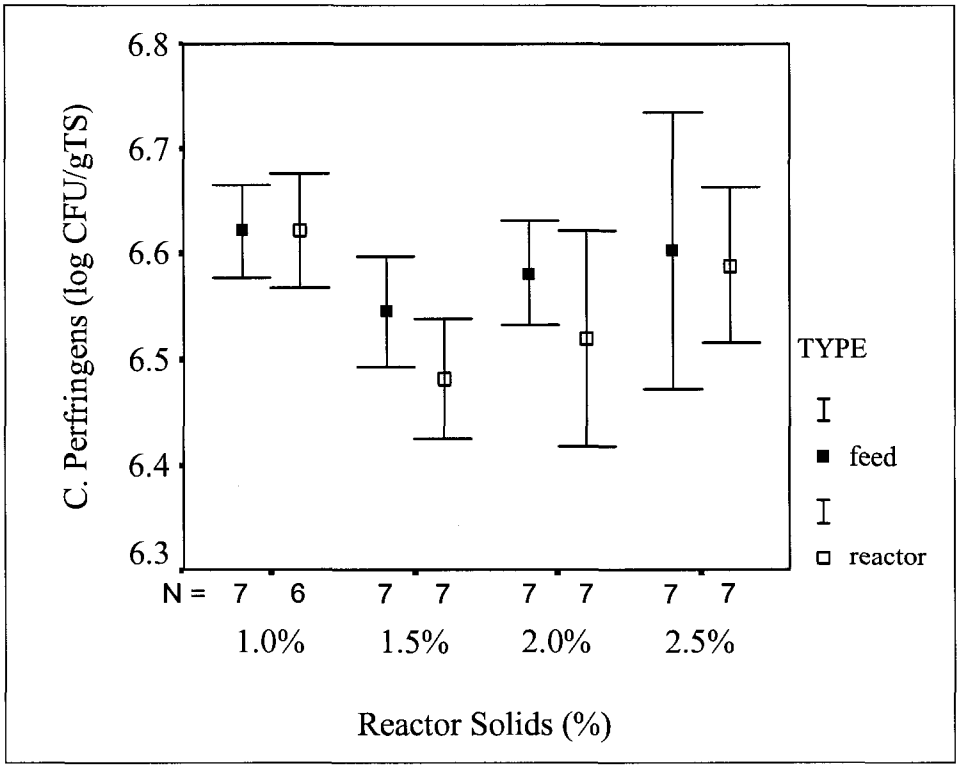

Figure 4.14: Error plot for 90% confidence interval around mean C. perfringens concentrations (log CFU/g TS) by pretreatment reactor feed solids in phase I for feed and reactor effluent samples

5. Results: Phase II

5.0 Results and Discussion: Phase II

In phase II of experimentation, the selected feed concentration of 1.5% from phase I was used in combination with an air flow rate of 0.06vvm to produce microaerobic conditions. The test conditions in this phase were feeding frequency (manipulated by feed volume) and hydraulic residence time (HRT). The resulting setup explored increasing contact time. A two-by-two factorial experimental design was employed with two feeding frequencies (using 50% and 25% reactor volume replacements) and two HRT (2d and 4d). For the 2d HRT reactors, a 25% reactor volume feed required feeding every 12h and the 50% reactor volume feed required feeding every day. For the 4d HRT reactors, a 25% reactor volume feed required feeding once per day and the 50% reactor volume feed required feeding every other day. In all cases the draw/fill method was used.

Some concentration of solids was observed in the reactors and was likely due to both evaporation and insufficient mixing. Neither the mean values over the time period monitored for total solids (TS), nor volatile solids (VS) results, showed any significant solids removal in the pretreatment reactors (Figure 5.1a and Figure 5.1b). However the VS/TS ratio did indicate that solids destruction tended to increase with contact time (Figure 5.1c). The suspended solids measurements showed similar trends (Figure 5.2a, b, c). Pairwise comparisons of the measured values for feed and effluent samples for respective sampling events was considered but deemed an inappropriate analysis due to the nature of semi-batch operation since both feed and effluent vary over time and the reactor contents are made up of multiple feed events.

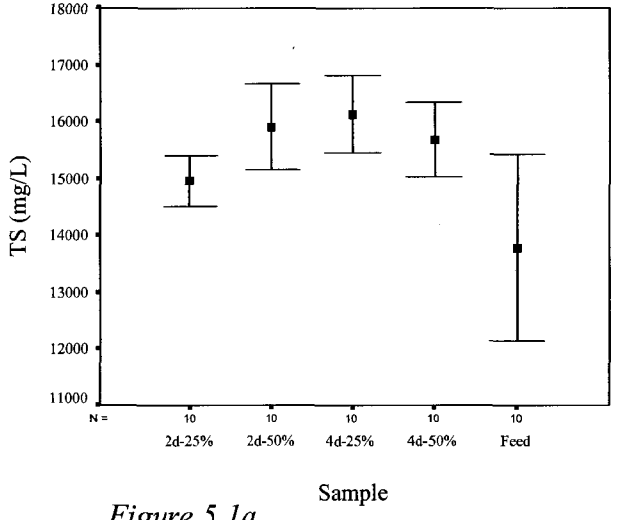

Figure 5.1a

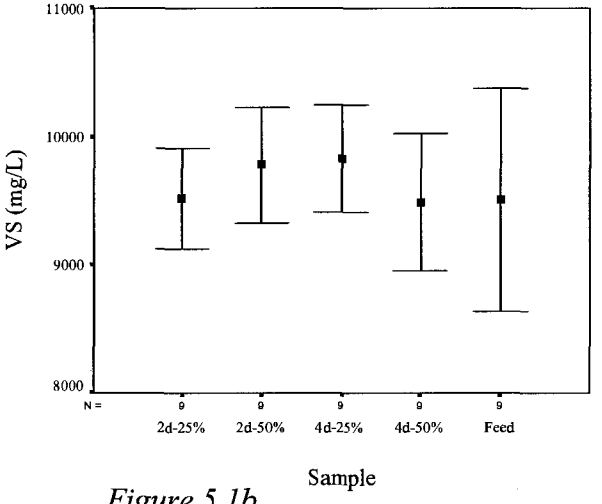

Figure 5.1b

Figure 5.1: Error plot for 90% confidence interval around mean total and volatile solids (mg/L) and VS/TS by pretreatment contact time in phase II for reactor effluent samples

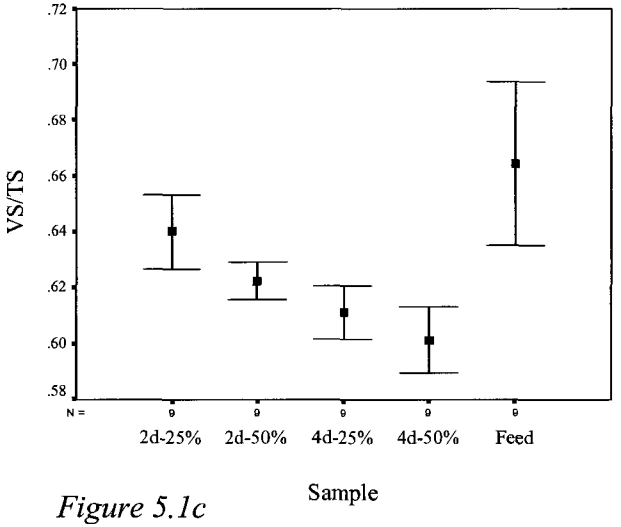

Figure 5.1c

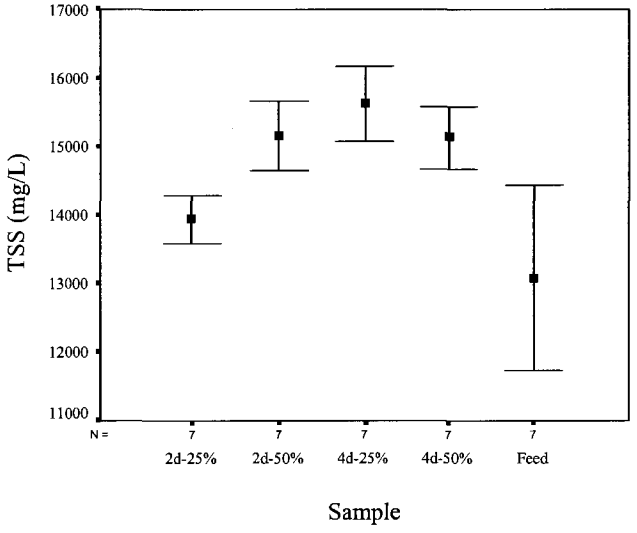

Figure 5.2a

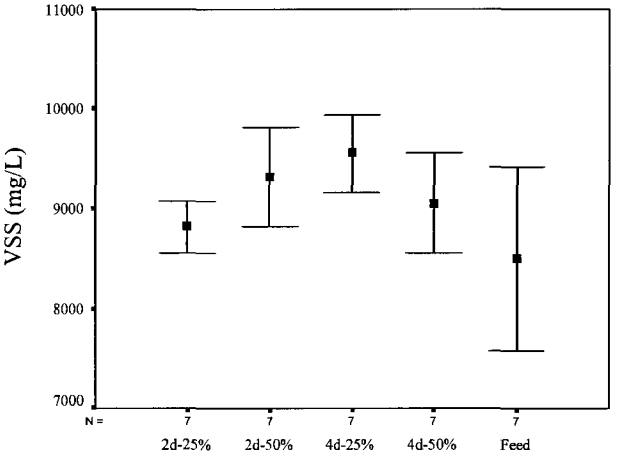

Figure 5.2b Sample

Figure 5.2: Error plot for 90% confidence interval around mean total and volatile suspended solids (mg/L) and VSS/TSS by pretreatment contact time in phase II for reactor effluent samples

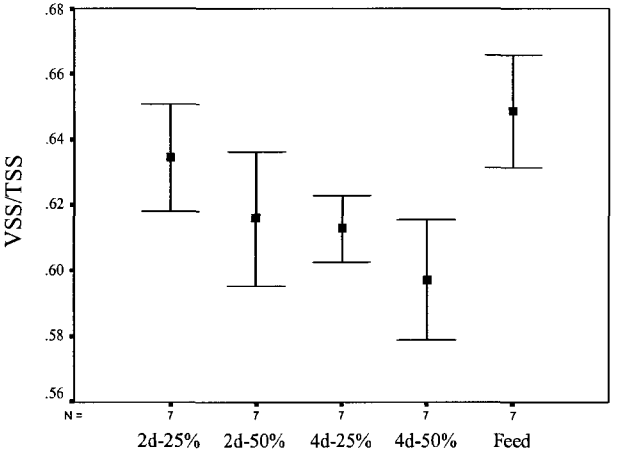

Figure 5.2c Sample

The oxidative-reductive potential (ORP) in the reactors tended to increase with contact time as shown in Figure 5.3. The mean ORP of the 2d HRT and 50% volume replacement reactor was higher than the similar conditions in phase I. This was attributed to the less degradable feed available in the summer months and therefore less biological aerobic activity to consume the dissolved oxygen (DO).

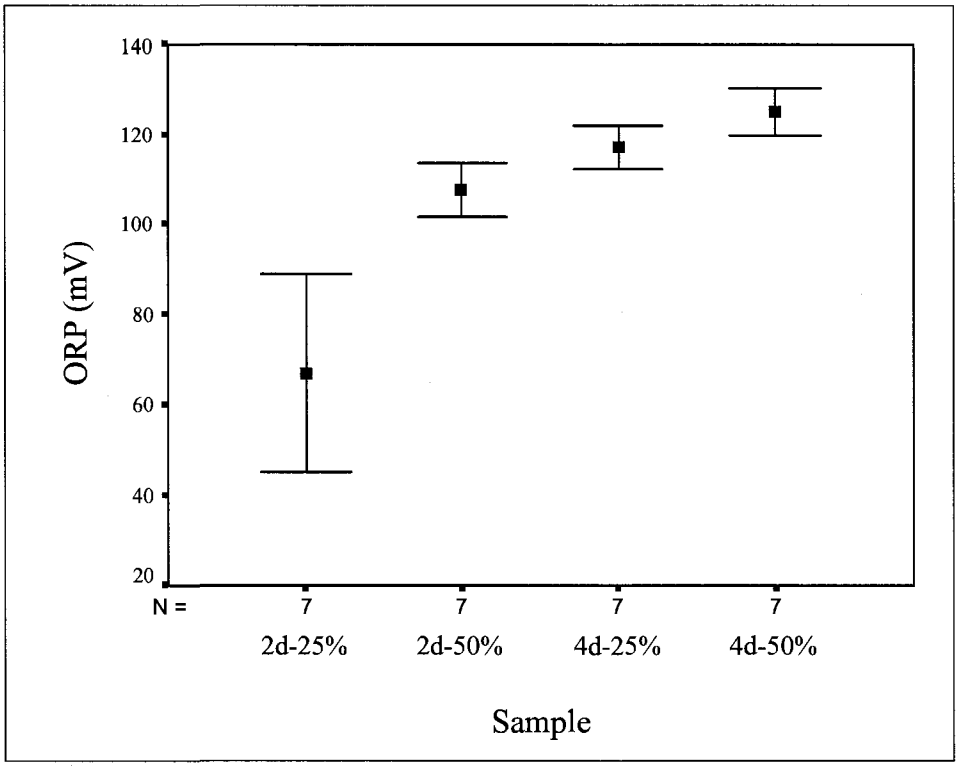

Figure 5.3: Error plot for 90% confidence interval around mean ORP (mV) by pretreatment contact time in phase II for reactor effluent samples

Although the ORP tended to increase with increased contact time, the DO followed a different trend where it was significantly lower for those reactors fed with 25% of the volume at the respective HRTs (Figure 5.4). These findings suggested that the biological activity at the time of sampling (prior to feeding) was higher for more

frequent feeding. For less frequent feeding (50% volume replacement) the 4d HRT reactor had the highest mean DO which suggested that biological activity was the least in this reactor just prior to feeding.

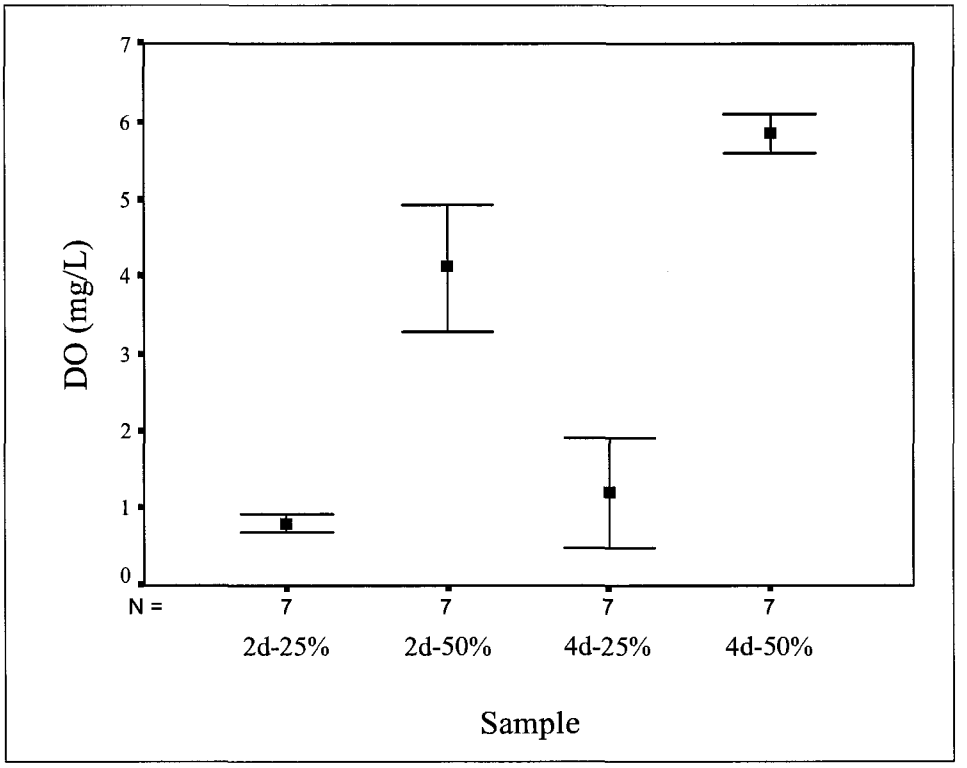

Figure 5.4: Error plot for 90% confidence interval around mean DO (mg/L) by pretreatment contact time in phase II for reactor effluent samples

In general the mean total Kjeldahl nitrogen (TKN) in the reactors was less than in the feed though not statistically significantly at the 90% confidence interval (Figure 5.5). No significant differences in mean TKN concentrations were observed among the reactors. Compared to phase I, which took place in winter months, the mean feed concentration of TKN for 1.5% total solids feed was approximately 250mg/L less in phase II. This indicated that nitrification/denitrification may have been more prevalent at the source plant during the summer. Ion chromatography showed positive values for

nitrate and nitrite species in the pretreatment reactors although the exact accuracy of those numbers is uncertain due to backpressure problems with the chromatography column. However, those findings confirmed that nitrification was likely occurring in the reactors.

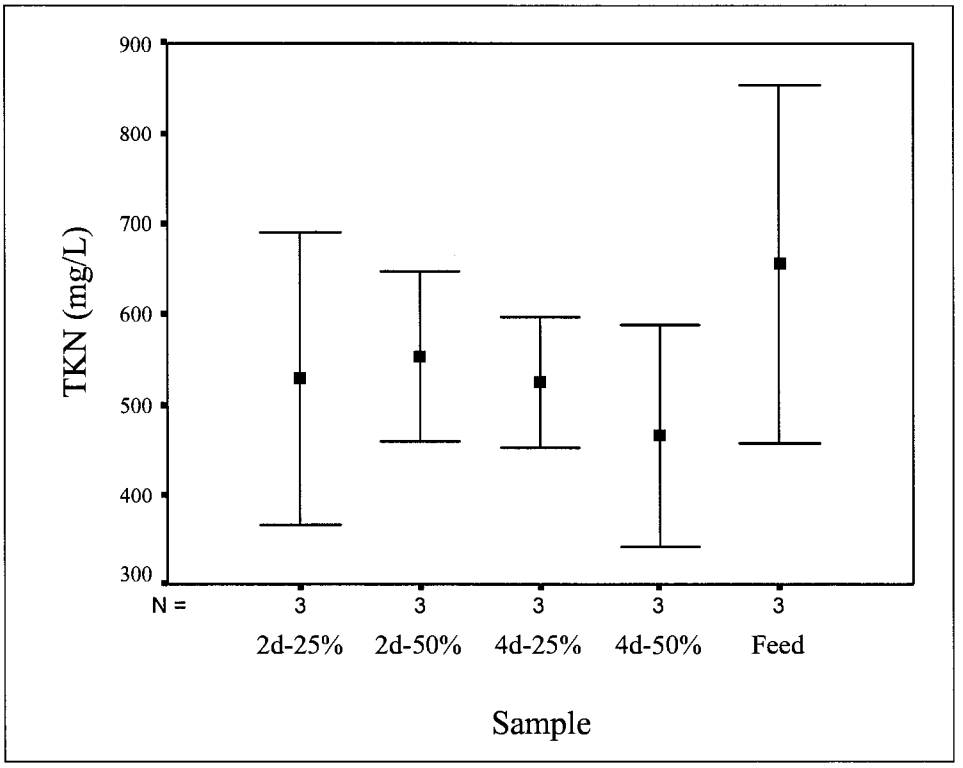

Figure 5.5: Error plot for 90% confidence interval around mean TKN (mg/L) by pretreatment contact time in phase II for feed and reactor effluent samples

The mean soluble TKN in the phase II feed sludge (~30mg/L) was approximately 55mg/L less than the 1.5% total solids feed in phase I (~85mg/L). This further indicated that nitrification/denitrification was more active in the warmer summer months than the cooler winter months at the source plant whose basins were open to the outside air. The soluble TKN results also showed more clearly that nitrogen transformation was occurring

in the pretreatment reactors where concentrations decreased from 30mg/L in the feed to less than 5mg/L in all pretreatment reactors (Figure 5.6). These results suggested that nitrification was occurring in the pretreatment reactors as expected by the observed elevated ORPs. Nitrification is an aerobic process and was not found to occur in phase I where mean ORPs were lower. It is also noteworthy that the large variation in soluble TKN in the feed was stabilized in the effluents of the pretreatment reactors.

Further evidence of nitrogen transformation was found in the measured mean ammonia values, where a significant drop was observed in all reactors as compared to the feed (Figure 5.7). Only the reactor with the shortest contact time had any appreciable ammonia remaining in the effluent on the order of 10mg/L. This reactor was also the least stable in producing consistent effluent in terms of ammonia concentration. The mean feed ammonia levels were approximately twice as high in phase II (~50mg/L) compared to phase I (~25mg/L) indicating that hydrolysis was more active at the source plant in phase II (during the summer). Also, the net ammonia removal was greater in phase II than phase I and has been explained by the likelihood of nitrification processes in phase II pretreatment where as ammonia was seen to accumulate in phase I pretreatment trials which were subject to lower ORP conditions.

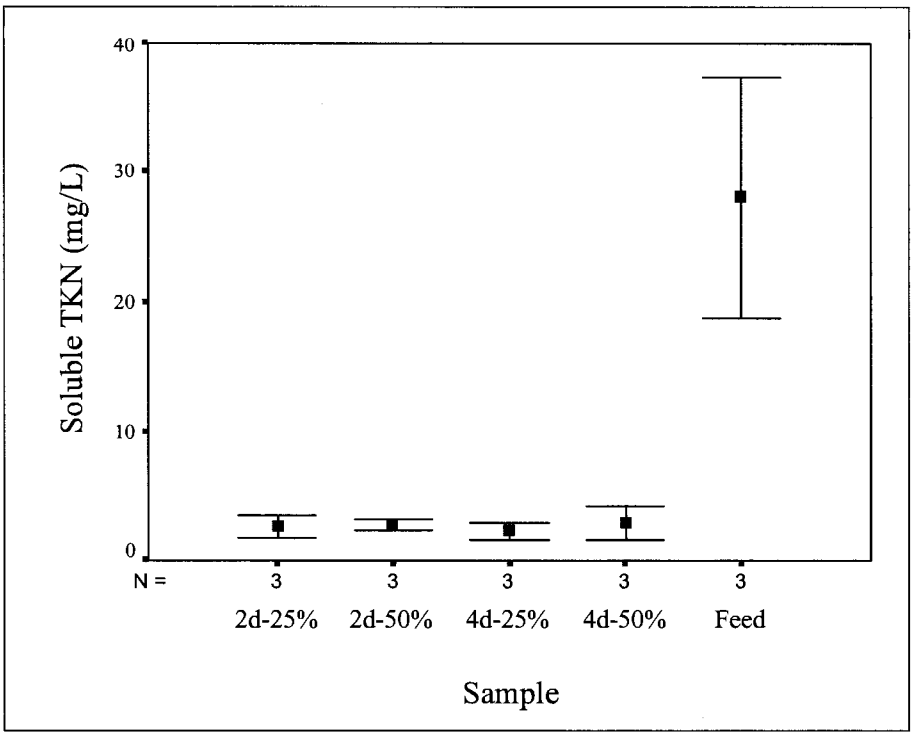

Figure 5.6: Error plot for 90% confidence interval around mean soluble TKN (mg/L) by pretreatment contact time in phase II for feed and reactor effluent samples

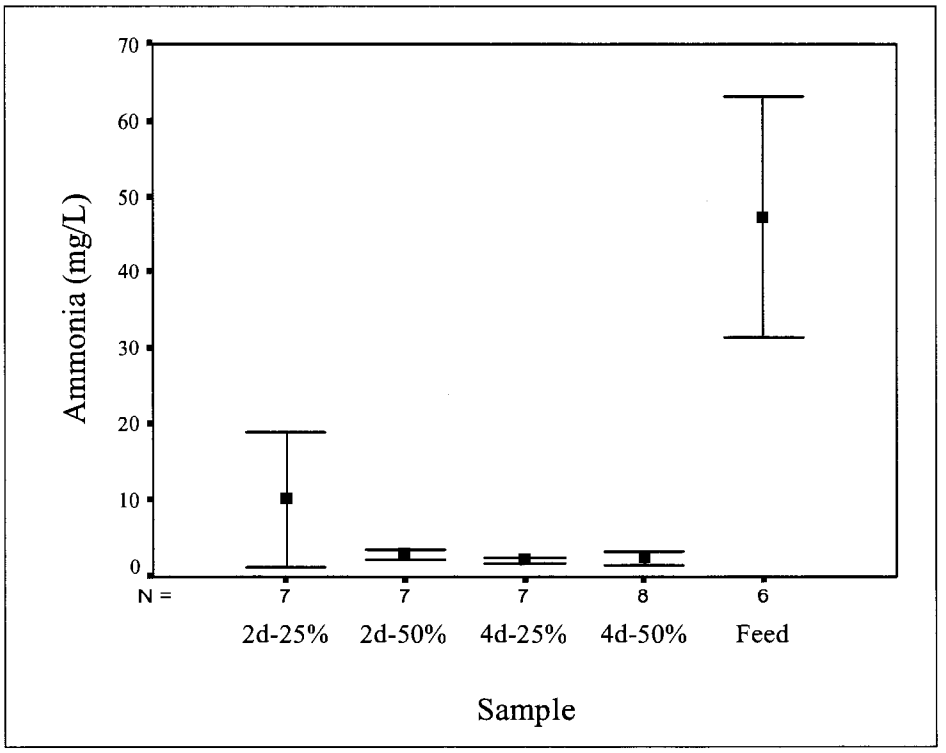

Figure 5.7: Error plot for 90% confidence interval around mean ammonia (mg NH_4^+/L) by pretreatment contact time in phase II for feed and reactor effluent samples

The decrease in ammonia in the effluents as compared to the feed in phase II could explain the similar trend observed in alkalinity (Figure 5.8). The mean values for alkalinity tended to decrease with increased contact time, however, not statistically significantly at the 90% confidence interval. The alkalinity in the pretreatment effluents was at least twice as low in phase II than phase I but the feeds were similar in both phases. VFAs were below the detection limit in phase II whereas they tended to accumulate up to 200mg/L under similar conditions in phase I. Given the absence of VFAs in phase II, they would not account for the drop in alkalinity. It is more likely that the oxidation of the alkalinity associated with the ammonia caused the drop in alkalinity in phase II. Figure 5.10 shows that similar to alkalinity, the pH also decreased with increased contact time but increased relative to the feed in phase II. It is expected that the mean value of VFAs measured in the feed samples (210mg acetate/L) may have depressed the feed pH slightly. Under the oxidizing conditions of pretreatment in this phase (2) of the experiment, the feed VFAs were likely quickly consumed, resulting in a more neutral pH in the effluents than in the feed. The depletion of soluble chemical oxygen demand (COD) after pretreatment confirmed that there was a degradation of VFAs and other soluble substrates in the pretreatment reactors, though it was not statistically significant among the reactors at the 90% confidence interval (Figure 5.9). Whereas there was no net consumption of soluble COD under similar conditions in phase I, soluble COD removal was apparent in phase II likely due to greater aerobic activity and no contribution to COD from VFAs.

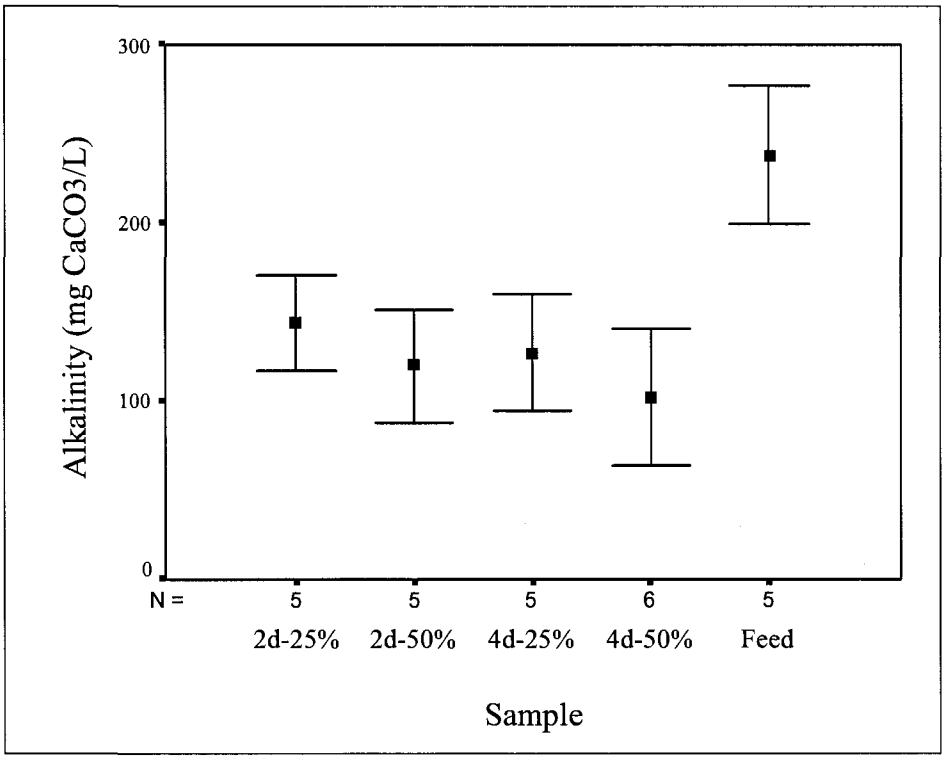

Figure 5.8: Error plot for 90% confidence interval around mean alkalinity (mg $CaCO_3$/L) by pretreatment contact time in phase II for feed and reactor effluent samples

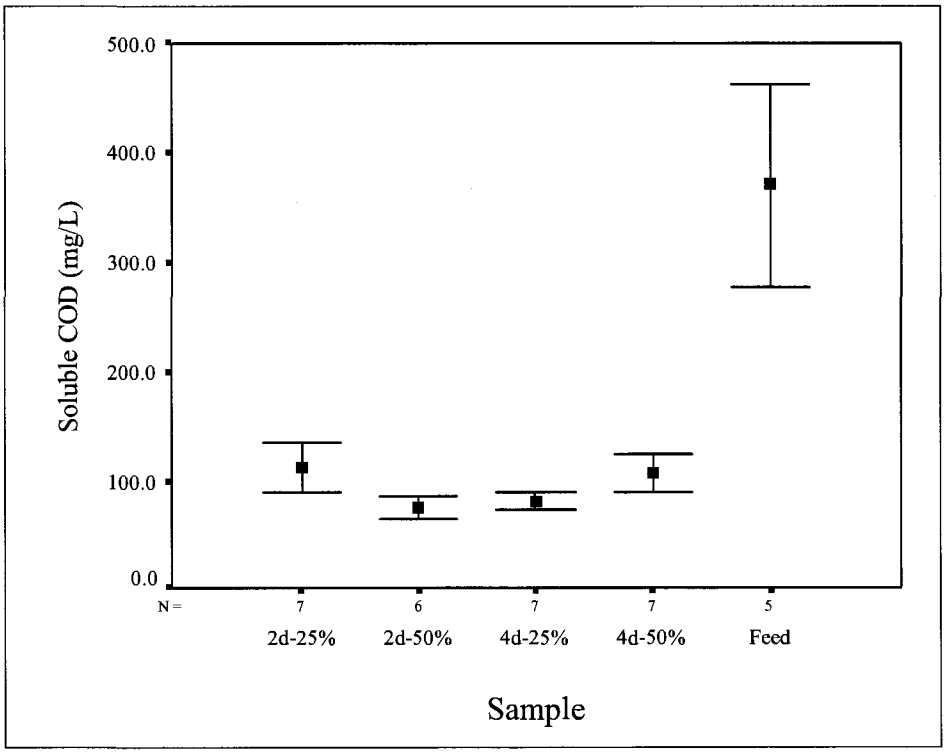

Figure 5.9: Error plot for 90% confidence interval around mean soluble COD (mg/L) by pretreatment contact time in phase II for feed and reactor effluent samples

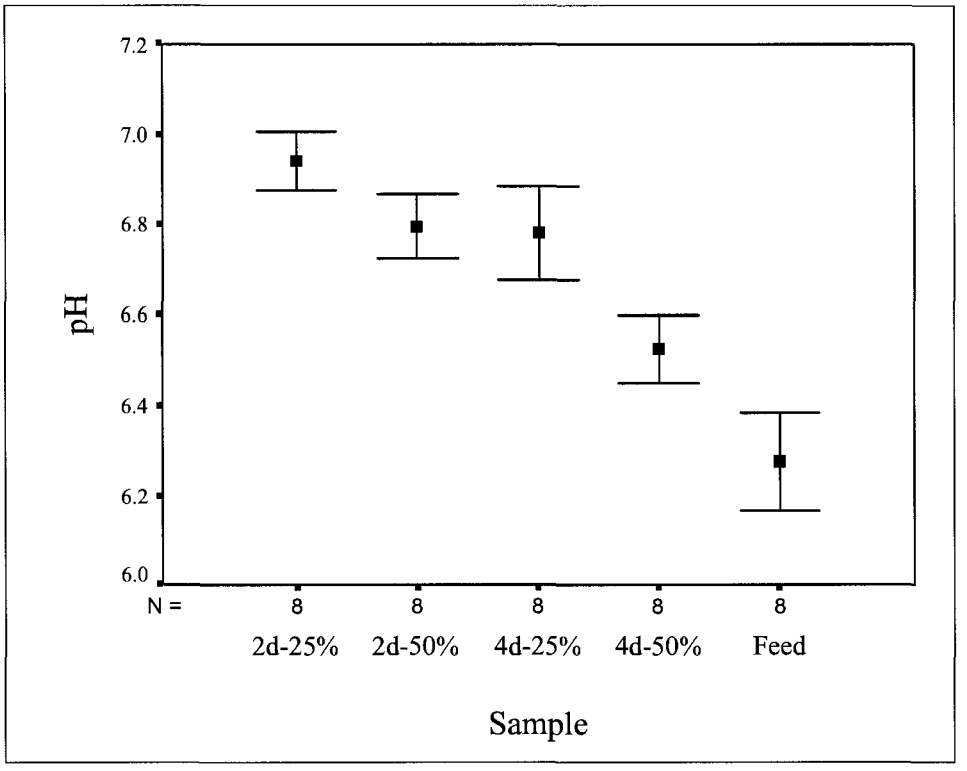

Figure 5.10: Error plot for 90% confidence interval around pH by pretreatment contact time in phase II for feed and reactor effluent samples

In general, the optimum contact time for pathogen destruction in the pretreatment conditions tested in phase II was the longest residence time coupled with less frequent feeding (4d HRT and 50% reactor volume replacement). This held true for fecal coliforms, *E. coli*, and *Shigella* spp (Figures 5.11, 5.12, and 5.14). It was suspected that both the predation and longer exposure to sub-optimal pH led to the increased pathogen destruction with contact time. These results are consistent with the literature where in general better pathogen removal occurs with longer exposure times with draw/fill semi-continuous feeding (Farrell et *al.*, (1988).

Although both the *Salmonella* spp. and *C. perfringens* mean concentrations tended to decrease slightly after pretreatment, there was no obvious trend with contact time (Figures 5.13 and 5.15). The minimal C. *perfringens* die-off, if taken as a model

indicator for *Ascaris* eggs die-off as reported by Bujoczek et *al.*,(2003), suggested that *Ascaris* eggs would not be significantly affected in microaerobic pretreatment of sludge under the conditions tested. Feed and effluent qualities were similar in phase I and phase II for C. *perfringens*. However, the mean log CFU/gTS feed concentrations for fecal coliforms, E. *coli, Salmonella* spp., and *Shigella* spp., were approximately 0.4, 0.9, 1.8, and 0.2 logs lower respectively in phase I than in phase II. Slightly greater mean log reductions were observed for fecal coliforms, *Salmonella* spp., and *Shigella* spp., in phase I under similar conditions studied in phase II (1.5% TS feed, 2d HRT, 50% volume replacement, 0.06vvm air supply). However, these differences were not statistically significant at the 90% confidence interval.

In all cases the pathogen destruction was less than a full log reduction, which suggested that pretreatment alone was insufficient to yield greater pathogen removal from the sludge tested. The longest contact time and feeding cycle was selected for further investigation in phase III of experimentation as it showed the highest potential for pathogen destruction. Multiple conditions were not chosen for further analysis due to practical restrictions on lab resources and available time for sample analysis.

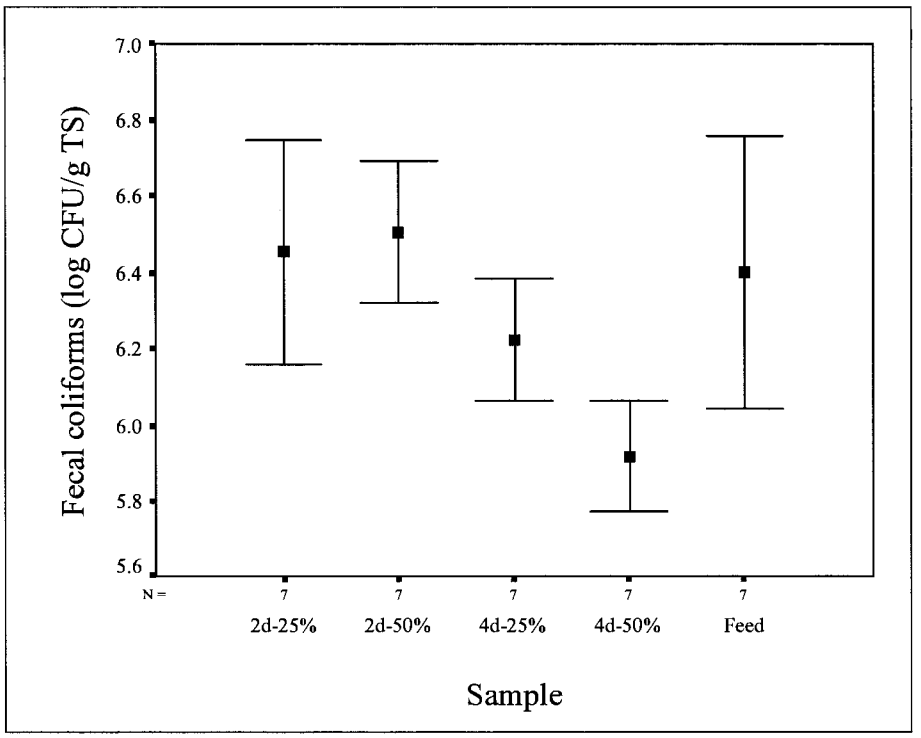

Figure 5.11: Error plot for 90% confidence interval around mean fecal coliform concentration (log CFU/g TS) by pretreatment contact time in phase II for feed and reactor effluent samples

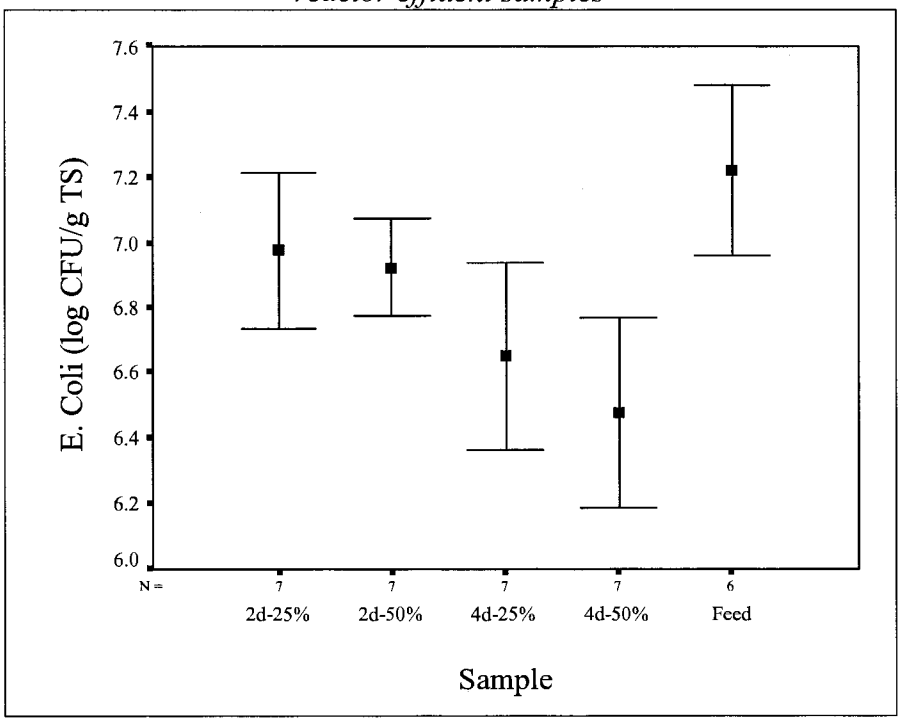

Figure 5.12: Error plot for 90% confidence interval around mean E. coli concentration (log CFU/g TS) by pretreatment contact time in phase II for feed and reactor effluent samples

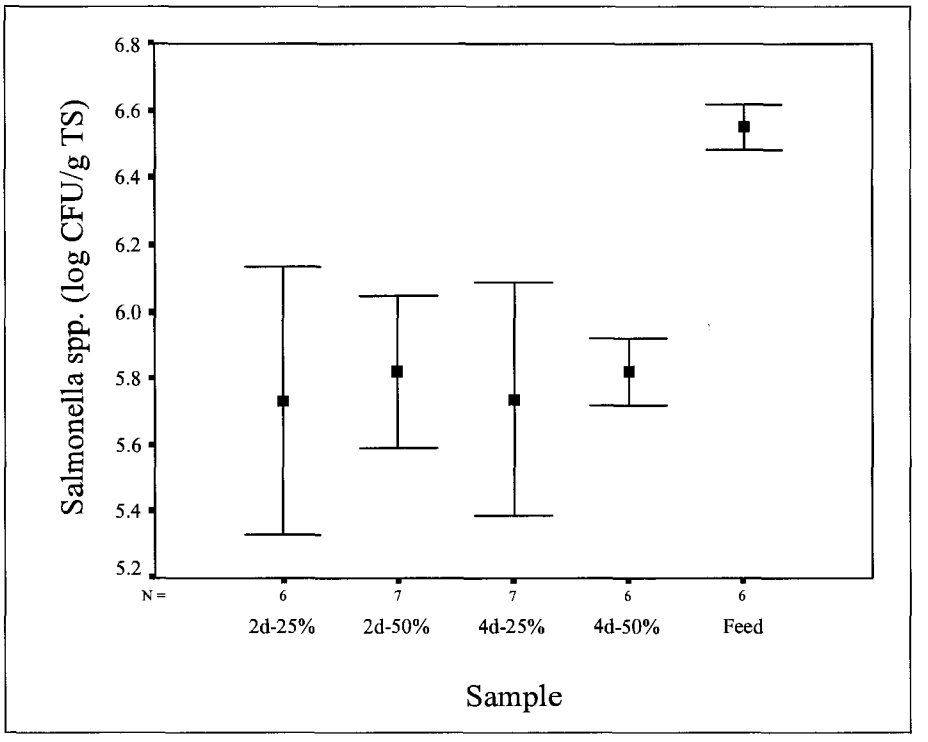

Figure 5.13: Error plot for 90% confidence interval around mean Salmonella spp. concentration (log CFU/g TS) by pretreatment contact time in phase II for feed and reactor effluent samples

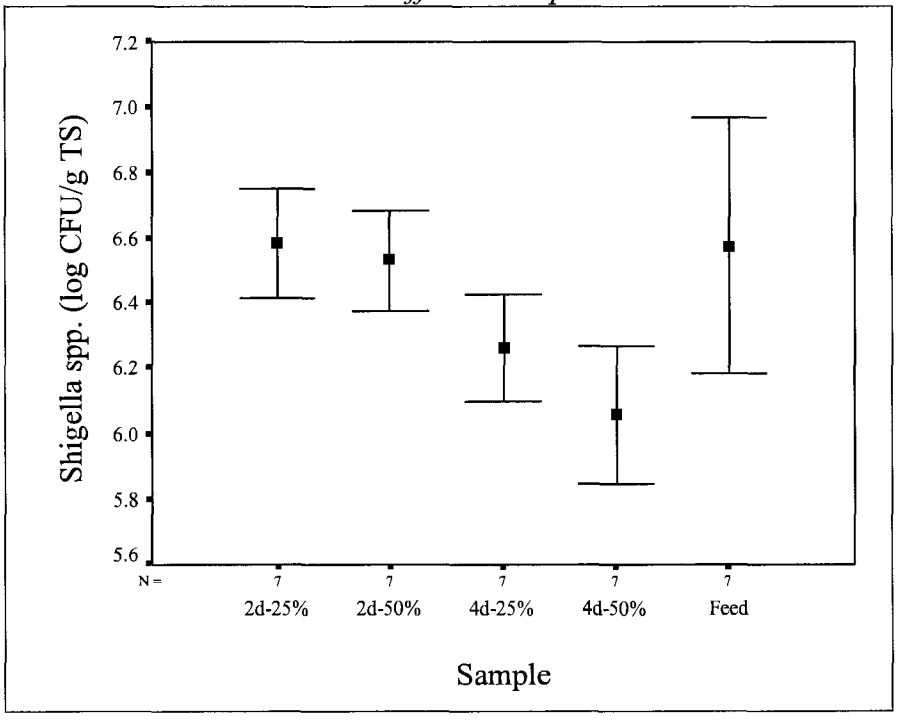

Figure 5.14: Error plot for 90% confidence interval around mean Shigella spp. concentration (log CFU/g TS) by pretreatment contact time in phase II for feed and reactor effluent samples

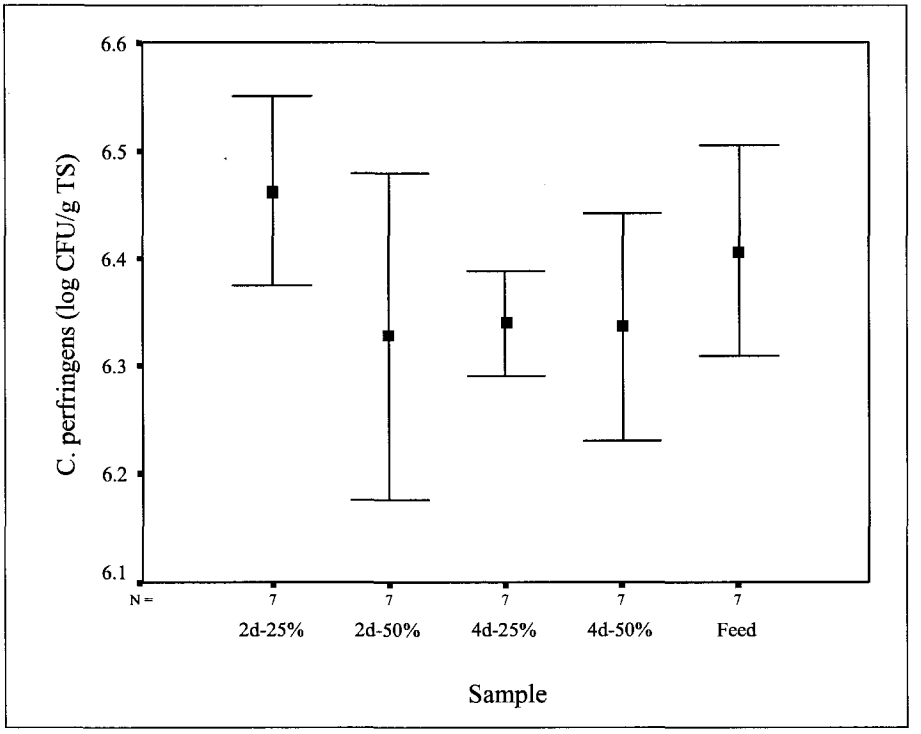

Figure 5.15: Error plot for 90% confidence interval around mean C. perfringens concentration (log CFU/g TS) by pretreatment contact time in phase II for feed and reactor effluent samples

6. Phase III: Results

6.0 Overview

Phase III of the experiments involved three treatment stages: feed preparation, pretreatment and digestion as previously described in the experimental section.

This phase examined the effect of temperature and feed shear on the performance of the microaerobic pretreatment system in a two-by-two factorial experimental design. The semi-batch pretreatment reactors were operated with 4d hydraulic residence times (HRT), aerated at 0.06vvm and fed 50% of their volumes with domestic sewage sludge with a solids concentration of 1.5%. The four reactors were operated under the conditions outlined in Table 6.0.1

Table 6.0.1: Phase III microaerobic pretreatment test conditions

	T = ambient	T = 35°C
Not sheared feed	R2	R4
Sheared feed	R1	R3

The results of the data analysis are presented by variable followed by a summary that discusses potential interactions. All of the data in phase III were treated in a similar fashion with the output of the statistical analysis performed with SPSS for Windows v.10.1 found electronically in Appendix C. The logic diagram for the data analysis is shown in Figure 6.0.1.

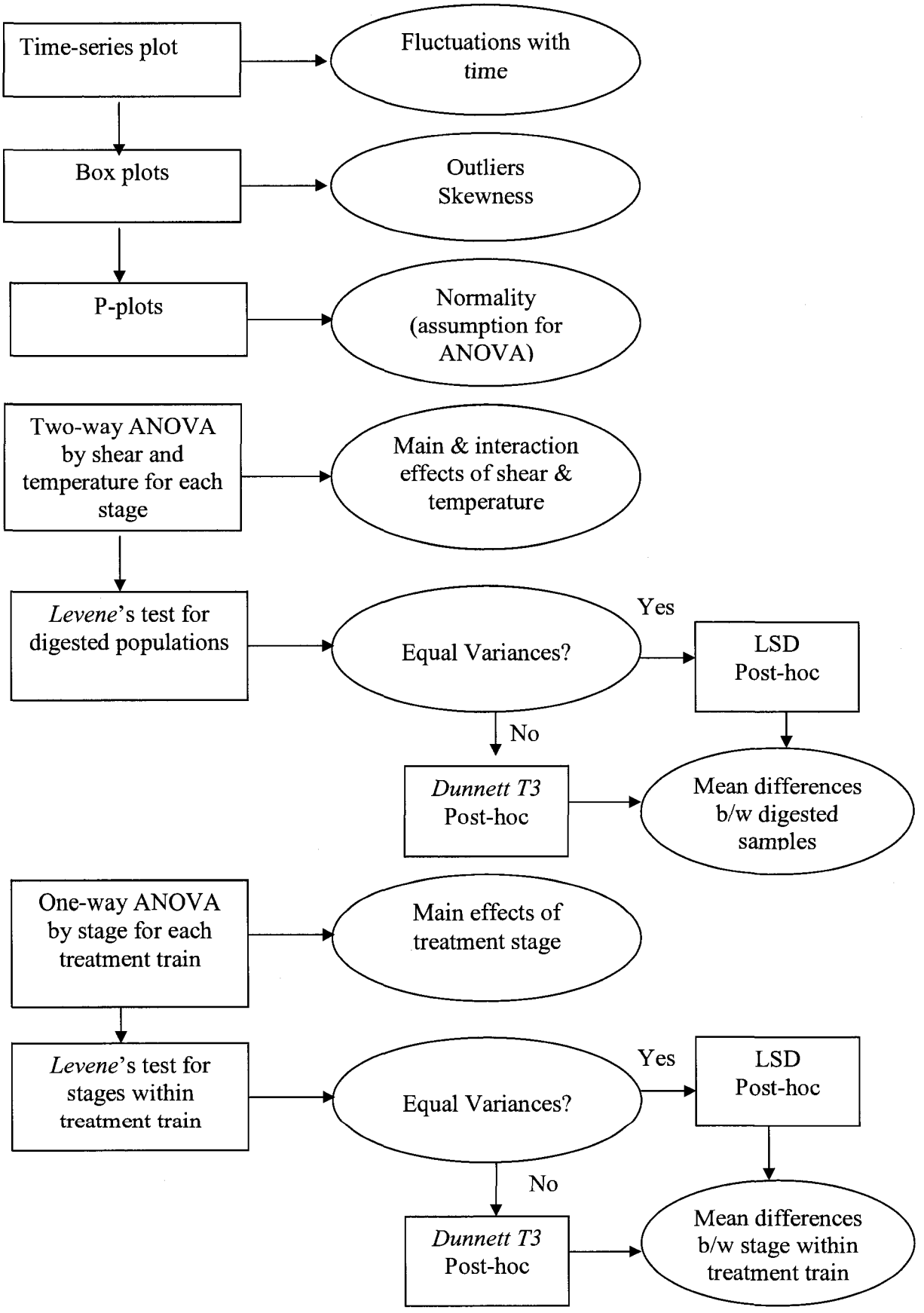

Figure 6.0.1: Logic diagram of data analysis for phase III

First, the data were plotted in line graphs by treatment stage and date to visualize any temporal fluctuations over the pseudo-steady state; and to highlight any trends in differences between the respective populations with time. Next, the data were presented in box plots to identify outliers and the extent if any of skewness in the data. In general, outliers in this phase were left in further analyses as they were deemed to represent inherent variability within the system. To further assess the assumption of normality upon which the subsequent analyses of variance (ANOVA) were based, p-plots were generated for each sample type population denoted by the label 'reactor'. However, despite any deviations from normal, the conclusions from the ANOVA were generally accepted given the inherent robustness of the test with similar sample sizes (Mendenhall et *al.*, 1999).

To easily compare means between all sample populations for a given measured variable, error plots were generated that showed the 90% confidence interval about the mean. This confidence interval was selected because it was expected to reveal significant effects but not underestimate the inherent variability in the biological systems. Formal comparisons of different factors were then performed first in two-way ANOVA by treatment stage ('feed', 'pretreated', or 'digested') with temperature and shearing as fixed factors and second in one-way ANOVA by system ('control', 'ambient-sheared', 'ambient-not sheared', '35°C-sheared', or '35°C-not sheared') with treatment stage as the fixed factor. These tests were also performed at the 90% confidence level.

The two-way analysis performed on the 'feed' stage revealed whether feed shearing had a significant impact on the given variable. The two-way analysis performed

on the 'pretreated' stage revealed whether temperature and shearing had any significant main or interaction effects on the given variable in pretreatment. Finally, the two-way analysis performed on the 'digested' stage showed whether temperature and feed shearing had a significant impact on the given variable in the pretreated and digested populations, which were also compared relative to the control.

The *Levene's* test for homogeneity of variances was performed at the 95% significance level for each set of populations in the treatment stage to assess the assumption of homogeneity of variances required for the ANOVA. In cases where the null hypothesis (that variances were equal) was accepted, homogeneity of variances was assumed and the least squares difference (LSD) comparison of means was used. However, for analysis of the 'digested' stage, where the null hypothesis was rejected and therefore variances deemed to be unequal at the 95% significance level, the *Dunnett T3* post-hoc comparison of means was used. Post-hoc tests were not performed for temperature or shear factors for the 'feed' and 'pretreated' stages since there was no difference in temperature in the feed stage and there were less than three groups in both cases. The mean differences were shown graphically by stage in profile plots typical of two-way factorial analysis with one factor (shearing) shown on the x-axis, the other factor (temperature) shown as separate lines, and the mean of the control for the digested stage analysis shown as a separate point.

To determine whether there were significant differences in the mean measurements taken across a given treatment system, one way ANOVAs were performed by system with the treatment stage as the fixed factor. The *Levene*'s test for homogeneity of variances was performed (at the 95% confidence interval) for the respective feed,

pretreated, and digested sample populations. Similarly to the two-way ANOVA, in cases where the null hypothesis was not rejected, homogeneity of variances was assumed and the least squares difference (LSD) post-hoc comparison of means was used. However, where the null hypothesis was rejected and therefore variances deemed to be unequal at the 95% significance level, the *Dunnett T3* post-hoc comparison of means was used. The mean differences including the bounds of the 90% confidence interval were tabulated using output from the appropriate post hoc test.

Pairwise comparisons between feed and effluent for respective sampling events were not done as this was not appropriate for a semi-batch operation.

6.1 Feed Shearing

The sheared reactor feed was prepared by blending it at high speed in a household blender for 10 minutes prior to feeding. The degree of solubilization after feed shearing was assessed in several ways. Analysis by x-ray particle diffraction was attempted. However, due to the high organic content of the samples, reasonable results were not achievable. Analysis by serial filtration yielded more reasonable results shown in Figure 6.1.1.

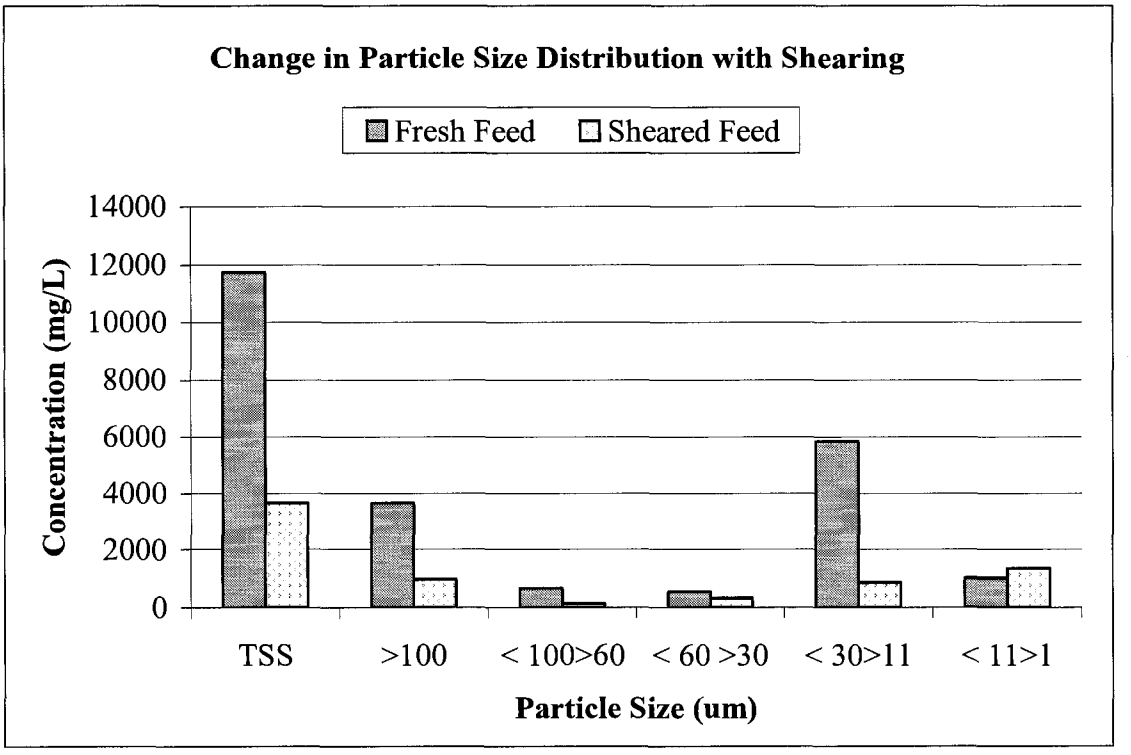

Figure 6.1.1: Particle size distribution of feed samples in phase III as determined by serial filtration

The two samples analyzed were identical despite the feed shearing. The test results (performed in duplicate) showed that at least 60% of the suspended solids were solubilized after feed shearing as indicated by the decrease in TSS of approximately 8000mg/L between fresh and sheared feed. However, these results seem impractically high and are interpreted with some reservation.

Other methods were used to quantify solubilization including assessing the ammonia, soluble COD, and soluble TKN values and were presented with the other data reported for the particular variable.

6.2 Dissolved Oxygen

The dissolved oxygen concentration was measured in the pretreatment and digester effluents over the course of the staged digestion trials (phase III). The measured values fluctuated as seen in the time-series plots in Figure 6.2.1, Figure 6.2.2, and also in Appendix C.

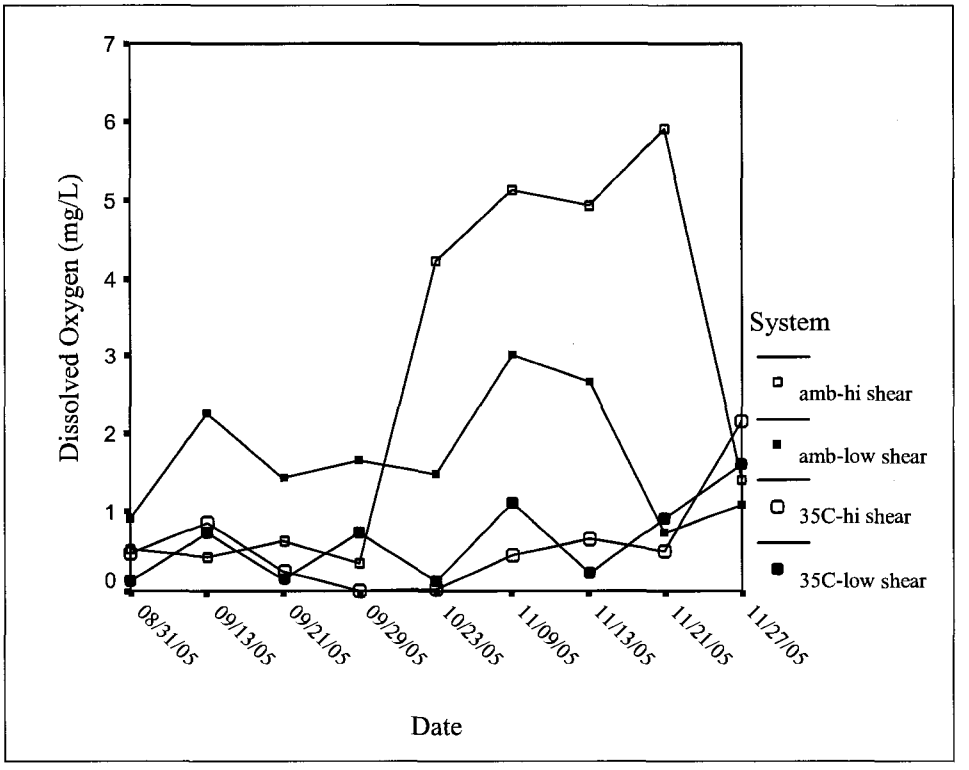

Figure 6.2.1: Time–series plot for dissolved oxygen concentrations (mg/L) measured in pretreatment effluents from phase III

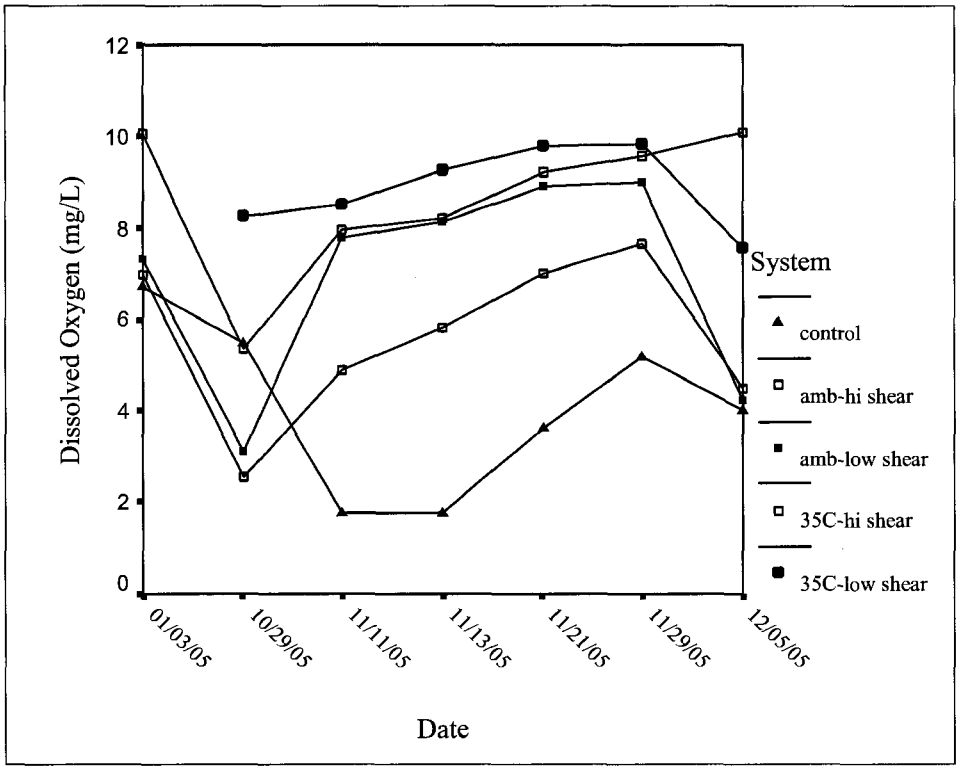

Figure 6.2.2: Time–series plot for dissolved oxygen concentrations (mg/L) measured in digested effluents from phase III

The sharp increase in DO observed in the high temperature, high shear feed reactor that occurred after start-up was attributed to the onset of foaming which would have increased the surface area of the air-liquid interface and therefore the rate of oxygen mass transfer into solution. The box plots of the DO concentrations for each sample type indicated two outliers and generally normal distributions (Figure 6.2.3 and Appendix C). The latter observation was confirmed by p-plots performed by sample set (Appendix C). The outliers were left in the data during further analyses as they were accepted as inherent variability of the system.

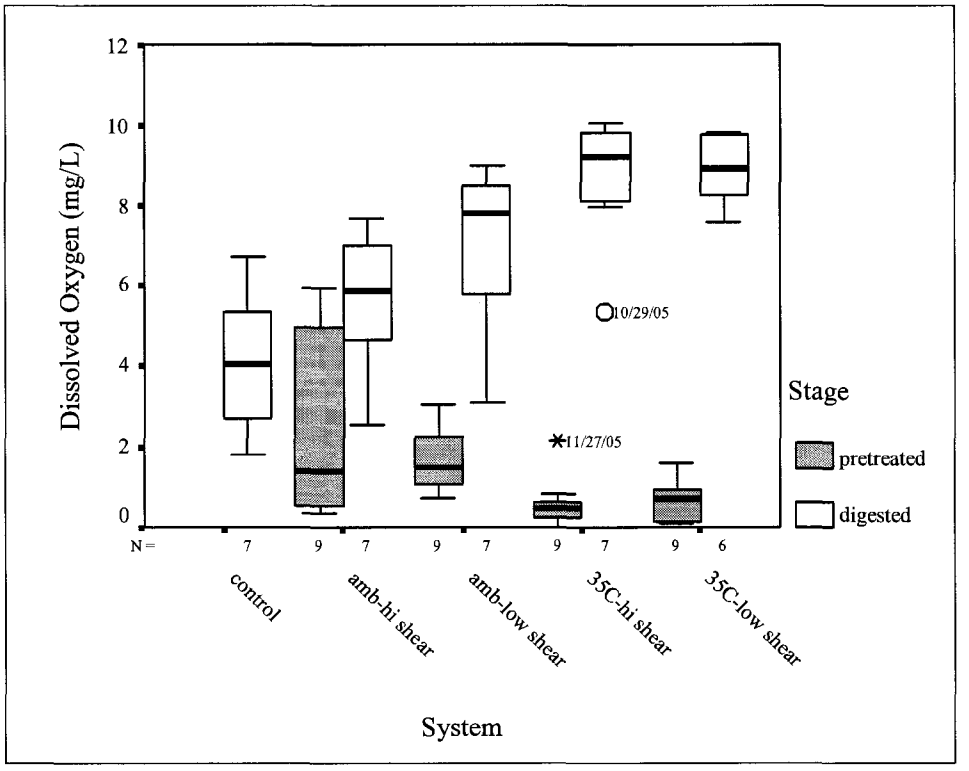

Figure 6.2.3: Box plots for dissolved oxygen concentration (mg/L) in pretreated and digested samples from phase III

It was found that for pretreated populations (Figure 6.2.4), the *Levene*'s test for homogeneity of variance (Appendix C) was significant, ($F(3,32)=31.881$, $p<0.05$), and so the null hypothesis was rejected and therefore the variances were not homogenous. The two-way ANOVA performed at the 90% significance level showed that:

1. Feed shearing had an insignificant effect on the DO in the pretreatment effluent ($F(1,32)=1.009$, $p=0.323$).

2. Higher pretreatment temperature was found to have a significant negative effect on the DO (-1.54 +/- 0.74 mg/L) in the pretreatment effluent ($F(1,32)=12.307$, $p<0.10$).

3. No significant interaction between the effect of pretreatment temperature and feed shearing on DO concentration in the pretreatment effluent was found (F(1,32)=1.189, p=0.284).

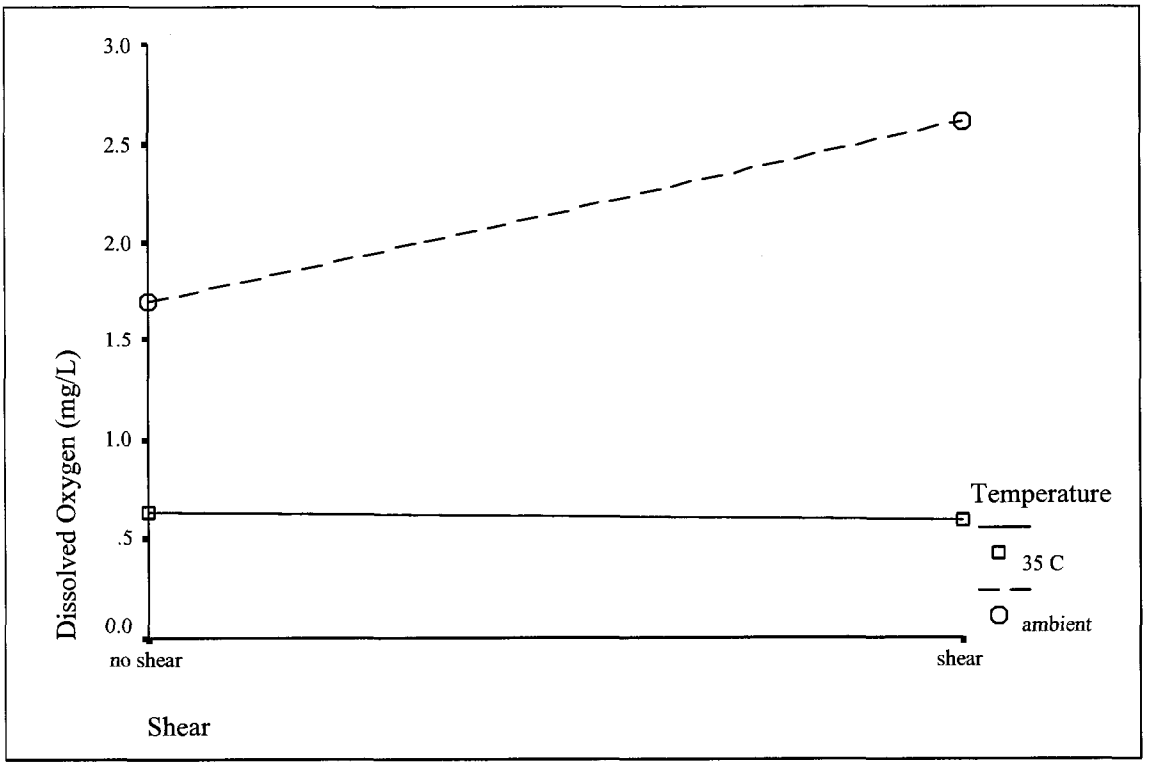

Figure 6.2.4: Means of DO (mg/L) for pretreated effluents in phase III

For digested populations (Figure 6.2.5), the *Levene*'s test for homogeneity of variance (Appendix C) was insignificant, (F(4,29)=1.189, p=0.337), so the null hypothesis was not rejected and therefore the variances were homogenous. The subsequent two-way ANOVA and LSD pairwise comparisons performed at the 90% significance level showed that:

1. Feed shearing had an insignificant effect on the DO in the digester effluent of pretreated samples F(1,29)=1.240, p=0.275).

2. Higher pretreatment temperature was found to have a significant positive effect on the DO in the final effluent (2.48 +/- 1.18mg/L) over ambient pretreatment, ($F(1,29)=12.746$, $p<0.10$); it was also found to have a greater positive effect over the control digester (4.68 +/- 1.44 mg/L). Pretreatment at ambient temperature had a significantly positive effect over the control (2.20 +/- 1.42mg/L) and on the DO of the digested effluents.

3. No significant interaction between the effect of pretreatment temperature and feed shearing on DO concentration in the digester effluent was found ($F(1,29)=(1.189)$, $p=0.284$).

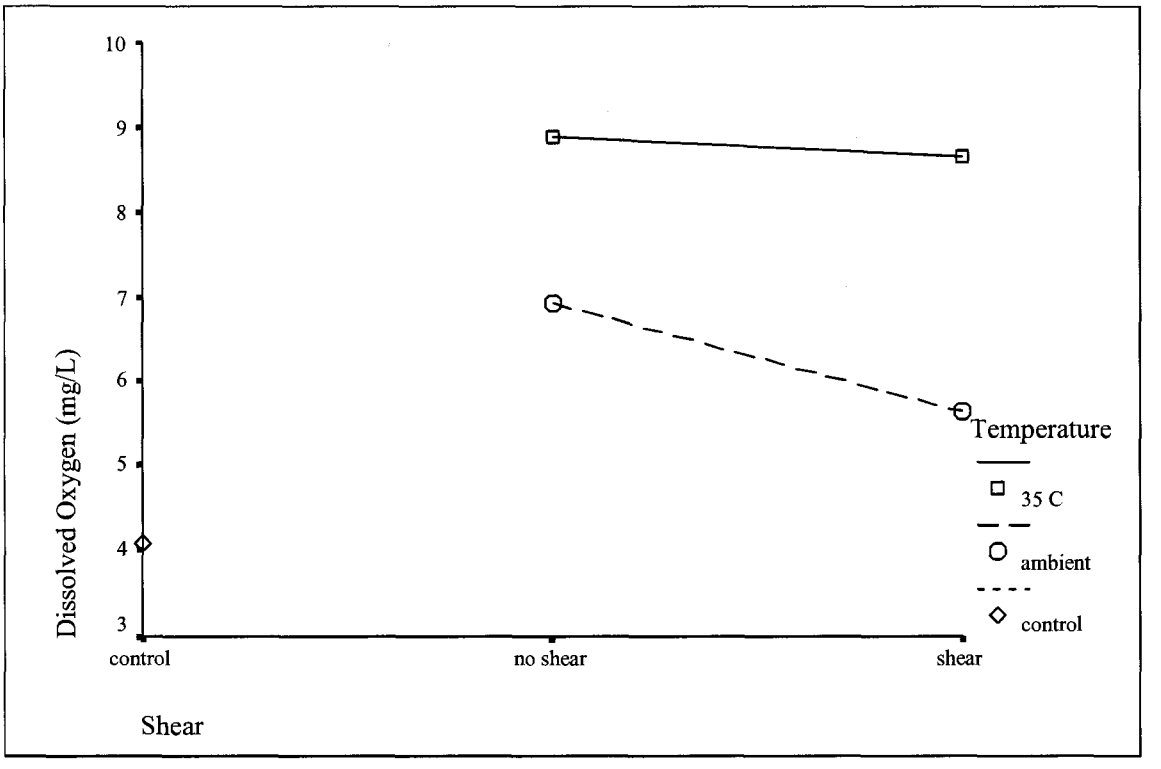

Figure 6.2.5: Means of DO (mg/L) for digested effluents in phase III

The mean values for DO by sample presented in Figure 6.2.6 and show the bounds of the 90% confidence interval. The depressed DO in the high temperature pretreatment reactors relative to those at ambient temperatures may have been caused by an increase in microbial activity expected at the elevated temperature. However, given insignificant differences in solids destruction, it was more likely that the cause was the decline in oxygen saturation concentration with increased temperature (which depressed the driving force for mass transfer). It was interesting to note that downstream (in ambient temperature digestion) the high temperature systems were at approximately 8mg/L and had significantly higher DO than the control which was at 4mg/L and slightly higher DO than the ambient pretreatment systems whose mean DO were between 5.8 to 6.4mg/L. Accordingly, these digesters also had decreased ORP as shown in the next section. The mean DO in the ambient temperature, low shear pretreatment reactor was 2-3mg/L less than that measured in the similar reactor studied in phase II. This may have been caused by a slightly higher biodegradable fraction in the feed available in phase III that took place in the fall. This may have also caused the observed lower pretreatment mean ORP in phase III than in phase II for the similar reactors studied in each phase..

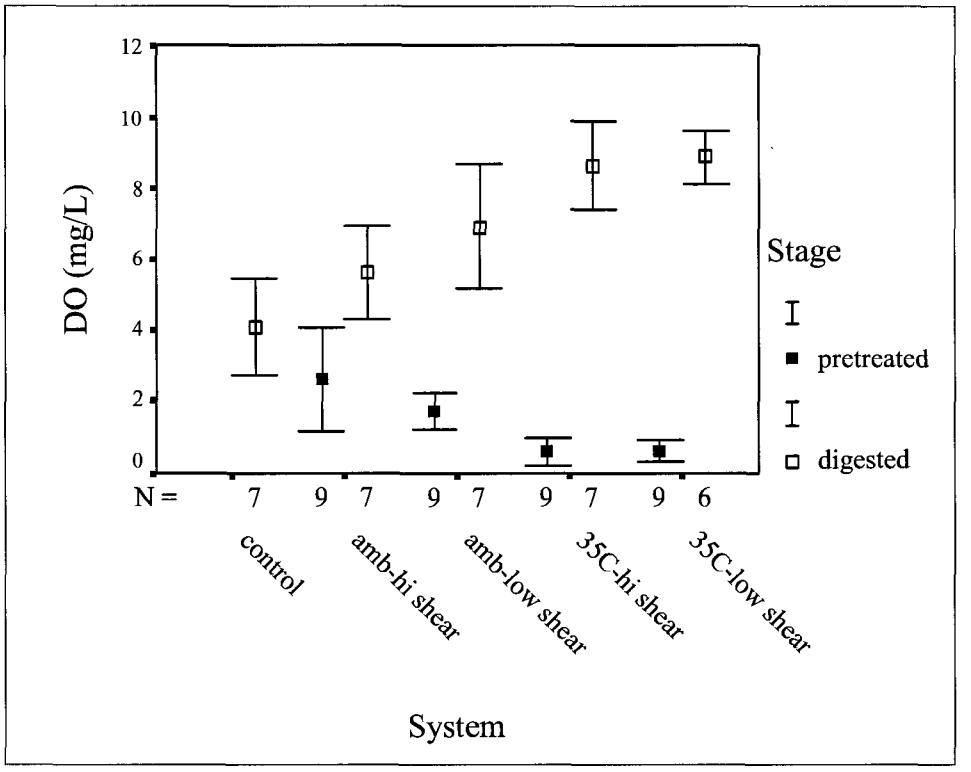

Figure 6.2.6: Error plot for 90% confidence interval around mean DO concentrations by system type and stage of treatment

The mean differences significant at the 0.1 level between the DO in pretreatment and digestion for each system are presented in Table 6.2.1. They show the greatest increases of 8.05 and 8.26mg/L occurred in the two high temperature systems with high or low shear feeds.

Table 6.2.1: Mean difference between stages significant at the 0.1 level for variable DO (mg/L)

	Pretreated-Digested
Control	n.a.
Ambient/high shear	3.02 +/- 1.91
Ambient/low shear	5.24 +/- 1.46
35°C/high shear	8.05 +/- 1.06
35°C/low shear	8.26 +/- 0.65

6.3 Oxidative-Reductive Potential (ORP)

The ORP was measured in the pretreatment and digester effluents over the course of the staged digestion trials (phase III). The measured values fluctuated as seen in the time-series plots in Appendix C with the number of successful samples indicated on the x-axis. The sharp increase in ORP observed in the high temperature, shear feed reactor was attributed to the onset of foaming which would have increased the surface area of the air-liquid interface and therefore the rate of oxygen mass transfer into solution. The box plots of the ORP measurements for each sample type tested indicate several outliers and generally normal distributions. The latter observation was confirmed by p-plots performed by sample set (Appendix C). The outliers were left in the data during further analyses as they were assumed to be associated with the inherent variability of the system.

It was found that for pretreated populations (Figure 6.3.1), the *Levene*'s test for homogeneity of variance (Appendix C) was insignificant, ($F(3,32)=1.262$, $p=0.304$), so the null hypothesis was not rejected and the variances were homogenous. The two-way ANOVA performed at the 90% significance level showed that:

1. Feed shearing had an insignificant effect on the ORP in the pretreatment effluent ($F(1,32)=0.093$, $p=0.762$).
2. Higher pretreatment temperature was found to have a significant negative effect on the ORP (-227.2 +/- 69.12 mV) in the pretreatment effluent compared to at ambient temperature ($F(1,32)=30.998$, $p<0.10$).

3. No significant interaction between the effect of pretreatment temperature and feed shearing on ORP concentration in the pretreatment effluent was found (F(1,32)=0.081, p=0.777).

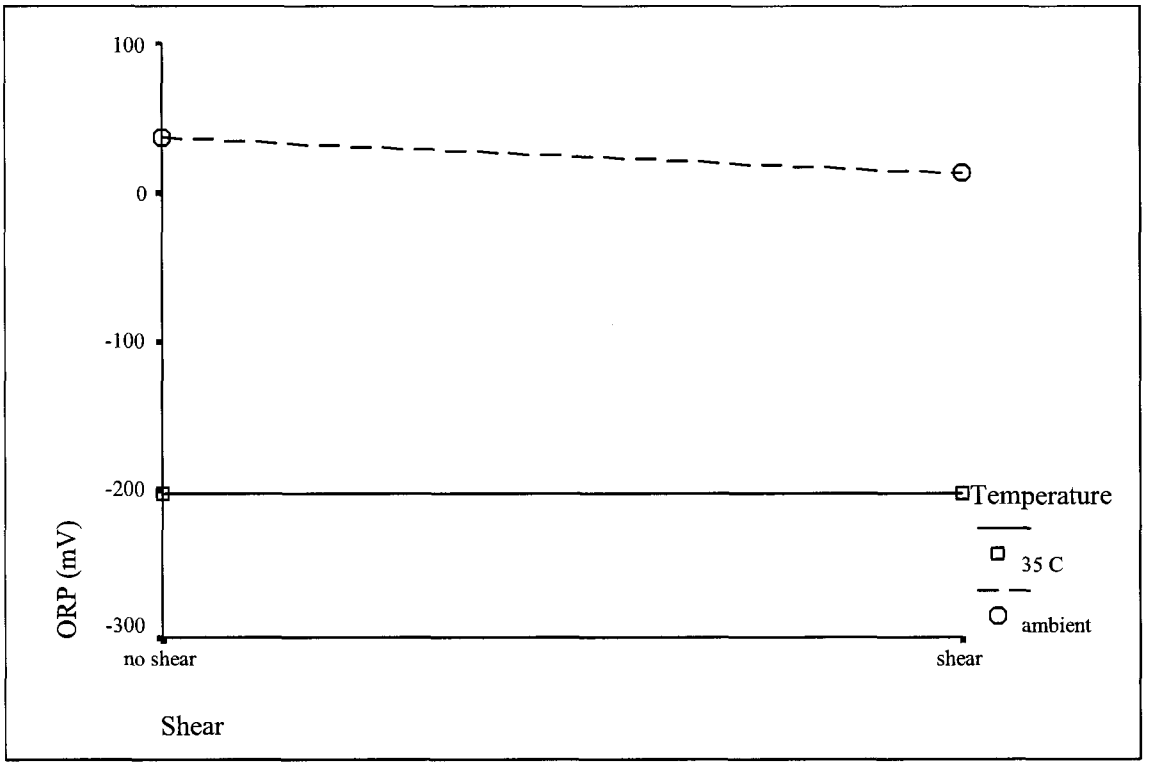

Figure 6.3.1: Means of ORP (mV) for pretreated effluents in phase III

For digested populations (Figure 6.3.2), the *Levene*'s test for homogeneity of variance (Appendix C) was insignificant, (F(4,34)=0.656, p=0.626), indicating that the null hypothesis could not be rejected and therefore the variances were homogenous. The two-way ANOVA and LSD pairwise comparisons performed at the 90% significance level showed that after fully aerobic digestion:

1. Feed shearing had an insignificant effect on the ORP in the final effluent F(1,34)=0.757, p=0.390).

2. Higher pretreatment temperature was found to have a significant positive effect on the ORP in the final effluent (53.7 +/- 38.4 mV) over ambient pretreatment ($F(1,34)=5.589$, $p<0.10$). It was also found to have a greater positive effect over the control digester (102.7 +/- 49.2 mV). Pretreatment at ambient temperature did not have a significant effect on the ORP of the digested effluent relative to the control.

3. No significant interaction between the effect of pretreatment temperature and feed shearing on ORP concentration was found ($F(1,34)=0.051$, $p=0.82$).

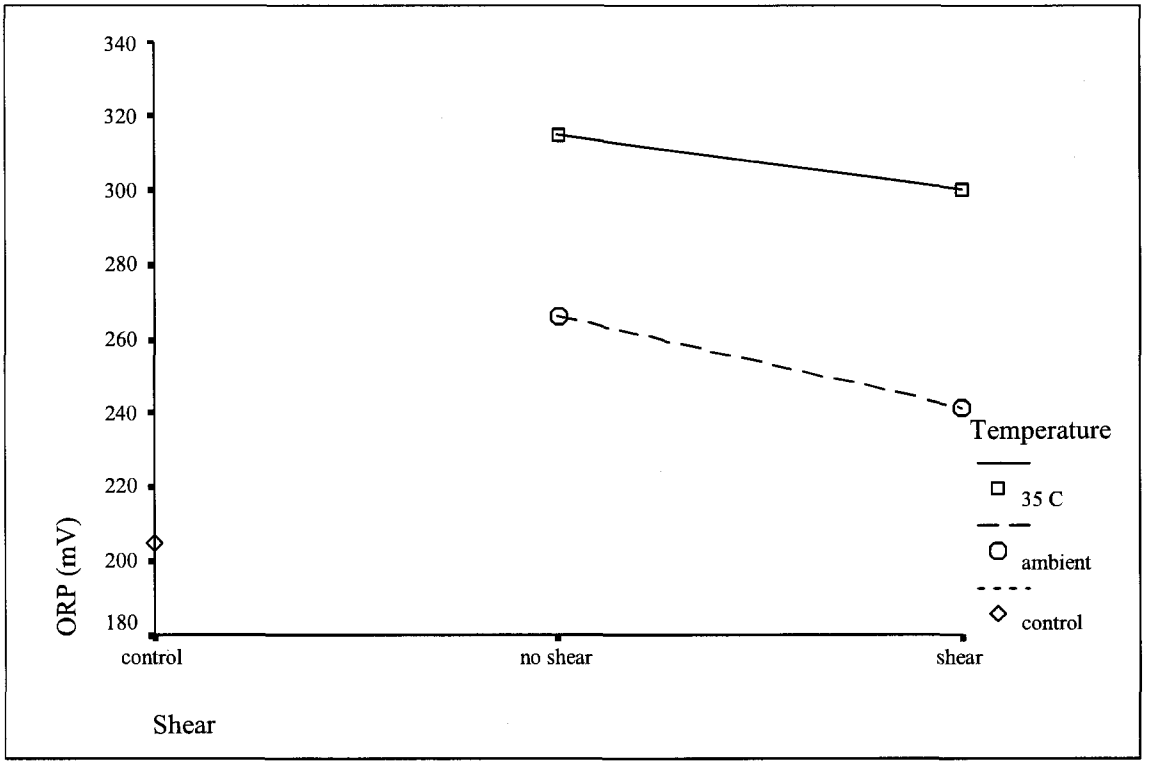

Figure 6.3.2: Means of ORP (mV) for digested effluents in phase III

Given equal variances, LSD post-hoc tests were performed to identify which means were significantly different (Appendix C). The mean values by sample were presented in Figure 6.3.3 and show the bounds of the 90% confidence interval.

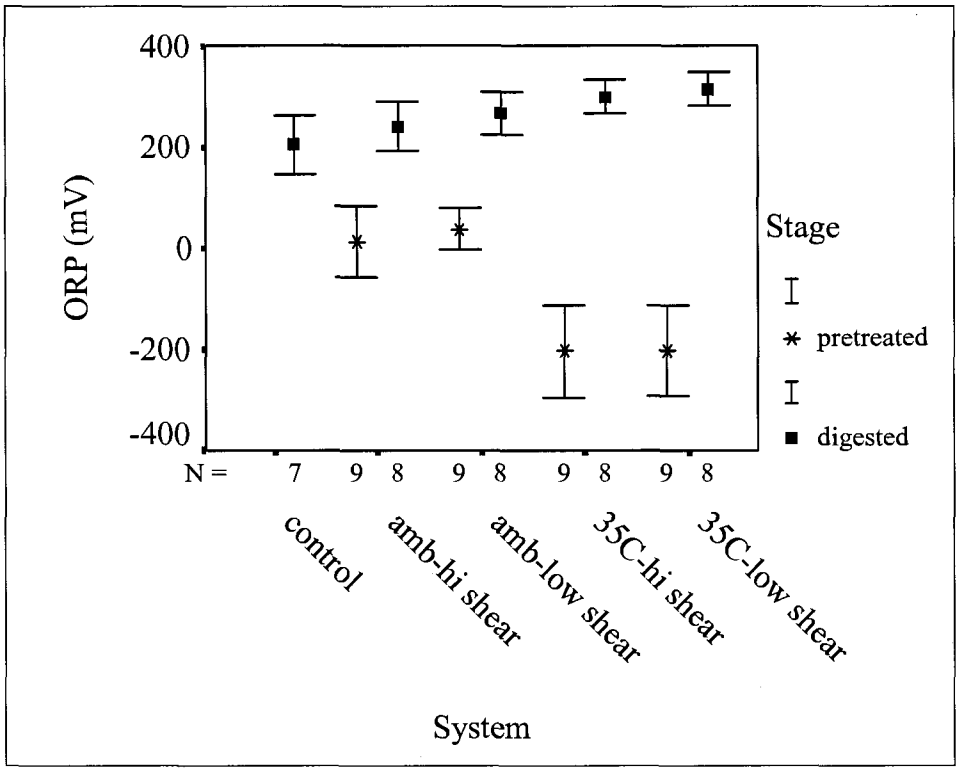

Figure 6.3.3: Error plot for 90% confidence interval around mean ORP (mV) by system type and stage of treatment

In general the mean ORP values of all pretreatment reactors were significantly less than all digesters (Table 6.3.1). As expected the ORP in the high temperature pretreatment reactor was significantly lower than in the ambient temperature reactors. LSD post-hoc tests showed a significant difference of 502.7mV ORP between the pretreatment and the digester effluents from the high shear, high temperature system; also, a significant difference of 516.5mV ORP at the 90% confidence interval between the pretreatment and digester effluents from the low shear, high temperature pretreatment reactor was found. Given verified equal variances and confirmation of temperature effects from the one-way ANOVA, the difference between both digesters receiving high temperature feed and the control was accepted as significantly different.

Table 6.3.1: Mean difference between stages significant at the 0.1 level for variable ORP (mV)

	Pretreated-Digested
Control	n.a.
Ambient/high shear	228.3 +/- 83.4
Ambient/low shear	229.1 +/- 56.0
35°C/high shear	502.7 +/- 95.9
35°C/low shear	516.5 +/- 94.3

The depressed mean ORP in the high temperature pretreatment reactor relative to those at ambient temperatures was in line with the depressed DO observed in those reactors. As previously noted, this may have been caused by an increase in microbial activity at the elevated temperature. However, given insignificant differences in solids destruction, it was more likely that the cause was the decline in oxygen saturation concentration with increased temperature (which depressed the driving force for mass transfer). The mean ORP downstream was higher in the digesters that were accordingly observed to have higher DO. The relationship between ORP and DO in fully aerobic digestion appears to be linear as shown in Figure 6.3.4. A linear regression analysis performed in Microsoft Excel 2000 shows a moderate ($R^2 = 0.74$) linear relationship for all fully aerobic digesters

$$DO = 0.0325\ (ORP) - 1.5932 \qquad [6.1]$$

where DO is in [mg/L] and ORP is in [mV]. Outliers that occurred on 12/20/05 were removed from the scatter plot.

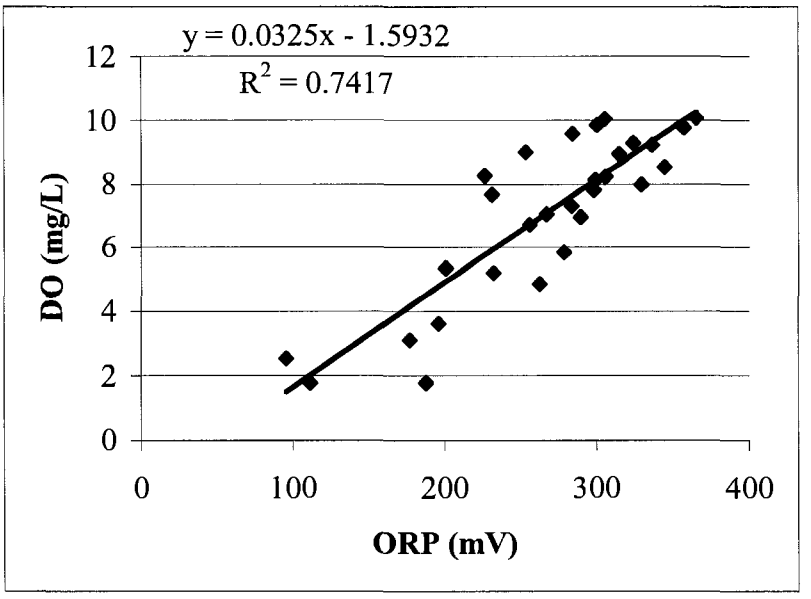

Figure 6.3.4: Scatter plot of ORP (mV) vs. DO (mg/L) for all digester sampling events

6.4 Total Kjeldahl Nitrogen (TKN)

The TKN was measured in the pretreatment and digester effluents over the course of the staged digestion trials (phase III). The measured values fluctuated as seen in the time-series plots in Appendix C. The box plots of the TKN measurements did not indicate outliers (though none were expected for n = 3) and indicated generally normal distributions about the mean. The latter observation was confirmed by p-plots performed by sample set (Appendix C).

For feed samples, the *Levene*'s test for homogeneity of variance (Appendix C) was insignificant, ($F(1,4)=0.296$, $p=0.616$), so the null hypothesis was not rejected and therefore the variances were homogenous. The two-way ANOVA performed at the 90% significance level showed that:

1. Feed shearing had an insignificant effect on the TKN in the sheared feed relative to the not sheared feed ($F(1,4)=0.052$, $p=0.831$).

For pretreated populations (see Figure 6.4.1), the *Levene*'s test for homogeneity of variance (Appendix C) was insignificant, ($F(3,12)=0.054$, $p=0.983$), so the null hypothesis was not rejected and therefore the variances were homogenous. The two-way ANOVA performed at the 90% significance level showed that:

1. Feed shearing had an insignificant effect on the TKN in the pretreatment effluent ($F(1,12)=0.782$, $p=0.394$).
2. Higher pretreatment temperature was found to have an insignificant effect on the TKN relative to pretreatment at ambient temperature in the pretreatment effluent ($F(1,12)=0.697$, $p=0.420$).
3. No significant interaction between the effect of pretreatment temperature and feed shearing on TKN concentration in the pretreatment effluent was found ($F(1,12)=0.113$, $p=0.743$).

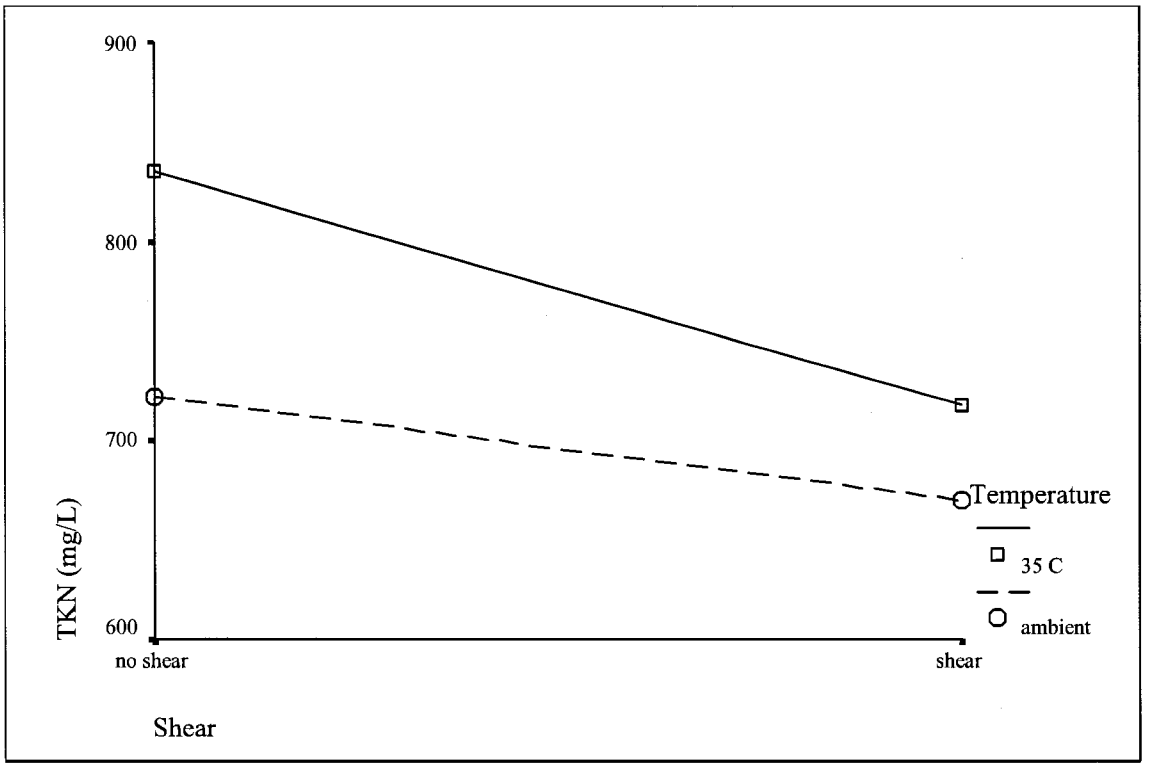

Figure 6.4.1: Means of TKN (mg/L) for pretreated effluents in phase III

For digested populations (Figure 6.4.2), the *Levene*'s test for homogeneity of variance (Appendix C) was significant, ($F(4,10)=3.489$, $p<0.05$), which indicated that the null hypothesis could be rejected and therefore the variances were not homogenous. The two-way ANOVA performed at the 90% significance level showed that:

After fully aerobic digestion (see Figure 6.4.2):

1. Feed shearing had an insignificant effect on the TKN in the final effluent $F(1,10)=0.078$, $p=0.786$).
2. Pretreatment temperature had an insignificant effect on the TKN in the final effluent $F(1,10)=0.053$, $p=0.823$).
3. No significant interaction between the effect of pretreatment temperature and feed shearing on TKN concentration was found ($F(1,10)=0.460$, $p=0.513$).

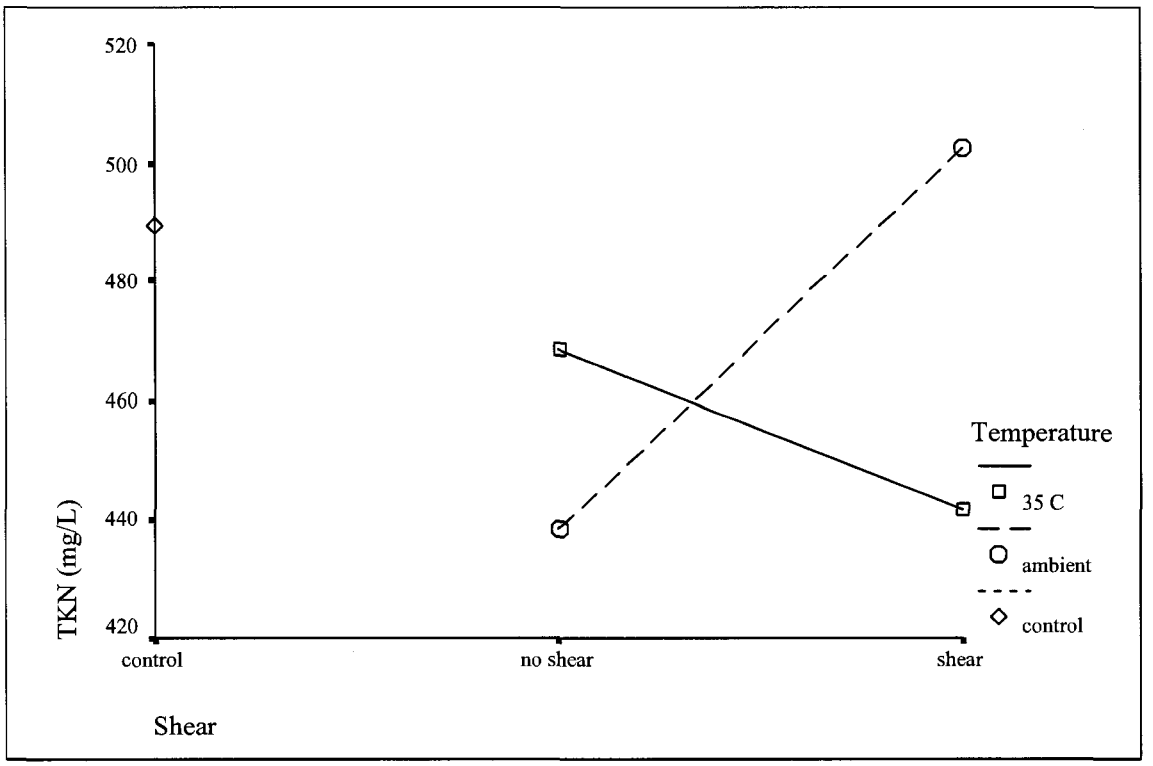

Figure 6.4.2: Means of TKN (mg/L) for digested effluents in phase III

In general the mean TKN values of all pretreatment reactors and feed were greater than all digesters in phase III (Figure 6.4.3). The difference was attributed to nitrification that was occurring in the digesters, which converted nitrogen to nitrate and nitrite species confirmed in subsequent measurements. The differences between TKN values at each stage are shown in Table 6.4.1 where significant at the 0.1 level.

The mean TKN values in the feed were slightly higher in phase III as in phase II though not significantly different at the 90% confidence interval. If the increase were real, this could be explained by a lesser extent of nitrification occurring at the source plant during the fall months versus summer months and hence more TKN in the feed in the fall (phase III). Also, TKN removal (based on mean values) was not observed after

pretreatment in phase III to the same extent as seen in phase II. It is hypothesized that nitrification was less prevalent in phase III pretreatment where the mean reactor ORP and DO levels were less than in phase II. However, the variability in the data is such that strong conclusions cannot be drawn regarding the mean differences of TKN between phases.

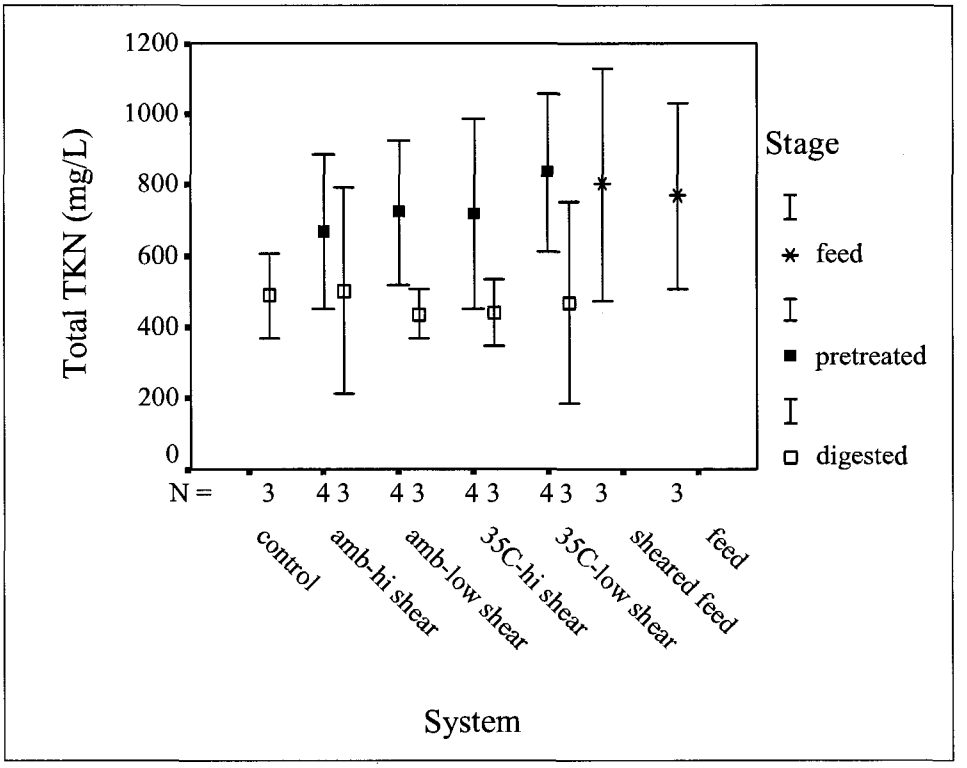

Figure 6.4.3: Error plot for 90% confidence interval around mean TKN (mg/L) by system type and stage of treatment

Table 6.4.1: Mean difference between stages significant at the 0.1 level for variable TKN (mg/L)

	Feed-Pretreated	Pretreated-Digested	Feed-Digested
Control	-	-	-278.7 +/- 208.32
Ambient/high shear	n.s.	n.s.	-298.3 +/- 284.4
Ambient/low shear	n.s.	-283.3 +/- 202.7	-329.7 +/- 216.7
35°C/high shear	n.s.	-275.8 +/- 264.5	-359.0 +/- 282.8
35°C/low shear	n.s.	-367.1 +/- 250.6	-299.7 +/- 267.9

n.s. = not significant

6.5 Soluble Total Kjeldahl Nitrogen (TKN)

The soluble TKN was measured in the pretreatment and digester effluents over the course of the staged digestion trials (phase III). The measured values fluctuated as seen in the time-series plots in Appendix C. The box plots of the soluble TKN measurements did not indicate outliers (though none were expected for n = 3) and showed generally normal distributions about the mean. The latter observation was confirmed by p-plots performed by sample set (Appendix C). The soluble TKN was measured in the pretreatment and digester effluents over the course of the staged digestion trials (phase III). The measured values fluctuated as seen in the time-series plots in Appendix C.

For feed samples, the *Levene*'s test for homogeneity of variance (Appendix C) was insignificant, ($F(1,6)=1.097$, $p=0.335$), so the null hypothesis was not rejected and therefore the variances were homogenous. The two-way ANOVA performed at the 90% significance level showed that:

1. Feed shearing had a significant positive effect (32.4 +/- 18.6mg/L) on the soluble TKN in the sheared feed relative to the not sheared feed ($F(1,6)=11.479$, $p<0.10$).

For pretreated populations (see Figure 6.5.1), the *Levene*'s test for homogeneity of variance (Appendix C) was significant, ($F(3,12)=4.747$, $p<0.05$), so the null hypothesis was rejected and therefore the variances were not homogenous. The two-way ANOVA performed at the 90% significance level showed that:

1. Feed shearing had an insignificant effect on the soluble TKN in the pretreatment effluent ($F(1,12)=0.306$, $p=0.590$).
2. Higher pretreatment temperature was found to have a significant positive effect on the soluble TKN relative to pretreatment at ambient temperature (192.0 +/- 136.3mg/L) in the pretreatment effluent ($F(1,12)=6.299$, $p<0.10$).
3. No significant interaction between the effect of pretreatment temperature and feed shearing on soluble TKN concentration in the pretreatment effluent was found ($F(1,12)=1.225$, $p=0.290$).

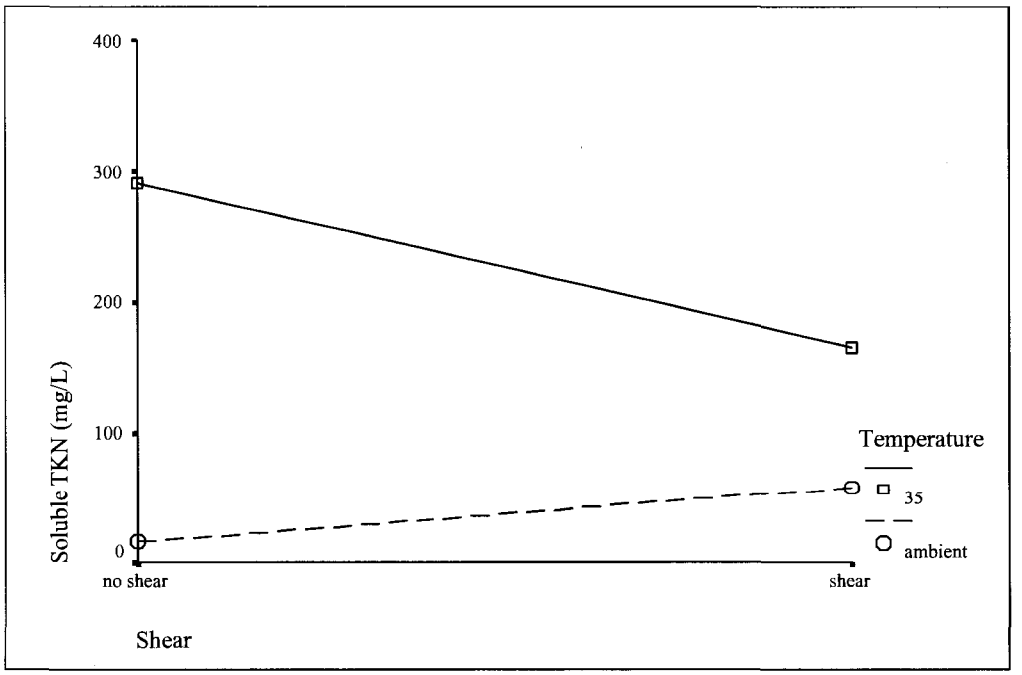

Figure 6.5.1: Means of soluble TKN (mg/L) for pretreated effluents in phase III

For digested populations (Figure 6.5.2), the *Levene*'s test for homogeneity of variance (Appendix C) was significant, ($F(4,10)=9.744$, $p<0.05$), so the null hypothesis was not rejected and therefore the variances were homogenous. The two-way ANOVA performed at the 90% significance level showed that after fully aerobic digestion:

1. Feed shearing had an insignificant effect on the soluble TKN in the final effluent $F(1,10)=0.081$, $p=0.782$).
2. Pretreatment temperature had an insignificant effect on the soluble TKN in the final effluent $F(1,10)=0.030$, $p=0.866$).
3. No significant interaction between the effect of pretreatment temperature and feed shearing on soluble TKN concentration was found ($F(1,10)=0.036$, $p=0.854$).

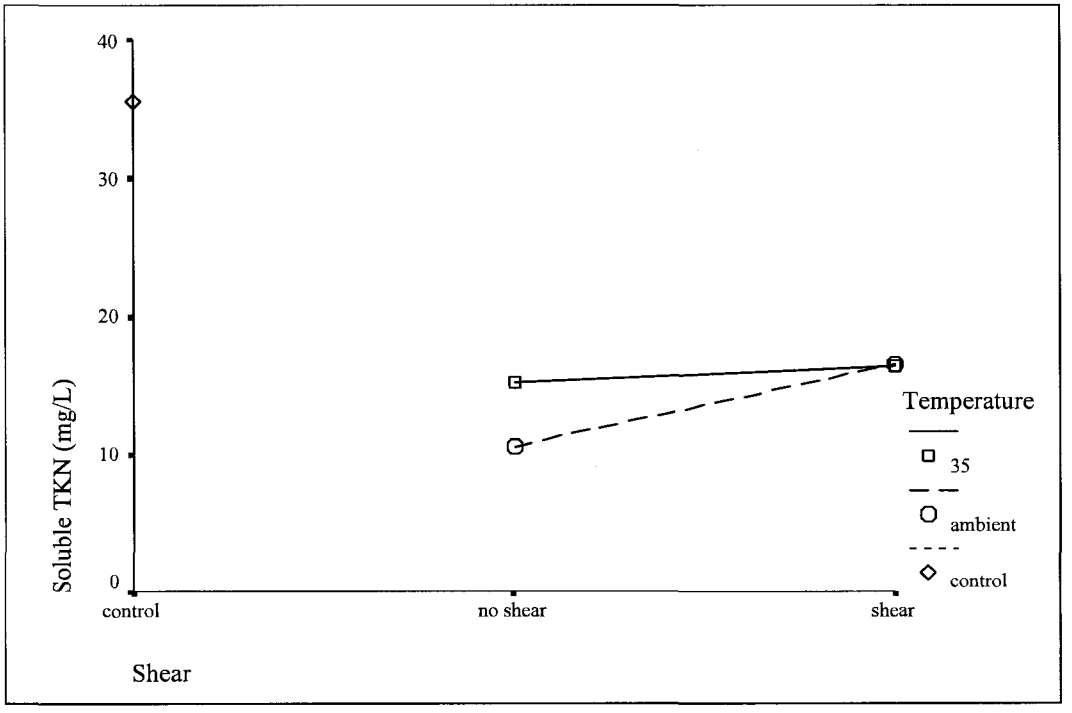

Figure 6.5.2: Means of soluble TKN (mg/L) for digested effluents in phase III

Although feed shearing had a significant effect on soluble TKN in the feed at the 90% confidence interval, significant differences in soluble TKN between pretreatment reactors and digesters respectively were not found. The mean soluble TKN values by sample presented in Figure 6.5.3 and show the bounds of the 90% confidence interval. The mean differences between stages were reported in Table 6.5.1 where significant at the 0.1 level. There was a large variance about the mean for pretreated effluent of the high-temperature, low feed shear system however for a sample population size of 4 it was not practical to identify and remove any outliers. In general the mean soluble TKN values of the high temperature pretreatment reactors and feed were greater than the respective downstream digesters indicating protein release in high temperature pretreatment and nitrification in subsequent digestion. Elevated nitrate and nitrite species in these digesters confirmed in that nitrification was taking place as later reported. At ambient temperatures soluble TKN in the pretreatment effluents and feed from phase II and phase III were similar.

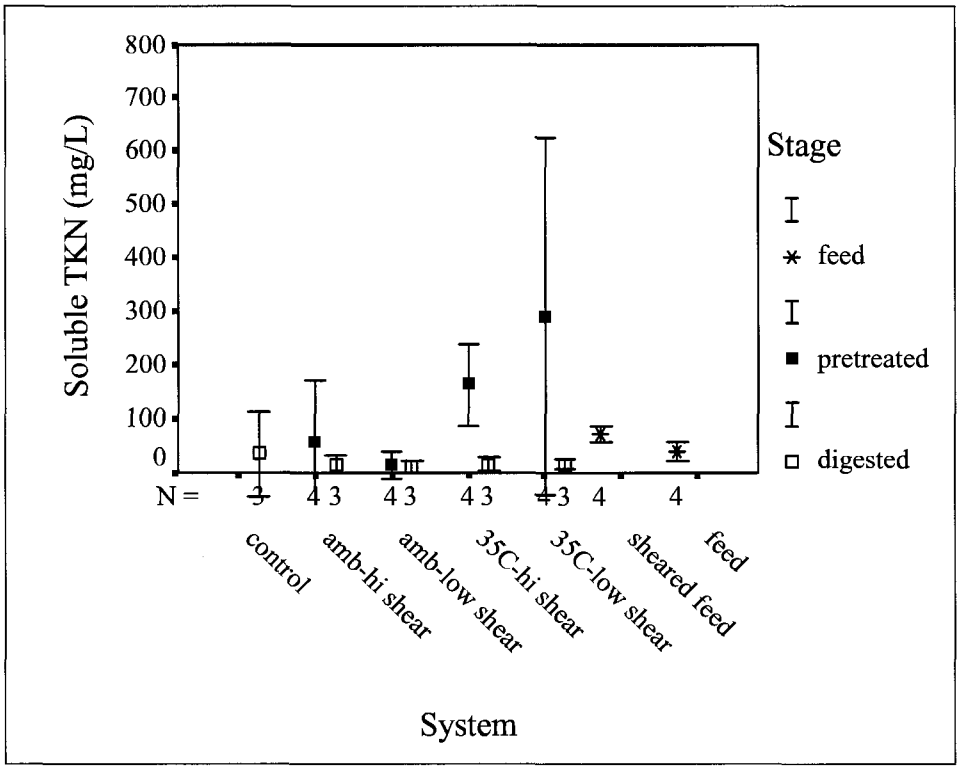

Figure 6.5.3: Error plot for 90% confidence interval around mean soluble TKN by system type and stage of treatment

Table 6.5.1: Mean difference between stages significant at the 0.1 level for variable soluble TKN (mg/L)

	Feed-Pretreated	Pretreated-Digested	Feed-Digested
Control	-	-	n.s.
Ambient/high shear	n.s.	n.s.	n.s.
Ambient/low shear	25.4 +/- 21.2	n.s.	30.1 +/- 22.9
35°C/high shear	-91.8 +/- 52.9	148.6 +/- 57.1	n.s.
35°C/low shear	-251.3 +/- 227.0	276.8 +/- 245.2	n.s.

n.s. = not significant

6.6 Ammonia

The ammonia concentration was measured in the pretreatment and digester effluents over the course of the staged digestion trials (phase III). The measured values fluctuated as seen in the time-series plots in Appendix C. The ammonia in the feed and sheared feed generally followed the same trends whose variations were attributed to fluctuations in the source feed solids and ammonia concentrations. In general, the high temperature pretreated effluents had consistently higher ammonia concentrations than the ambient temperature reactors. The ambient-high shear fed pretreatment reactor had negligible ammonia in general, whereas the low shear reactor maintained levels similar to the inlet feed. The ammonia in the digesters started high and then decreased likely as nitrification reached a steady state. As such the first observation was left out of further analyses. The box plots of the ammonia measurements indicated two outliers in the ambient high shear pretreated effluent data. The extreme outlier in the middle (09/29/05) was left out of further mean comparisons and ANOVAs. The box plots also showed generally normal distributions about the mean. The latter observation was confirmed by p-plots performed by sample set (Appendix C).

For feed samples, the *Levene*'s test for homogeneity of variance (Appendix C) was insignificant, ($F(1,14)=0.033$, $p=0.859$), so the null hypothesis was not rejected and therefore the variances were homogenous. The two-way ANOVA performed at the 90% significance level showed that:

1. Feed shearing had an insignificant effect on the ammonia in the sheared feed relative to the not sheared feed ($F(1,14)=0.928$, $p=0.352$).

For pretreated populations (see Figure 6.6.1), the *Levene*'s test for homogeneity of variance (Appendix C) was significant, ($F(3,34)=9.131$, $p<0.05$), so the null hypothesis was rejected and therefore the variances were not homogenous. The two-way ANOVA performed at the 90% significance level showed that:

1. Feed shearing had an insignificant effect on the ammonia in the pretreatment effluent ($F(1,34)=0.956$, $p=0.335$).
2. Higher pretreatment temperature was found to have a significant positive effect on the ammonia relative to pretreatment at ambient temperature (187.0 +/- 28.7mg/L) in the pretreatment effluent ($F(1,34)=121.192$, $p<0.10$).
3. A significant interaction between the effect of pretreatment temperature and feed shearing on ammonia concentration in the pretreatment effluent was found ($F(1,34)=3.398$, $p<0.10$).

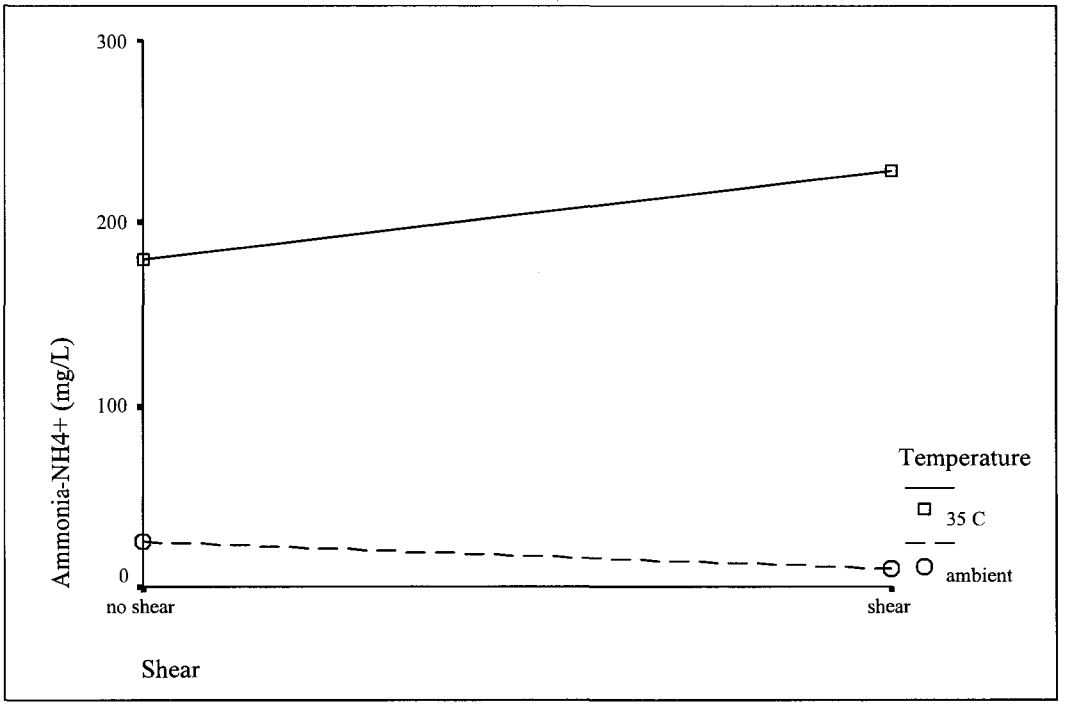

Figure 6.6.1: Means of ammonia (mg NH_4^+/L) for pretreated effluents in phase III

For digested populations (Figure 6.6.2), the *Levene*'s test for homogeneity of variance (Appendix C) was significant, ($F(4,25)=20.882$, $p<0.05$), so the null hypothesis was rejected and therefore the variances were not homogenous. The two-way ANOVA performed at the 90% significance level showed that after fully aerobic digestion:

1. Feed shearing had an insignificant effect on the ammonia in the final effluent $F(1,25)=0.342$, $p=0.564$).
2. Pretreatment temperature had an insignificant effect on the ammonia in the final effluent $F(1,25)=0.239$, $p=0.629$).
3. No significant interaction between the effect of pretreatment temperature and feed shearing on ammonia concentration was found ($F(1,25)=0.075$, $p=0.786$).

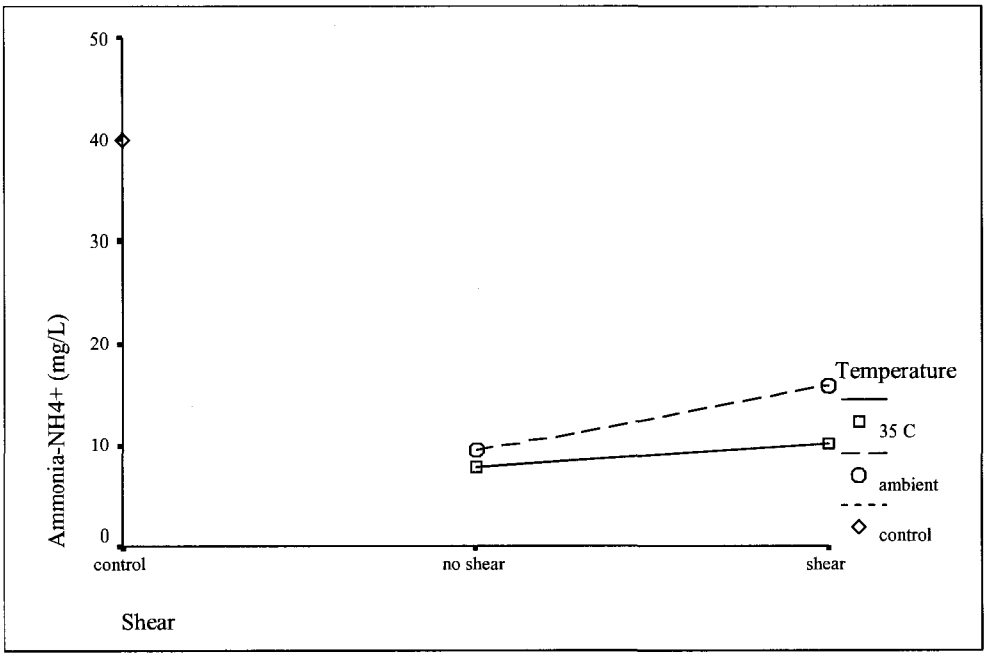

Figure 6.6.2: Means of ammonia (mg NH_4^+/L) for digested effluents in phase III

In general the ammonia levels were constant across all microaerobic pretreatment reactors and aerobic digesters except in the high temperature pretreatment reactors where the values increased substantially as shown in Figure 6.6.3. The mean differences between stages are reported in Table 6.6.1. The respective ammonia release of 218 and 172mg/L in both the high and low shear high temperature pretreatment reactors indicate that hydrolysis was enhanced in these reactors relative to those at ambient temperatures. The mean effluent ammonia levels from pretreatment at ambient temperatures were similar (<40mg/L) in phase I, II, and III compared to effluents pretreatment at high temperature (>150mg/L).

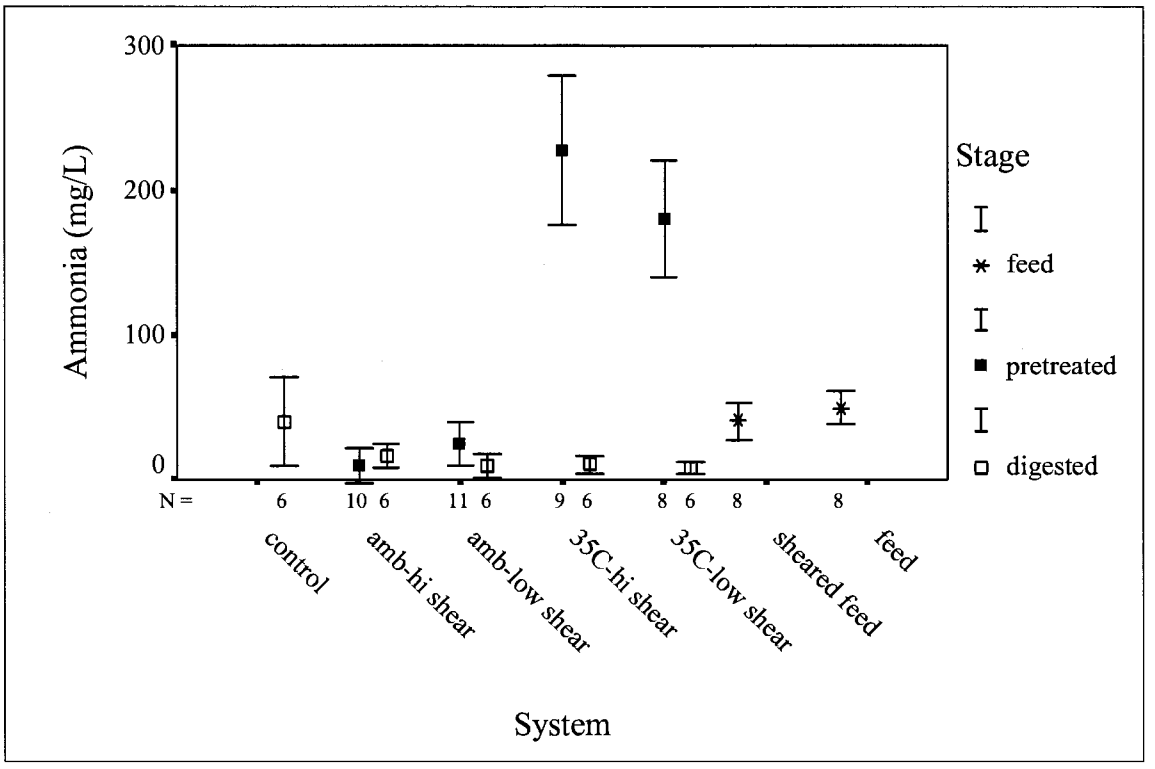

Figure 6.6.3: Error plot for 90% confidence interval around mean ammonia (mg NH_4^+/L) by system type and stage of treatment

Table 6.6.1: Mean difference between stages significant at the 0.1 level for variable ammonia-NH_4^+ (mg/L)

	Feed-Pretreated	Pretreated-Digested	Feed-Digested
Control	-	-	n.s.
Ambient/high shear	39.6 +/- 21.1	n.s.	33.4 +/- 17.6
Ambient/low shear	n.s.	n.s.	31.0 +/- 18.9
35°C/high shear*	-178.7 +/- 69.1	217.8 +/- 68.6	39.1 +/- 16.6
35°C/low shear*	-139.6 +/-55.9	172.2 +/- 55.1	32.6 +/- 17.7

*Dunnet T3 for unequal variances
n.s. = not significant

6.7 Combined Nitrite and Nitrate (mg NO_3/L)

The combined nitrate and nitrite concentration (mg NO_3/L) was measured in the feed, pretreatment and digester effluents over the course of the staged digestion trials (phase III). The measured values fluctuated as seen in the time-series plots in Appendix C. In general, combined nitrate and nitrite concentrations were consistently higher in the digesters fed with high temperature pretreated sludge where the low shear system was the greater of the two. The box plots of the combined nitrate and nitrite (mg NO_3^-/L) measurements indicated several outliers. However, since the number of samples was limited, all measurements were included in the analysis. The box plots also showed generally normal distributions about the mean except in those reactors whose mean was close to zero. The latter observation was confirmed by p-plots performed by sample set (Appendix C).

For feed samples, the *Levene*'s test for homogeneity of variance (Appendix C) was significant, ($F(1,6)=8.118$, $p<0.05$), so the null hypothesis was rejected and therefore

the variances were not homogenous. The two-way ANOVA performed at the 90% significance level showed that:

1. Feed shearing had an insignificant effect on the combined nitrate and nitrite (mg NO_3^-/L) in the sheared feed relative to the not sheared feed ($F(1,6)=0.898$, $p=0.380$).

For pretreated populations (see Figure 6.7.1), the *Levene*'s test for homogeneity of variance (Appendix C) was significant, ($F(3,15)= 59.981$, $p<0.05$), so the null hypothesis was rejected and therefore the variances were not homogenous. The two-way ANOVA performed at the 90% significance level showed that:

1. Feed shearing had an insignificant effect on the combined nitrate and nitrite (mg NO_3^-/L) in the pretreatment effluent ($F(1,15)= 2.531$, $p=0.132$).
2. Pretreatment temperature was found to have an insignificant effect on the combined nitrate and nitrite (mg NO_3/L) in the pretreatment effluent ($F(1,15)=2.825$, $p=0.113$).
3. No significant interaction between pretreatment temperature and feed shearing on the effect of combined nitrate and nitrite (mg NO_3/L) concentration in the pretreatment effluent was found ($F(1,15)=2.505$, $p<0.134$).

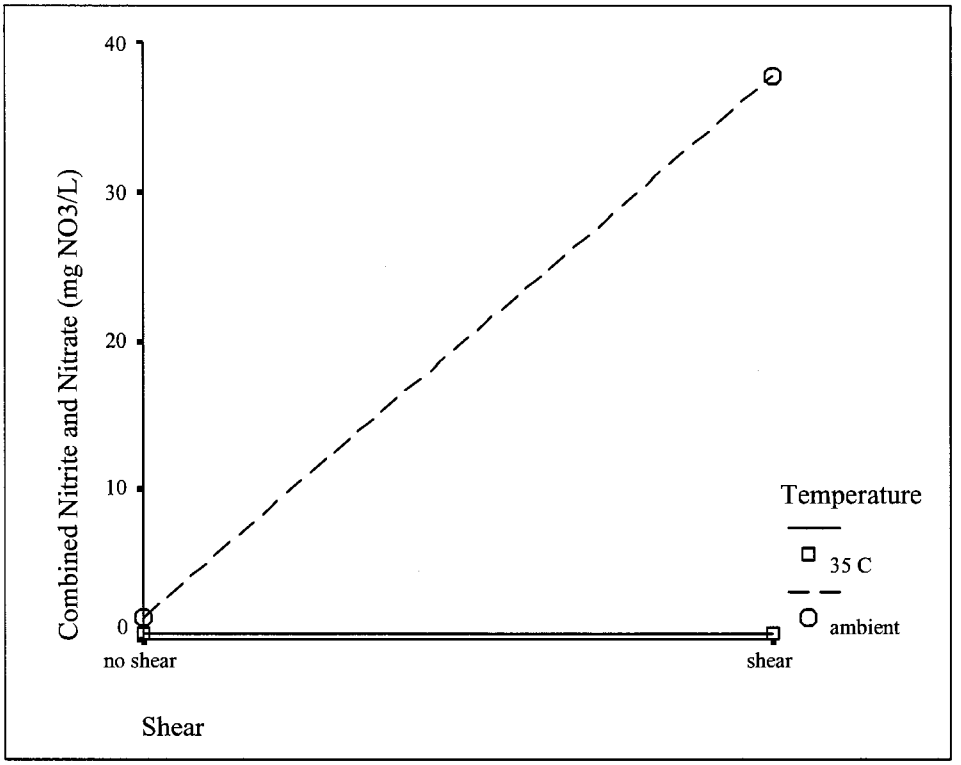

Figure 6.7.1: Means of combined nitrate and nitrite concentration (mg NO_3^-/L) for pretreated effluents in phase III

For digested populations (Figure 6.7.2), the *Levene*'s test for homogeneity of variance (Appendix C) was insignificant, ($F(4,20)=2.275$, $p=0.097$), so the null hypothesis was not rejected and therefore the variances were homogenous. The two-way ANOVA performed at the 90% significance level showed that after fully aerobic digestion:

1. Feed shearing had an insignificant effect on the combined nitrate and nitrite concentration in the final effluent $F(1,20)=1.122$, $p=0.302$).

2. Higher pretreatment temperature was found to have a significant positive effect on the combined nitrate and nitrite in the final effluent (153.7 +/- 48.4mg NO_3/L) over ambient pretreatment ($F(1,38)=5.589$, $p<0.10$). It was also found to have a

greater positive effect over the control digester (203.1 +/- 59.2mg NO_3/L). Pretreatment at ambient temperature did not have a significant effect on the combined nitrite and nitrate concentration in the digested effluent relative to the control.

3. No significant interaction between the effect of pretreatment temperature and feed shearing on combined nitrate and nitrite concentration was found ($F(1,20)=0.632$, $p=0.436$).

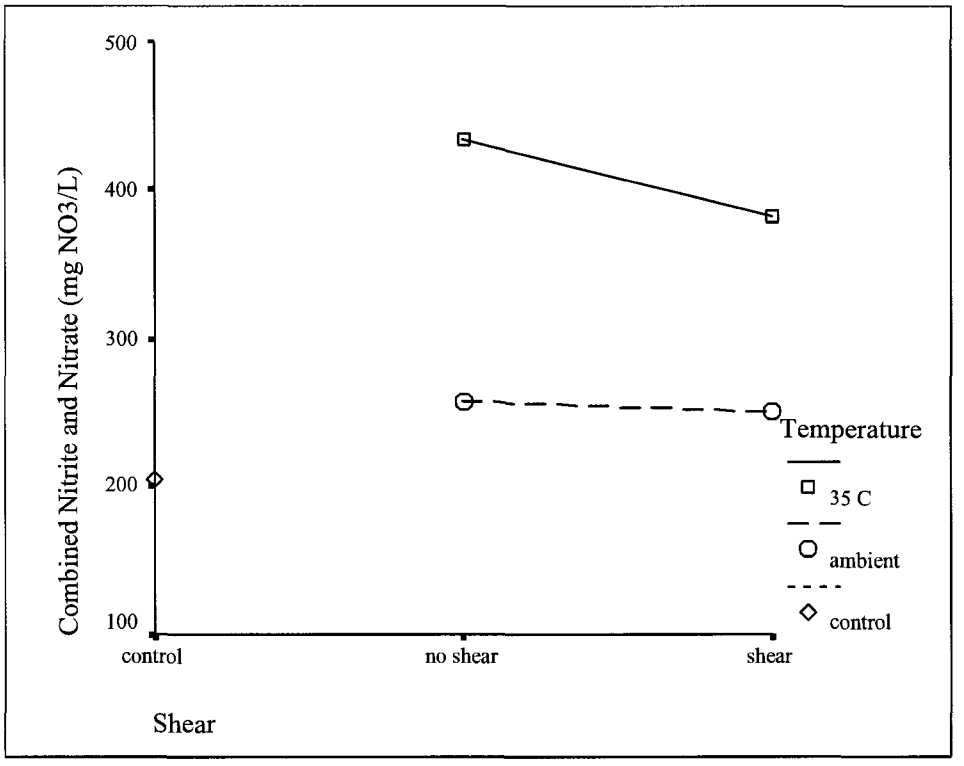

Figure 6.7.2: Means of combined nitrate and nitrite concentration (mg NO_3^-/L) for digested effluents in phase III

The combined nitrite and nitrate levels were higher in the digested effluents from the systems pretreated at higher temperatures than in those pretreated at ambient temperatures and the control (Figure 6.7.3). The mean differences between stages (Table

6.7.1) also indicated increased nitrification downstream of high temperature pretreatment. It is likely that the added substrate for nitrifiers (ammonia) produced upstream facilitated the increased levels of oxidized nitrogen species observed downstream in the high temperature systems. This mechanism was expected to have caused a statistically significant depression in the pH of those digesters that received high temperature pretreated sludge as later reported. The general lack of oxidized nitrogen species in the pretreatment effluents suggest that either microaerobic conditions in phase III were well-suited to simultaneous nitrification and denitrification (where nitrates are reduced to nitrogen gas), or that nitrification was not occurring taking place at all. The slight depletion in ammonia and soluble TKN suggest the former.

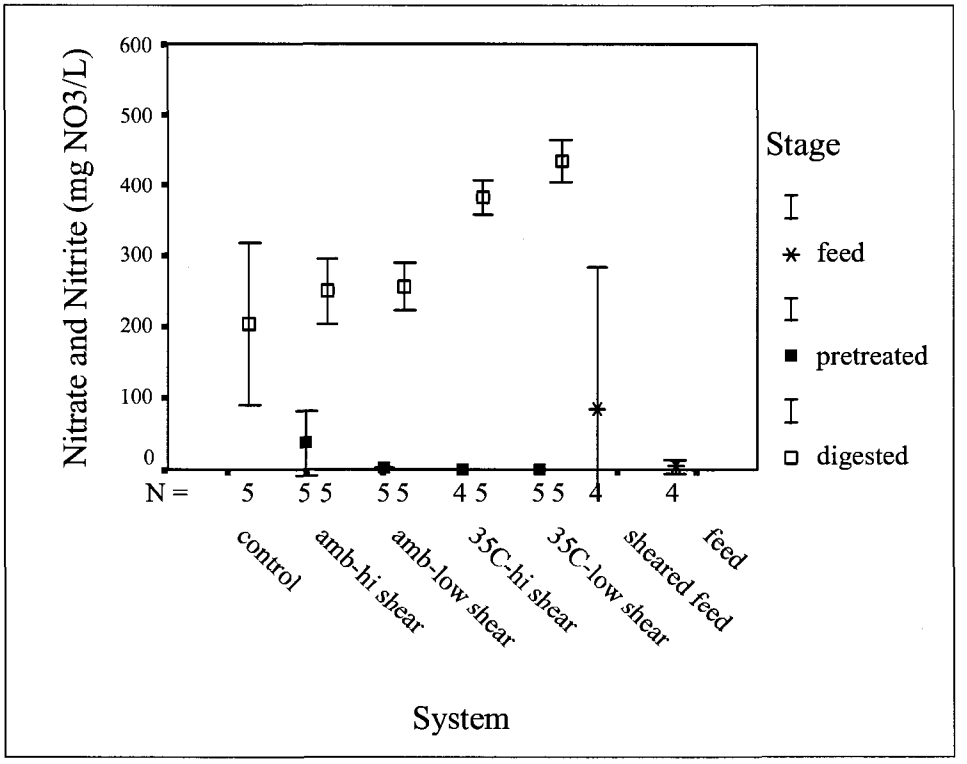

Figure 6.7.3: Error plot for 90% confidence interval around mean total nitrate and nitrite (mg NO_3^-/L) by system type and stage of treatment

Table 6.7.1: Mean difference between stages significant at the 0.1 level for variable total nitrate and nitrite (mg NO_3^-/L)

	Feed-Pretreated	Pretreated-Digested	Feed-Digested
Control	-	-	-200.4 +/- 115.4
Ambient/high shear*	n.s.	-212.8 +/- 75.8	n.s.
Ambient/low shear	n.s.	-256.5 +/- 46.4	-253.5 +/- 46.0
35°C/high shear*	n.s.	-381.6 +/- 35.7	-296.9 +/- 283.5
35°C/low shear*	n.s	-433.7 +/- 42.7	-429.5 +/- 42.2

*Dunnet T3 for unequal variances
n.s. = not significant

6.8 Total Volatile Fatty Acids (VFAs)

The total VFA concentrations were measured in the feed, pretreatment and digester effluents over the course of the staged digestion trials (phase III). The measured values fluctuated as seen in the time-series plots in Appendix C. In general the VFAs were similar in both the sheared and not sheared feed. The VFAs in the high temperature pretreatment reactors were in general higher than the ambient temperature reactors. Total VFAs were below the detection limit in the digesters and were not reported. The box plots of the VFAs measurements indicated several outliers. However, all measurements were included in the analysis as the fluctuations were attributed to the inherent variability in the system. The box plots also showed generally normal distributions about the mean. The latter observation was confirmed by p-plots performed by sample set (Appendix C).

For feed samples, the *Levene*'s test for homogeneity of variance (Appendix C) was insignificant, ($F(1,7)=0.300$, $p=0.601$), so the null hypothesis was not rejected and therefore the variances were assumed homogenous. The two-way ANOVA performed at the 90% significance level showed that:

1. Feed shearing had an insignificant effect on the VFAs in the sheared feed relative to the not sheared feed ($F(1,7)=0.005$, $p=0.948$).

For pretreated populations (see Figure 6.8.1), the *Levene*'s test for homogeneity of variance (Appendix C) was significant, ($F(3,24)= 11.996$, $p<0.05$), so the null hypothesis was rejected and therefore the variances were not assumed homogenous. The two-way ANOVA performed at the 90% significance level showed that:

1. Feed shearing had an insignificant effect on the total VFA concentration in the pretreatment effluent ($F(1,24)= 0.777$, $p=0.387$).
2. Higher pretreatment temperature had a significant positive effect (464.0 +/- 159.7mg acetate/L) on the total VFA concentration in the pretreatment effluent ($F(1,24)=24.717$, $p<0.10$).
3. No significant interaction between the effect of pretreatment temperature and feed shearing on VFA in the pretreatment effluent was found ($F(1,24)=0.683$, $p=0.417$).

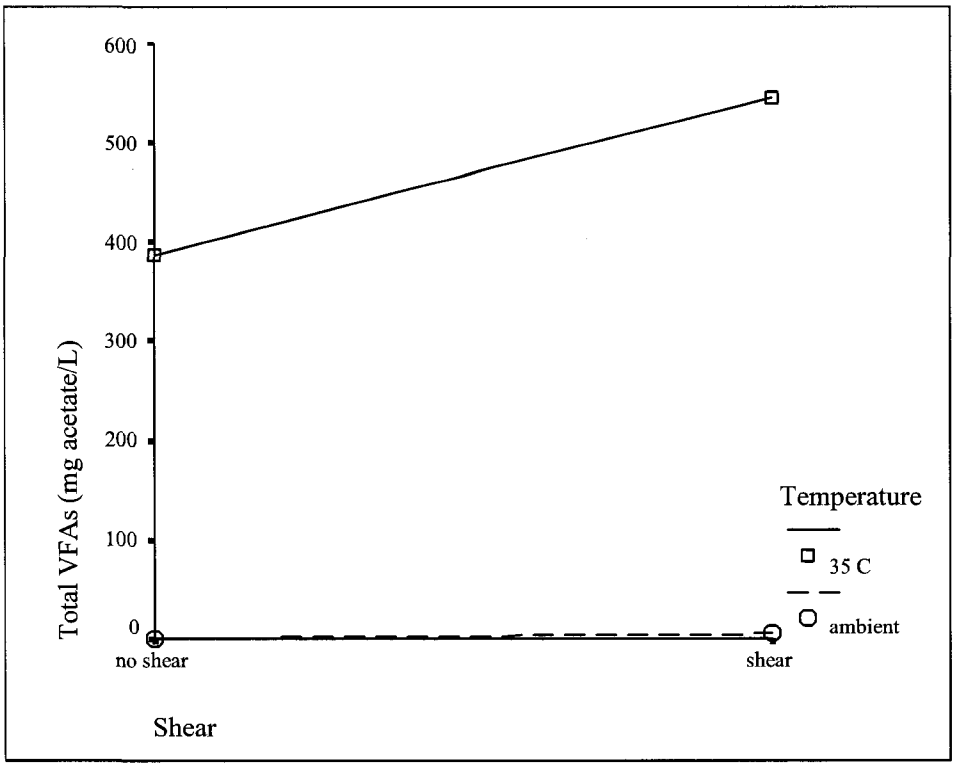

Figure 6.8.1: Means of total VFA (mg acetate/L) for pretreated effluents in phase III

In general there were large variations in the total VFAs in the feed samples that were subsequently diminished after pretreatment (Figure 6.8.2). Only the high temperature reactors were found to maintain low levels of VFAs as expected under low DO and ORP conditions. The ambient temperature reactors did not maintain detectable levels of VFAs; similarly, VFAs were not detectable after aerobic digestion as they were likely consumed by opportunistic aerobes. The differences between each stage are shown in Table 6.8.1 where the difference between low shear feed and sludge pretreated at ambient temperature was statistically significant at the 0.1 level. As in phase II, VFA accumulation was insignificant after pretreatment at ambient temperature. The mean levels of VFAs accumulated in the high temperature pretreatment reactors were similar to those found in phase I at ambient temperature where the ORPs were also similar.

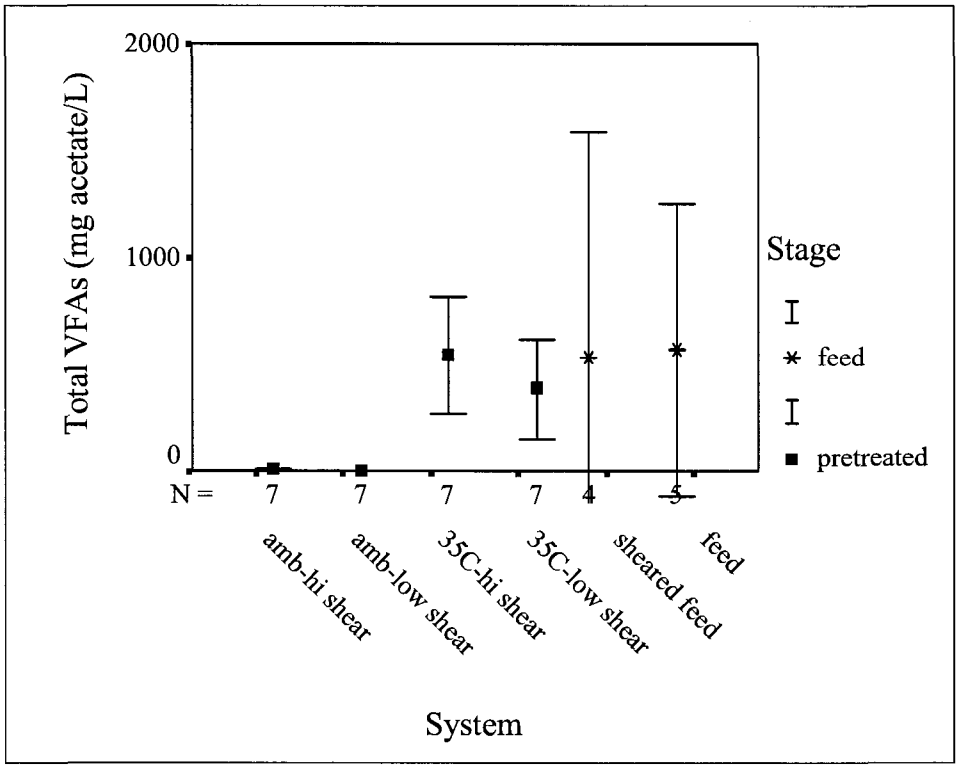

Figure 6.8.2: Error plot for 90% confidence interval around mean total VFA (mg acetate/L) by system type and stage of treatment

Table 6.8.1: Mean difference between stages significant at the 0.1 level for variable total VFA (mg acetate/L)

	Feed-Pretreated
Ambient/high shear	n.s.
Ambient/low shear	568.6 +/- 483.7
35°C/high shear	n.s.
35°C/low shear*	n.s.

*Dunnet T3 for unequal variances
n.s. = not significant

6.9 Alkalinity

Alkalinity was measured in the feed and pretreated samples over the course of the staged digestion trials (phase III). The measured values fluctuated as seen in the time-series plots in Appendix C. In general the alkalinity was similar in the sheared and not sheared feed. The alkalinity in the high temperature pretreatment reactors was in general higher than the ambient temperature reactors. Also the alkalinity was below the detection limit in the digesters and therefore was not reported. The box plots of the alkalinity measurements did not indicate any outliers and also showed generally normal distributions about the mean. The latter observation was confirmed by p-plots performed by sample set (Appendix C).

For feed samples, the *Levene*'s test for homogeneity of variance (Appendix C) was significant, ($F(1,8)=6.586$, $p<0.05$), so the null hypothesis was rejected and therefore the variances were not assumed to be homogenous. The two-way ANOVA performed at the 90% significance level showed that:

1. Feed shearing had an insignificant effect on the alkalinity in the sheared feed relative to the not sheared feed ($F(1,8)=1.292$, $p=0.289$).

For pretreated populations (see Figure 6.9.1), the *Levene*'s test for homogeneity of variance (Appendix C) was significant, ($F(3,22)= 3.837$, $p<0.05$), so the null hypothesis was rejected and therefore the variances were not assumed to be homogenous. The two-way ANOVA performed at the 90% significance level showed that:

1. Feed shearing had an insignificant effect on the alkalinity in the pretreatment effluent ($F(1,22)=0.980$, $p=0.333$).

2. Higher pretreatment temperature had a significant positive effect (271.6 +/- 110.2mg $CaCO_3$/L) on the alkalinity in the pretreatment effluent ($F(1,22)=17.917$, $p<0.10$).

3. No significant interaction between the effect of pretreatment temperature and feed shearing on alkalinity in the pretreatment effluent was found ($F(1,22)=1.061$, $p=0.336$).

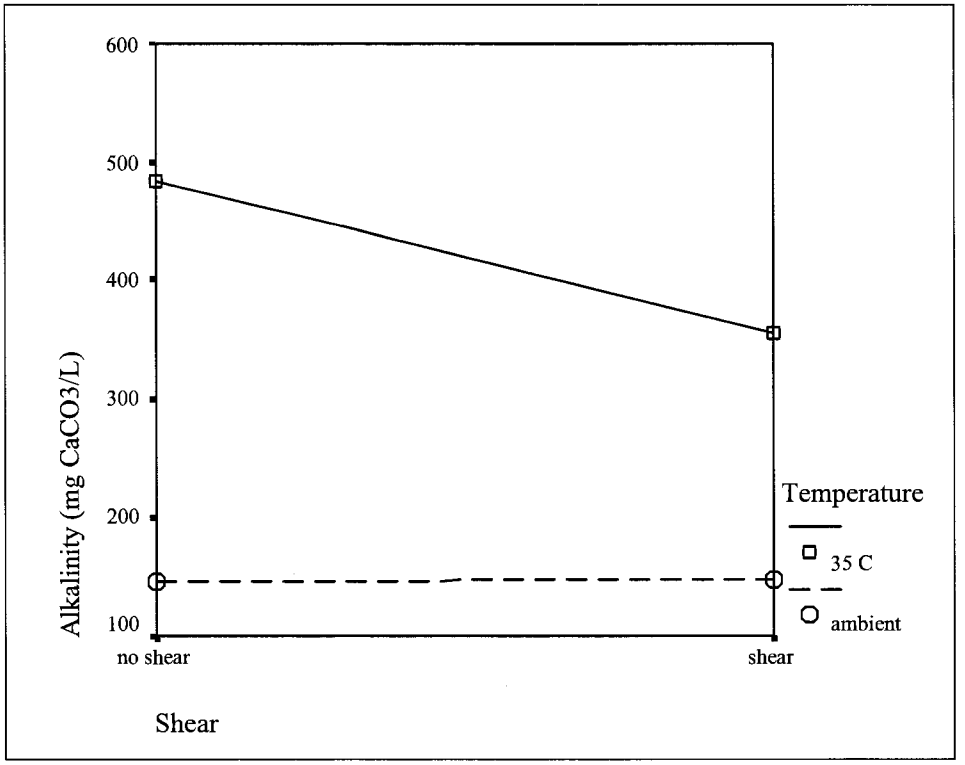

Figure 6.9.1: Means of alkalinity (mg $CaCO_3$/L) for pretreated effluents in phase III

The increased alkalinity in the high temperature pretreatment reactors relative to the feed and ambient pretreatment reactors (Figure 6.9.2) was attributed to the increased ammonia release observed at 35°C. The pH levels of all of the digesters were in general

below the first endpoint of the titration to determine alkalinity (pH 5.1) and therefore it was not detectable in the final effluents. At ambient temperature, the mean alkalinity in the pretreatment reactors in phase III were similar and had values in between the higher levels found in phase I and lower levels found in phase II; but these were not statistically different at the 90% confidence interval from effluents from either phase.

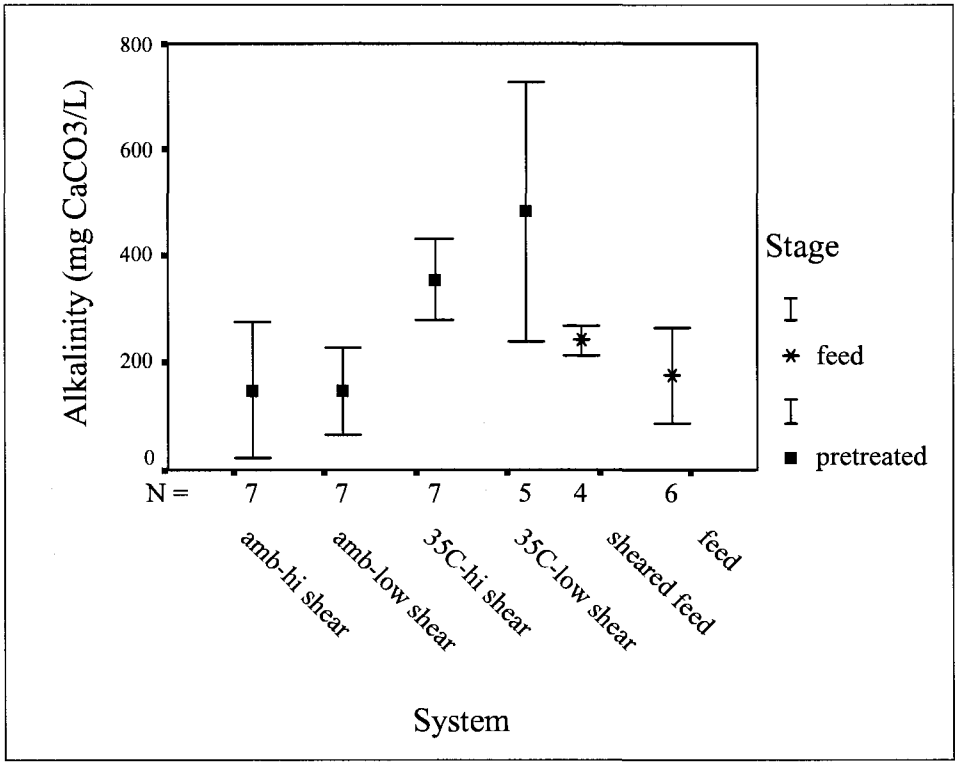

Figure 6.9.2: Error plot for 90% confidence interval around mean alkalinity (mg $CaCO_3$/L) by system type and stage of treatment

The mean differences between stages for each system confirm that alkalinity increased statistically significantly at the 0.1 level relative to the feed only after pretreatment at 35°C and not at ambient temperature as shown in Table 6.9.1

Table 6.9.1: Mean difference between stages significant at the 0.1 level for variable alkalinity (mg $CaCO_3$/L)

	Feed-Pretreated
Ambient/high shear	n.s.
Ambient/low shear	n.s.
35°C/high shear	112.6 +/- 97.7
35°C/low shear	306.0 +/- 209.6

n.s. = not significant

6.10 Soluble COD

The soluble COD was measured in the pretreatment and digester effluents over the course of the staged digestion trials (phase III). The measured values fluctuated as seen in the time-series plots in Appendix C. In general, sheared and not sheared feeds followed similar trends but the soluble COD in the sheared feed was typically slightly higher than the not sheared feed which indicated that feed shearing resulted in solubilization. The high temperature pretreatment reactors had consistently higher soluble COD values than the ambient temperature reactors though there were no obvious trends between those receiving sheared versus not sheared feed. The box plots of the soluble COD measurements indicated several outliers and generally normal distributions about the mean. The latter observation was confirmed by p-plots performed by sample set (Appendix C). The outliers were left in further analyses, as they were associated with the variability within the incoming feed and within the systems themselves.

For feed samples, the *Levene*'s test for homogeneity of variance (Appendix C) was insignificant, ($F(1,16)=0.152$, $p=0.702$), so the null hypothesis was not rejected and

therefore the variances were homogenous. The two-way ANOVA performed at the 90% significance level showed that:

1. Feed shearing had a significant positive effect (256.5 +/- 170.1 mg/L) on the soluble COD in the sheared feed relative to the not sheared feed ($F(1,16)=6.864$, $p<0.10$).

For pretreated populations (see Figure 6.10.1), the *Levene*'s test for homogeneity of variance (Appendix C) was insignificant, ($F(3,32)=0.785$, $p<0.511$), so the null hypothesis was not rejected and therefore the variances were homogenous. The two-way ANOVA performed at the 90% significance level showed that:

1. Feed shearing had an insignificant effect on the soluble COD in the pretreatment effluent ($F(1,32)=0.895$, $p=0.351$).
2. Higher pretreatment temperature was found to have a significant positive effect on the soluble COD relative to pretreatment at ambient temperature (684.4 +/- 70.4mg/L) in the pretreatment effluent ($F(1,32)=271.530$, $p<0.10$).
3. No significant interaction between pretreatment temperature and feed shearing on the effect of soluble COD concentration in the pretreatment effluent was found ($F(1,32)=0.049$, $p=0.847$).

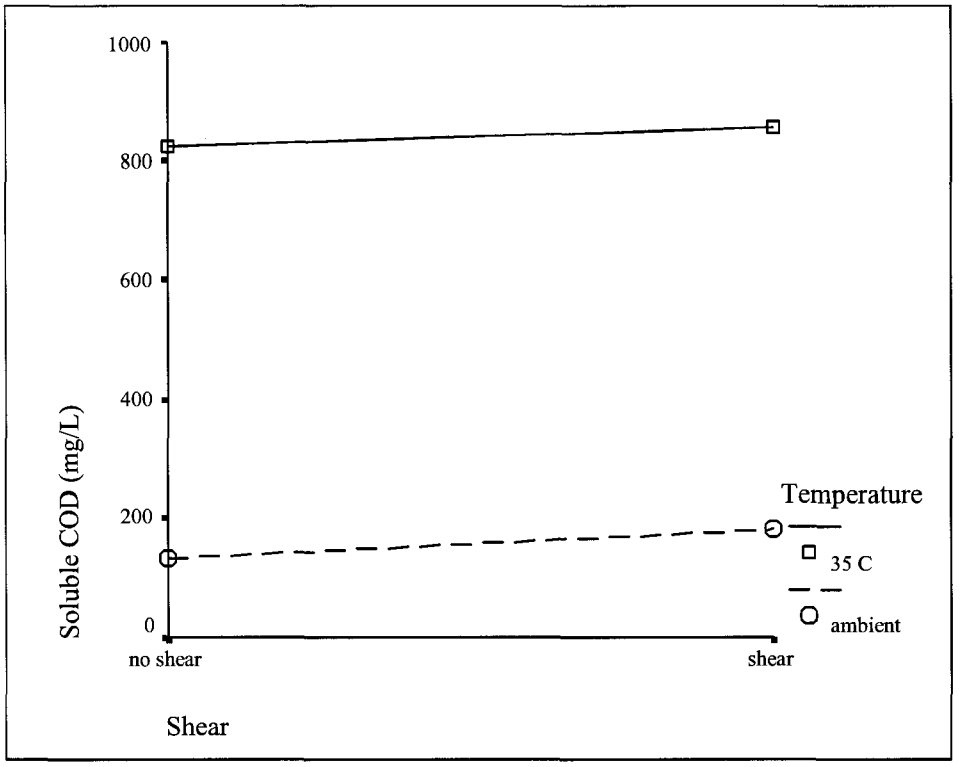

Figure 6.10.1: Means of soluble COD (mg/L) for pretreated effluents in phase III

For digested populations (Figure 6.10.2), the *Levene's* test for homogeneity of variance (Appendix C) was significant, ($F(4,15)=6.455$, $p<0.05$), so the null hypothesis was rejected and therefore the variances were not assumed to be homogenous. The two-way ANOVA performed at the 90% significance level showed that after fully aerobic digestion (Figure 6.10.2):

1. Feed shearing had an insignificant effect on the COD in the final effluent $F(1,15)=0.004$, $p=0.953$).

2. Pretreatment temperature had an insignificant effect on the COD in the final effluent $F(1,15)=1.051$, $p=0.321$). However, the *Dunnett T3* post-hoc test showed a significant difference between the soluble COD concentrations in the digesters

that received pretreated sludge under ambient versus high temperature conditions (72.1 +/- 39.1mg/L).

3. No significant interaction between pretreatment temperature and feed shearing on the effect of COD concentration was found (F(1,15)=0.077, p=0.785).

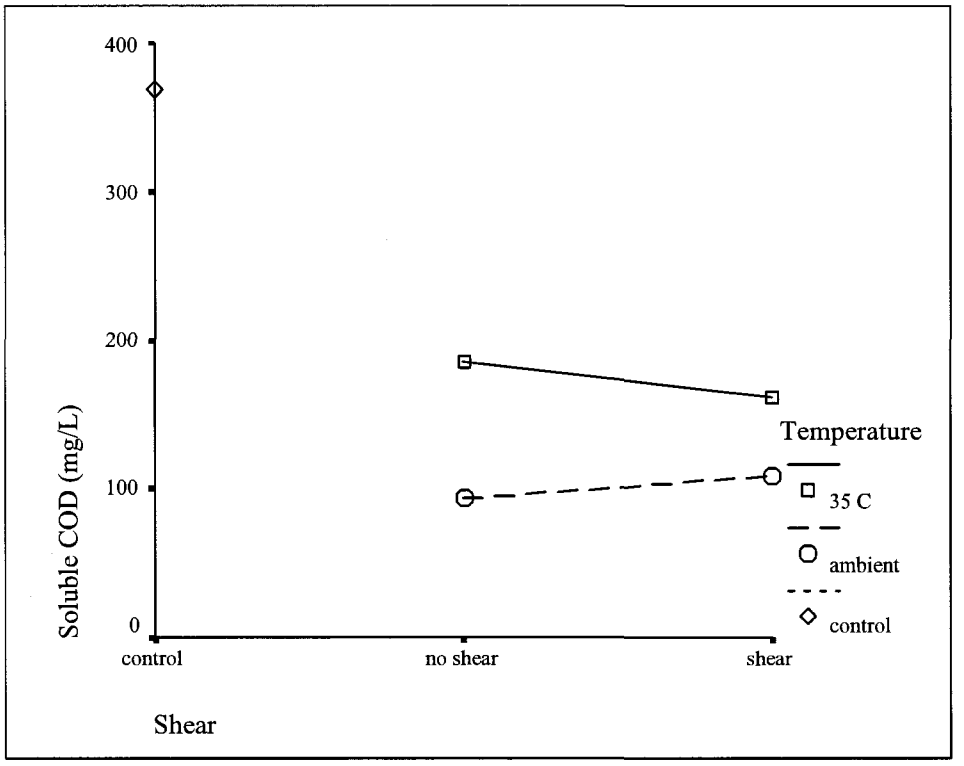

Figure 6.10.2: Means of soluble COD (mg/L) for digested effluents in phase III

Soluble COD was consumed in the ambient temperature pretreatment reactors while the release of COD associated with hydrolysis was greater than what was consumed at 35°C (Figure 6.10.3). Downstream, the soluble COD stabilized to low levels with little variation in pretreated digesters, whereas a higher mean value and greater variation was observed in the control digester suggesting that microaerobic

pretreatment facilitated a more consistent consumption of soluble COD in aerobic digestion.

In both ambient and 35°C pretreatment the mean difference between the sheared feed and its downstream digester effluent was greater than in the not sheared feed and its downstream digester effluent which suggested that there was greater soluble COD removal with sheared feed (Table 6.10.1).

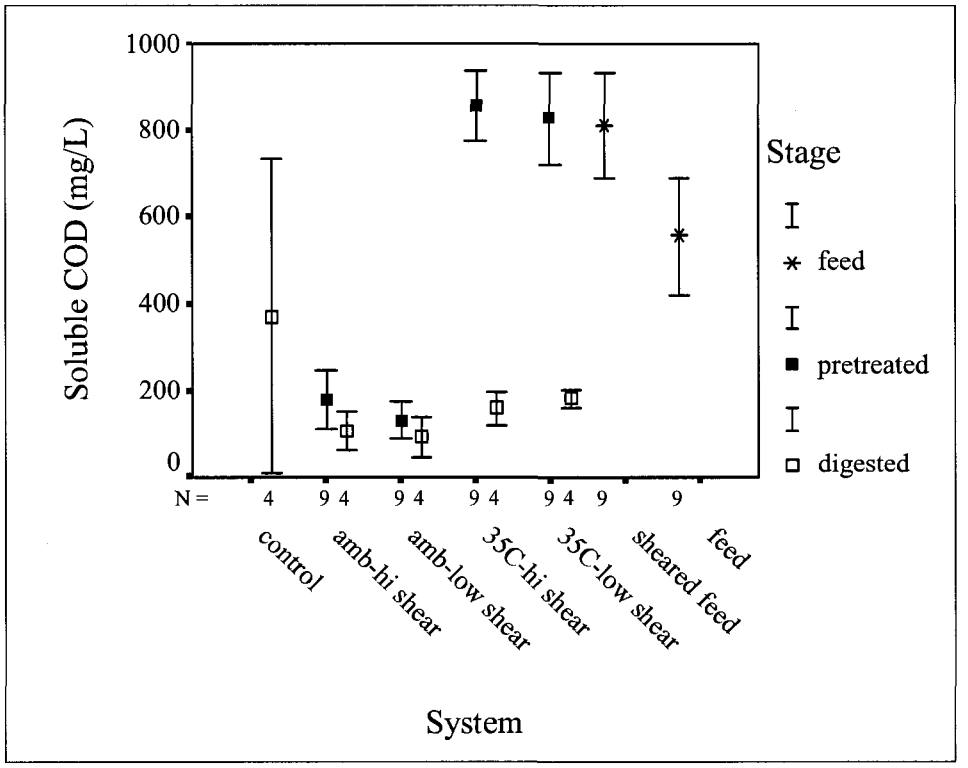

Figure 6.10.3: Error plot for 90% confidence interval around mean soluble COD (mg/L) by system type and stage of treatment

Table 6.10.1: Mean difference between stages significant at the 0.1 level for variable soluble COD (mg/L)

	Feed-Pretreated	Pretreated-Digested	Feed-Digested
Control	-	-	n.s.
Ambient/high shear	-630.0 +/- 154.1	n.s.	-702.8 +/- 120.9
Ambient/low shear	-421.9 +/- 120.4	n.s.	-460.9 +/- 153.4
35°C/high shear	n.s.	-694.9 +/- 116.4	-649.6 +/- 161.4
35°C/low shear	271.7 +/-210.2	-641.0 +/- 140.5	-369.3 +/- 213.9

n.s. = not significant

6.11 pH

The pH was measured in the feed, pretreatment and digester effluents over the course of the staged digestion trials (phase III). The measured values fluctuated as seen in the time-series plots in Appendix C. In general, the pH was slightly higher in the sheared than in the not sheared feed. The pH in the high temperature pretreatment reactors was in general higher than the ambient temperature reactors. Also, the pH measurements were consistently lower in the digesters fed with high temperature pretreated sludge. The box plots of the pH measurements indicated several outliers. However, all measurements were included in the analyses as the fluctuations were attributed to the inherent variability in the system. The box plots also showed generally normal distributions about the mean. The latter observation was confirmed by p-plots performed by sample set (Appendix C).

For feed samples, the *Levene*'s test for homogeneity of variance (Appendix C) was insignificant, ($F(1,15)=0.105$, $p=0.751$), so the null hypothesis was not rejected and

therefore the variances were assumed homogenous. The two-way ANOVA performed at the 90% significance level showed that:

1. Feed shearing had an insignificant effect on the pH in the sheared feed relative to the not sheared feed ($F(1,15)=0.671$, $p=0.425$).

For pretreated populations (see Figure 6.11.1), the *Levene*'s test for homogeneity of variance (Appendix C) was insignificant, ($F(3,40)= 2.276$, $p=0.094$), so the null hypothesis was not rejected and therefore the variances were assumed homogenous. The two-way ANOVA performed at the 90% significance level showed that:

1. Feed shearing had an insignificant effect on the pH in the pretreatment effluent ($F(1,40)=1.129$, $p=0.294$).
2. Higher pretreatment temperature had a significant positive effect (0.71 +/- 0.17) on the pH in the pretreatment effluent ($F(1,40)=47.452$, $p<0.05$).
3. No significant interaction between pretreatment temperature and feed shearing on the effect of pH in the pretreatment effluent was found ($F(1,40)=2.597$, $p=0.115$).

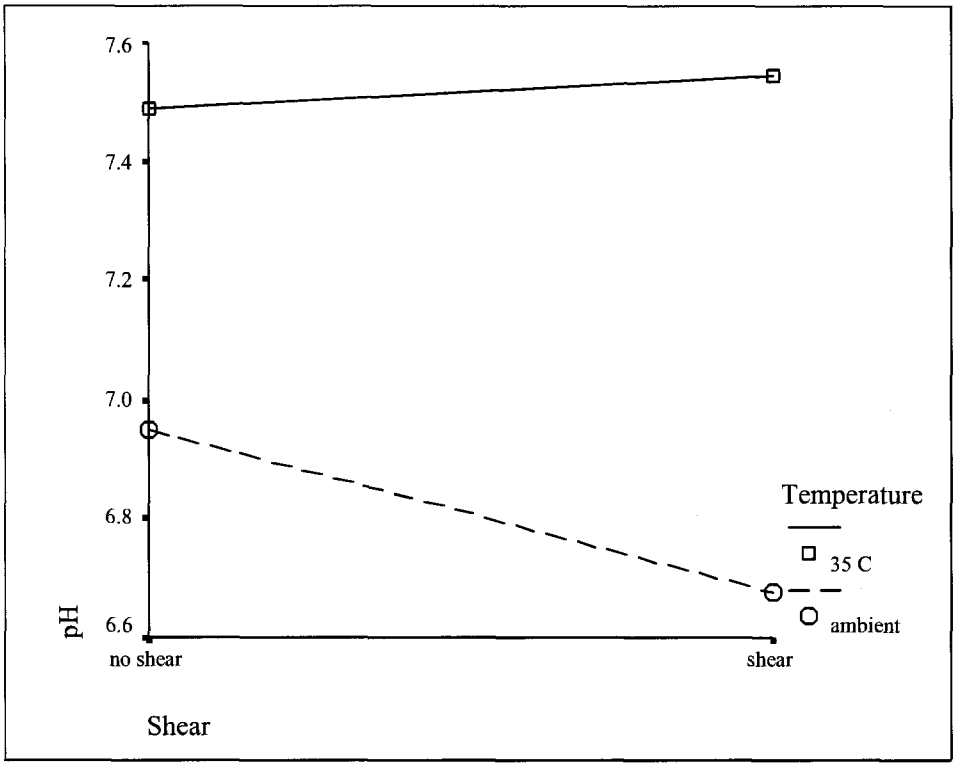

Figure 6.11.1: Means of pH for pretreated effluents in phase III

For digested populations (Figure 6.11.2), the *Levene*'s test for homogeneity of variance (Appendix C) was insignificant, ($F(4,34)=2.340$, $p=0.075$), so the null hypothesis was not rejected and therefore the variances were homogenous. The two-way ANOVA performed at the 90% significance level showed that after fully aerobic digestion:

1. Feed shearing had a significant positive effect (0.39 +/- 0.38) on the pH in the final effluent relative to final effluents from not sheared feeds $F(1,34)=3.115$, $p<0.10$). It was also found to have a negative effect over the control digester (–0.86 +/- 0.48). Pretreatment with not sheared feed had a significant negative effect (-1.26 +/- 0.48) on the pH in the digested effluent relative to the control.

2. Higher pretreatment temperature had a significant negative effect (-1.14 +/- 0.38) on the pH in the final effluent relative to final effluents pretreated at ambient temperature $F(1,34)=26.511$, $p<0.10$). It was also found to have a greater negative effect over the control digester (–1.63 +/- 0.48). Pretreatment at ambient temperature had a significant negative effect (-0.49 +/- 0.48) on the pH in the digested effluent relative to the control.

3. No significant interaction between the effect of pretreatment temperature and feed shearing on pH was found ($F(1,34)=1.461$, $p=0.235$).

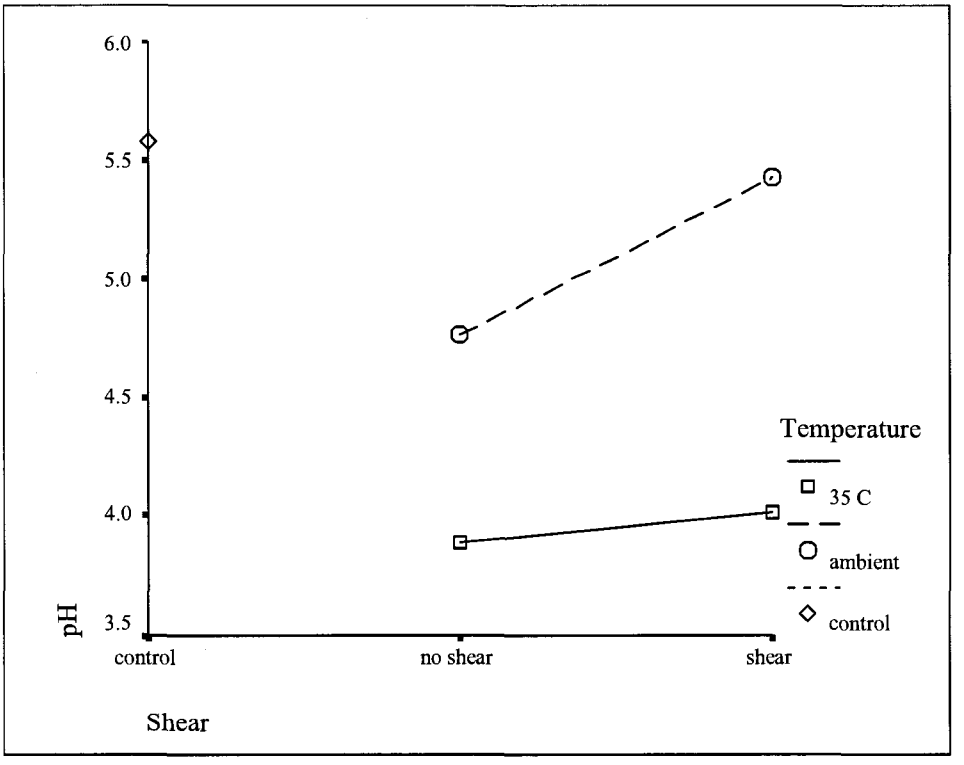

Figure 6.11.2: Means of pH for digested effluents in phase III

The mean values of pH by system and stage are shown in Figure 6.11.3 with associated error bars about the 90% confidence interval. The pH in high temperature pretreatment tended to increase the more so than at ambient temperature and may be explained by the accumulation of ammonia caused by hydrolysis in those reactors. pH tended drop in digestion more so in the high temperature pretreatment systems. This can be explained by nitrification activity, which would remove ammonia and cause pH depression.

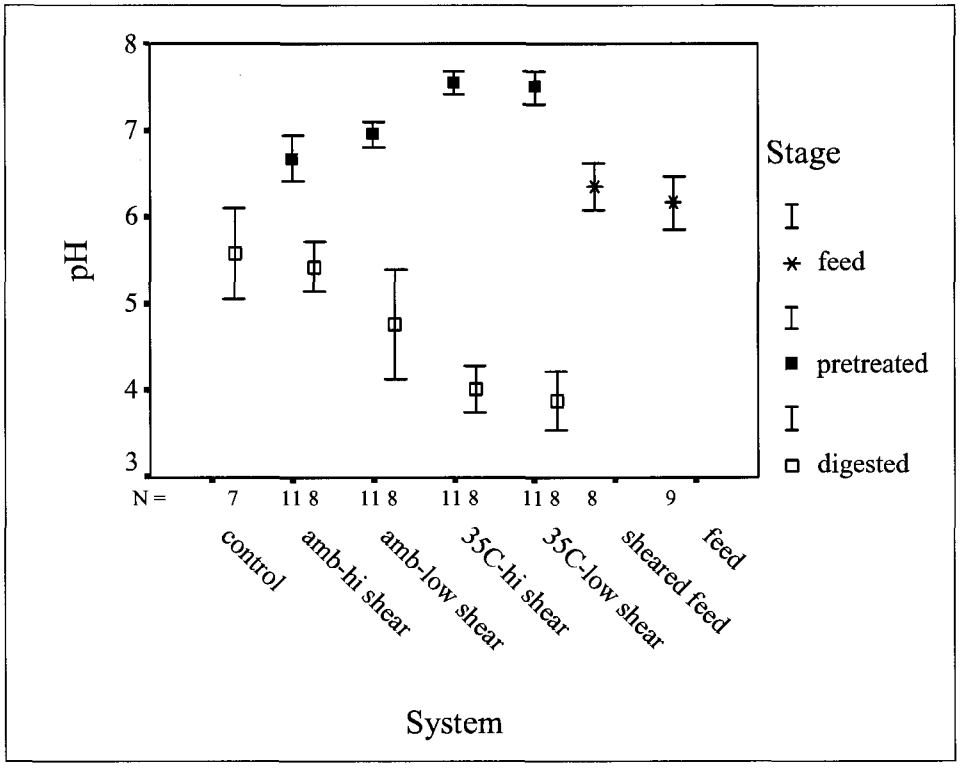

Figure 6.11.3: Error plot for 90% confidence interval around mean pH by system type and stage of treatment

Table 6.11.1: Mean difference between stages significant at the 0.1 level for variable pH

	Feed-Pretreated	Pretreated-Digested	Feed-Digested
Control	-	-	0.58 +/- 0.53
Ambient/high shear	n.s.	1.25 +/- 0.35	0.92 +/- 0.37
Ambient/low shear*	-0.79 +/- 0.43	2.18 +/- 0.86	1.39 +/- 0.89
35°C/high shear	-1.21 +/- 0.28	3.54 +/- 0.28	2.33 +/- 0.30
35°C/low shear	-1.33 +/- 0.34	3.60 +/- 0.36	2.27 +/- 0.37

*Dunnet T3 for unequal variances
n.s. = not significant

6.12 Fecal Coliforms

The fecal coliform concentration was measured and normalized with respect to total solids in the feed, pretreatment and digester effluents over the course of the staged digestion trials (phase III). The measured values fluctuated as seen in the time-series plots in Appendix C. In general, the fecal coliform concentrations were similar in the sheared and not sheared feeds. The fecal coliform concentration in the high temperature pretreatment reactors was in general slightly lower than the ambient temperature reactors. Also the fecal coliform concentration measurements were consistently lower in the digesters fed with high temperature pretreated sludge and the control. The box plots of the fecal coliform concentration measurements indicated several outliers. However, all measurements were included in the analysis as the fluctuations were attributed to the inherent variability in the system. The box plots also showed generally normal distributions about the mean. The latter observation was confirmed by p-plots performed by sample set (Appendix C).

For feed samples, the *Levene*'s test for homogeneity of variance (Appendix C) was insignificant, ($F(1,10)=0.966$, $p=0.349$), so the null hypothesis was not rejected and

therefore the variances were assumed homogenous. The two-way ANOVA performed at the 90% significance level showed that:

1. Feed shearing had an insignificant effect on the fecal coliform concentration in the sheared feed relative to the not sheared feed ($F(1,10)=0.479$, $p=0.505$).

For pretreated populations (see Figure 6.12.1), the *Levene*'s test for homogeneity of variance (Appendix C) was insignificant, ($F(3,17)= 2.545$, $p=0.090$), so the null hypothesis was not rejected and therefore the variances were assumed homogenous. The two-way ANOVA performed at the 90% significance level showed that:

1. Feed shearing had an insignificant effect on the fecal coliform concentration in the pretreatment effluent ($F(1,17)=1.028$, $p=0.325$).
2. Higher pretreatment temperature had an insignificant effect on the fecal coliform concentration in the pretreatment effluent ($F(1,17)=2.062$, $p=0.169$).
3. No significant interaction effect was found between pretreatment temperature and feed shearing on fecal coliform concentration in the pretreatment effluent ($F(1,17)=0.276$, $p=0.606$).

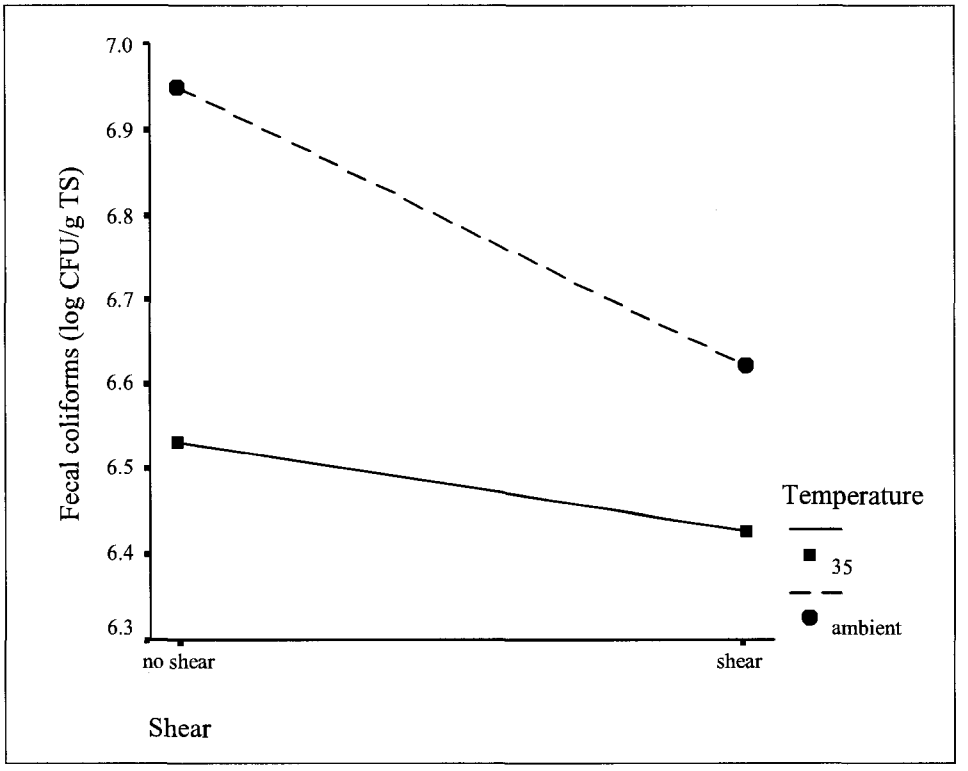

Figure 6.12.1: Means of fecal coliforms (log CFU/g TS) for pretreated effluents in phase III

For digested populations (Figure 6.12.2), the *Levene*'s test for homogeneity of variance (Appendix C) was significant, ($F(4,32)=7.802$, $p<0.05$), so the null hypothesis was rejected and therefore the variances were not assumed homogenous. The two-way ANOVA performed at the 90% significance level showed that:

After fully aerobic digestion (see Figure 6.12.2):

1. Feed shearing had a insignificant effect on the fecal coliform concentration in the final effluent relative to final effluents from not sheared feeds $F(1,32)=0.329$, $p=0.570$).

2. Higher pretreatment temperature had a significant negative effect (-2.34 +/- 0.63 log CFU/g TS) on the fecal coliform concentration in the final effluent relative to

final effluents pretreated at ambient temperature $F(1,32)=79.354$, $p<0.10$). It was also found to have a greater negative effect over the control digester (–3.06 +/- 0.67 log CFU/g TS). Pretreatment at ambient temperature had a significant negative effect (-0.72 +/- 0.49 log CFU/g TS) on the fecal coliform concentration in the digested effluent relative to the control.

3. No significant interaction between pretreatment temperature and feed shearing on the effect of fecal coliform concentration was found ($F(1,32)=0.003$, $p=0.960$).

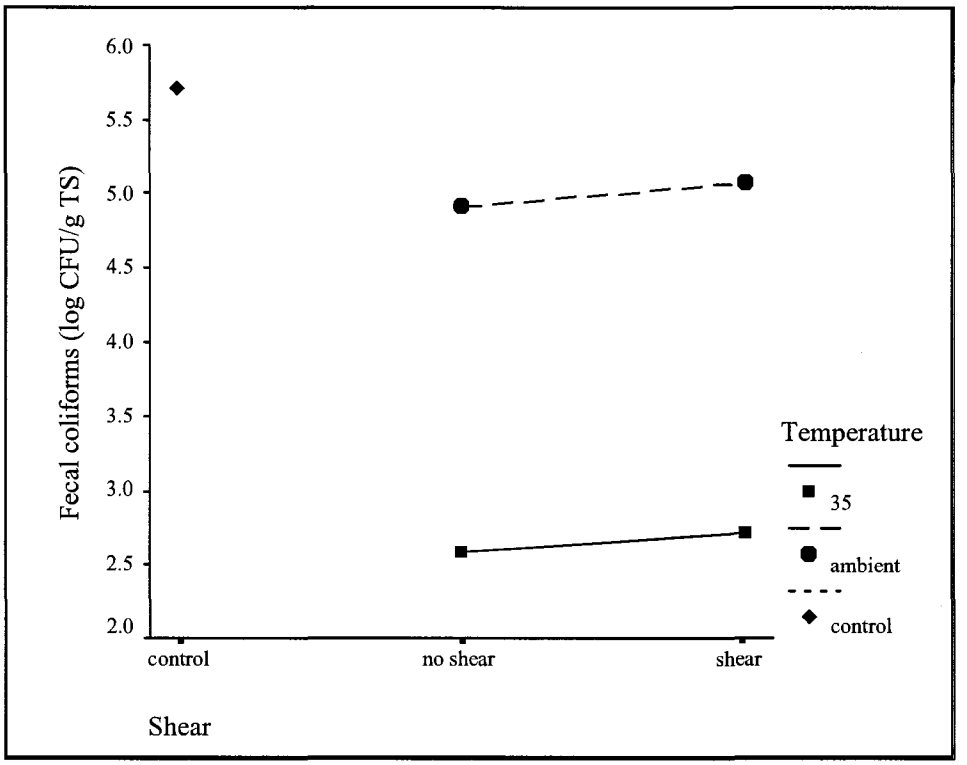

Figure 6.12.2: Means of fecal coliforms (log CFU/g TS) for digested effluents in phase III

The inactivation of fecal coliforms in the control digester was 1.5 +/- 0.5 log reductions. In all cases, the mean reduction for pretreated digester effluents was greater

than aerobic digestion alone (Figure 6.12.3). However the difference in reduction between control and digester effluents was only significant for the digester effluents pretreated at high temperature up to 4.2 +/- 0.8 and 4.6 +/- 1.2 log reductions. It is interesting that although the differences between feed and pretreated effluents were not in general significant, the impact further downstream became more pronounced. This indicates that microaerobic pretreatment in itself does not necessarily inactivate pathogens but that it induces some mechanism to make conditions further downstream less hospitable to pathogens. Also, that higher temperature pretreatment was more effective than low temperature pretreatment of aerobically digested sludge for fecal coliform removal. This pattern is similar for other pathogens monitored as further discussed in subsequent sections.

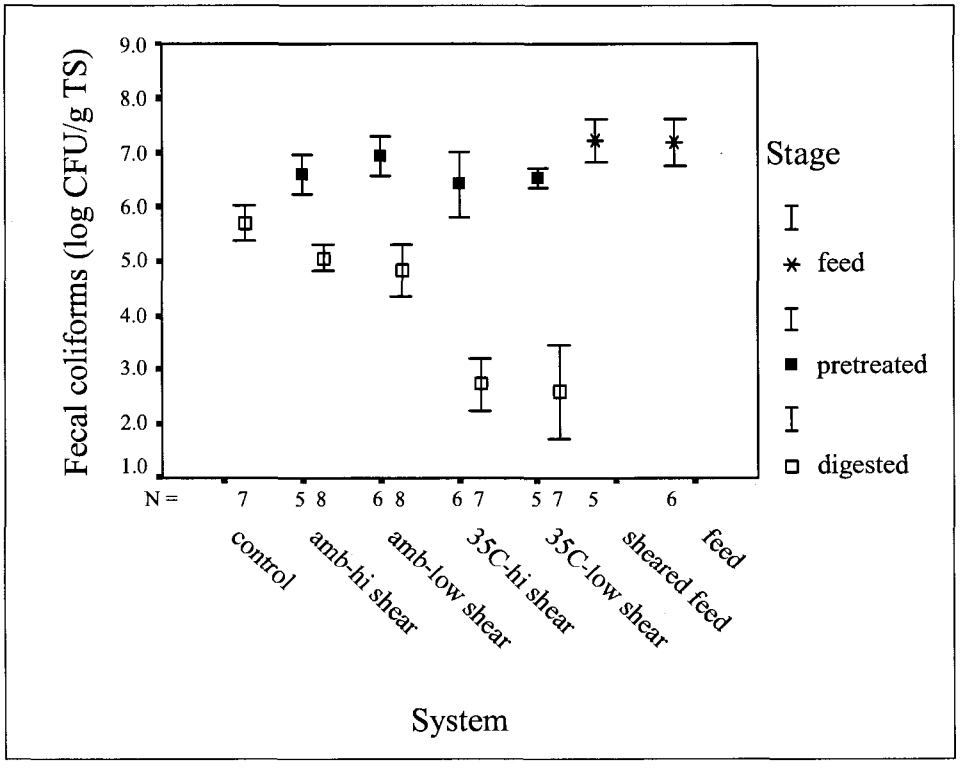

Figure 6.12.3: Error plot for 90% confidence interval around mean fecal coliforms (log CFU/g TS) by system type and stage of treatment

Table 6.12.1: Mean difference between stages significant at the 0.1 level for variable fecal coliforms.

	Feed-Pretreated	Pretreated-Digested	Feed-Digested
Control	-	-	1.48 +/- 0.49
Ambient/high shear	n.s.	1.55 +/- 0.58	1.83 +/- 0.54
Ambient/low shear	n.s.	2.04 +/- 0.56	2.29 +/- 0.56
35°C/high shear	n.s.	3.71 +/- 0.79	4.18 +/- 0.75
35°C/low shear*	0.66 +/- 0.62	3.60 +/- 1.19	4.61 +/- 1.23

*Dunnet T3 for unequal variances
n.s. = not significant

4.13 *E. coli*

The *E. coli* concentrations were measured and normalized with respect to total solids in the feed, pretreatment and digester effluents over the course of the staged digestion trials (phase III). The measured values fluctuated as seen in the time-series plots in Appendix C. In general, the *E. coli* concentrations were similar in the sheared and not sheared feeds. The *E. coli* concentration in the high temperature pretreatment reactors was in general slightly lower than the ambient temperature reactors. Also the measured *E. coli* concentrations were consistently lower in the digesters fed with high temperature pretreated sludge and the control. The box plots of the *E. coli* concentration measurements indicate several outliers. However, all measurements were included in the analysis as the fluctuations were attributed to the inherent variability in the system. The box plots also show generally normal distributions about the mean. The latter observation was confirmed by p-plots performed by sample set (Appendix C).

For feed samples, the *Levene*'s test for homogeneity of variance (Appendix C) was insignificant, ($F(1,13)=0.083$, $p=0.778$), so the null hypothesis was not rejected and therefore the variances were assumed to be homogenous. The two-way ANOVA performed at the 90% significance level showed that:

1. Feed shearing had an insignificant effect on the *E. coli* concentration in the sheared feed relative to the not sheared feed ($F(1,13)=0.045$, $p=0.836$).

For pretreated populations (see Figure 6.13.1), the *Levene*'s test for homogeneity of variance (Appendix C) was insignificant, ($F(3,21)= 0.155$, $p=0.925$), so the null hypothesis was not rejected and therefore the variances were assumed homogenous. The two-way ANOVA performed at the 90% significance level showed that:

1. Feed shearing had an insignificant effect on the *E. coli* concentration in the pretreatment effluent ($F(1,21)=0.057$, $p=0.814$).
2. Higher pretreatment temperature had an insignificant effect on the *E. coli* concentration in the pretreatment effluent ($F(1,21)=2.266$, $p=0.147$).
3. No significant interaction effect was found between pretreatment temperature and feed shearing on *E. coli* concentration in the pretreatment effluent ($F(1,21)=0.505$, $p=0.485$).

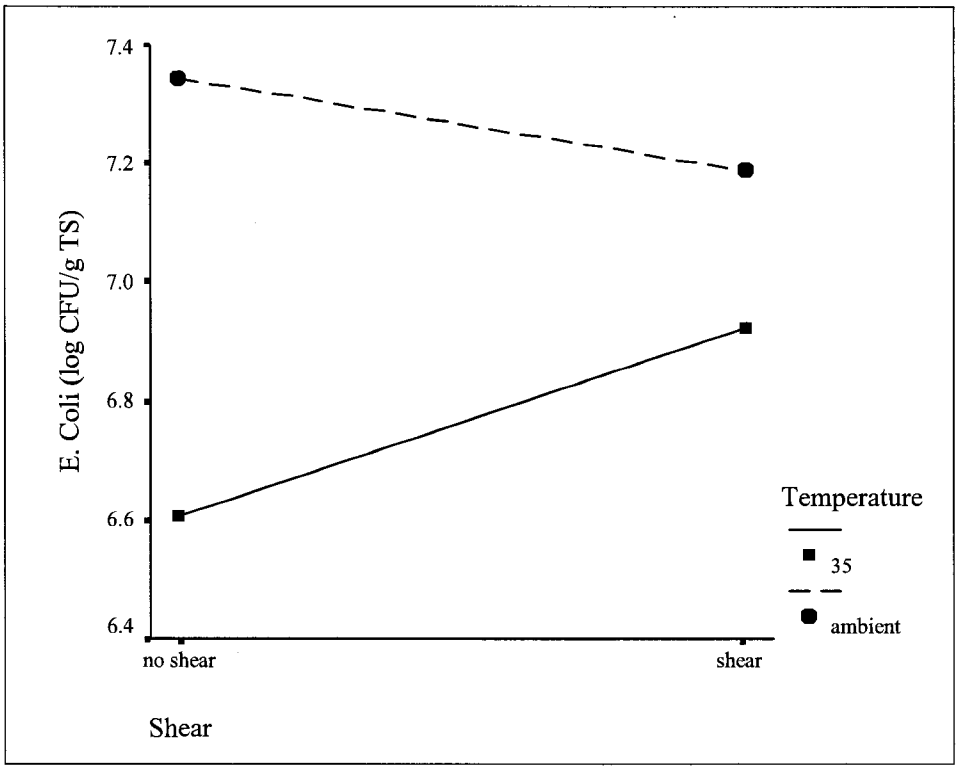

Figure 6.13.1: Means of E. coli (log CFU/g TS) for pretreated effluents in phase III

For digested populations (Figure 6.13.2), the *Levene*'s test for homogeneity of variance (Appendix C) was insignificant, ($F(4,30)=2.513$, $p=0.062$), so the null hypothesis was not rejected and therefore the variances were assumed homogenous. The two-way ANOVA performed at the 90% significance level showed that:

After fully aerobic digestion (see Figure 6.13.2):

1. Feed shearing had a insignificant effect on the *E. coli* concentration in the final effluent relative to final effluents from not sheared feeds $F(1,30)=0.051$, $p=0.823$).

2. Higher pretreatment temperature had a significant negative effect (-0.92 +/- 0.40 log CFU/g TS) on the *E. coli* concentration in the final effluent relative to final effluents pretreated at ambient temperature $F(1,30)=15.397$, $p<0.10$). It was also

found to have a greater negative effect over the control digester (–1.43 +/- 0.49 log CFU/g TS). Pretreatment at ambient temperature had a significant negative effect (-0.51 +/- 0.49 log CFU/g TS) on the *E. coli* concentration in the digested effluent relative to the control.

3. No significant interaction between pretreatment temperature and feed shearing on the effect of *E. coli* concentration was found (F(1,30)=0.143, p=0.708).

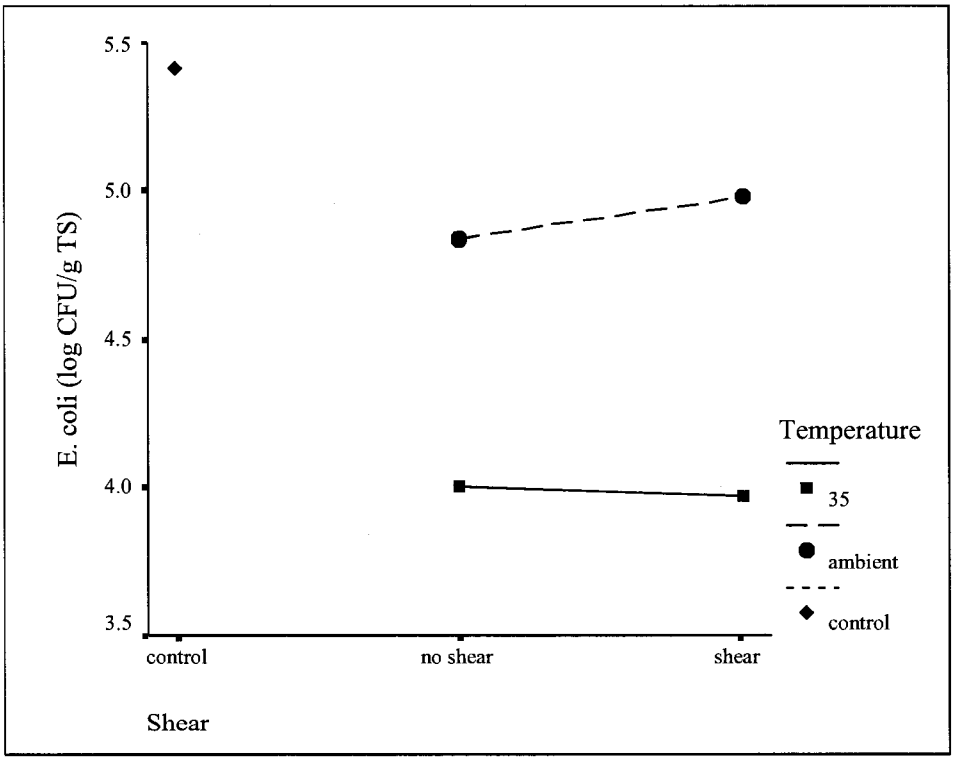

Figure 6.13.2: Means of E. coli (log CFU/g TS) for digested effluents in phase III

The inactivation of *E. coli* in the control digester was 1.6 +/- 0.8 log reductions (Table 6.13.1). In all cases, the mean reduction for pretreated digester effluents was greater than aerobic digestion alone (Figure 6.13.3). However the difference in reduction between control and digester effluents was only significant for the digester effluents pretreated at high temperature up to 3.1 +/- 0.7 and 3.1 +/- 0.7 log reductions. Similarly to fecal coliforms, the differences between feed and pretreated effluents were not in general significant, but the impact of pretreatment further downstream became more pronounced. It was found that higher temperature pretreatment was more effective than low temperature pretreatment and no pretreatment of aerobically digested sludge for *E.coli* removal.

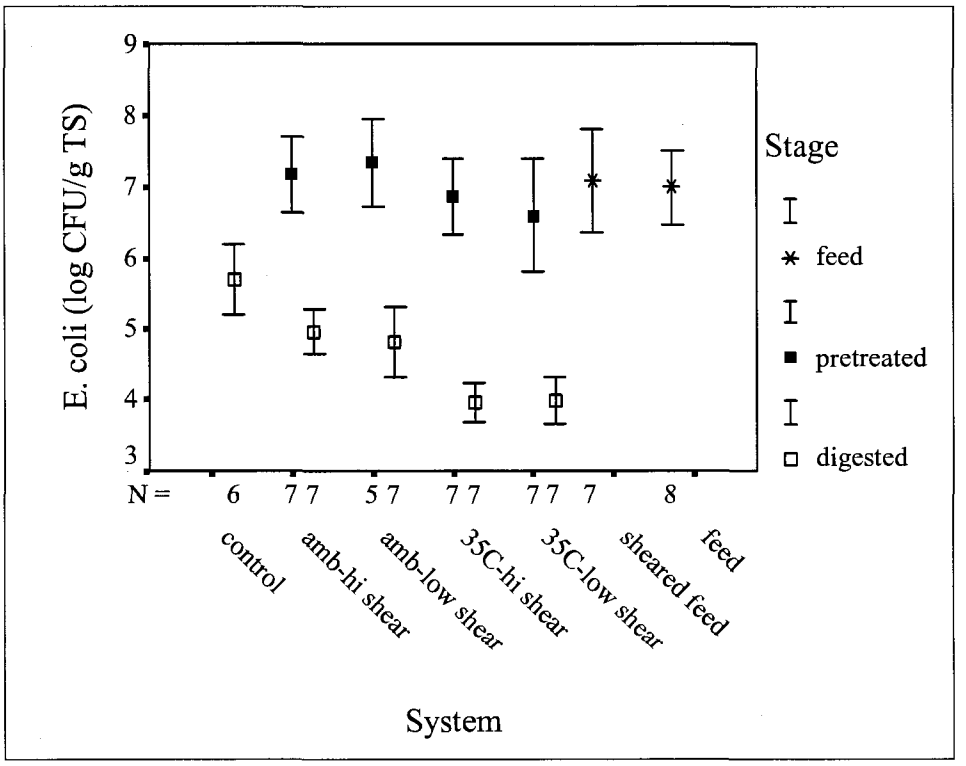

Figure 6.13.3: Error plot for 90% confidence interval around mean E. coli (log CFU/g TS) by system type and stage of treatment

Table 6.13.1: Mean difference between stages significant at the 0.1 level for E. coli (log CFU/g TS)

	Feed-Pretreated	Pretreated-Digested	Feed-Digested
Control	-	-	1.58 +/- 0.79
Ambient/high shear	n.s.	2.21 +/- 0.69	2.12 +/- 0.54
Ambient/low shear	n.s.	2.51 +/- 0.71	2.17 +/- 0.63
35°C/high shear	n.s.	2.96 +/- 0.72	3.13 +/- 0.70
35°C/low shear	n.s.	2.61 +/- 0.74	3.00 +/- 0.71

n.s. = not significant

6.14 *Salmonella* spp.

The *Salmonella* spp. concentrations were measured and normalized with respect to total solids in the feed, pretreatment and digester effluents over the course of the staged digestion trials (phase III). The measured values fluctuated as seen in the time-series plots in Appendix C. In general, the *Salmonella* spp. concentrations were similar in the sheared and not sheared feeds. The *Salmonella* spp. concentration in the high temperature pretreatment reactors was in general slightly lower than the ambient temperature reactors. Also, the *Salmonella* spp. concentrations were consistently lower in the digesters fed with high shear and high temperature pretreated sludge and the control. The box plots of the *Salmonella* spp. concentration measurements indicate two outliers. However, all measurements were included in the analysis as the fluctuations were attributed to the inherent variability in the system. The box plots also show generally normal distributions about the mean. The latter observation was confirmed by p-plots performed by sample set (Appendix C).

For feed samples, the *Levene*'s test for homogeneity of variance (Appendix C) was insignificant, ($F(1,11)=0.679$, $p=0.427$), so the null hypothesis was not rejected and therefore the variances were assumed homogenous. The two-way ANOVA performed at the 90% significance level showed that:

1. Feed shearing had an insignificant effect on the *Salmonella* spp. concentration in the sheared feed relative to the not sheared feed ($F(1,11)=0.065$, $p=0.803$).

For pretreated populations (see Figure 6.14.1), the *Levene*'s test for homogeneity of variance (Appendix C) was insignificant, ($F(3,23)= 1.376$, $p=0.275$), so the null hypothesis was not rejected and therefore the variances were assumed homogenous. The two-way ANOVA performed at the 90% significance level showed that:

1. Feed shearing had an insignificant effect on the *Salmonella* spp. concentration in the pretreatment effluent ($F(1,23)=0.192$, $p=0.665$).
2. Higher pretreatment temperature had an insignificant effect on the *Salmonella* spp. concentration in the pretreatment effluent ($F(1,23)=0.787$, $p=0.384$).
3. No significant interaction effect was found between pretreatment temperature and feed shearing on *Salmonella* spp. concentration in the pretreatment effluent ($F(1,23)=0.609$, $p=0.443$).

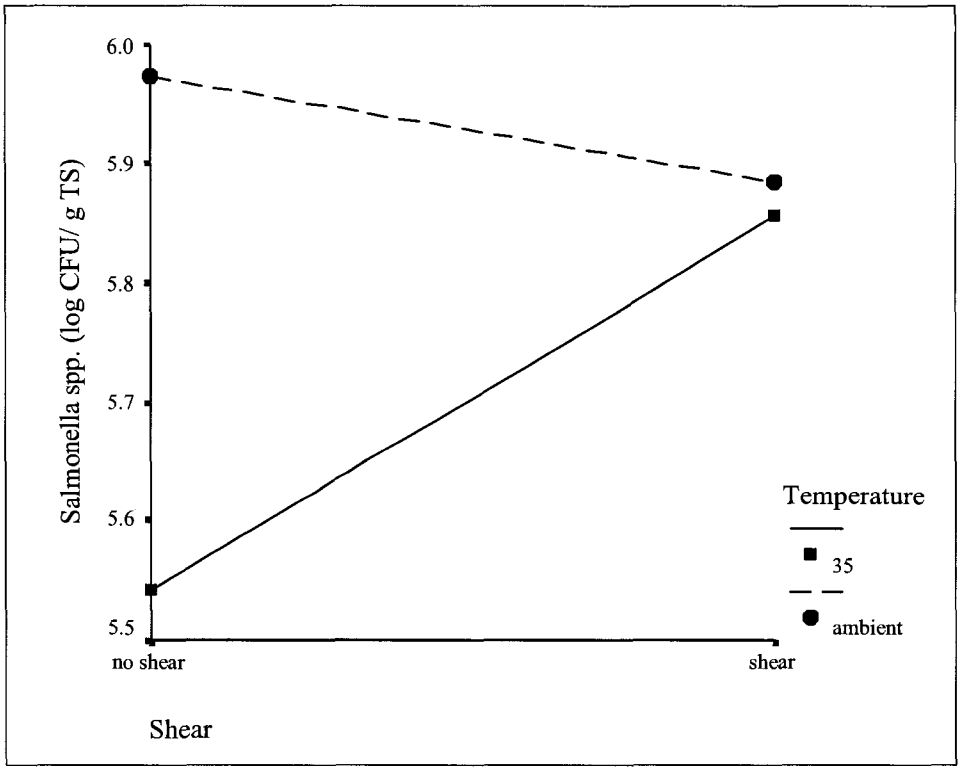

Figure 6.14.1: Means of Salmonella spp. (log CFU/g TS) for pretreated effluents in phase III

For digested populations (Figure 6.14.2), the *Levene*'s test for homogeneity of variance (Appendix C) was insignificant, ($F(4,26)=1.634$, $p=0.196$), so the null hypothesis was not rejected and therefore the variances were assumed homogenous. The two-way ANOVA performed at the 90% significance level showed that:

After fully aerobic digestion (see Figure 6.14.2):

1. Feed shearing had a insignificant effect on the *Salmonella* spp. concentration in the final effluent relative to final effluents from not sheared feeds $F(1,26)=32.123$, $p=0.108$).
2. Higher pretreatment temperature had a significant negative effect (-1.33 +/- 0.40 log CFU/g TS) on the *Salmonella* spp. concentration in the final effluent relative

to final effluents pretreated at ambient temperature $F(1,26)=32.123$, $p<0.10$). It was also found to have a greater negative effect over the control digester (–1.28 +/- 0.50 log CFU/g TS).

3. A significant interaction between pretreatment temperature and feed shearing on the effect of *Salmonella* spp. concentration was found ($F(1,26)=20.128$, $p<0.10$).

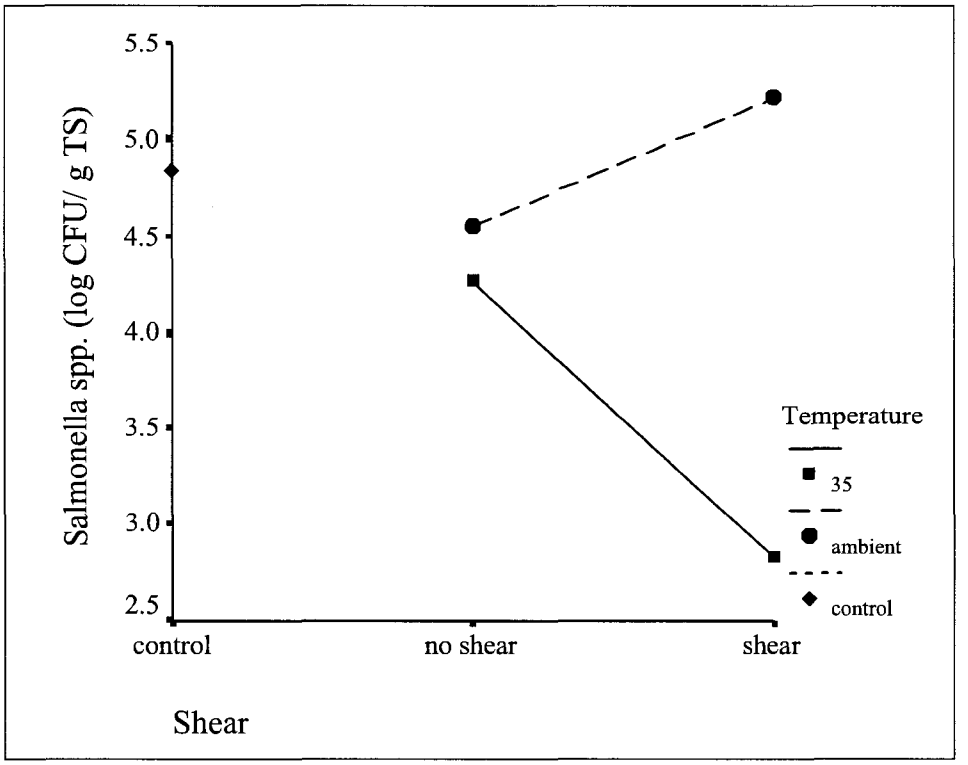

Figure 6.14.2: Means of Salmonella spp. (log CFU/g TS) for digested effluents in phase III

The inactivation of *Salmonella* spp. in the control digester was 1.7 +/- 0.7 log reductions (Table 6.14.1). In all cases except the ambient high shear pretreated system,

the mean reduction for pretreated digester effluents was greater than aerobic digestion alone (Figure 6.14.3). However the difference in reduction between control and digester effluents was only significant for the digester effluents pretreated at high temperature up to 4.6 +/- 0.5 and 2.2 +/- 0.7 log reductions. The differences between feed and pretreated effluents for *Salmonella* spp. removal were up to 1.0 log reduction for the high temperature low shear condition. Interestingly, the high shear condition appeared to be more hospitable to *Salmonella* spp. where no significant reduction was observed after ambient microaerobic pretreatment and only 0.6 +/- 0.5 log reductions were observed at the 35°C. However, this trend was reversed further downstream where the digester effluent from the high shear, high temperature system was significantly lower than all other final effluents with an observed log reduction of 3.6 +/- 0.5 from the original feed. It may be that shearing the feed compromised the *Salmonella* spp. in such a way to make it more susceptible to inactivation under the conditions in digestion. The impact of pretreatment became more pronounced further downstream as was found for the other bacteria measured. Higher temperature pretreatment was more effective than low temperature pretreatment of aerobically digested sludge for *Salmonella* spp. removal.

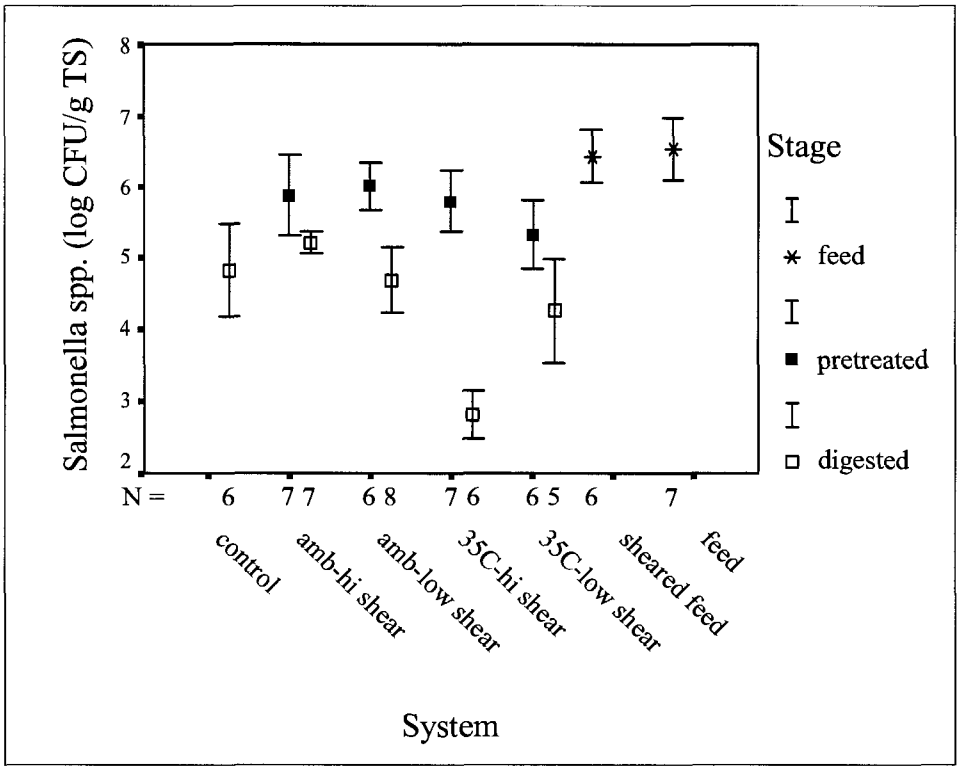

Figure 6.14.3: Error plot for 90% confidence interval around mean Salmonella spp. (log CFU/g TS) by system type and stage of treatment

Table 6.14.1: Mean difference between stages significant at the 0.1 level for variable Salmonella spp.

	Feed-Pretreated	Pretreated-Digested	Feed-Digested
Control	-	-	1.68 +/- 0.70
Ambient/high shear*	n.s.	n.s.	1.23 +/- 0.52
Ambient/low shear	0.54 +/- 0.51	1.42 +/- 0.51	1.97 +/- 0.51
35°C/high shear	0.58 +/- 0.53	3.03 +/- 0.53	3.61 +/- 0.53
35°C/low shear	0.97 +/- 0.67	1.27 +/- 0.73	2.24 +/- 0.73

*Dunnet T3 for unequal variances
n.s. = not significant

4.15 *Shigella* spp.

The *Shigella* spp. concentrations were measured and normalized with respect to total solids in the feed, pretreatment and digester effluents over the course of the staged digestion trials (phase III). The measured values fluctuated as seen in the time-series plots in Appendix C. In general, the *Shigella* spp. concentrations were slightly higher in the sheared than in the not sheared feeds. The concentrations were similar in all pretreatment reactors and were consistently lower in the digesters fed with high temperature pretreated sludge and the control. The box plots of the *Shigella* spp. concentration measurements indicate one outlier which was included in subsequent analysis. The box plots also show generally normal distributions about the mean. The latter observation was confirmed by p-plots performed by sample set (Appendix C).

For feed samples, the *Levene*'s test for homogeneity of variance (Appendix C) was insignificant, ($F(1,7)=1.478$, $p=0.264$), so the null hypothesis was not rejected and therefore the variances were assumed homogenous. The two-way ANOVA performed at the 90% significance level showed that:

1. Feed shearing had an insignificant effect on the *Shigella* spp. concentration in the sheared feed relative to the not sheared feed ($F(1,7)=0.007$, $p=0.937$).

For pretreated populations (see Figure 6.15.1), the *Levene*'s test for homogeneity of variance (Appendix C) was insignificant, ($F(3,10)= 2.363$, $p=0.133$), so the null hypothesis was not rejected and therefore the variances were assumed homogenous. The two-way ANOVA performed at the 90% significance level showed that:

1. Feed shearing had an insignificant effect on the *Shigella* spp. concentration in the pretreatment effluent (F(1,10)=0.111, p=0.746)
2. Higher pretreatment temperature had an insignificant effect on the *Shigella* spp. concentration in the pretreatment effluent (F(1,10)=0.120, p=0.736).
3. No significant interaction effect was found between pretreatment temperature and feed shearing on *Shigella* spp. concentration in the pretreatment effluent (F(1,10)=1.767, p=0.213).

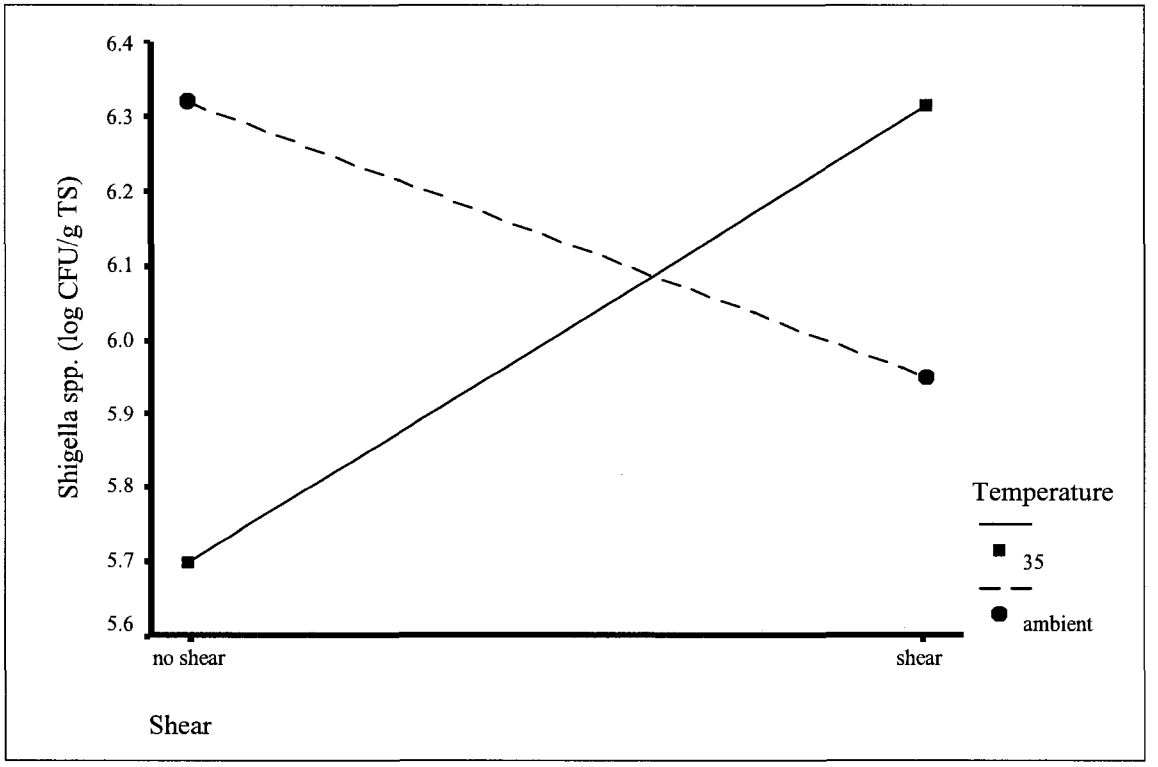

Figure 6.15.1: Means of Shigella *spp. (log CFU/g TS) for pretreated effluents in phase III*

For digested populations (Figure 6.15.2), the *Levene*'s test for homogeneity of variance (Appendix C) was insignificant, (F(4,13)=1.257, p=0.336), so the null

hypothesis was not rejected and therefore the variances were assumed homogenous. The two-way ANOVA performed at the 90% significance level showed that:

After fully aerobic digestion (see Figure 6.15.2):

1. Feed shearing had a insignificant effect on the *Shigella* spp. concentration in the final effluent relative to final effluents from not sheared feeds $F(1,13)=0.010$, $p=0.923$).
2. Higher pretreatment temperature had a significant negative effect (-1.31 +/- 0.42 log CFU/g TS) on the *Shigella* spp. concentration in the final effluent relative to final effluents pretreated at ambient temperature $F(1,13)=30.437$, $p<0.10$). It was also found to have a greater negative effect over the control digester (–1.71 +/- 0.56 log CFU/g TS).
3. A significant interaction between pretreatment temperature and feed shearing on the effect of *Shigella* spp. concentration was found ($F(1,13)=3.971$, $p<0.10$).

The inactivation of *Shigella* spp. in the control digester was 1.8 +/- 0.8 log reductions (Table 6.15.1). In all cases, the mean reduction for pretreated digester effluents was greater than aerobic digestion alone (Figure 6.15.3). However the difference in reduction between control and digester effluents was only significant for the digester effluents pretreated at high temperature up to 3.7 +/- 0.9 and 3.3 +/- 1.1 log reductions. Figure 6.15.3 and Table 6.15.1 show that the differences between feed and pretreated effluents for *Shigella* spp. removal were up to 1.4 +/- 1.0 log reductions for the high temperature

low shear condition however this difference was not significant for the high shear condition.

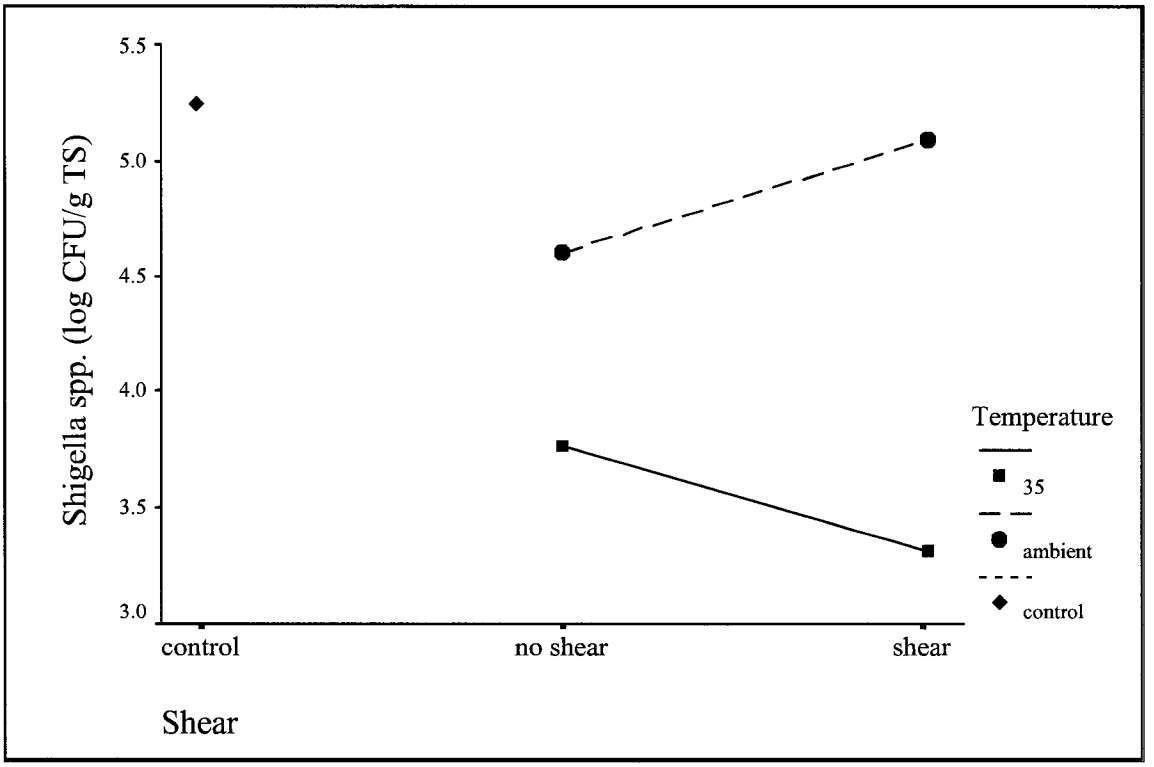

Figure 6.15.2: Means of Shigella *spp. (log CFU/g TS) for digested effluents in phase III*

However, the digester effluents from the high temperature high shear system saw the largest decline in *Shigella* spp. at 3.7 +/- 0.9, a behaviour similar to the *Salmonella* spp. trends. At ambient temperatures, *Shigella* spp. were decreased in pretreatment by 1.0 +/- 0.9 and 0.7 +/- 0.6 for the high and low shear conditions, respectively. The impact of pretreatment became more pronounced further downstream as was found for the other bacteria measured. Higher temperature pretreatment was more effective than low temperature pretreatment of aerobically digested sludge for *Shigella* spp. removal.

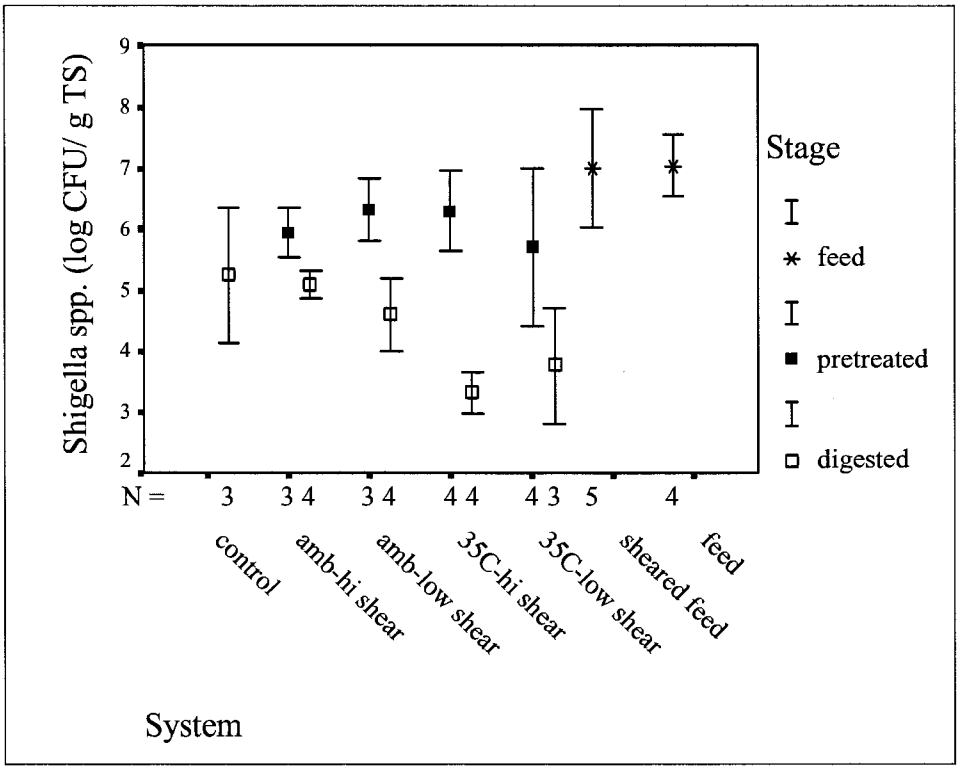

Figure 6.15.3: Error plot for 90% confidence interval around mean Shigella spp. (log CFU/g TS) by system type and stage of treatment

Table 6.15.1: Mean difference between stages significant at the 0.1 level for variable Shigella spp.

	Feed-Pretreated	Pretreated-Digested	Feed-Digested
Control	-	-	1.80 +/- 0.82
Ambient/high shear	1.05 +/- 0.93	n.s.	1.91 +/- 0.86
Ambient/low shear	0.72 +/- 0.62	1.72 +/- 0.62	2.45 +/- 0.58
35°C/high shear	n.s.	3.00 +/- 0.92	3.68 +/- 0.88
35°C/low shear	1.35 +/- 1.02	1.93 +/- 1.10	3.28 +/- 1.10

*Dunnet T3 for unequal variances
n.s. = not significant

6.16 Summary of Phase III

The results of the two way ANOVAs comparing high shear, high temperature pretreated systems and the control are summarized in Table 6.16.1.

Feed shearing significantly increased (at the 0.1 level) soluble TKN and COD by 32.4 +/- 18.4mg/L and 256.5 +/- 170.1mg/L. However no other variables monitored on the feed were affected by shearing. The impacts of solubilization on other process variables were not as pronounced as expected however the outcomes reinforced the validity temperature effects by providing duplicate systems at high and ambient temperatures.

The main effect of shear on pretreated effluents for all measured variables was insignificant at the 0.1 level. However, higher temperature (35°C) caused significant negative differences in DO and ORP attributed to differences in oxygen saturation concentration and enhanced biological activity at with increased temperature. It also caused positive significant differences in soluble TKN, ammonia, total VFAs, alkalinity, soluble COD and pH which indicated increased rates of cellular hydrolysis and fatty acid fermentation. No significant difference in pathogens was observed after pretreatment alone.

For digested effluents, feed shearing showed only one positive main effect and that was on pH. It is possible that the enhanced hydrolysis caused by shearing contributed to ammonia release that caused an increase in pH. For all pretreated digester effluents, destruction of *E.coli* and fecal coliforms was enhanced compared to the control. This enhancement was more pronounced for digester effluents pretreated at high temperature up to 3.1 and 1.4 log reductions more over the control for fecal coliforms and

E. coli, respectively. *Salmonella* and *Shigella* removals were not significantly different in the control and digester effluents pretreated at ambient temperatures but were enhanced by 1.3 and 1.7 log reductions respectively with higher pretreatment temperatures possibly caused by depressed pH observed in the digesters downstream in those systems. Interestingly, a strong interaction effect was found between high feed shear and pretreatment temperature on the enhancement of *Salmonella* spp. inactivation.

It is hypothesized that the significantly lower pH in the high temperature pretreated digesters enhanced pathogen removal. These digesters also had significantly higher nitrate and nitrite concentrations indicating that there was nitrification, which is known to cause pH depression. Looking further upstream the significantly higher ammonia levels were present in the high temperature pretreatment reactors likely caused by enhanced hydrolysis. It is hypothesized that the additional substrate for nitrification provided from high temperature pretreatment promoted downstream nitrification and in turn pH depression, which enhanced pathogen removal. The mean differences between systems of measured values are summarized in Table 6.16.1 where significant at the 90% confidence interval with key values pertaining to the above hypothesis are shown in boldface.

*Table 6.16.1: Summary of significant mean differences (0.1 level) between treatment systems by treatment stage and variable**

	Feed	Pretreated Effluents		Digested Effluents			
	Main Effects	Main Effects		Main Effects		Temperature Effects	
Variable	Shear – No Shear	Shear -- No Shear	High Temp – Amb. Temp	Shear – No Shear	High Temp – Amb.Temp	High Temp - Control	Amb. Temp – Control
DO (mg/L)	n.a.	n.s	-1.54 +/-0.74	n.s.	2.48 +/- 1.18	4.68 +/- 1.44	2.20 +/- 1.42
ORP (mV)	n.a.	n.s	-227.2 +/1 69.1	n.s.	53.7 +/- 38.4	102.7 +/- 49.2	n.s
TKN (mg/L)	n.s.	n.s	n.s	n.s	n.s	n.s	n.s
sTKN (mg/L)	32.4 +/1 18.4	n.s.	192 +/- 136.3	n.s.	n.s.	n.s.	n.s.
Ammonia (mg/L)	n.s.	n.s.	**187.0 +/- 28.7**	n.s.	n.s.	n.s.	n.s.
Nitrate (mg/L)	n.s.	n.s.	n.s.	n.s.	153.7 +/- 48.4	**203.1 +/- 59.2**	n.s.
Total VFA (mg acetate/L)	n.s.	n.s.	464.0 +/- 159.7	n.d.	n.d.	n.d.	n.d.
Alkalinity (mg $CaCO_3$/L)	n.s.	n.s.	271.6 +/- 110.2	n.d.	n.d.	n.d.	n.d.
Soluble COD (mg/L)	256.5 +/- 170.1	n.s.	684.4 +/- 70.4	n.s.	72.1 +/- 39.1	n.s.	n.s.
pH	n.s.	n.s.	0.71 +/- 0.17	0.39+/-0.38	-1.14 +/- 0.38	**-1.63 +/- 0.48**	-0.49 +/- 0.48
Fecal coliforms (log CFU/g TS)	n.s.	n.s.	n.s.	n.s.	-2.34 +/- 0.44	**-3.06+/- 0.67**	-0.72 +/- 0.55
E. coli (log CFU/g TS)	n.s.	n.s.	n.s.	n.s.	-0.92 +/- 0.40	**-1.43 +/- 0.49**	-0.51 +/- 0.49
Salmonella spp. (log CFU/g TS)	n.s.	n.s.	n.s.	n.s.	-1.33 +/- 0.40	**-1.28 +/- 0.50**	n.s.
Shigella spp. (log CFU/g TS)	n.s.	n.s.	n.s.	n.s.	-1.31 +/- 0.42	**-1.71 +/- 0.56**	n.s.

* *Key values in bold for the hypothesis of enhanced hydrolysis in pretreatment leading to low pH in digestion and hence greater pathogen removal observed in the final effluent for high temperature pretreatment systems*

n.s. = not significant at the 0.1 level

n.d = not detected

7. Conclusions

7.0 Conclusions

The investigation of microaerobic pretreatment was trialed as a novel approach to staged digestion for improved pathogen removal. Microaerobic pretreatment alone was investigated in three phases of the experiments and did not result in inactivation greater than one log reduction for any bacteria monitored.

In Phase I where feed solids concentration was varied across four reactors, the lowest solids loading of 1.1% TS showed the best removal rates of pathogens. In Phase II, contact time was evaluated in terms of feeding frequency and residence time. It was found that less frequent feeding and longer residence times were more effective in removing pathogens as expected from the reactor kinetics and suggested by the literature.

The impact after digestion was found to be significant in Phase III for fecal coliforms, *E.coli, Salmonella* spp. and *Shigella* spp. It appeared that changes to the sludge matrix in microaerobic pretreatment improved digester performance in terms of pathogen removal.

Operating variables were monitored to gain an understanding of the factors impacting performance. Statistical analyses were performed at the 90% confidence interval to determine which if any factors differed significantly between systems and stages. The major findings were that mesophilic pretreatment (35°C) with air supplied at 0.06vvm yielded significantly higher ammonia levels after pretreatment than did pretreatment at ambient temperatures. This was hypothesized to account for the significantly greater extent of nitrification observed in downstream aerobic digestion over the effluents from digestion without pretreatment and those that were pretreated under

ambient microaerobic conditions. Accordingly, the pH in those digesters was significantly lower than after digestion without pretreatment and after digestion with ambient microaerobic pretreatment. This enhanced depression in pH was hypothesized to account for the significantly enhanced inactivation in pathogens.

7.1 Phase 1

In the first phase of experimentation, a fixed airflow of 0.06vvm (350mL/min) was supplied while feed solids concentration was varied from 1.0% - 2.5% across four pretreatment reactors at ambient temperature. These reactors had hydraulic residence times (HRT) of 2 days and were fed daily in semi-batch mode (with draw/fill of 50% of the reactor volume used per feed).

1. Minimal solids destruction occurred in the 2d residence time.
2. A step down in ORP from approximately –100mV to –250mV was found between the 1.0% and 1.5% reactors and the ORP in the 1.5% - 2.5% reactors were similar.
3. The total TKN increased with feed solids concentration and was similar in the respective feed and effluent samples, which indicated that there were minimal nitrogen losses through nitrification/denitrification.
4. A two to threefold increase in soluble TKN and ammonia was observed in the three highest solids and most reducing reactors indicating that hydrolysis was more prevalent there than under the more oxidizing conditions of the 1.0% solids reactor.
5. The total VFA concentrations from fermentation increased under more reducing conditions reaching 600mg acetate/L in the reactor fed with 2.5% total solids feed.

6. The VFA concentrations were associated with the alkalinity and pH in the reactor effluents.
7. The soluble COD increased in the reactor feeds with increased solids concentration. The 1.0% solids reactor achieved some net soluble COD removal. However, the higher solids reactors remained insignificantly different between the respective feed and effluent samples. It was hypothesized that the contribution of VFA production to soluble COD masked the COD removed and kept the net soluble COD balance fairly even except in the 1.0% reactor where there was negligible production of VFAs, and there was a net loss in soluble COD.
8. The alkalinity in the feed samples was constant across all reactors. The maximum effluent alkalinity was from the 1.5% pretreatment reactor. The alkalinity levels were likely affected negatively by increased VFA concentrations (which dominated in the 2.0% and 2.5% feed solids reactors) and positively by increased ammonia concentrations (which dominated in the 1.5% feed solids reactor).
9. The pH levels measured in the feed samples were on average approximately 6.4. In the reactor effluent samples, the pH tended to increase significantly at the 90% confidence interval relative to the feed pH and followed a similar profile to the alkalinity with the highest mean observed in the 1.5% feed solids reactor.
10. The optimum feed concentration for *E. Coli*, fecal coliforms, and *C. perfringens* mean log removal was 1.5% solids.
11. There was a greater variability in the effluent fecal coliforms concentrations in the 1.5% feed solids reactor than in the feeds and in the effluents of the 2.0% and 2.5% feed solids reactors.

12. None of the fecal coliform or *C. perfringens* destructions observed after pretreatment were significant at the 90% confidence interval.
13. *E. coli* showed a significant decrease at the 90% confidence interval in the 1.0% and 1.5% feed solids reactors.
14. The highest destructions observed for *Shigella* spp. and *Salmonella* spp. based on mean log reductions of CFU/g TS were found in the 1.0% and 1.5% feed solids reactors though the *Salmonella* spp destruction was not significant at the 90% confidence interval in the 1.5% feed reactor. A significant decrease was also observed at the 90% confidence interval for *Shigella* spp. only in the 2.5% feed solids reactor.
15. In general the log reductions of pathogens were small or negligible after pretreatment only, especially for C. *perfringens*, which was expected to be resistant to removal.
16. he resistance of C. *perfringens* indicator in microaerobic pretreatment of sludge suggests that *Ascaris* ova if present would also be persistent under these conditions as reported by Bujoczek et al., (2001).

7.2 Phase II

In phase II of experimentation, the selected feed concentration of 1.5% from phase I was used in combination with an air flow rate of 0.06vvm to produce microaerobic conditions. The test conditions in this phase were feeding frequency (manipulated by feed volume) and hydraulic residence time (HRT). The resulting setup explored increasing contact time. A two-by-two factorial experimental design was employed with two feeding frequencies (using 50% and 25% reactor volume replacements) and two HRT (2d and 4d). For the 2d HRT reactors, a 25% reactor volume

feed required feeding every 12h and the 50% reactor volume feed required feeding every day. For the 4d HRT reactors, a 25% reactor volume feed required feeding once per day and the 50% reactor volume feed required feeding every other day. In all cases the draw/fill method was used.

1. The oxidative-reductive potential (ORP) in the reactors tended to increase with contact time.
2. The mean ORP of the 2d HRT and 50% volume replacement reactor was higher than the similar conditions in phase I. This was attributed to the less degradable feed available in the summer months and therefore less biological aerobic activity to consume the dissolved oxygen (DO).
3. ORP tended to increase with increased contact time, the DO followed a different trend where it was significantly lower for those reactors fed with 25% of the volume at the respective HRTs.
4. In general, the mean TKN in the reactors was less than in the feed though not statistically significantly at the 90% confidence interval. No significant differences in mean TKN concentrations were observed between reactors. Ion chromatography showed positive values for nitrate and nitrite and confirmed that nitrification was likely occurring in the reactors.
5. The soluble TKN results indicated that nitrogen transformation was occurring in the pretreatment reactors as compared to the feed where concentrations decreased from 30mg/L in the feed to less than 5mg/L in all pretreatment reactors. Nitrification is an aerobic process and was not found to occur in phase I where mean ORPs were lower than in phase II.

6. Mean ammonia values dropped significantly in all reactors as compared to the feed, which indicated nitrification occurred. Only the reactor with the shortest contact time had any appreciable ammonia remaining in the effluent on the order of 10mg/L. This reactor was also the least stable in producing consistent effluent in terms of ammonia concentration.
7. The decrease in ammonia in the effluents compared to the feed explained a similar trend observed in alkalinity. The mean values for alkalinity tended to decrease with increased contact time, however, not statistically significantly at the 90% confidence interval. VFAs were below the detection limit in the effluent samples and did not account for the drop in alkalinity.
8. pH decreased with increased contact time and increased relative to the feed. It is expected that the mean value of VFAs measured in the feed samples (210mg acetate/L) depressed the feed pH. Under the oxidizing conditions of pretreatment in this phase of the experiment, VFAs were likely quickly consumed, which resulted in a more neutral pH in the effluents than in the feed.
9. The depletion of soluble COD after pretreatment confirmed that there was a degradation of VFAs and other soluble substrates in the pretreatment reactors, though it was not statistically significant among the reactors at the 90% confidence interval.
10. In general, the optimum contact time for pathogen destruction in the pretreatment conditions tested in phase II was the longest residence time coupled with less frequent feeding (4d HRT and 50% reactor volume replacement). This held true for fecal coliforms, *E. coli*, and *Shigella* spp.

11. It was suspected that both the predation and longer exposure to sub-optimal pH led to the increased pathogen destruction with contact time. These results are consistent with the literature where in general better pathogen removal occurs with longer exposure times with draw/fill semi-continuous feeding (Farrell et *al.*, (1988).
12. Although both the *Salmonella* spp. and *C. perfringens* mean concentrations tended to decrease slightly after pretreatment, there was no obvious trend with contact time. The minimal C. *perfringens* die-off, if taken as a model indicator for *Ascaris* eggs die-off as reported by Bujoczek et *al.* (2003), suggested that *Ascaris* eggs would not be significantly affected in microaerobic pretreatment of sludge under the conditions tested.
13. In all cases the pathogen destruction was less than a full log reduction, which suggested that pretreatment alone was insufficient to yield greater pathogen removal from the sludge tested.
14. The longest contact time and feeding cycle was selected for further investigation in phase III of experimentation as it showed the highest potential for pathogen destruction.

7.3 Phase III

Phase III of the experiments involved three treatment stages: feed preparation, pretreatment and digestion as previously described in the experimental section. This phase examined the effect of temperature and feed shear on the performance of the microaerobic pretreatment system in a two-by-two factorial experimental design. The

semi-batch pretreatment reactors were operated with 4d hydraulic residence times (HRT), aerated at 0.06vvm and fed 50% of their volumes with domestic sewage sludge with a solids concentration of 1.5%. The detailed findings and conclusions from phase III are:

1. Higher pretreatment temperature was found to have a significant negative effect on the DO (-1.54 +/- 0.74 mg/L) in the pretreatment effluent ($F(1,32)=12.307$, $p<0.10$). The depressed DO in the high temperature pretreatment reactors relative to those at ambient temperatures was attributed to a decline in oxygen saturation concentration with increased temperature (which depressed the driving force for mass transfer).

2. Higher pretreatment temperature was found to have a significant positive effect on the DO in the final effluent (2.48 +/- 1.18mg/L) over ambient pretreatment, ($F(1,29)=12.746$, $p<0.10$); it was also found to have a greater positive effect over the control digester (4.68 +/- 1.44 mg/L). Pretreatment at ambient temperature had a significantly positive effect over the control (2.20 +/- 1.42mg/L) and on the DO of the digested effluents. In ambient temperature digestion, the high temperature systems were at approximately 8mg/L and had significantly higher DO than the control which was at 4mg/L and slightly higher DO than the ambient pretreatment systems whose mean DO were between 5.8 to 6.4mg/L.

3. Higher pretreatment temperature was found to have a significant negative effect on the ORP (-227.2 +/- 69.12 mV) in the pretreatment effluent compared to at ambient temperature ($F(1,32)=30.998$, $p<0.10$). Higher pretreatment temperature was found to have a significant positive effect on the ORP in the final effluent (53.7 +/- 38.4 mV) over ambient pretreatment ($F(1,34)=5.589$, $p<0.10$). It was

also found to have a greater positive effect over the control digester (102.7 +/- 49.2 mV). Pretreatment at ambient temperature did not have a significant effect on the ORP of the digested effluent relative to the control.

4. A linear regression analysis performed on D and ORP data showed a moderate ($R^2 = 0.74$) linear relationship for all fully aerobic digesters:

$$DO = 0.0325\ (ORP) - 1.5932$$

where DO is in [mg/L] and ORP is in [mV].

5. Feed shearing had a significant positive effect (32.4 +/- 18.6mg/L) on the soluble TKN in the sheared feed relative to the not sheared feed ($F(1,6)=11.479$, $p<0.10$) which indicated solubilization as a result of feed shearing.

6. Higher pretreatment temperature was found to have a significant positive effect on the soluble TKN relative to pretreatment at ambient temperature (192.0 +/- 136.3mg/L) in the pretreatment effluent ($F(1,12)=6.299$, $p<0.10$) which indicated that hydrolysis occurred more readily at higher temperature.

7. Higher pretreatment temperature was found to have a significant positive effect on the ammonia relative to pretreatment at ambient temperature (187.0 +/- 28.7mg/L) in the pretreatment effluent ($F(1,34)=121.192$, $p<0.10$) which indicated that hydrolysis occurred more readily at higher temperature.

8. A significant interaction between the effect of pretreatment temperature and feed shearing on ammonia concentration in the pretreatment effluent was found ($F(1,34)=3.398$, $p<0.10$).

9. Higher pretreatment temperature was found to have a significant positive effect on the combined nitrate and nitrite in the final effluent (153.7 +/- 48.4mg NO_3/L) over ambient pretreatment ($F(1,38)=5.589$, $p<0.10$). It was also found to have a greater positive effect over the control digester (203.1 +/- 59.2mg NO_3/L). Pretreatment at ambient temperature did not have a significant effect on the combined nitrite and nitrate concentration in the digested effluent relative to the control.

10. Higher pretreatment temperature had a significant positive effect (464.0 +/- 159.7mg acetate/L) on the total VFA concentration in the pretreatment effluent ($F(1,24)=24.717$, $p<0.10$) which agreed with the ORP levels for these systems.

11. Higher pretreatment temperature had a significant positive effect (271.6 +/- 110.2mg $CaCO_3$/L) on the alkalinity in the pretreatment effluent ($F(1,22)=17.917$, $p<0.10$) which was related to the ammonia release in those reactors.

12. Feed shearing had a significant positive effect (256.5 +/- 170.1 mg/L) on the soluble COD in the sheared feed relative to the not sheared feed ($F(1,16)=6.864$, $p<0.10$) which indicated solubilization as a result of feed shearing.

13. Higher pretreatment temperature was found to have a significant positive effect on the soluble COD relative to pretreatment at ambient temperature (684.4 +/- 70.4mg/L) in the pretreatment effluent ($F(1,32)=271.530$, $p<0.10$) which indicated that hydrolysis occurred more readily at higher temperature.

14. Higher pretreatment temperature had a significant positive effect (0.71 +/- 0.17) on the pH in the pretreatment effluent ($F(1,40)=47.452$, $p<0.05$) which was related to the ammonia release in those reactors.

15. Feed shearing had a significant positive effect (0.39 +/- 0.38) on the pH in the final effluent relative to final effluents from not sheared feeds $F(1,34)=3.115$, $p<0.10$). It was also found to have a negative effect over the control digester (–0.86 +/- 0.48). Pretreatment with not sheared feed had a significant negative effect (-1.26 +/- 0.48) on the pH in the digested effluent relative to the control.

16. Higher pretreatment temperature had a significant negative effect (-1.14 +/- 0.38) on the pH in the final effluent relative to final effluents pretreated at ambient temperature $F(1,34)=26.511$, $p<0.10$). It was also found to have a greater negative effect over the control digester (–1.63 +/- 0.48). Pretreatment at ambient temperature had a significant negative effect (-0.49 +/- 0.48) on the pH in the digested effluent relative to the control. pH tended drop in digestion more so in the high temperature pretreatment systems. This can be explained by nitrification activity, which would remove ammonia and cause pH depression.

17. Higher pretreatment temperature had a significant negative effect (-2.34 +/- 0.63 log CFU/g TS) on the fecal coliform concentration in the final effluent relative to final effluents pretreated at ambient temperature $F(1,32)=79.354$, $p<0.10$). It was also found to have a greater negative effect over the control digester (–3.06 +/- 0.67 log CFU/g TS). Pretreatment at ambient temperature had a significant negative effect (-0.72 +/- 0.49 log CFU/g TS) on the fecal coliform concentration

in the digested effluent relative to the control. Sub-optimal pH was hypothesized to account for differences in pathogen removal.

18. The inactivation of fecal coliforms in the control digester was 1.5 +/- 0.5 log reductions. In all cases, the mean reduction for pretreated digester effluents was greater than aerobic digestion alone. The difference in reduction between control and digester effluents was only significant for the digester effluents pretreated at high temperature up to 4.2 +/- 0.8 and 4.6 +/- 1.2 log reductions.

19. Higher pretreatment temperature had a significant negative effect (-0.92 +/- 0.40 log CFU/g TS) on the *E. coli* concentration in the final effluent relative to final effluents pretreated at ambient temperature $F(1,30)=15.397$, $p<0.10$). It was also found to have a greater negative effect over the control digester (–1.43 +/- 0.49 log CFU/g TS). Pretreatment at ambient temperature had a significant negative effect (-0.51 +/- 0.49 log CFU/g TS) on the *E. coli* concentration in the digested effluent relative to the control. Sub-optimal pH was hypothesized to account for differences in pathogen removal.

20. The inactivation of *E. coli* in the control digester was 1.6 +/- 0.8 log reductions. In all cases, the mean reduction for pretreated digester effluents was greater than aerobic digestion alone. The difference in reduction between control and digester effluents was only significant for the digester effluents pretreated at high temperature up to 3.1 +/- 0.7 and 3.1 +/- 0.7 log reductions. Higher temperature pretreatment was more effective than low temperature pretreatment and no pretreatment of aerobically digested sludge for *E.coli* removal.

21. Higher pretreatment temperature had a significant negative effect (-1.33 +/- 0.40 log CFU/g TS) on the *Salmonella* spp. concentration in the final effluent relative to final effluents pretreated at ambient temperature $F(1,26)=32.123$, $p<0.10$). It was also found to have a greater negative effect over the control digester (–1.28 +/- 0.50 log CFU/g TS). Sub-optimal pH was hypothesized to account for differences in pathogen removal.

22. A significant interaction between pretreatment temperature and feed shearing on the effect of *Salmonella* spp. concentration was found ($F(1,26)=20.128$, $p<0.10$).

23. The inactivation of *Salmonella* spp. in the control digester was 1.7 +/- 0.7 log reductions. In all cases except the ambient high shear pretreated system, the mean reduction for pretreated digester effluents was greater than aerobic digestion alone. The difference in reduction between control and digester effluents was only significant for the digester effluents pretreated at high temperature up to 4.6 +/- 0.5 and 2.2 +/- 0.7 log reductions.

24. The differences between feed and pretreated effluents for *Salmonella* spp. removal were up to 1.0 log reduction for the high temperature low shear condition. The high shear condition appeared to be more hospitable to *Salmonella* spp. where no significant reduction was observed after ambient microaerobic pretreatment and only 0.6 +/- 0.5 log reductions were observed at the 35°C.

25. The digester effluent from the high shear, high temperature system had significantly less *Salmonella* spp. than all other final effluents with an observed log reduction of 3.6 +/- 0.5 from the original feed. Shearing the feed mave have

compromised the *Salmonella* spp. in such a way to make it more susceptible to inactivation under the conditions in digestion. The impact of pretreatment became more pronounced further downstream as was found for the other bacteria measured. Higher temperature pretreatment was more effective than low temperature pretreatment of aerobically digested sludge for *Salmonella* spp. removal.

26. Higher pretreatment temperature had a significant negative effect (-1.31 +/- 0.42 log CFU/g TS) on the *Shigella* spp. concentration in the final effluent relative to final effluents pretreated at ambient temperature $F(1,13)=30.437$, $p<0.10$). It was also found to have a greater negative effect over the control digester (–1.71 +/- 0.56 log CFU/g TS). Sub-optimal pH was hypothesized to account for differences in pathogen removal.

27. The inactivation of *Shigella* spp. in the control digester was 1.8 +/- 0.8 log reductions (Table 6.15.1). In all cases, the mean reduction for pretreated digester effluents was greater than aerobic digestion alone (Figure 6.15.3). The difference in reduction between control and digester effluents was only significant for the digester effluents pretreated at high temperature up to 3.7 +/- 0.9 and 3.3 +/- 1.1 log reductions.

28. The differences between feed and pretreated effluents for *Shigella* spp. removal were up to 1.4 +/- 1.0 log reductions for the high temperature low shear condition. This difference was not significant for the high shear condition. The digester effluents from the high temperature high shear system saw the largest decline in *Shigella* spp. at 3.7 +/- 0.9, a behaviour similar to the *Salmonella* spp. trends. At

ambient temperatures, *Shigella* spp. were decreased in pretreatment by 1.0 +/- 0.9 and 0.7 +/- 0.6 for the high and low shear conditions, respectively.

7.4 Recommendations

In order to further validate this option, testing at pilot and full-scale should be conducted with the aim of equal to better pathogen removal rates as those found under the best conditions at lab-scale. A draw/fill approach should be used and mechanical mixing provided where necessary. The main operating variables that yielded the most valuable information in determining the state of the system were temperature, ORP, DO, pH, and ammonia. These variables can be conveniently monitored online with appropriate probes and tracked in a distributed control system. The establishment of target ORP, DO, and temperature ranges can be used to control air supply.

7.5 Technical Risks and Issues

There are several technical risks that may arise from full-scale operation of microaerobic pretreatment of aerobically digested sludge which can be mitigated by various design options and due diligence. For example, it is expected that at full scale foaming issues may occur as were observed at lab-scale. To mitigate this foam cutters and/or antifoam agents could be considered.

In terms of sludge quality, the stability of the system in delivering consistent quality sludge should be determined by long-term monitoring at full-scale prior to consideration of disposal options that require stringent quality criteria such as land application.

Although odour issues are not generally a major problem in aerobic digestion, the reducing conditions of microaerobic digestion can lead to the evolution of sulphides in which case open digesters should be avoided and an off-gas capture system including scrubbing may be required.

Finally, the pH in digestion after pretreatment was found to drop as low as pH 3.5, at this low pH certain heavy metals come out of solution. Testing for these compounds should be performed on digested sludge and considered in determining final disposal alternatives.

7.6 Future Study

In light of the hypotheses stated regarding the mechanisms that promoted enhanced pathogen removal, several opportunities for future study present themselves. Namely, the investigation of the impact on digester effluent of enhanced hydrolysis by other means such as enhanced shearing, or higher solids loading could be investigate. The shear rates applied in this study were insufficient to generate significantly higher ammonia levels. However, more aggressive means could improve this outcome. Low temperature options that promote and confirm this mechanism would offer more economical means of achieving pathogen removal.

Finally, higher volume-to-surface-area ratios at larger scale are expected to improve the exothermic heat retention of the system. Therefore pilot and full-scale testing of microaerobic pretreatment may promote autothermal activity, which would also defer energy requirements for mesophilic microaerobic pretreatment.

8. References

Anderson, G.K., and Yang, G., (1992). Determination of bicarbonate and total volatile acid concentration in anaerobic digesters using a simple titration. *Water Environment Research*, vol. 64., no.1, pp. 53-59.

APHA (1998). Standard Methods for the Examination of Water and Wastewater, 20th edition. *American Public Health Association.*

Arunachalam, R.S., Shah, H.K., and Ju, L-K., (2004). Aerobic sludge digestion low dissolved oxygen concentration. *Water Environment Research,* vol.76, no.5, pp.453-462.

Bujoczek, G., R.S. Reimers, J. Oleszkiewicz., (2001). Abiotic factors affecting inactivation of pathogens in sludge. *Water Science Technology*, vol.44, no.10, pp. 79-84.

Carrington, E.G., Davis, R.D., Hall, J.E., Pike, E.B., Smith, S.R. and Unwin, R.J., (1998). Review of the scientific evidence relating to the controls on the agricultural use of sewage sludge. Report DETR 4415/3 [part1] and Report DETR 4454/4 [part 2].

Chu, A., and Mavinic, D.S., (1998). The effects of macromolecular substrates and a metabolic inhibitor on volatile fatty acid metabolism in thermophilic aerobic digestion. *Water Science and Technology,* vol.38 no.2 pp-55-61.

Chu, A., Mavinic, D.S., Kelly, H.G., and Ramey, W.D., (1994). Volatile fatty acid production in thermophilic aerobic digestion of sludge. *Water Resources*, vol.28, no.7, pp.1513-1522.

Dumontet, S., Dinel, H. and S.B. Baloda., (1999). Pathogen reduction in sewage sludge by composting and other biological treatments: A review. *Biological Agriculture and Horticulture*, vol.16, pp. 409-430.

EC (European Communities) (2001). Evaluation of Sludge Treatments for pathogen reduction. *Office of the Official Publications of the European Community.*

Farrell, J.B., Erlap, A.E., Rickabaugh, J., Freedman, D., and Hayes, S., (1988). Influence of feeding procedure on microbial reductions and performance of anaerobic digestion. *Journal of the Water Pollution Control Federation,* vol.60, no.5, pp.635-644.

Feacham, R.G., Bradley, D.J., Garelick, H. and Mara, D.D., (1983). Health aspects of excreta and wastewater management. *John Wiley and Sons.*

Fukushi, K., Babel, S., and Burakrai, S., (2002). Survival of *Salmonella* spp. in a simulated acid-phase anaerobic digester treating sewage sludge. *Bioresource Technology*, vol.86, pp. 53-57.

Horan, N.J., Fletcher, L., Betmal, S.M., Wilks, S.A., and Keevil, C.W., (2004). Die-off of enteric bacterial pathogens during mesophilic anaerobic digestion. *Water Research*, vol.38, pp. 113-1120.

Jones, P., and Martin, M. (2003). A review of the literature on the occurrence and survival of pathogens of animals and human in green compost. *The Waste and Resources Action Program.*

Jones, P. W. 1976. The effect of temperature, solids content and pH on the survival of salmonellas in cattle slurry. *British Veterinary Journal,* vol.132, pp. 284-293.

Kelly, H.G., Melcer, H., and Mavinic, D.S., (1993). Autothermal thermophilic aerobic digestion of municipal sludges: a one-year, full-scale demonstration project. *Water Environment Research,* vol.65, no.7, pp.849-861.

Hasegawa, S., Shiota, N., Katsura, K., and Akashi, A., (2000). Solubilizatlion of organic sludge by thermophilic aerobic bacteria as a pretreatment for anaerobic digestion. *Water Science and Technology*, vol.41, no.3, pp. 163-169.

Lee, K.M., Brunner, C.A., Brunner, Farrell, J.B., and Erlap, A.E., (1989). Destruction of enteric bacteria and viruses during two-phase digestion. *Journal of the Water Pollution Control Federation,* vol.61, no.8, pp. 1421-1428.

Mavinic, D.S., Mahendraker, V., Sharma, A., and Kelly, H.G., (2001) Effect of Microaerophilic conditions on autothermal thermophilic aerobic digestion process. *Journal of Environmental Engineering*, vol.127, no.4, pp. 311-316.

McIntosh, K.B., and Oleszkiewicz, J.A., (1997). Volatile fatty acid production in aerobic thermophilic pre-treatment of primary sludge. *Water Science and Technology*, vol.36, no.11, pp. 189-196.

Mendenhall, W., Beaver, R.J., Beaver, B.M., (1999). Introduction to Probability and Statistics, Tenth Edition. *Brooks/Cole Publishing Company.*

Metcalf & Eddy, Tchobanoglous, G., Burton, F.L., Burton, F., Stensel H.D, (2002) *Wastewater Engineering: Treatment and Reuse,* McGraw-Hill.

Obeta Ugwuanti, J., Harvey, L.M., and McNeil, B., (2004). Effect of aeration rate and waste load on evolution of volatile fatty acids and waste stabilization during thermophilic aerobic digestion of a model high strength agricultural waste. *Bioresource Technology.*

Pagilla, K.R., Craney, K.C., and Kido, W.H., (1996). Aerobic thermophilic pretreatment of mixed sludge for pathogen reduction and Norcadia control. *Water Environment Research*, vol. 68, no.7, pp. 1093-1098.

Parmar, N., Singh, A., and Ward, O.P., (2001). Characterization of the combined effects of enzyme, pH, and temperature treatments for removal of pathogens from sewage sludge. *World Journal of Microbiology & Biotechnology*, vol.17. pp 169-172.

Ponugoti, P. R., Dahab, M.F., and Surampalli, R., (1997). Effects of different biosolids treatment systems on pathogen and pathogen indicator reduction, *Water Environment Research*, vol.69 no.7, pp. 1195-1206.

Sahlstrom, L., Aspan, A., Bagge, E., Danielsson-Tham, M-L., and Albihn, A., (2004). Bacterial pathogen incidences in sludge from Swedish sewage treatment plants. *Water Research*, vol.38, pp. 1989-1994.

Salsali, H.R., Parker, W.J, and Sattar, S.A., (2005). Impact of concentration, temperature, and pH on inactivation of *Salmonella* spp. by volatile fatty acids in anaerobic digestion. *Canadian Journal of Microbiology*, vol.52, pp. 279-286.

Schafer, P.L., and Farrell, J.B., (2000). Turn up the heat. *Water Environment Technology*, vol.12, pp. 27-35.

Singh, L., Sai Ram, M., Alam, S.I., and Maurya, M.S., (1994). *Inactivation of pathogens during aerobic and anaerobic treatments at low temperatures. Bulletin of Environmental Contaminant Toxicology*, vol.54, pp. 472-478.

Strauch, D., (1991). Microbiological treatment of municipal sewage waste and refuse as a means of disinfection prior to recycling in agriculture. *Studies in Environmental Science*, vol. 42, pp. 121-136.

Strauch, D., (1998). Pathogenic micro-organisms in sludge. Anaerobic digestion and disinfection methods to make sludge usable as a fertiliser. *European water Management*, vol. 1 pp.212-26.

Surampalli, R.Y., Banerji, S.K., and Chen, J.C., (1994). Micobiological stability of wastewater sludges from activated sludge systems. *Bioresource Technology*, vol.49, pp. 203-207.

US EPA, (1999). Control of pathogens and vector attraction in sewage sludge. *Environmental regulations and technology.* Report EPA/625/R-92/013.

Ward, A., Stensel, H.D., Ferguson, J.F., Ma, G., and Hummel, S., (1998). Effect of autothermal treatment on anaerobic digestion in dual digestion process. *Water Science and Technology*, vol.38, no.8-9, pp. 435-442.

Ward, A., Stensel, H.D., Ferguson, J.F., Ma, G., and Hummel, S., (1999) Preventing growth of pathogens in pasteurized digester solids. *Water Environment Research*, vol.71, no.2.

Zabranska, J., Dohanyos, H., Jenicek, P., Ruzickova, H., and Ranova, A., (2003). Efficiency of autothermal thermophilic aerobic digestion and thermophilic anaerobic digestion of municipal wastewater sludge in removing *Salmonella* spp. and indicator bacteria. *Water Science and Technology*, vol.47, no.3, pp. 151-156.

Zhou, J., and Mavinic, D.S., (2003). Pollution reduction at wastewater treatment facilities through thermophilic sludge digestion. *Water Science and Technology*, vol.48, no.3, pp. 57-63.