PRODUCING ENHANCED QUALITY BIOSOLIDS THROUGH ANAEROBIC DIGESTER OPTIMIZATION

By

Hamidreza Salsali

A thesis submitted to the
Faculty of Graduate Studies and Research
In partial fulfillment of the requirements for the degree of

Doctor of Philosophy

In
Environmental Engineering

Carleton University, Ottawa, Canada
April 2006

The Doctor of Philosophy in Environmental Engineering is a joint program with the University of Ottawa, administered by the Ottawa-Carleton Institute for Environmental Engineering.

© Copyright, Hamidreza Salsali, 2006

Library and
Archives Canada

Bibliothèque et
Archives Canada

Published Heritage
Branch

Direction du
Patrimoine de l'édition

395 Wellington Street
Ottawa ON K1A 0N4
Canada

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence

ISBN: 978-0-494-16675-8

Our file Notre référence

ISBN: 978-0-494-16675-8

NOTICE:

The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

AVIS:

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protègent cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

Canada

ABSTRACT

The primary objective of this research was the development of a better understanding of the operation of anaerobic digesters in single and multi stage configurations, to improve process performance and pathogen destruction rate.

In each study phase, tested anaerobic digestion system was compared to single stage mesophilic digestion at the same HRT. During single stage temperature-HRT study the effect of elevated operating temperatures, and HRT on pathogen reduction was evaluated. In addition, an evaluation of pathogen reduction was assessed in digesters that were operated in a series configuration. The fate of indicator bacteria and bacterial pathogens was investigated in this study.

Results of the single stage study demonstrated that with the exception of *Clostridium perfringens* the concentrations of the microbial species in the digester effluents decreased as the temperature of operation increased. Operation at 55°C and 62°C resulted in essentially non-detectable concentrations of fecal coliforms, *E. coli* and *Salmonella* spp.. Operation at 42°C and 49°C resulted in incremental decreases in effluent concentrations as compared to operation at 35°C. With the exception of operation at 62°C, the effluent concentrations of *Clostridium perfringens* were observed to increase relative to the feed concentrations. The results suggest that *Clostridium perfringens* is not a useful indicator organism for the fate of pathogens in anaerobic digestion.

Staged operation of the digesters resulted in substantial improvements in pathogen destruction as compared to single stage digestion for all of the indicators. With the exception of *E. coli* all of the indicators were reduced to a greater extent in three stage

digestion as compared to two stage digestion. With the exception of *Salmonella* spp. all of the indicators were removed to a greater extent when the first stage of the three stage system was operated at elevated temperature as compared to the three stage system that was operated at 35°C. This study revealed that fecal streptococci and *Clostridium perfringens* were more resistant to inactivation in the digesters as compared to Fecal coliform, *E. coli*. and *Salmonella* spp.

In addition, Denaturing Gradient Gel Electrophoresis (DGGE) testing of the digester effluents was performed to evaluate the impact of staging and temperature on the diversity of the microbial populations. DGGE testing revealed that staged operation resulted in a more diverse bacterial population that that observed in single stage digesters that were operated under conventional conditions. The results of this study indicate that DGGE analysis can be a suitable and sensitive tool to support established engineering tools in the investigation of biological treatment processes.

ACKNOWLEDGMENTS

I would like to express my deepest gratitude and thanks to my supervisor, Professor Wayne J. Parker for giving me the opportunity to work in the wonderland of Anaerobic Digestion. His intense understanding of science and broad knowledge in the field of Anaerobic Digestion has been a valuable source of inspiration and motivation for my study. Without his encouraging supervision, continuous support, constructive discussions, and their creation of a free stimulating academic atmosphere, I would never have succeeded in accomplishing this work. His care and support in my daily life will always be remembered fondly.

I am also greatly indebted to the technical assistance of Dr. Marie Tudoret Chow and my sincere thanks to Ken McMartin, Pierre Trudel, and Jim Whitehorne for creating the wonderful, enjoyable atmosphere in everyday work at the Civil and Environmental Engineering Laboratories and for their kindest help and friendship.

I wish also, to thank the reviewers appointed by the Department of Civil and Environmental Engineering, for their constructive review of the manuscript.

My warmest thanks to my parents for their unlimited love, care and support. I also grateful to my wife's parents, my brothers and sisters, for their encouragement and help in many practical ways.

Last but no least, my deepest and most loving thanks go to my wife Mrs. Hanan Salsali for her support and love. She is also one of my best mentors who introduced me to the field of Microbiology and taught me much about science in practice. I can say she is the fountain of what I am today. I also grateful to my cute little sons Ali, and Amir for bring me the happiness and challenge of being a father.

TABLE OF CONTENTS

ABSTRACT	ii
ACKNOWLEDGMENTS	iv
TABLE OF CONTENTS	v
LIST OF TABLES	ix
LIST OF FIGURES	x
LIST OF ABBREVIATIONS	xiii
LIST OF APPENDICES	xiv
 CHAPTER 1 INTRODUCTION AND ABJECTIVES	 1
 CHAPTER 2 LITERATURE REVIEW	 4
2.1 Indicator and Pathogenic Organisms Found in Municipal Wastewater	4
2.1.1 Pathogenic Bacteria	5
2.1.2 Indicator Organisms	6
2.2 Parameters Affecting the Killing or Inactivation of Pathogens	8
2.2.1 Temperature	8
2.2.2 Moisture	12
2.2.3 pH	13
2.2.4 Digester Hydraulics and Mixing	13
2.2.5 Free Ammonia and Non-ionized Volatile Fatty Acids	14
2.2.6 Feeding protocol	17
2.3 Pathogen Reduction in Sludge Treatment Systems	19
2.3.1 Anaerobic Digestion	19
2.3.1.1 Solids Retention Time	21
2.3.1.2 Temperature	22
2.3.1.3 Fate of Pathogens	23
2.3.2 Two-Phase Anaerobic Digestion Process	28
2.3.2.1 Fate of Pathogens	30

2.3.3	Temperature-Phased Anaerobic Digestion	33
2.3.3.1	Advantages of temperature-phased anaerobic digestion systems	34
2.3.3.2	Fate of Pathogens	36
2.3.4	Three Phase Digestion	39
2.4	Detection and Enumeration Techniques of Bacterial Pathogen in Sludge	41
2.4.1	Conventional Technique	41
2.4.2	Molecular Technique	44
2.5	Summary	48
CHAPTER 3 EXPERIMENTAL MATERIALS AND MAETHODS		50
3.1	Source of Sludge	50
3.2	Digester Design	50
3.3	Start up Procedure and Operation	51
3.4	Experimental Plan	52
3.4.1	Single Stage Temperature-HRT studies	52
3.4.2	Staging of Digesters	53
3.5	Sampling Plan	55
3.6	Physical and Chemical Analyses	57
3.6.1	Temperature	57
3.6.2	pH	57
3.6.3	Total and Volatile Solids	57
3.6.4	Total and Volatile Suspended Solids	57
3.6.5	Alkalinity and Volatile Acids	58
3.6.6	Individual Organic Acids	58
3.6.7	Chemical Oxygen Demand	58
3.6.8	Gas Production and Biogas Composition (CH ₄ , CO ₂)	59
3.6.9	Ammonia Nitrogen (NH ₃ -N)	59
3.6.10	Dewaterability	60
3.7	Bacteriological Analysis	60
3.7.1	Collection of Samples	61
3.7.2	Glassware and Culture Media Preparation	61
3.7.3	Sample Dilutions	62
3.7.4	Method Detection Limit	62
3.8	Analytical Procedures	63
3.8.1	Fecal Coliform	63
3.8.2	<i>E.coli</i>	63
3.8.3	Fecal Streptococci	64
3.8.4	<i>Salmonella</i> spp.	64
3.8.5	<i>C. perfringens</i>	64
3.9	Quality Controls	64
3.10	Fatty Acid Disinfection Study	65

3.11	Microbial Community Study	67
3.11.1	Temperature Configuration	68
3.11.2	DNA Extraction	68
2.11.3	Polymerase Chain Reaction (PCR) Amplification of DNA	69
3.11.4	DGGE	69
3.11.5	Image Analyses	70
CHAPTER 4 PERFORMANCE EVALUATION OF SINGLE AND STAGED ANAEROBIC DIGESTION OPERATION		72
4.1	Feed Characteristics	72
4.2	Performance Evaluation of Single, Two and Three Stage Anaerobic Digestion Process	74
4.2.1	Digester Solid Analysis	76
4.2.1.1	Single Stage Temperature- HRT Studies	76
4.2.1.2	Effect of Staging on Solids Reduction	81
4.2.2	Digesters Volatile Fatty Acid Analysis	92
4.2.2.1	Effect of Single Stage Temperature-HRT Studies on VFA Concentration	93
4.2.2.2	Effect of Temperature Staging on VFA Concentration	99
4.2.3	Ammonia Analysis	107
4.2.3.1	Single Stage Temperature-HRT Studies	108
4.2.3.2	Effect of Staging on Ammonia Concentration	110
4.2.4	Alkalinity Analysis	113
4.2.4.1	Single Stage Temperature-HRT Studies	113
4.2.4.2	Effect of Staging on Alkalinity Concentrations	115
4.2.5	pH Analysis	119
4.2.5.1	Single Stage Temperature-HRT Study	119
4.2.5.2	Effect of Temperature Staging on pH Values	120
4.2.6	Methane Yield Analysis	122
4.2.6.1	Single Stage Temperature-HRT Studies	122
4.2.6.2	Effect of Temperature Staging on Methane Yield	125
4.2.7	Dewaterability Analysis	128
4.2.7.1	Single Stage Temperature-HRT Studies	129
4.2.7.2	Effect of Staging on Dewaterability	130
4.2.8	COD Analysis	133
4.2.8.1	Effect of Single Stage Temperature-HRT Studies on COD Concentration	134
4.2.8.2	Effect of Staging on COD	136
4.3	Evaluation of Single, Two and Three Stage Anaerobic Digestion for Pathogen Reduction.	142
4.3.1	Fecal Coliform Analysis	143
4.3.1.1	Effect of Single Stage Temperature and HRT on FC Reduction	143
4.3.1.2	Effect of the Temperature Staging on FC Destruction	147

4.3.2	<i>E.coli</i> Analysis	154
4.3.2.1	Effect of Temperature and HRT on <i>E.coli</i> Inactivation	154
4.3.2.2	Effect of Temperature Staging on <i>E.coli</i> Destruction	158
4.3.3	<i>Salmonella</i> spp. Analysis	163
4.3.3.1	Effect of Single Stage Temperature and HRT on <i>Salmonella</i> spp.	164
4.3.3.2	Effect of Temperature Staging on <i>Salmonella</i> spp. Inactivation	167
4.3.4	Fecal Streptococci Analysis	172
4.3.4.1	Effect of Temperature and HRT on FS Inactivation	172
4.3.4.2	Effect of the Temperature Staging on FS Inactivation	175
4.3.5	<i>Clostridium perfringens</i> Analysis	181
4.3.5.1	Effect of Single Stage Temperature and HRT on <i>Clostridium perfringens</i>	181
4.3.5.2	Effect of Temperature Staging on <i>Clostridium Perfringens</i> Inactivation	183
CHAPTER 5 IMPACT OF VOLATILE FATTY ACIDS ON THE INACTIVATION OF <i>SALMONELLA</i> AND <i>C.PERFRINGENS</i>		189
5.1	Characteristics of digester effluent	190
5.2	Inactivation of <i>Salmonella</i> spp. by Volatile Fatty Acids: Effects of Concentration, Temperature and pH	191
5.3	Inactivation of <i>Clostridium perfringens</i> by Volatile Fatty Acids Mixture: Effects of Concentration, Temperature and pH	210
CHAPTER 6 MICROBIAL DIVERSITY OF STAGED OPERATION OF ANAEROBIC DIGESTION OF MUNICIPAL SEWAGE SLUDGE		218
6.1	Microbial Diversity In Two Stage Study	219
6.2	Microbial Diversity In Three Stage Study	223
CHAPTER 7 CONCLUSIONS AND ENGINEERING SIGNIFICANCE		228
7.1	Conclusions	228
7.1.1	Single Stage Temperature-HRT studies	228
7.1.2	Staging of Digesters	229
7.1.3	Impact of Volatile Fatty Acids on the Inactivation of <i>Salmonella</i> spp. and <i>Clostridium perfringens</i>	230
7.1.4	Microbial Diversity of Staged Operation of Anaerobic Digestion of Municipal Sewage Sludge	231
7.2	Engineering Significance	231
CHAPTER 8 ASPECTS FOR FUTURE RESEARCH		232
CHAPTER 9 REFERECES		233

LIST OF TABLES

Table 2-1: Major Pathogens in Municipal Wastewater and Sewage Sludge	5
Table 2-2: Environmental and Operating Conditions During Anaerobic Digestion of Municipal Sludges	21
Table 2-3: Purposes of Each of the Digesters in Three Stage Digestion	39
Table 3-1: Frequency of Sampling of Analytical Parameters	56
Table 3-2: Method Detection Limit for Biological Parameters	63
Table 3-3: Control Microorganisms for Microbiological Tests	65
Table 3-4: Temperature Profiles of Digestion Systems	68
Table 4-1: Feed Sludge Characteristics	73
Table 4-2: Summary of Operating Phases of Single Stage Temperature-HRT Studies	74
Table 4-3: Summary of Operating Phases of Multistage Studies	76
Table 4-4: Average Total Solids (TS) and Total Suspended Solids (TSS) Concentration (mg/L)	76
Table 4-5: Average First Order Rate Constant (d^{-1})	80
Table 4-6: Average Steady State TS and TSS Removal Efficiency	82
Table 4-7: Calculated First Order Reaction Rate Coefficients (d^{-1})	85
Table 4-8: Average Steady State TS and TSS Removal Efficiency	87
Table 4-9: Calculated First Order Reaction Rate Coefficients ¹ (d^{-1})	91
Table 5-1: Characterization of Feed Sludge and Digester Effluents	191
Table 5-2: Average Decay Coefficients (d^{-1})	203
Table 5-3: Characteristics of Short-Chain VFA's	207
Table 5-4: Concentration of the Nonionized Form IVFA's at pH=5.5	208
Table 5-5: Average Decay Coefficients for <i>C. perfringens</i> (d^{-1})	214
Table 6-1: Similarity Coefficients (C_s) in Two Stage Study	222
Table 6-2: Similarity Coefficient (C_s) From Three Stage Study	227

LIST OF FIGURES

Figure 2-1: Time-Temperature Requirements to Produce Sludge Which is Virtually Pathogen-Free	9
Figure 3-1: Schematic Drawing of Lab Digesters	51
Figure 3-2: Reactor Configurations and Temperatures	54
Figure 4-1: Impact of HRT and Temperature on Solids Removal	78
Figure 4-2: Solids Removal Versus Two Stage Temperature Configuration	83
Figure 4-3: Solids Removal Versus Two Stage Temperature Configuration	88
Figure 4-4: Impact of HRT and Temperature on TVFA Concentration	94
Figure 4-5: Individual Fatty Acid Distribution Versus Temperature and HRT	98
Figure 4-6: TVFA Concentration Versus Two Stage Temperature Configuration	100
Figure 4-7: Individual Fatty Acid Distribution Versus Two Stage Temperature Configuration	102
Figure 4-8: TVFA Concentration Versus Three Stage Temperature Configuration	104
Figure 4-9: Individual Fatty Acid Distribution Versus Three Stage Temperature Configuration	106
Figure 4-10: Impact of HRT and Temperature on Ammonium	108
Figure 4-11: Ammonium Concentration Versus Two Stage Temperature Configuration	110
Figure 4-12: Ammonium Concentration Versus Three Stage Temperature Configuration	112
Figure 4-13: Impact of HRT and Temperature on Alkalinity	114
Figure 4-14: Alkalinity Concentrations Versus Two Stage Temperature Configuration	116
Figure 4-15: Alkalinity Concentrations Versus Three Stage Temperature Configuration	118
Figure 4-16: Impact of HRT and Temperature on pH Value	119
Figure 4-17: pH Values Versus Two Stage Temperature Configuration	120
Figure 4-18: pH Values Versus Three Stage Temperature Configuration	121
Figure 4-19: Methane Yield Versus HRT and Temperature	123
Figure 4-20: Methane Yield Versus Two Stage Temperature Configuration	126
Figure 4-21: Methane Yield Versus Three Stage Temperature Configuration	127
Figure 4-22: Dewaterability Versus HRT and Temperature	129
Figure 4-23: Dewaterability Versus Two Stage Temperature Configuration	131
Figure 4-24: Dewaterability Versus Three Stage Temperature Configuration	132
Figure 4-25: COD Versus HRT and Temperature	135
Figure 4-26: COD Versus Two Stage Temperature Configuration	137
Figure 4-27: COD Versus Three Stage Temperature Configurations	140
Figure 4-28: Effect of Temperature and HRT on (a) FC Densities, and (b) FC log Reduction in Digesters	144

Figure 4-29: FC Decay Rate Constant (d^{-1})	146
Figure 4-30: FC Destruction Versus Two Stage Temperature Configuration	148
Figure 4-31: FC Decay Rate Constant Versus Two Stage Temperature Configurations	149
Figure 4-32: FC in Three Stage Digester (a) Log Reductions in Digesters (b) Log Reductions Versus Temperature	151
Figure 4-33: FC Decay Rate Constant Versus Three Stage Temperature Configurations	153
Figure 4-34: Effect of Temperature and HRT on (a) <i>E.coli</i> Densities, and (b) <i>E.coli</i> Log Reduction in Digesters	155
Figure 4-35: <i>E.coli</i> Decay Rate Constant Versus Temperature and HRT	157
Figure 4-36: <i>E. coli</i> Log Reductions Versus Two Stage Temperature Configuration	158
Figure 4-37: <i>E.coli</i> Decay Rate Constant Versus Two Stage Temperature Configuration	159
Figure 4-38: <i>E. coli</i> in Three Stage Digester (a) Log Concentrations (b) Log Reductions Versus Three Stage Temperature Configuration	161
Figure 4-39: <i>E.coli</i> Decay Rate Constant Versus Three Stage Temperature Configuration	162
Figure 4-40: Effect of Temperature and HRT on (a) <i>Salmonella</i> Densities, and (b) <i>Salmonella</i> Log Reduction in Digesters	165
Figure 4-41: <i>Salmonella</i> Decay Rate Constant Versus Temperature and HRT	166
Figure 4-42: <i>Salmonella</i> Log Reductions Versus Two Stage Temperature Configuration	167
Figure 4-43: <i>Salmonella</i> Decay Rate Constant Versus Two Stage Temperature Configuration	168
Figure 4-44: <i>Salmonella</i> in Three Stage Digester (a) Log Reductions in digesters (b) Log Reductions Versus Temperature	170
Figure 4-45: <i>Salmonella</i> Decay Rate Constant Versus Three Stage Temperature Configuration	172
Figure 4-46: Effect of Temperature and HRT on (a) FS Densities, and (b) FS Log Reduction in Digesters	173
Figure 4-47: FS Decay Rate Constant Versus Temperature and HRT	175
Figure 4-48: FS Log Reductions Versus Two Stage Temperature Configuration	176
Figure 4-49: FS Decay Rate Constant Versus Two Stage Temperature Configuration	177
Figure 4-50: FS in the Three Stage Digester (a) Log Reductions in Digesters (b) Log Reductions Versus Temperature	178
Figure 4-51: FS Decay Rate Constant Versus Two Stage Temperature Configuration	180
Figure 4-52: <i>Clostridium perfringens</i> Concentrations Versus HRT and Temperature	181

Figure 4-53: <i>Clostridium perfringens</i> Decay Rate Constant Versus Temperature and HRT	182
Figure 4-54: <i>Clostridium perfringens</i> Removal Versus Two Stage Temperature Configuration	184
Figure 4-55: <i>Clostridium perfringens</i> Decay Rate Constant Versus Two Stage Temperature Configuration	185
Figure 4-56: <i>Clostridium perfringens</i> Removal Versus Three Stage Temperature Configuration	186
Figure 4-57: <i>Clostridium perfringens</i> Decay Rate Constant Versus Three Stage Temperature Configuration	188
Figure 5-1: Average Reduction of <i>Salmonella</i> in Different Concentration of Acids at 35°C	193
Figure 5-2: Average Reduction of <i>Salmonella</i> in Different Concentration of Acids at 42°C	196
Figure 5-3: Average Reduction of <i>Salmonella</i> in Different Concentration of Acids at 49°C	198
Figure 5-4: <i>Salmonella</i> Removal Efficiencies	199
Figure 5-5: Observed and Model Predicted Fraction Remaining Versus Time	201
Figure 5-6: Impact of pH and Temperature on <i>Salmonella</i> Concentrations	205
Figure 5-7: Impact of varying pH on Noionized Concentration of VFA's	208
Figure 5-8: <i>Clostridium perfringens</i> Concentrations at (a) 35°C, (b) 42°C, (c) 42°C and (d) 55°C Versus Acid Concentration	212
Figure 5-9: Observed and Model Predicted Fraction Remaining Versus Time	213
Figure 5-10: Ratio of Final to Initial Concentrations of <i>Clostridium perfringens</i>	215
Figure 6-1: DGGE Images from Two Stage Study	221
Figure 6-2: DGGE Images from Three Stage Study	224

LIST OF ABBREVIATIONS

2PAD	Two-Phase Anaerobic Digestion
CFU	Colony Forming Units
COD	Chemical Oxygen Demand (mg/L)
CST	Capillary Suction Time (s)
CSTR	Completely Stirred Tank Reactor
DGGE	Denaturing Gradient Gel Electrophoresis
DNA	Deoxyribonucleic Acid
HRT	Hydraulic Retention Time (day)
PCR	Polymerase Chain Reaction
SRT	Solids Retention Time (day)
STD	Standard Deviation
TPAD	Temperature-Phase Anaerobic Digestion
TS	Total Solids (mg/L)
TSS	Total Suspended Solids (mg/L)
TVFA	Total Volatile Fatty Acids (mg/L)
V	Volume (m ³)
VFA	Volatile Fatty Acids (g COD/L)
VS	Volatile Solids (mg/L)
VSS	Volatile Suspended Solids (mg/L)
<i>E.coli</i>	<i>Escherichia Coli</i>
TC	Total Coliforms
FC	Fecal Coliforms
FS	Fecal Streptococci

LIST OF APPENDICES

Appendix A: Raw Data for Single Stage Temperature-HRT Study	243
Appendix B: Raw Data for Staging of Digesters	261
Appendix C: Raw Data for Volatile Fatty Acids Study	281
Appendix D: Results of Statistical Analysis	297
Appendix E: Others	306

CHAPTER 1

INTRODUCTION AND OBJECTIVES

Sludge treatment and disposal is one of the most important and challenging steps in municipal wastewater treatment systems. The U.S. Environmental Protection Agency (EPA) has reported that approximately 6.9 million tons of biosolids were generated in the United States in 1999 and generation is expected to double by the year 2010 (U.S. EPA, 1999). About 60 percent was beneficially used and 40 percent disposed of in landfills or by incineration. Disposal options however, are often limited. For example ocean disposal has been eliminated. Cost and air pollution considerations have curtailed the use of incineration, and transportation costs and the lack of available sites have lessened the popularity of land filling (Ponugoti *et al.*, 1997). Beneficial use of biosolids typically involves some type of land application, composting, or use as landfill cover.

Land application is based on the belief that biosolids should be considered a resource rather than a waste (Ponugoti *et al.*, 1997). However, land application includes a potential risk to public health from heavy metals or toxic organics and from pathogens that may be present in the biosolids (Bitton, 1994). In many communities heavy metals and toxic compounds in biosolids may be reduced through source control (Bitton, 1994). However, biosolids originating from wastewater treatment plants contain many human pathogens, from infected individuals which can not be controlled at the source and present health hazards to the general public (Sidhu *et al.*, 2001). In order to protect public health, the U.S. EPA enacted 40 CFR part 503 which governs land application of biosolids. This regulation protects public health by regulating biosolids pathogen density

and requiring vector attraction reduction before land application. The U.S.EPA part 503 regulations classify biosolids in two categories Class A and Class B, based on pathogen density and land application restrictions. Class B sludge has a Fecal Coliform (FC) density less than 2×10^6 MPN/g TS, and land application is restricted. Class A sludge criteria, as specified in U.S.EPA part 503 regulation, are as follows: FC limit of less than 1000 MPN/g TS or less than three *Salmonella* per 4 g, less than one virus per 4 g, and less than one viable helminth ovum per 4 g dry weight of biosolids (U.S. EPA, 1999).

Class A levels allow unrestricted use of biosolids. The evaluation of processing options typically begins with a screening of possible alternatives based on ability to meet class A pathogen criteria, reliability, proven technology, cost, and familiarity (Krugel *et al.* 1998).

Several technologies can be used to reduce pathogens. These have been categorized as processes to significantly reduce pathogens (PSRP) and processes to future reduce pathogens (PFRP) (McFarland, 2000).

The disadvantages associated with some of these new technologies include the requirement for significant capital investment in new equipment, increased energy costs or decreased energy availability and in some cases reduced process stability. Although there is significant public pressure, there is no clear scientific evidence that it is necessary to produce biosolids of this quality for land application purposes. Hence, it is difficult to justify the substantial costs of these new technologies when the need to produce pathogen-free biosolids for direct agricultural land application has not been scientifically proven.

An alternative approach is to examine whether there are modifications to the operating practices of existing digesters, which would result in the production of a much-improved quality of biosolids without incurring significant costs associated with the new generation of digestion processes. This would ideally optimize the operation of the digesters thereby making use of the substantial existing infrastructure associated with tankage and in some cases co-generation facilities. An improved quality of biosolids with respect to pathogen content would result in reduced risks of exposure associated with land application. This study addresses an evaluation of modifications in operating strategies and operational configuration to examine the potential for producing a higher quality of biosolids with relatively little capital expenditure and in an energy efficient manner.

The overall objective of this research was to examine operational strategies that might be employed to improve pathogen destruction rate through anaerobic digester optimization. Keeping the above consideration in mind, the following specific objectives were formulated in the design of this study:

1. Conduct bench scale experimentation to evaluate the effect of elevated operating temperatures and HRT on pathogen destruction.
2. Evaluate pathogen reduction in digesters that are in a series configuration.
3. Perform modeling of pathogen fate.

The aim was to obtain a better understanding of the operation of anaerobic digesters in single and multi stage configurations, to improve process performance and pathogen destruction rate.

CHAPTER 2

LITERATURE REVIEW

2.1 Indicator and Pathogenic Organisms Found in Municipal Wastewater Sewage Sludge

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

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

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

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

National Research Council, 1996

2.1.1 Pathogenic Bacteria

The pathogenic bacteria in wastewater and wastewater sludge have been classified into two categories based on their significance to human health: (1) of major concern, and (2) of minor concern (Kowal, 1983): The major concern category included *Salmonella sp.*, *Shigella sp.*, *Vibrio cholerae*, *E. coli* (pathogenic strains), *Leptospira sp.*, and *Yersinia enterocolitica*. All of these bacteria may have symptomless infections and human carrier states, and many have important nonhuman reservoirs as well. The minor concern organisms included *Aeromonas sp.*, *Clostridium perfringens*, and *Pseudomonas aeruginosa*. The significance for human health of *Salmonella*, and *Clostridium*

perfringens as two of the most common and widely distributed food borne pathogen, is briefly considered.

Salmonella

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

Clostridium perfringens

Clostridium perfringens is an anaerobic, gram-positive, sporeforming, and heat-resistant bacteria. It is widely distributed in the environment and frequently occurs in the intestines of humans and many domestic and feral animals. Spores of the organism persist in soil, sediments, and areas subject to human or animal fecal pollution.

2.1.2 Indicator Organisms

The analytical techniques for detection and enumeration of pathogenic bacteria (e.g., *Salmonella*), viruses, and cysts of protozoan parasites in wastewater and accompanying sludge are time consuming procedures, require well trained technicians, and usually are expensive. Therefore, other organisms have been used as indicators of pathogens. The best indicator organisms of the potential presence of pathogens are the facultative enteric organisms. Commonly used microbial indicators are indicated in the following sections.

Total Coliforms (TC)

The total coliform group includes the aerobic and facultative anaerobic, gram negative, non spore forming, rod-shaped bacteria that ferment lactose with gas production within 48h at 35°C (Bitton, 1994). This group includes *Escherichia Coli*, *Enterobacter*, *Klebsilla*, and *Citrobacter*. These coliforms are discharged in high numbers (2×10^9 coliforms/day/capita) in human and animal feces, but not all of them are of fecal origin.

Fecal Coliforms (FC)

FC are thermotolerant coliforms and include all coliforms that can ferment lactose with gas production within 48h at 44.5°C. The presence of FC indicates the presence of fecal material from warm blooded animals. However, human and animal sources of contamination cannot be differentiated. FC display a survival pattern similar to that of bacterial pathogens but their usefulness as indicators of protozoan or viral contamination is limited (Bitton, 1994). Farrah and Bitton, (1984) have shown that if the FC reduction in aerobic treatment of sewage sludge was 2-log, then a reduction of *Salmonella* and enteric viruses, respectively, would be about 1.5 log and 1.3 log.

Fecal Streptococci (FS)

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

2.2 Parameters Affecting the Killing or Inactivation of Pathogens

The survival of microorganisms in sludge treatment is a function of a number of factors, which include the local temperature, local antagonistic conditions, and the characteristics of the species or genera. The lethal effect on the population of specific microorganisms is a gradual one and generally the kinetics follow the exponential law of disinfection as given by equation 2-1 (Chick's law);

$$X_t/X_0 = e^{-k(t-t_0)} \quad (2-1)$$

Where X_t/X_0 is the surviving fraction, X_0 is the original population after the time interval $(t-t_0)$, and k is the specific decay rate.

2.2.1 Temperature

Pathogens are inactivated during exposure to heat, which to be effective and relatively rapid must be above their optimum growth temperature. The period of exposure required for disinfection is dependent on the temperature and on the species of the organism. Feacham *et al.* (1983) determined the thermal death curve of a number of enteric pathogens. These have been collated into a single graph by Strauch (1998), which indicates a 'safety zone' where, if the operating parameters were above the minimum requirements, the resultant sludge would be virtually pathogen-free (Figure 2-1). These data suggest that the operating parameters should be in excess of 7 minutes at 70°C; 30 minutes at 65°C; 2 hours at 60°C; 15 hours at 55°C or 3 days at 50°C.

Figure 2-1: Time-Temperature Requirements to Produce Sludge, Which is Virtually Pathogen-Free (Strauch, 1998).

The data of Strauch was based on using pure cultures of the microorganisms. The U.S. EPA have produced formulae to determine the time-temperature regimes for thermally treated “Class A” sludge, (U.S. EPA, 1999). These are dependent upon the solids content of the sludge and are subsequently described. If the solids content of the sludge is 7% or more

$$D = (131.7 \times 10^6) / 10^{0.14t} \quad (2-2)$$

Where D is time in days and t is temperature in °C. Contact time should be for not less than 30 minutes and the temperature should be not less than 50°C. If the sludge particles are small and are heated by hot gas or immiscible fluid the heating time should be not less than 15 seconds; If the solids content of the sludge is less than 7% the exposure is calculated from

$$D = (50.07 \times 10^6) / 10^{0.14t} \quad (2-3)$$

Contact time should be for not less than 30 minutes and the temperature should be not less than 50°C. Whilst the formulae are in agreement with the graph of Strauch at the higher temperatures there is a wide divergence at lower temperatures. For sludges of less than 7% solids the U.S. EPA time-temperature conditions are: 30 minutes at 67°C; 1 hour at 65°C; 4.8 hours at 60°C; 1 day at 55°C; and 5 days at 50°C. For sludges with high solids content the requirements range from 30 minutes at 70°C to 13 days at 50°C.

The differences seem to arise because the U.S. EPA developed a linear model, using a logarithmic scale for time, and incorporated a margin of safety, whereas it can be seen from the work of Strauch (Figure 2-1) that the boundary of the safety zone is an irregular curve influenced by different sensitivities of different microorganisms and is not directly proportional to either time or temperature. Using equation 2-3, a temperature of 55°C yields a contact time of 23.97 hours for complete pathogen destruction. A 10 percent temperature increase would reduce the time of contact by 500 percent.

Sanders *et al*, (1979) studied the fate of *poliomyelitis* virus type I during anaerobic digestion of waste activated sludge. The digesters were bench scale "draw and fill" CSTRs. Viral reductions were determined in the digesters and controls exposed to the same time-temperature conditions were compared. The results showed that viral decay was a direct function of temperature. As temperature increased from 34°C to 50°C the importance of heat in the loss of viruses also increased. At 50°C, temperature accounted for nearly 100 percent of the 7 log reduction measured. At this temperature, the *poliomyelitis* virus decay was so rapid that heat seemed to be the only major factor.

Carrington (1984) studied the impact of sludge pasteurization followed by mesophilic anaerobic digestion on the viability of *Ascaris* ova. Pasteurization was carried out in heated beakers by stirring raw sewage sludge after a known number of *Ascaris* ova had been added. No *Ascaris* ova survived after 55°C was maintained for about 2 hours. The authors mention that because of the resistance of *Ascaris* to heat it is likely that such treated sludge would also be free of other pathogens. Pasteurization at 47°C for 3 hours and 50°C for 2 hours had no detectable effect upon viability, but after subsequent digestion the ova showed a decrease in viability.

The sludge treatment processes that operate at mesophilic temperatures (33-37°C) were developed for the stabilisation of sludge. The mean retention time in such plants is usually in the region of 12-15 days, but to enable the process to operate on a continuous basis there must be an addition of fresh material in the form of raw sludge, normally at least once every day. For maximum reduction of the numbers of pathogens the removal of treated sludge must take place before the addition of raw sludge. This still means, however, that a fraction of the withdrawn sludge will have been exposed to the digesting conditions for 24 hours or less. The implications of mean retention time are discussed later (Carrington, 2001).

It can be seen from Figure 2-1 that prolonged exposure to mesophilic temperatures will have an effect on the survival of pathogens. The simple effect of temperature will be enhanced by the acids and other antagonistic compounds developed during the digestion process (Carrington, 2001). However, these factors either alone or in combination may impact upon the injury of microorganisms in anaerobic digestion. The

degree of injury will likely vary and will be a function of the specific microorganisms susceptibility (Goepfert and Hicks, 1969; Abdul and Lloyd, 1985; Fukushi *et al*, 2003)

2.2.2 Moisture

In general, simple drying is not an effective means of minimising the pathogen content of sludges, although reducing the moisture content can enhance the effects of other treatments. To be most effective the initial reduction in water content should be carried out relatively slowly. Most microorganisms cannot grow in substrates where the water activity has been reduced to below 0.9 (Singleton and Sainsbury, 1987). Water activity (A_w) is a measure of the water available to the microorganisms and equates to 1/100th of the relative humidity within the sludge particle and not the water content of the body of the sludge. In practice it is very difficult to measure water activity in sludge particles and is not a suitable parameter for the routine monitoring of sludge from treatment processes such as thermal drying.

2.2.3 pH

Slaked lime

The raising of the pH of sludge to at least pH 12 by the use of slaked lime has the effect of suspending microbiological activity and reducing the hazard from pathogens by 2 log or more (Strauch, 1998). Carrington *et al*, (1998), reviewing the work of others suggested a pH of 12 for 2 hours will reduce many pathogens to insignificant numbers. Because the activity of other organisms is only suspended, offensive odours may be produced, and such sludge should be utilised within two days.

pH and temperature

When quicklime or similar substances, (e.g. pulverised fuel ash or cement kiln dust), are mixed with sludge, in addition to the raising of the pH there is an autothermic effect and the temperature of the mixture is raised to between 55°C and 70°C. Strauch (1998), describing earlier studies found that a combination of pH 12 and temperatures in the range 60-70°C destroyed *Ascaris* ova and a range of viruses within 24 hours. The combined effects of increased temperature and pH resulting from the addition of CaO in the sludge may be comparable to conventional pasteurization processes which require a temperature of 70°C.

2.2.4 Digester Hydraulics and Mixing

Eliminating pathogen short-circuiting is paramount for proper pathogen reduction through anaerobic digesters (Kennedy *et al*, 1997). Staging of anaerobic digesters is a first course of action to reduce pathogen bypassing. For instance, the city of Vancouver in Canada operates multiple stage thermophilic digesters at the Annacis Island and Lions Gate WWTP (Schafer and Farrell, 2000).

The two-phase digestion process applies staging by treating sludge in two separate digesters connected in series. A thermophilic anaerobic digester needs to be completely mixed so that heat transfer is rapid and evenly spread throughout the volume of sludge (Carrington, 1984). Some researchers blame poor mixing conditions as the cause of significant divergence in pathogen elimination between bench-scale and full-scale applications of thermophilic digestion (Carrington and Harman, 1982)

2.2.5 Free Ammonia and Non-ionized Volatile Fatty Acids

Free Ammonia

Ward and Ashley (1976) studied the heat inactivation of *poliomyelitis* virus in wastewater sludge. Samples of raw sludge and digested sludge from the Albuquerque Sewage Treatment Plant were subjected to temperatures of 43°C, 47°C and 51°C for 200 minutes. The reduction rates of *poliomyelitis* virus observed in the digested sludge samples that were exposed to higher temperatures were faster than the raw sludge sample exposed to the similar temperature range. It was suggested that a virucidal agent present in the supernate of the digested sludge caused a significant acceleration of the heat inactivation of *poliomyelitis* virus.

In subsequent research Ward and Ashley (1977) found that the virucidal agent they had discovered was ammonia. Additional work from the same authors revealed that the free ammonia was responsible for the virus inactivation. The authors recommended a minimum pH of 8.0 for anaerobically digested sludge to significantly inactivate *poliomyelitis* virus. They claimed that the density of poliovirus was decreased by 3-log after incubation at 21°C for 72 hours in digested sludge with 279 mg/L of free ammonia and a pH of 9.0.

Fenters *et al.* (1979) examined the inactivation of *poliomyelitis* virus by components present in anaerobically digested sludge. After 2 days of incubation at 35°C in the sludge supernate containing 39 mg/L of free ammonia *poliomyelitis* virus was reduced to undetectable levels. In a recent study conducted by Pecson *et al.* (2003) the effect of ammonia, temperature, pH, and exposure time on the inactivation of the eggs of the wastewater pathogen, *Ascaris* was examined. Inactivation of *Ascaris* was evaluated at different pH's (7, 9, and 11), ammonia concentrations (0, 10, 100, and 1000 mg/L as

N), and temperatures (from 32-52°C). *Ascaris* eggs were heated for 24 hours in various concentrations of ammonia and at different pH levels, rinsed, incubated in optimum growing conditions, and viewed microscopically for development. They observed that, pH had no effect on inactivation, while increasing temperature did with the inactivation occurring over a narrow threshold temperature range. Within this range, a supplementary inactivation was seen in eggs suspended in high ammonia, and high pH solutions. This supplementary inactivation was directly related to the concentration of unionized NH_3 present in the solution. They concluded that these findings may help explain the wide range of times needed for *Ascaris* inactivation in sludge treatment processes. Sludges that attain higher temperatures, that have higher NH_3 concentrations, and that do not allow volatilization of gaseous NH_3 (such as in covered storage tanks), would be expected to have increased inactivation by this mechanism. Accordingly, the time needed for inactivation in these conditions should be shorter.

Non-ionized Volatile Fatty Acids

The concentration of free ammonia and non-ionized VFA in anaerobic digesters varies with the pH and temperature of the sludge Ferran *et al.* (2002). The toxic effects of these substances will therefore change based on the digester operating conditions. At low pH the non-ionized VFA are the dominant species while at pH above 7.5 the uncharged or free ammonia prevails. Increased temperature significantly enhances the ratio of free ammonia to total ammonia. Conversely, the ratio of non-ionized VFA to total VFA does not benefit from thermophilic temperatures. Goepfert and Hicks (1969) studied the survival of *Salmonella typhimurium* in the presence of formic, acetic, propionic and butyric acid. Different pH values, acid concentrations and temperatures

were tested. The reduction of *Salmonella typhimurium* increased at lower pH with constant acetic acid concentration keeping with the principle that it is the non-ionized form of the acid that is bactericidal. A 3 log reduction of *Salmonella typhimurium* was measured after 24 hours of incubation at 37°C in the presence of 366 mg/l of non-ionized acetic acid. The sensitivity of *Salmonella typhimurium* increased as the chain length and concentration of the acid increased. Finally, an increase in temperature enhanced the killing rates.

Abdul and Lloyd (1985) studied the fate of pathogens in laboratory model anaerobic digesters. Anaerobically grown cultures of various strains of *E.coli* received additions of volatile fatty acids. All the data were obtained at a pH of 8.0, which is typical of conventional anaerobic digesters. A concentration of 120 mmol/l of acetic acid inhibited the growth of two of the three strains of *E.coli*. The fatty acid toxicity increased with increasing chain length. Further examination of the samples with inhibited growth showed a complete loss of viability. The results obtained in this study contradict the previous theory that only the non-ionized form of the acids is bactericidal.

Fukushi *et al.* (2003) studied the impact of pH and volatile acid concentration independently on the survival of *Salmonella* spp. in raw waste activated sludge. A mixture of acetic, propionic and butyric acids was dosed into the sludge and the pH was established by the addition of NaOH and HCl. A range of pH's varying from 3.5-5.5 was studied over an incubation period of 10 days at a temperature of 37°C. There was no difference in *Salmonella* response when concentrations varying from 2500-7500 mg/L were employed with a common initial pH of 5.5. In all cases substantial reductions in concentrations did not occur until approximately day 8 of the incubation. When dosed

with 9300 mg/L of acid mixture, the bottles with a pH of 5 and less demonstrated a complete removal of *Salmonella* in 2 days. The results suggest that the acid phase digester of a two phase digestion system would need to operate at pH's that are lower than those normally achieved in practice in order to obtain a substantial reduction in *Salmonella* sp. at a temperature of 37°C. An accumulation of VFA's may however contribute to a fractional inactivation of pathogens.

Puchajda and Oleszkiewicz (2004) investigated the ability of acid-phase digestion to effectively inactivate FC's at mesophilic (38°C) and low-mesophilic temperatures (21°C) and over a range of solids contents (approximately 3.7%, 6.0% and 8.0% TS). They noted that low-mesophilic digesters lowered FC levels to non detectable levels (Class A), while effluent from acid-digesters operated at mesophilic temperatures showed FC's at level of between Class A and Class B limit. They concluded that the acidic stress conditions from non-ionized VFA contributed to effective inactivation of FC's in low-mesophilic (21°C) conditions. An increase in organic loading resulted in elevated levels of non-ionized VFA release in low-mesophilic and subsequently higher FC inactivation as compared to the mesophilic digester.

2.2.6 Feeding Protocol

Intermittently fed "draw and fill" anaerobic digesters have been shown to considerably reduce pathogen bypassing. Farrel *et al.* (1988) observed that changing the feeding pattern from draw/fill (D/F) to fill/draw (F/D) affected the reduction of fecal coliforms and virus counts, but did not affect volatile solids destruction. Reductions of both bacterial and virus counts were greater using the D/F feeding mode than the F/D pattern, but the effect was greater for bacteria than for viruses. The experiments were

carried out at 35°C and a mean HRT of 14 days. Reductions of 2.85, 2.40, and 2.29 logs were reported for TC, FC and FS, respectively, during D/F operation which, under the F/D operations, reductions of 1.28, 1.16, and 1.21 for TC, FC, and FS was reported. Virus inactivation was 0.97 and 0.74 logs, respectively, for D/F and F/D patterns. A material balance on the number of organisms assuming first order kinetics led to the development of equation 2-4 by Ginnivan *et al.* (1980). Equation 2-4 may be used to calculate a rate constant from digester feed and product concentrations or digester performance knowing the rate constant.

$$(v/V)(C_f/C_d) = [1 + (v/V)]e^{k\theta} - 1 \quad (2-4)$$

Where v = volume withdrawn and added, V = digester volume before volume v is fed, k is the first order rate constant, θ is the time from addition to withdrawal, C_f is the microbial density in feed (CFU/100 ml) and C_d is the microbial density in sludge withdrawn (CFU/100 ml). Ginnivan and co-workers suggests that the use of equation 2-4 at retention times other than the HRT at which the data were collected (i.e., 14 days) is not recommended unless data on the effect of HRT (shorter or longer) on bacterial inactivation rates are determined. Ferran *et al.* (2002) used equation 2-4 to study the effect of feeding frequency on pathogen inactivation in draw and fill operation. In their modeling they varied the frequency of feeding and hence the duration of the hold period. The results of this modeling exercise indicated that the fewer the number of daily feedings the greater the reduction in fecal coliform. A low number of daily feedings improves fecal coliform kill by increasing the batch holding time. A high number of daily feedings causes the digested product to be contaminated with sludge that has been digested only a short time therefore seriously compromising effective pathogen reduction.

2.3 Pathogen Reduction in Sludge Treatment Systems

Sludge is the largest residue by volume that is removed from the wastewater at treatment facilities. Sludge treatment is an expensive and complex process. Municipal sludge contains approximately 93 to 99 percent water (by weight) and 1 to 7 percent solids. Unprocessed sewage sludge may contain large numbers of pathogenic organisms (e.g., bacteria, viruses, protozoa, and helminths). The principal methods of sludge processing and management include preliminary treatment, sedimentation, thickening, stabilization, conditioning, disinfection, dewatering, heat drying, thermal reduction (incineration), beneficial use, and ultimate disposal (Metcalf and Eddy, 2003).

Stabilization includes processes that primarily break down the organic fraction of the sludge in order to reduce the mass and produce an aesthetically acceptable (odorless) sludge. In addition, reduction in pathogens and vector attraction are paramount in biosolids that are to be land applied or surface applied under the current regulations. The major sludge stabilization processes include anaerobic digestion, aerobic digestion, composting, alkaline (lime) stabilization, and air-drying. The effectiveness of anaerobic digestion stabilization processes in reducing the densities of indicator bacteria and pathogenic organisms in municipal wastewater sludge is discussed below.

2.3.1 Anaerobic Digestion

Biological stabilization of sludge solids by anaerobic digestion is frequently employed prior to disposal and/or land application of biosolids. Anaerobic digestion of municipal wastewater sludge involves the transformation of complex organic solids in the absence of dissolved oxygen to gaseous end products such as methane and carbon dioxide and to an innocuous sludge. A net reduction in odours, pathogen concentration,

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

The design parameters for anaerobic digestion include HRT, volatile solids loading, temperature, and mixing. Digestion performance usually is based on volatile solids destruction and gas or methane production. Malina (1992) reported optimum and extreme environmental conditions for maximum methane production in anaerobic digesters (Table 2-2). The parameters that provide an insight into the conditions of the environment early enough to avert digester failure are the volatile acid concentrations, alkalinity, pH, and the carbon dioxide content of the gas. These parameters are interrelated and one variable may directly or indirectly affect the others. Therefore, close monitoring of these variables is essential to the control of an anaerobic system. The design and operating variables during anaerobic digestion are discussed subsequently.

2.3.1.1 Hydraulic Retention Time

The primary objective of anaerobic digestion is the destruction of volatile solids (organic matter). The key to efficient digestion is the development and maintenance of a large, stable, viable population of methane-forming bacteria. Therefore, the most important parameter for system design and operation is the solids retention time (SRT). Anaerobic digesters are commonly operated without recycle; therefore, the terms HRT and SRT will be used alternatively.

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

Variable	Optimum	Extreme
pH	6.8-7.4	6.4-7.8
Oxidation Reduction Potential (ORP)(mV)	-520 to -530	-490 to -550
Volatile Acids (mg/L as acetic acid)	50-500	>2,000
Alkalinity (mg/L as CaCO ₃)	1500-3,000	1,000-5,000
Temperature Mesophilic Thermophilic	30-35°C (86-95 °F) 50-56°C (122-132 °F)	20-40°C 68-104°F 45-60°C (113-140 °F)
HRT (day)	10-15	7-30
Gas Composition Methane (CH ₄) (%v) Carbon Dioxide (CO ₂)(%v)	65-70 30-35	60-75 25-40

A minimum SRT is essential in the anaerobic digestion process to ensure that the necessary microorganisms are being produced at the same rate as they leave the system in the digested sludge. The system fails from washout of the microbial population of methanogens at SRT below a critical value. This critical SRT depends on the generation time of the methane-forming bacteria (i.e., the time required to double the microbial population of methane-forming bacteria) (Bitton, 1994). The generation times for methane-forming bacteria range from less than two days to 30 days at a temperature of 35°C depending on the organism.

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

2.3.1.2 Temperature

Anaerobic digesters generally are operated in either the mesophilic temperature range 30-38°C (86 to 100°F) or the thermophilic range 50-60°C (122 to 140°F). Most digesters have been designed to operate in the mesophilic range, however, considerable research is being undertaken to evaluate the potential of the thermophilic range. Thermophilic microorganisms are considered to be more sensitive to temperature changes than mesophilic organisms. Several advantages have been reported for thermophilic digestion over mesophilic digestion, including increased degree of organic matter destruction, improved dewaterability characteristics, and increased destruction of pathogenic bacteria (Parkin and Owen, 1986). Limitations of the process that have been

reported include extreme sensitivities of the organisms to the defined temperature range, a higher net energy input (consequently higher operation costs) than for mesophilic digestion, and the production of biosolids with a comparatively more offensive odour (WEF, 1995).

2.3.1.3 Fate of Pathogens

Berg and Berman (1980) found that temperature was the primary factor in the die-off of indicator bacteria at an HRT of 20 days. The reduction of indicator organisms at 49°C was greater than at 35°C. At mesophilic conditions, viruses, FC, TC and FS were reduced approximately 1 log, 2 log, 3 log and 2 log respectively.

Carrington and Harman (1984) estimated the decimal decay-rates for *Salmonella duesseldorf* using equation 2-4 developed by Ginnivan *et al.*, (1980). They observed inactivation rates for *Salmonella* were greater in heated anaerobic digesters. Decay rates for *Salmonella duesseldorf* were greater at 48°C ($k = 3.4 \text{ d}^{-1}$ at 20 days HRT) than at 35°C ($k = 1.15 \text{ d}^{-1}$ at 20 days HRT). Pike *et al.* (1988) also reported that the inactivation rates for *Salmonella duesseldorf* were greater at 49°C than at 35°C. The results showed that at 35°C *Salmonella duesseldorf* densities were reduced by 91% at a 10 day SRT and 99.8% at a 20 day SRT. The decimal decay rates for *Salmonella* followed first order kinetics at mesophilic conditions.

Stukenberg *et al.* (1994) found that the effectiveness of mesophilic anaerobic digestion in reducing FC densities averaged 2 logs in 54 wastewater treatment plants (WWTP). Approximately 47 out of the 54 plants (87%) showed FC densities below the limit established by current regulations (40 CFR Part 503) for Class B biosolids (i.e., 2×10^6 or 6.3 logs MPN/g TS). Ponugoti *et al.* (1997) reported that FC, FS, and

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

Aitken and Mullenix (1992) examined the fate of FC and FS in batch and semi-batch bench scale reactors operating at a temperature of 55°C. Effluent FC had a concentration of approximately 10 MPN/g TS while FS concentrations ranged from 10^2 - 10^3 MPN/g TS. The results of this study differed from those of Berg and Berman (1980) in that FS were found to be more resistant to disinfection than FC. Oropeza *et al.* (2001) reported the removal of pathogens and parasites during the start-up and stabilization phases of a laboratory scale thermophilic anaerobic digester and compared the results with that of mesophilic conditions. The results showed that, in both stages, the anaerobic thermophilic digester presented higher removal efficiencies of pathogens and parasites, than the mesophilic digester.

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

At 55°C and 20 day HRT's, the densities of indicator and pathogenic bacteria were reduced to levels that met the EPA Part 503 requirements for Class A biosolids. Sanders *et al.* (1979) found that viral recovery was a direct function of temperature. As the temperature increased from 34°C to 37°C and to 50°C, the importance of heat in the loss of recoverable virus also increased. Retention times of 5, 10, and 15 days had a smaller but direct effect on viral recovery in the mesophilic range. The effect of retention time decreased as temperature increased. Detention time had the greatest effect on viral recovery at 34°C. The results indicated that the effects of volatile solids loading on viral removal were not conclusive.

Nielsen and Petersen (2000) reported on the concentrations of *Salmonella* and FS after thermophilic digestion in a full scale digester in Holbaek, Denmark. These concentrations were found to be <2 and <10 MPN/100mL respectively. These concentrations were 1-2 orders of magnitude less than those that were observed when the same digesters were operated mesophilically. The Holbaek plant was operated at a temperature range of 50-55°C and an HRT of 12 days.

Iranpour *et al.* (2002) described the start up and operation of a full-scale thermophilic digester at 55°C at the Terminal Island Treatment Plant, Los Angeles, USA. The digester was fed in batches and in some instances the feed period overlapped with the draw period. However, it was noted that the digesters were not completely mixed and operated in somewhat of a dispersed plug flow mode. It is believed that this may have limited to some extent the bleed through of untreated sludge to the digester effluent. After startup was completed the digester was found to maintain FC concentrations of 100 MPN/g TS and *Salmonella* concentrations were less than 3 MPN/4g TS.

Aitken, *et al*, (2003) investigated the removal of pathogens and indicator organisms at laboratory scale (20 L) in a completely-mixed thermophilic anaerobic digester operated at 55°C and a four-day HRT. Digester feed and effluent samples were analyzed for these two species as well as *Salmonella* spp., FC, *Clostridium perfringens* spores, and somatic and male-specific coliphages.

The effluent did not contain detectable concentrations of *Ascaris*, poliovirus or *Salmonella*, with removals across the digester of greater than 2 log greater than 3 log and nearly 4 log, respectively. Removal of FC exceeded 4 log but the effluent biosolids contained slightly greater than 1000 MPN/g TS. They noted that concentrations of *Clostridium perfringens* were essentially unchanged across the continuous digester, therefore they concluded that *Clostridium perfringens* spores are not useful indicators of pathogen removal by thermophilic anaerobic digestion at temperatures at or below 55°C.

Aitken and co-worker observed that the coliphages were removed in the continuous digester but to a lesser extent than poliovirus. The results of Aitken study was consistent with observations made by others that coliphages are more heat-resistant than enteric viruses (Mocé-Llivina *et al*, 2003). Male-specific coliphages were removed more consistently and extensively (2.4 to 2.6 log) than somatic coliphages across the digester. These data suggested that somatic coliphages may be more heat-resistant than male-specific coliphages, as has been observed at 60°C (Mocé-Llivina *et al*, 2003), but both groups appear to contain a relatively heat-resistant fraction.

Berg and Berman (1980) found that temperature was the primary factor in the die-off of viruses. At 49°C and 20 days of HRT, the reduction of viruses was greater than that observed at 35°C. Under mesophilic conditions, viruses were reduced by more than 1.0

log units whereas with thermophilic digestion, the numbers of viruses were reduced by 2 to 3 logs. The authors concluded that neither TC, FC, nor FS were good indicators for the presence of viruses in mesophilic or thermophilic digested anaerobic sludges.

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

Pike *et al.* (1988) reported that the inactivation rate for *Ascaris* ova was greater at 49°C than at 35°C. Mesophilic anaerobic digestion (35°C) had little effect on viability of *Ascaris* ova. Lee *et al.* (1989) indicated that anaerobic digestion at 53°C and a HRT of 10 days reduced the densities of *Ascaris* eggs to essentially undetectable levels. Stadterman *et al.* (1995) studied reduction in *Cryptosporidium parvum* oocysts viability through a bench scale mesophilic anaerobic digester that was seeded with *Cryptosporidium parvum* oocysts. They observed *Cryptosporidium* oocysts exposed to anaerobically digested sludge disappeared rapidly. Fifty percent inactivation levels were achieved between 1 and 2 hours and 99.98% of the oocysts were inactivated after 24 hours. Chauret *et al.* (1999) studied the fate of *Cryptosporidium* oocysts during mesophilic anaerobic digestion however, no statistically significant reduction was observed.

In summary, there is a large body of evidence on the fate of pathogens in mesophilic and thermophilic anaerobic digestion. In general, it can be expected that the reduction in microbial indicators will be in the range of 1-3 log under mesophilic condition while thermophilic digestion can produce sludges that have pathogen

concentrations below detectable concentrations. Viruses and helminthes are more resistant to reduction in mesophilic anaerobic digestion than thermophilic digestion. There is less data available on the fate of protozoan pathogens and there appears to be considerable uncertainty in the fate of these organisms under mesophilic and thermophilic anaerobic digestion. It is also important that attention is paid to the design and operation of the digesters to insure that all of the sludge is exposed to the temperature for a sufficient time period. In this regard the use of innovative process configurations such as staged operation and SBR operation can enhance the biosolids quality.

2.3.2 Two-Phase Anaerobic Digestion Process

In conventional anaerobic digestion, the acid forming and the methane forming microorganisms are kept together in a single reactor system and there is a delicate balance between these two groups of organisms, because both groups differ widely in terms of physiology, nutritional needs, growth kinetics and sensitivity to environmental conditions. Problems encountered with stability and controls in conventional design applications have led researchers to new solutions. Pohland and Ghosh (1971) first proposed the physical separation of acid formers and methane formers in two separate reactors, where optimum environmental conditions for each group of organisms would be provided to enhance the overall process stability and control. Methods for phase separation include physical separation with membrane barriers, poisoning or nutrition inhibition of one set of microorganisms, and kinetic control, which can be achieved by controlling first- and second-phase reactor retention times. The first phase provides hydrolysis and acidification, and the second phase maximizes gas production. A typical two-phase digestion design has a first stage with a 1 to 2 day HRT followed by a 10 to 14

day second stage. The short HRT results in washout of the relatively slow-growing methanogens from the first reactor and hence there is an accumulation of acids in this reactor and a decrease in the pH. The pH of the first stage reactor is often less than 6 and the volatile acids will often accumulate to more than 6000 mg/L (Metcalf and Eddy, 2003). Literature suggests that two-phase digestion will likely meet both the pathogen reduction requirements for fecal coliform and the Vector Attraction Reduction (VAR) requirements for Class A solids if both phases are operated at thermophilic temperatures (U.S. EPA, 1992).

The claimed advantages of phase separation are:

1. Increased stability with better control of acid phase.
2. Higher organic loading rates.
3. Increased specific activity of methanogens leading to an increase in methane production rates.
4. Increased overall COD and VS reduction efficiencies.

Speculated disadvantages of phase separation include:

1. Hydrogen build-up in the first-phase reactor during the acid formation to levels inhibitory to acid producing bacteria.
2. Elimination of possible interdependent nutritional requirements of acid and methane formers.

2.3.2.1 Fate of Pathogens

Gabb *et al.* (2000) studied the reduction of FC, *Salmonella*, enteric virus and viable helminth ova through a two-phase thermo-meso anaerobic digestion process. The digesters employed were bench scale CSTR's that were drawn and filled every 1 to 6 hours. The thermophilic digester operated at 50°C with a detention time of 2 days while the mesophilic digester operated at 35°C with a HRT of 13 days. The raw sludge was seeded with each of the pathogens mentioned above in order to demonstrate sufficient reduction. The process did not consistently achieve the Class A *Salmonella*, virus and viable helminth criterion although these pathogens were reduced by 4.4 log, 2 to 4 log and 2 log respectively. Most of the helminth ova destruction took place in the thermophilic first phase.

Lee *et al.* (1989) evaluated pathogen reduction in conventional and two-phase anaerobic digestion under mesophilic and thermophilic conditions and at residence times of 10 and 20 days. The draw and fill operation was performed three times daily to simulate semi-continuous operation. Three indicator bacteria and one virus FC, *E.coli*, FS, and enterovirus were chosen for the determination of microbial density. They found that thermophilic anaerobic digestion at 53°C reduced indicator bacteria and enterovirus to undetectable levels for an SRT as low as 10 days. In this study mesophilic, two-phase anaerobic digestion achieved greater log bacterial density reduction than conventional digestion by 0.81 for FC, 0.74 for EC, and 0.49 for FS, at SRTs of 10 and 20 days, respectively. No difference in the viral density reduction was observed between the conventional and the two-phase digesters at both 10 and 20 day SRT's under mesophilic conditions. The 20 day HRT operation achieved approximately 60% more viral

destruction than 10 day HRT operation in both conventional and two phase digestion under thermophilic conditions.

Huyard *et al.* (2000) investigated the impact of different temperature and feeding sequences on destruction of pathogens with a two phase anaerobic digestion pilot process. The acidogenic reactor operated over a temperature range of 55°C to 60°C with an HRT of 2 ± 0.1 days. The partially digested sludge was pumped from the acidogenic reactor to the methanogenic reactor that was operated at 37°C with an HRT of 10 ± 0.5 days. The pilot unit was operated with the acidogenic reactor at a temperature of 55°C with 2, 4 and 12 feedings per day, and a temperature of 60°C with 2 and 4 feedings per day. The sludge was not preheated in this study and hence there were substantial temperature drops of 8-14 degree in the acid phase digester because of the cooler sludge entering the acid digester

FC reduction rates were higher when the acid reactor temperature was set at 60°C as compared to 55°C and when the pilot unit was fed two times per day rather than four times. For a feeding strategy of 4 feedings per day and a temperature of 55°C reduction of polio virus and *Ascaris* ova was found to average 4 log and 2.61 log, respectively.

De Leon and Jenkins (2002) evaluated acid phase digestion at an HRT of 2 days and temperatures of 35°, 48°, 52° and 62°C (System 1) and a mesophilic acid-thermophilic gas system (System 2) at bench scale, The thermophilic digester in system 2 was evaluated at temperatures of 48°, 52° and 62°C while the mesophilic digester was operated at 35°C. A control was operated with the second stage digester operated at a mesophilic temperature of 35°C. In system 2 the HRT's were 2 days and 15 days in the first and second stage digesters respectively.

The system 1 digesters achieved average effluent FC concentrations that were less than 1000 MPN/g TS at temperatures of 52°C and 62°C. The 35°C and 48°C conditions resulted in FC concentrations that were approximately $10^{3.7}$ and $10^{3.1}$ MPN/g TS respectively. In system 2 the acid phase digester achieved a 1.5 log reduction in FC concentrations. After the second stage thermophilic digester the FC concentrations were less than 1000 MPN/g TS for all the temperatures that were evaluated. Interestingly, the mesophilic control digester also achieved this level of FC concentrations.

A full-scale operation study was conducted by McCarthy *et al*, (2004) to compare performance of two mesophilic anaerobic digesters operated in series to one mesophilic anaerobic digester operated at a solids retention time equivalent to the series digesters. The average digester temperature for the study was 35°C to 36°C and the SRT in each digester was maintained between 17 and 21 days. Loading to the primary digester varied from 2.43 to 4.5 kg VS/m³/day. Loading to the secondary digester was approximately 1.13 kg VS/ m³/day. The control digester was operated at a SRT between 29 and 45 days and was loaded between 1.29 and 2.10 kg VS/ m³/day. They observed that for both tests the results for the control digester were similar to the results for the first digester in series. In significant contrast, the results for the second digester were lower by 1.5 to 2 log units for FC.

It is important to establish the ratio of HRTs between the first and second stage digesters that is required to attain an acid phase digester. In this regard Puchajda and Oleszkiewicz (2003) evaluated the impact of different retention times on hydrolysis and acidification rates of a thermophilic anaerobic digester. Three lab scale anaerobic digestion systems were evaluated in their study.

A temperature phased anaerobic digestion (TPAD) was compared with a single phase thermophilic digester (TAD) and single phase mesophilic digester (MAD). In phase 1 of the experiments, the HRT was 15 d in both the TAD and MAD digesters and 5 d + 10 d was employed for the TPAD. In phase 2, the HRT was 13 d in the MAD and TAD and 3 + 10 d was employed in TPAD. In phase 3, the HRT was set at 11 d in the MAD and TAD, and 1 + 10 d was employed in TPAD.

They observed that hydrolysis and acidification rates decreased exponentially with an increase in HRT from 1 to 5 days. Additionally hydrolysis was found to be faster than acidification. Therefore they concluded that at short retention times acidification may be the limiting step in the first reactor of the two-phase process.

In summary, studies reveal that acid phase digestion at mesophilic temperatures may reduce pathogens somewhat more than that of mesophilic methanogenic digestion. This can presumably be attributed to the inhibitory effect of volatile fatty acids. However, the effluent from a mesophilic acid-phase digester alone will not be pathogen-free. It would appear that at least one of the digesters must be operated at thermophilic temperatures to ensure pathogen-free biosolids.

2.3.3 Temperature-Phased Anaerobic Digestion

This treatment involves the stabilization of organic waste using a thermophilic methanogenic reactor (typically operating at 55°C) followed by a mesophilic methanogenic reactor (typically 35°C). Doing so has been shown to combine the advantages of stand-alone thermophilic and mesophilic systems while overcoming the drawbacks associated with each. Thermophilic systems are known to achieve high rates of volatile solids (VS) and pathogen destruction, however are often considered to be less

stable than mesophilic systems and can contain high concentrations of potentially odour-causing volatile fatty acids (VFAs) in effluent streams. Mesophilic reactors can reduce VFAs to low levels, but only achieve low VS and pathogen destruction and may have problems with foaming.

To date, temperature-phased systems have been seen to consistently out-perform single reactor systems in all parameters compared (Han and Dague 1997).

2.3.3.1 Advantages of Temperature-Phased Anaerobic Digestion Systems

The advantages of temperature-phased processes over single-stage mesophilic and thermophilic anaerobic systems become obvious when the drawbacks of each single system are discussed. Temperature-phase systems are known to diminish foaming and odour problems and enable pathogen levels to be reduced below the class A biosolids standard (Han and Dague, 1997). Another advantage of temperature-phase systems is that they allow for treatment of wastes with high organic loads at HRTs shorter than conventional single-stage anaerobic digestion (i.e. have a greater system capacity) due to the faster reaction rates achieved at the higher temperature in the first thermophilic stage (Han and Dague, 1996).

TPAD systems have been found to have an increased rate of hydrolysis in the first stage. This increases the overall efficiency of the digestion process, as for wastes with high concentrations of particulate organic matter (e.g. wastewater solids and MSW), it is assumed to be the rate-limiting step when cellulose used as a sole carbon source (Chyi and Dague, 1994). The increased destruction of VS leads to higher biogas (methane and carbon dioxide) production than single-stage systems, leading to greater potential for energy recovery (Han and Dague, 1997). It has been suggested that the temperature-

phased system is so successful because of the mutually beneficial relationships between acidogenic, acetogenic and methanogenic bacterial populations in the first stage and this type of syntrophy may be required to achieve maximum destruction of organics and concomitant production of methane (Han and Dague, 1997).

Cheunbarn *et al.* (2000) investigated anaerobic thermophilic treatment followed by mesophilic anaerobic digestion (AnTAnM) and mesophilic anaerobic digestion followed by thermophilic anaerobic treatment (AnMAnT) to achieve a Class A sludge product and to enhance the sludge treatment in terms of VS destruction, gas production, sludge dewaterability, and process stability. The lab-scale AnTAnM system consisted of a 4-L thermophilic reactor (operating volume = 1.5 L) followed by a 12-L mesophilic digester (operating volume = 10 L), and the lab-scale AnMAnT system consisted of a 12-L mesophilic digester (operating volume = 10 L) followed by a 4-L thermophilic reactor (operating volume = 1.5 L). A 12-L lab-scale anaerobic mesophilic digester (operating volume = 10 L) was used as the control system.

The HRT in the thermophilic reactors was maintained at 1 day and in the mesophilic digesters at 14 day HRT for both the AnTAnM and the AnMAnT systems. The control mesophilic digester was operated at a 15 day HRT. All systems reduced VS in the feed sludge greater than 38% (Class A sludge vector attraction reduction requirement). The average VS reductions by the AnTAnM system (61%) and AnMAnT system (63%) were approximately the same and were higher than the VS reduction in the control anaerobic digester (50%). Average methane production from the AnTAnM mesophilic digester ($0.66 \pm 0.10 \text{ m}^3/\text{kg VS destroyed}$) was higher than that of the AnMAnT ($0.51 \pm 0.06 \text{ m}^3/\text{kg VS destroyed}$) and the control anaerobic mesophilic

digesters ($0.52 \pm 0.03 \text{ m}^3/\text{kg}$ VS destroyed). The average supernatant COD in the AnTAnM system product sludge ($10,500 \pm 200 \text{ mg/L}$) and AnMAnT system product sludge ($10,200 \pm 150 \text{ mg/L}$) were approximately the same and were significantly lower than the supernatant COD in the control anaerobic digester ($14,100 \pm 350 \text{ mg/L}$). All three systems were fed with sludge containing an average supernatant COD of $22,500 \text{ mg/L}$. The dewaterability of the product sludges, measured as time to filter, was 207 s for the AnMAnT and 244 s for the AnTAnM. Both the AnMAnT and AnTAnM product sludges had filter times lower than the control product sludge (364 s). These results indicate mesophilic digestion followed by thermophilic digestion gives better performance in terms of product sludge dewaterability than thermophilic followed by mesophilic digestion.

2.3.3.2 Fate of Pathogens

Cheunbarn *et al.* (2000) observed that both the AnTAnM and the AnMAnT systems reduced FC density from 10^8 MPN/g TS to $<10^3 \text{ MPN/g TS}$ (Class A sludge limit) at all times. Furthermore, the FC density in the AnMAnT system product sludge was consistently lower than that in the AnTAnM product sludge. The differences were statistically significant according to a paired comparison Student's *t*-test at 95% confidence interval. These results suggest that, if pathogen reduction is the main objective of the dual-stage system, AnMAnT gives a better performance than other systems. *Salmonella* density in the feed sludge during these experiments was between 2 and 12 MPN/4g TS during three of eight sampling events and $<1 \text{ MPN/4g TS}$ at all other times. *Salmonella* density in the AnTAnM and AnMAnT system effluent, and the control anaerobic digester product sludge was $<1 \text{ MPN/4g TS}$ at all times.

Han *et al.* (1997) described bench scale testing of two TPAD systems and compared their performance to a conventional mesophilic digester. In TPAD system A the volumes of the first and second stage digesters were 4 and 10 L respectively while in system B the volume of the first digester was 2 L. Hence, the ratio of residence times in the first digester and the second digester was different between the two systems. The mesophilic digester had a volume of 14 L. The TPAD digesters were operated at 55°C and 35°C respectively. The TPAD digesters were operated over a range of HRT's from 11-28 days while the mesophilic digester was operated over a range of HRT's from 24 to 40 days. System A generally performed superior to System B with respect to solids reduction and biogas production. Both TPAD systems performed better than the conventional mesophilic digester with respect to these parameters. The inlet concentrations of TC's and FC's were approximately 10^8 MPN/g TS and 10^7 MPN/g TS respectively.

In system A, the effluent from the first stage contained approximately 250 MPN/g TS and 200 MPN/g TS of TC and FC respectively. The effluent from the second stage contained approximately 200 MPN/g TS and 175 MPN/g TS of TC and FC respectively. The effluent from the conventional mesophilic digester contained approximately 10^7 MPN/g TS and 10^6 MPN/g TS of TC and FC respectively. In all of the systems the effluent concentrations of pathogen indicators were observed to be independent of HRT.

In a subsequent study, Santha *et al.* (2003) evaluated the performance and operational stability of a TPAD system modified to operate in the sequential-batch mode. The sequential-TPAD system, fed with a 40:60 mixture (dry weight basis) of primary sludge and thickened waste activated sludge at 5.5% total solids, showed stable performance at system retention times as short as 12 days. The thermophilic stage of the

TPAD system operated in the draw-fill-hold mode conformed to the U.S.EPA time-temperature requirements at 55°C. The capability of the system to effectively reduce FC, *E.coli*, and *Salmonella* pathogens to non-detectable levels of less than IMPN/gTS was established by testing. They also evaluated critical applicability issues, such as digester heating, feed and discharge rates, and system configurations that have to be addressed while contemplating full-scale implementation.

The effect of feed solids concentration on the FC concentration over the operation of both the thermophilic and the mesophilic digester treating mixture of primary and waste activated sludge was evaluated by Vandeburgh and Ellis, (2002). System HRT was maintained at 20 days, with 5.7 days in the thermophilic reactor and 14.3 days in the mesophilic reactor. The feed solids concentrations explored in this study were 4.4, 4.9, 5.7, and 7.9% TS. Even though performance data indicated that the FC concentration of both the thermophilic and the mesophilic samples increased at the highest feed solids concentration but the TPAD process in this study met the criteria for Class A biosolids production.

In summary, temperature phased anaerobic digestion has been found to produce a biosolids product that is high quality with respect to pathogen content. Almost all studies on TPAD operation have examined systems that were designed as new facilities or have been substantial systems upgrades. However there is less evidence in the literature of wastewater treatment plants reconfiguring existing digesters for TPAD operation.

2.3.4 Three Phase Digestion

A review of the research being done in the field of thermophilic digestion revealed that multiple phased digestion could address concerns with respect to odour control and pathogen content. The two most promising multi phased processes were: 1) acid-gas phased digestion and, 2) temperature phased digestion. The Inland Empire Utilities Association conducted a full scale test of a three stage digestion process that included a combination of the acid/gas and temperature phased digestion processes (Drury *et al*, 2002). In the three-stage digestion process the first digester was an acid digester, followed by a thermophilic gas digester, and concluded with a mesophilic gas digester. The purpose of each of the three stages of digestion is shown in Table2-3.

Table 2-3: Purposes of Each of the Digesters in Three Stage Digestion.

Stage	Purpose
Mesophilic Acid	<ol style="list-style-type: none"> 1. Maximize solubilisation of solids 2. Maximize the production of volatile acids 3. Enhance pathogen reduction
Thermophilic Gas	<ol style="list-style-type: none"> 1. Further solubilisation of solids 2. Further production of volatile acids 3. Maximize the production of gas 4. Maximize pathogen reduction
Mesophilic Gas	<ol style="list-style-type: none"> 1. Remove odorous compounds 2. Improve sludge dewaterability 3. Further production of gas 4. Insure process stability 5. Further pathogen reduction

Drury *et al.* (2002) investigated two different configurations of three phase digestion. In Sequence 1 the second-stage thermophilic digester was operated with a continuous feed and at temperatures of 45°, 48° and 50°C. The HRTs in the digesters of Sequence 1 were in the range 2.5-3.5 days, 14-16 days and 5-6 days respectively. In Sequence 2, the second stage digester was operated in semi-batch mode at a temperature of 56°C. The feeding sequence for this digester included feed, hold and draw intervals of 4, 17 and 3 hours respectively. The HRT's in the digesters were in the range 2.5-3.5 days, 18-20 days and 13-17 days respectively. Hence, Sequence 1 had a total HRT of approximately 23 days while Sequence 2 had an HRT of approximately 38 days.

Sequence operation at temperatures below 50°C did not result in FC concentrations that were consistently less than 1000 MPN/g TS. Hence a majority of testing was performed at the higher temperature. Under these conditions all samples for Helminth ova, *Salmonella* and enteric viruses were below the method detection limits (Drury *et al.*, 2002). All of the FC concentrations for Sequence 2 were below the method detection limit while 90% of samples from Sequence 1 were below this level. Effluent concentrations of FC were consistently less than the US EPA Class A requirements of 1000 MPN/g TS (Drury *et al.* 2002). The 3 phase digestion process implemented by IEUA is an example of how facilities can reconfigure existing equipment to achieve a high quality biosolids product. The fact that similar results were obtained with continuous and semi-batch reactors operating at considerably different HRT's and temperatures indicates that existing plants may consider different options depending on the design of the digesters that are in the plant.

It should be noted that the facility apparently had considerable excess digester capacity as evidenced by the extended HRT's that were employed.

2.4 Detection and Enumeration Techniques of Bacterial Pathogens in Sludge

Microbial monitoring and identification is essential to identify sources of contamination and to establish the level of treatment necessary to ensure a safe environment. The molecular era that emerged in the 1980s resulted in sequence based molecular methods for detecting pathogens. Rapid alternative approaches were then developed to allow microbial identification directly from specimens. Bacterial detection methods can be divided into two categories, conventional and rapid techniques. Conventional culture methods usually involve a time consuming enrichment step, cost effectiveness, minimal required training and sometimes a pre-enrichment step to stabilize damaged cells. Three to five days are typically required for isolation and identification because of the need for culture on selective media and confirmation of the suspect isolate by biochemical and immunological tests. Rapid methods often rely on at least one enrichment step with immunological detection of antigens or amplification of unique segments of nucleic acid by polymerase chain reaction (PCR) technology (Bitton, 1994)

In this part of the study potential microbial detection methods used for sludge samples is reviewed.

2.4.1 Conventional Technique

Pour plate technique

Cultural methods, or those that grow bacteria in a prepared medium, have been used for bacterial pathogen detection and enumeration for over a century.

Multiple-tube fermentation technique

The technique of enumerating coliforms, *E.coli*, *Salmonella*, FS, and *Clostridium perfringens* by means of multiple-tube fermentation (MTF) has been used for many years as a water and wastewater quality monitoring method. The method consists of inoculating a series of tubes with appropriate decimal dilutions of the water or wastewater sample. Production of gas, acid formation or abundant growth in the test tubes after a certain period of time at a design incubation temperature constitutes a positive presumptive reaction.

The results of the MTF technique are expressed in terms of the most probable number (MPN) of microorganisms present. This number is a statistical estimate of the mean number of microorganisms in the sample.

Many factors may significantly affect microbial detection by MTF, especially during the presumptive phase. Interference by high numbers of other bacteria (Seidler *et al.* 1981; Means and Olson, 1981), as well as the inhibitory nature of the media (McFeters *et al.*, 1982), have been identified as factors contributing to underestimates of these microorganisms abundance. The MTF technique lacks precision in qualitative and quantitative terms. MTF is easy to implement and can be performed by a technician with basic microbiological training, but the method can become very tedious and labour intensive since many dilutions have to be processed for each water or wastewater sample. However, it is also relatively inexpensive, as it requires unsophisticated laboratory equipment. Nevertheless, this method is extremely time-consuming, requiring 48 h for presumptive results, and necessitates a subculture stage for confirmation which could take up to a further 48 h.

Membrane filter technique

The membrane filter (MF) technique is fully accepted and approved as a procedure for monitoring microbial quality in many countries. This method consists of filtering a homogenized sludge sample on a sterile filter with a 0.45-mm pore size which retains bacteria, incubating this filter on a selective medium and enumerating typical colonies on the filter. Many media and incubation conditions for the MF method have been tested for optimal recovery of the previously described microorganisms (Rice *et al.*, 1987). With the acceptance of MF as a technique of choice for monitoring microbial quality (APHA .1995), questions regarding interference with detection and the accuracy of the enumeration have arisen. The presence of high numbers of background heterotrophic bacteria was shown to decrease bacterial recovery by MF (Burlingame *et al.*, 1984).

The predominant concern about MF is its inability to recover stressed or injured microorganisms. A number of chemical and physical factors in the environment can cause sublethal injury to the microorganisms, resulting in a damaged cell unable to form a colony on a selective medium.

The high number of modified media in use is a reflection of the fact that no universal medium currently exists which allows optimal enumeration of various species of one group, originating from different environments and present in a wide variety of physiological states. A significant advantage of the MF technique over the MTF method is that with MF, the examination of larger volumes of sample is feasible, which leads to greater sensitivity and reliability. MF also offers a quantitative enumeration comparatively to the semi quantitative information given by the MTF method. MF is a relatively simple method to use and many samples can be processed in a day with limited

laboratory equipment by a technician with basic microbiological training. Nevertheless, since this method is not sufficiently specific, a confirmation stage is needed, which could take a further 24 h after the first incubation period on selective media.

Massa *et al.*, (2001) compared the faecal streptococci count on 25 samples of polluted waters obtained with three techniques: MPN, MF and PP methods. Since the PP method was found to be as good as the other techniques, it may be preferred in polluted waters. It is more economical in terms of both time and materials than the MPN count, and it is as accurate as the MF count. In addition during bacterial detection method development of this study MF method was compared against MPN method for detection and enumeration of *Salmonella* spp. We used Statistical methods included ANOVA to address if there was a significant difference between the results from MPN and MF methods. Statistically significant relationship was found between the two *Salmonella* detection techniques. The result of statistical analysis is presented in Appendix D.

2.4.2 Molecular Technique

Molecular methods have been developed to increase the rapidity of analysis. They are able to achieve a high degree of sensitivity and specificity without the need for a complex cultivation and additional confirmation steps. Consequently, some of these methods permit the detection of specific culture able and/or non-culture able bacteria within hours, instead of the days required with the traditional methods. Several molecular methods applied to the specific detection of coliforms, *E.coli*, *Salmonella*, FS, and *Clostridium perfringens* in wastewaters and drinking waters are discussed here.

Immunological Techniques

Immunological methods are based on the specific recognition between antibodies and antigens and the high affinity that is characteristic of this recognition reaction. Depending on the taxonomic level of the targeted antigens, immunological methods permit detection of antigens at family, genus, species or serotype levels. Two types of antibodies can be produced: polyclonal and monoclonal antibodies, whichever are more specific to the target organism. The properties of the antigen–antibody complex can be used (Bitton, 1994):

1. To perform an immunocapture of cells or antigens by enzyme-linked immunosorbent assay (IMS or ELISA), or
2. To detect targeted cells by immunofluorescence assay (IFA) or immuno-enzyme assay (IEA).

Hubner *et al.* (1992) refined the ELISA with monoclonal antibodies for the routine detection of Enterobacteriaceae and showed a degree of similarity of 98% with the standard MTF method. The ELISA is a rapid, simple and quite sensitive test, which allows the detection of less than 10^{-9} g of antigenic-protein (Stryer, 1988). However, assay limitations are often associated with the specificity of the antibody used, the concentration of both antibody and antigen and the type of reaction solution used. In addition, the solid matrix such as sludge often leads to non-specific binding of the antigen or of the second antibody (Kfir and Genthe, 1993). This method is very useful for testing monoclonal antibodies or detecting spiked microorganisms. Nevertheless, application of ELISA to the detection of specific cells from a natural contaminated sample is limited (Hanai *et al.* 1997). High levels of non-targeted microflora and diverse materials

associated with the sample may interfere on the level of the specificity of the ELISA method. In addition, because of cross-reactivity with other bacterial strains, the use of immunological methods for the detection of coliforms and *E.coli* has not yet been successful. The application of monoclonal antibodies to the detection of specific organisms in a natural environment, particularly in wastewater samples probably needs more investigation directed towards antibody specificity and bacterial abundance in a wastewater sample.

Polymerase chain reaction techniques

PCR allows a DNA target fragment to be amplified by cycling replication. This replication, which can be performed in vitro or in situ, consists of a chain reaction catalyzed by a DNA polymerase (Taq polymerase) and the use of oligonucleotidic primers. Detection specificity depends on the degree of homology and complementarity between target and primer and on hybridization temperature. The cycling of PCR results in an exponential amplification of the amount of the target sequence and significantly increases the probability of detecting a rare sequence or relatively low numbers of target microorganisms in a sample (Waage *et al.*, 1999).

The PCR amplification process involves: (i) a DNA denaturation from double-to single-stranded DNA, (ii) annealing primers to the single-stranded DNA at a specific hybridization temperature, and (iii) primer extension by a DNA Taq polymerase. Several PCR protocols have been developed for detection of TC, FC *E.coli* and related microorganisms and pathogens including *Salmonella* spp. To detect low levels of pathogens or indicator microorganisms in wastewater samples, a concentration step such

as filtration must first be performed. Bej *et al.* (1991) reported using filtration methods and releasing the DNA by freeze–thaw cycling without affecting the PCR amplification. Despite its sensitivity, it is difficult to use PCR for the quantification of microorganisms. Two techniques have mainly been developed for DNA quantification by PCR: the most-probable-number PCR (Toranzos *et al* 1993) and the competitive PCR (addition of a DNA competitor for the PCR stage to calibrate the PCR efficiency) (Zachar *et al.* 1993). To our knowledge, these techniques have not been applied up to now for the quantification of microorganisms in wastewater.

Despite the fact that PCR is recognized as a highly specific detection and identification method, it can present some limitations when applied to the detection of those cells in a natural sample that are viable or metabolically active without being cultureable. Indeed, PCR amplification applies to nucleic acid extracted from viable and cultureable, viable and non-cultureable or dead cells. Consequently, PCR-based assays cannot provide information on the physiological status of targeted cells. Another limitation inherent to PCR analysis of environmental samples is the frequent inhibition of the enzymatic reaction: humic substances are known as polymerization enzyme (Taq-polymerase) inhibitors. The presence of these elements in a wastewater sample can therefore considerably decrease the amplification yield of PCR applied to the detection of greatly diluted bacteria. They can also limit the revealing of the amplification products.

In summary, based on the review of the current literatures, both a standard culture method and PCR techniques were applied to each sample to detect and quantify microbial dynamic population of the investigated bacterial pathogen in this study.

2.5 Summary

In the above review, the importance of pathogen control in residues of the anaerobic digestion of municipal sludge was assessed. Pathogens in biosolids are a continuing issue for wastewater treatment plant operators when land application is being considered as a method for beneficial reuse of these materials. While thermophilic digestion has demonstrated capability of reducing pathogens to a point suitable for production of Class A biosolids, the EPA has not listed it as an alternative technology suitable for this purpose because of operational stability issues (Metcalf and Eddy, 2003). Fluctuating volatile fatty acid (VFA) levels, odorous effluent, and foaming problems have all been attributed to thermophilic digester operation (Metcalf and Eddy, 2003). A better understanding of optimal operating conditions may one day minimize such problems.

Acid/gas phased digestion may have certain advantages over single stage digestion. However, the suitability of acid/gas phased digestion will depend on the nature of the waste being treated as certain wastes may upset environmental conditions that are important for methane formation (Fox and Pohland, 1994). Acid/gas phased digestion may be conducted at thermophilic temperatures, such as in the 2PAD system, but studies are unclear as to whether the advantages in such systems come from temperature variation or phase separation.

Temperature phased digestion takes advantage of phase separation and thermophilic digestion. While the EPA lists it as a suitable technology for producing Class A biosolids, relatively few full scale installations are in operation in the United States and Canada. Studies suggest that the TPAD process can alleviate some of the problems associated with single stage thermophilic digestion, such as high effluent VFA.

Furthermore, TPAD digesters that utilize a first stage thermophilic digester may be appropriate for treating certain wastes due to the advantages of thermophilic temperatures and partial phase separation.

Previous studies, indicated that staging operation provide increased waste stabilization as compared to single stage processes. Therefore, research contained herein was the first direct comparison of single and multistage operation of anaerobic digestion with conventional mesophilic anaerobic digestion process. In this regard, the research conducted as part of this thesis is unique.

CHAPTER 3

EXPERIMENTAL MATERIALS AND METHODS

3.1 Source of Sludge

The primary and thickened waste activated sludge employed in this study was obtained from the Robert O. Pickard Environmental Centre (ROPEC) in Ottawa, Ontario, Canada. Conventional activated sludge process at ROPEC operates at 3-5 days SRT to generate the Thickened Waste Activated Sludge (TWAS) with total solid concentration of approximately 5-6%. The sludge was generally received once a week and stored at 4°C in closed container. A 1:1 (v/v) mixture of primary sludge and TWAS was mixed in the feed buckets prior to use, while the remainder of the sludge was kept at 4°C.

3.2 Digester Design

The experiments were carried out with cylindrical PVC digesters that each had a capacity of 20 L. The digester contents were mixed by an externally mounted variable-speed motor. The digesters were heated with electrical heat tracing that was controlled by a local temperature controller. The mixing system was sealed with oil seals to maintain anaerobic conditions. The shafts were rotated at a speed of approximately 40 rpm. The gas produced during the digestion was collected in a wet-tip gas meter with a separate gas collection system connected to each individual digestion unit. A diagram of the experimental apparatus is shown in Figure 3-1. Each digester had two sampling ports for both withdrawing and feeding. Another port on each digester was located in the headspace for gas collection and analysis.

In each feeding sequence a sample of digested sludge was withdrawn from the bottom of the digester after recording the volume of gas produced. As the sample of digested sludge was withdrawn, the gas that was produced and collected in the tedlar bag replaced the volume in the digester. Feed sludge was added by gravity through the feed port in top of the digester. After addition of the feed material, the gas was displaced into tedlar bags

Figure 3-1: Schematic Drawing of Lab Digesters.

3.3 Start up Procedure and Operation

The start up of the digesters was rapid with 15 L of active mesophilic digested sludge from ROPEC employed as an inoculum and 5 L of 1:1 (v/v) mix of primary sludge and TWAS. After the feed and digested sludge (inoculum) were placed in the laboratory digesters the temperature was rapidly raised to the target temperature (Ahrling, 1994). No feed sludge was added to the digester for a period of 2-4 day while the

performance of the digesters was monitored for both process stability (pH, VFA, alkalinity, and gas production) and solid reduction parameters. The target volume of feed sludge was added to the digester as gas production increased and the VFA concentration level dropped to below 1000 mg/L. Each digester was fed once daily as required during start-up and during steady state operation. The specified volume of sludge was fed each day to each digester to maintain the target HRT. The steady state operation period was defined as the period following three detention times of operation at the given operational conditions.

3.4 Experimental Plan

This section addresses the bench-scale screening study that was performed to identify the most promising alternative for future investigation where facilities have excess heating capacity for their existing digesters. The effects of temperature, staging, and HRT on anaerobic digestion performance were examined. A simultaneous evaluation of all these parameters in combination was not possible; hence the experiments were divided into the following components.

3.4.1 Single Stage Temperature-HRT studies

In this study, five bench scale anaerobic digesters were employed in parallel. Five operating temperatures that spanned the range of operating temperatures from mesophilic to thermophilic conditions were studied. The digesters were operated at conventional mesophilic (35°C) and thermophilic (55°C, 62°C) temperatures as well as temperatures between these endpoints (42°C, 49°C). HRTs of 20, 15 and 5 days, that represent respectively typical, minimum and potential acid-phase digester values for this operating parameter were evaluated at these temperatures.

3.4.2 Staging of Digesters

It is known that the operation of completely mixed reactors in series will approach plug flow conditions and thereby limit the effects of short-circuiting, that may result in the bleed-through of pathogens in a single, completely mixed reactor. It was hypothesized that an additional benefit of reactors in series operation would be an increase in the resilience of the system.

The part of this study focused on a comparison between two stage and three stage reactors in series and discussed performance with respect to solids destruction and pathogen reduction. The temperature configurations that were tested in this study are summarized in Figure 3-2.

The total HRT during the staging study was 15 days with HRTs of 5 days for the first stage and 10 days in the second stage of the two stage systems and 5 days for each of the stages of the three stage configurations. Each reactor had a working volume of 20 L and was operated at a constant temperature that ranged from 35°C to 49°C according Figure 3-2. In identifying potential temperature sequences, it was decided that mesophilic digesters would not be employed in front of thermophilic digesters.

Two stage configurations

Three stage configurations

Figure 3-2: Reactor Configurations and Temperatures.

This is because the mesophilic digester placed down stream can minimize disadvantages such as odors commonly associated with the effluent from thermophilic units (Metcalf and Eddy, 2003).

From Figure 3-2 it can be seen that four two stage and five three stage configurations were examined. The performances of these sequences were compared against a single stage control that operated at 35°C. The temperatures of the digesters were rapidly raised to the target temperatures prior to evaluating each series operation. The maximum temperatures considered for first and second stage of this study were 49°C and 42°C respectively, as this operating temperature may result in lower odors generated, and provide better volatile solids destruction than mesophilic digester.

The performance of two and three stage operation was assessed through regular monitoring for process stability and solid reduction parameters. These parameters are further described in Section 3.5. In summary these tests evaluated the impact of staging and the impact of differing levels of heat/energy availability in the two and three stage operation and various temperature regimes on staged operation of anaerobic digestion.

3.5 Sampling Plan

Table 3-1 shows the sampling frequency of each parameter during start-up and once steady state conditions were established for each operating conditions. During start up periods regular monitoring was performed to characterize process stability and volatile solid reduction. The pH, VFA's and alkalinity of the effluent of each digester was measured to monitor the digester stability.

Table 3-1: Frequency of Sampling of Analytical Parameters.

Parameters	Sampling frequency
Temperature	Daily
pH	Daily
Gas production	Daily
Biogas quality	Three times a week
Alkalinity	Three times a week
Volatile organic acids	Three times a week
Individual organic acids	Seven samples during steady state
TCOD & S COD	Seven samples during steady state
Capillary Suction Time (CST)	Three times a week
Solid analysis (TS, VS, TSS, and VSS)	Three times a week
Ammonia –nitrogen	Three times a week
TKN	Seven samples during steady state

Total solids (TS) and volatile solids (VS) removals, gas production, biogas composition (methane and carbon dioxide content), TCOD and SCOD were tested as the primary indicators of solid reduction. After each system reached steady state, samples were taken from the influent and effluent of each digester on seven different days and were analyzed for TS, VS, TCOD, SCOD, alkalinity, total and individual organic acids, gas production and composition. The effluent from the digesters was also analyzed for CST and nutrients

such as ammonia nitrogen and TKN to compare the performance of each digester in terms of effluent quality. All measurements were determined in duplicate.

3.6 Physical and Chemical Analyses

The raw sludge was thoroughly mixed prior to analysis. The following physical and chemical analyses were performed in this study:

3.6.1 Temperature

The temperature of the anaerobically digested sludge samples was determined immediately after collection during each experiment using an online temperature probe.

3.6.2 pH

The pH of the anaerobically digested sludge was determined immediately after sample collection. A pH meter (Corning Model 340 with a glass electrode) was used for the analysis.

3.6.3 Total and Volatile Solids

The TS and VS concentrations of the sludge were determined by placing a known volume of each sludge type in a porcelain dish and following the procedures established in the Standard Methods (APHA, 1995). TS were those remaining after the water was evaporated by heating the sludge sample at 103°C overnight. After the TS were determined, the dried residue was placed in a 550°C furnace for sixty minutes to burn off the VS. The ash was cooled in a desiccator before weighing.

3.6.4 Total and Volatile Suspended Solids

TSS and VSS concentrations were measured according to Standard Methods (APHA, 1995). Whatman glass fiber filter paper with a pore size of 0.45µm was used to filter TSS and VSS samples.

3.6.5 Alkalinity and Volatile Acids

The concentrations of alkalinity and volatile acids were determined by titration. A 50-ml portion of the supernatant that had been separated from the anaerobically digested sludges by centrifuging at 3,800 rpm for 15 minutes was used for this analysis. The alkalinity and total fatty acids were estimated by titration of the liquid fraction of the sludge sample with 0.1 N sulphuric acid according to the technique of Anderson and Yang (1992). The alkalinity and organic acids were reported as the concentration in milligrams per liter (mg/l) as bicarbonate (HCO_3^-) and acetic acid, respectively.

3.6.6 Individual Organic Acids

Individual organic acids were determined in membrane-filtered samples by ion chromatography. An ion chromatograph (Lachat Instrument, USA) that was equipped with a 7.5 x 500mm Lachat Instrument column (part #28070) was employed. The ion chromatograph was operated at an eluent flow of 0.5 ml/min and the eluent consisted of 0.8 mM sulphuric acid.

Samples were taken from the lab digesters through the sampling ports and centrifuged at 15,000 rpm for 45-60 minutes. Five mL of centrate was filtered through a 0.22 μm glass fiber filter into an auto sampler vial, and then acidified with 100 μL of 10 N H_2SO_4 (This was found to bring the pH of the samples to ≤ 3). The samples were then stored at 4°C until analysis. Samples were not stored more than three days.

3.6.7 Chemical Oxygen Demand

The chemical oxygen demand of digested and feed sludge was measured according to Standard Methods (APHA, 1995). Samples were mixed in a vortex mixer

prior to removing 2 mL with a 5-mL disposable pipet. Samples were diluted 20 to 50 times before adding 2 mLs of diluted sample to the COD vials for digestion on the heating block (150°C for 2 hours). After digestion, the samples were removed from the heating block and cooled to room temperature and then read on a Perkin-Elmer 2380 spectrophotometer at 600 nm. The spectrophotometer was calibrated with standards that were generated from a potassium hydrogen phthalate (KHP) solution.

Soluble COD was measured in the same manner as total COD, except samples were centrifuged at 15,000 rpm for 20 minutes, then filtered through a 0.45 µm filter before analysis.

3.6.8 Gas Production and Biogas Composition (CH₄, CO₂)

The volume of biogas was measured every day using a wet-tip gas meter. The biogas composition was measured at least three times a week. A volume of 0.5 ml of biogas that was withdrawn from the gas sampling ports was injected into a gas chromatograph model SRI 8610C which was equipped with 0.9 m X 0.32 cm spherocarb column model SRI (1001120) and a thermal conductivity detector for biogas composition analysis. Helium was used as the carrier gas and the column was operated at a temperature of 99°C. Calibration curves were created by multiple injections of a standard gas containing CH₄ and CO₂.

3.6.8 Ammonia Nitrogen (NH₃-N)

The ammonia nitrogen of digested and feed sludge samples were measured according to Standard Methods (APHA, 1995). A VWR Symphony NH₃ probe and Corning 340 meter were used for the analysis. The pH of the samples was first adjusted to greater than 12 by adding 1 mL of 10 N NaOH to 50 mL of sample. The sample was

tested immediately while being mixed by a magnetic stirrer. Ammonium chloride was used as a standard to prepare calibration curves. The values were measured as mV and were converted to concentrations using the calibration curve.

3.6.9 Dewaterability

Dewaterability of the digested sludge was measured by an OFITE model 294-00 capillary suction timer and was expressed as seconds. Whatman No. 17 chromatography grade filter paper was used in dewaterability analysis.

3.7 Bacteriological Analysis

In this study the behavior of microbial indicators during steady state periods was examined. During steady state, grab samples were taken from the influent and effluent of each digester on seven different days and were tested for bacteriological analysis. The microbial indicators investigated in this study consisted of:

- Coliform group; The FC group and *E.coli* (which is one species of bacteria in the fecal group) were used as indicator organisms. The sole use of *E.coli* as an indicator of fecal pollution has been proposed (Bitton, 1994), since it can be easily distinguished from other members of the FC group. FC display a survival pattern similar to that of bacterial pathogens, but their usefulness as indicators of protozoan or viral contamination is limited (Bitton, 1994). FC are commonly tested as part of U.S. EPA requirements for biosolids classification.
- Fecal streptococci; Members of this group persist but do not reproduce well in the environment. A subgroup of the FS group, the enterococci, has been suggested as useful for indicating the presence of viruses (Bitton, 1994). In this study, the behaviour of fecal streptococci as an alternative indicator was compared with

fecal coliform to assess its reliability as a microbial indicator during anaerobic digestion process.

- *Salmonella*; *Salmonella* are enterobacteriaceae that are widely distributed in the environment and include more than 2,000 serotypes. They are the most predominant pathogenic bacteria in wastewater and they cause typhoid and paratyphoid fever and gastroenteritis. *Salmonella* are commonly tested as part of the USEPA Class A biosolids requirements.
- *Clostridium perfringens*; This microorganism is an anaerobic gram positive, spore forming, rod-shaped bacterium that produces spores that are quite resistant to environmental stresses and to disinfection processes. In this study, the behavior of *C. perfringens* was assessed as this group forms highly resistant endospores that can be expected to survive for long periods under the extreme operational conditions.

3.7.1 Collection of Samples

Samples from of the digester feeds and effluents were collected two times per week during steady state operation. The bacteriological analysis of samples was performed at the Centre for Environmental Microbiology that is located at the University of Ottawa. The digester samples were collected in 50-mL sterilized tubes and stored at 4°C prior to microbial analysis. The pH of the tested culture media was adjusted to 7.5 ± 0.2 at 25 °C with 1 N HCl solution.

3.7.2 Glassware and Culture Media Preparation

Sampling bottles, pipettes, dilution water and all of the culture media were autoclaved at 121°C and 15 psi for 15 minutes. All Petri dishes used in the bacteriological

analyses were pre-sterilized and constructed of disposable plastic. The culture media that were employed for the microbial analyses were prepared according to the manufacturers protocols.

3.7.3 Sample Dilutions

Preliminary tests were conducted to determine the dilution factors for enumeration of specific microorganism and to verify the test procedures. The procedure consisted of diluting a series of the sludge sample with sterile phosphate buffer dilution water. The samples were then mixed at 4°C on a shaker (200rpm) for 15 min. Following shaking, the samples was filtered through a membrane filter according to Standard Methods (APHA, 1995), and then subsequently plated for enumeration on various agar media. After the agar was incubated for 24 to 48 hrs, a plate which had between 30 and 100 colonies was selected for counting and were reported as log colony forming unit (CFU) per gram dry weight of sludge samples.

3.7.4 Method Detection Limit

The Method Detection Limit (MDL) is the lowest detectable concentration of microorganisms that can be measured with greater than 90 % recovery from specific medium. This is important because it can never be determined if a pollutant was not present, only that it was not detected. Detection limits for microorganisms evaluated in this study are noted in Table 3-2.

Serial dilution of specific microorganism was prepared to make a concentration near (within one to five times) the expected detection limit. Seven or more replicates of this sample were analyzed and the MDL was determined using Student t-test at the 99% confidence level. Reporting limits take into account the sample size, matrix effects, and any dilution factors.

Table 3-2: Method Detection Limit for Biological Parameters.

Parameter	Method	Units	Detection Limit
Fecal coliform	Membrane Filtration	CFU/100ml	1000
<i>E.coli</i>	Membrane Filtration	CFU/100ml	1000
Fecal streptococci	Membrane Filtration	CFU/100ml	100
<i>Salmonella</i>	Membrane Filtration	CFU/100ml	100
<i>C. perfringens</i>	Membrane Filtration	CFU/100ml	1000

3.8 Analytical Procedures

3.8.1 Fecal Coliform

The fecal coliform tests were performed using the membrane filtration procedure (APHA, 1995). A 5-10 ml sample of diluted sludge sample was filtered through a 0.45µm membrane filter using standard techniques. The filter was then transferred to a 50-mm petri plate containing M-FC agar (Difco, BD Biosciences, Canada) and incubated at 42°C ± 0.5°C for 24 hours for enumeration of fecal coliform. The fecal coliform bacteria would appear; as blue colonies.

3.8.2 *E.coli*

The membrane filtration method provides a direct count of *E.coli* in sludge based on the development of colonies that grow on the surface of a membrane filter. A diluted sludge sample was filtered through the membrane, which retained the bacteria. After filtration, the membrane containing the bacteria was placed on a modified mFC basal media with BCIG that is a selective and differential medium, (Difco, BD Biosciences, Canada). The samples were then incubated at 35 ± 0.5°C for 24 h. The target colonies on modified agar were a blue color after the incubation

3.8.3 Fecal Streptococci

The MF test was utilized for assaying the fecal streptococci. After filtration, the membrane retaining the bacteria was placed on a selective KF Agar (Difco, BD Biosciences, Canada) and incubated for 48hrs at $35\pm 0.5^{\circ}\text{C}$. Pink and red colonies were counted as *Streptococcus*.

3.8.4 *Salmonella* spp.

Appropriate volumes of sludge were filtered through a $0.45\ \mu\text{m}$ gridded sterile membrane to give 30-100 colonies on the membrane surface. The filter was transferred to a petri dish with SS agar (Difco, BD Biosciences, Canada) medium. The culture plates were inverted and incubated at $37\pm 0.5^{\circ}\text{C}$ for 24 hrs. Colonies of *Salmonella* had a black centre. Confirmation that the observed colonies were *Salmonella* spp. was achieved by examining typical colonies using test method API 20E (Biomérieux 2010).

3.8.5 *Clostridium perfringens*

A volume of sludge sample was filtered and the membrane filter ($0.45\ \mu\text{m}$) placed on the surface of SPS agar (Merck Laboratories, Germany) containing sulphite and iron (III). The agar medium was then incubated in anaerobic chamber at 37°C . *Clostridium perfringens* usually produce black colonies as a result of the reduction of sulphite to sulphide which reacts with the iron (III) salt. In this test both spores and vegetative *Clostridium perfringens* were grown on the SPS agar.

3.9 Quality Controls

The microbiological analytical procedure was assessed for quality control. Each of the culture media was tested with appropriate reference strains of target bacteria (positive control) and non-target bacteria (negative control). The control microorganisms

utilized in the examinations of indicator bacteria were provided by the Centre for Research on Environmental Microbiology, University of Ottawa, Ontario, Canada, and are shown in Table 3-3.

Table 3-3: Control Microorganisms for Microbiological Tests.

Group	Control Microorganisms	
	Positive	Negative
Faecal streptococci	<i>Enterococcus faecalis</i>	<i>Escherichia coli</i>
Fecal coliforms	<i>Escherichia coli</i>	<i>Streptococcus faecalis</i>
<i>Salmonella</i>	<i>Salmonella typhimurium</i>	<i>Escherichia coli</i>
<i>Escherichia coli</i>	<i>Escherichia coli</i>	<i>Salmonella typhimurium</i>
<i>Clostridium perfringens</i>	<i>Clostridium sporogenes</i>	<i>Escherichia coli</i>

3.10 Fatty Acid Disinfection Study

The reduction in pathogens in anaerobic digestion technologies is generally attributed to the impact of their exposure to elevated temperatures. However, many of the digestion technologies expose the pathogenic bacteria to high concentrations of organic acids. While the impacts of exposure time and temperature on pathogen removal have been documented to a great extent, little attention has been paid to the presence of other antimicrobial agents such as organic acids. In addition, the impact of organic acids on pathogens has been examined at mostly mesophilic temperatures (Abdul and Lloyd, 1985; Kunte *et al.*, 2000) and their impact at elevated temperatures has received less attention.

In this study the organic acids of interest were dosed into sludges that were produced by anaerobic digestion of municipal wastewater treatment plant sludges. Acetic acid, propionic acid, butyric acid or a mixture of the three acids were employed in this study as the major volatile fatty acids which are produced through anaerobic digestion of

organic matter. The series of bench-scale anaerobic digesters described in section 3.4.1 provided the digested sludges. The target temperatures for the digesters were 35°C, 42°C, 49°C, and 55°C respectively. The digesters were operated at a 15 day HRT and were fed daily.

Samples collected from the bench scale digesters were transferred to 100 ml sterile flasks that had been flushed with nitrogen gas for 3 minutes to remove oxygen. The treatment flasks contained either acetic acid, propionic acid, butyric acid or a mixture of the three acids which were diluted in deionized water while control flasks contained no added acid. An equimolar solution was employed for the experiments that employed mixed acids to evaluate the inhibitory effect of different types of organic acids on bacterial inactivation.

The treatments that were evaluated in the first phase of the study consisted of dosing the digester effluents with either 750, 1500, 3000 or 6000 mg/L of either acetic, propionic or butyric acids or a mixture of the three fatty acids. Volatile fatty acid concentrations of 6000 mg/L and 750 mg/L, represent typical concentration of organic acid in acid phased digestion and conventional anaerobic digester where operated at elevated temperature. Each condition was assessed in duplicate.

In the first phase of the experiments the pH of the flasks was adjusted to 5.5 using a 1 M hydrochloric acid solution. The samples were then incubated at either 35°C, 42°C, 49°C, or 55°C for a period of 24 hours. The incubation temperature for a sample was the same temperature as that of the digester from which it was obtained. Prior to sampling the flasks at incubation times of 0, 4, 10 and 24 hours they were stirred thoroughly for 15

seconds. Samples that were withdrawn from the flasks were characterized with respect to total volatile organic acids, pH, *C. perfringens* and *Salmonella* spp. concentrations.

In the second phase of the study the impact of varying pH from 4.5 to 7.5 while dosing with a mixture containing 750 mg/L of an equimolar mixture of acetic, propionic, and butyric acids on inactivation of *C. perfringens*, and *Salmonella* spp. was examined. All test conditions that were evaluated in this phase were conducted in duplicate and the flasks were sampled at incubation times of 0, 12 and 24 hours. Samples that were withdrawn from the flasks were characterized with respect to total volatile organic acids, pH, *C. perfringens* and *Salmonella* spp. concentrations.

3.11 Microbial Community Study

Despite considerable interest in the microbiology of the anaerobic digestion processes, relatively little is known about the relationship between the structure of the microbial consortia and the functional characteristics of the anaerobic digesters. Our knowledge of microbial diversity and its role in anaerobic digestion process has been limited by the constraints of traditional microbiological techniques as only 0.1 – 1% of the microbial population is cultivable (Bitton, 1994).

The approach presented in this part of the study involved the use of PCR-based gradient gel electrophoresis for the generation of 16S-rDNA and subsequent image analysis as a novel strategy to study microbial diversity during staged operation of anaerobic digestion of sewage sludge at mesophilic operating temperature.

3.11.1 Temperature Configuration

The temperature history and flow configurations that were tested in this study are summarized in Table 3-4. The significance of the prior operation will be subsequently discussed in chapter 6.

Table 3-4: Temperature Profiles of Digestion Systems.

Parameter	Single-stage	Two-stage (System III and IV)		Three-stage (Systems I and II)		
Reactor		R1	R2	R1	R2	R3
HRT	15	5	10	5	5	5
Temperature (°C)	35	35	35 (III)	35	35	35 (I)
		42	35 (IV)	42	35	35 (II)
Previous operating temperature		49	35 (III)	55	49	42 (I)
		35	35 (IV)	62	42	35 (II)

3.11.2 DNA Extraction

Samples for analysis of microbial diversity were placed in sterile polypropylene tubes, and stored at -20°C until DNA extraction was performed. DNA from the sludge samples was obtained after removing the potential PCR inhibitors (Ammonia, VFA), and ethanol precipitation using a previously described protocol (Purohit *et al.*, 2003). The DNA extraction protocol is summarized as follows,

1. Sludge samples with a mass of 10 mg on a wet basis were placed in a 1.5 ml Eppendorf tube.
2. Centrifuge at 2500xg for 10 min and wash pellet twice with double distilled water
3. Suspend pellet in 250 µl 50 mM Tris with pH 9.0 and incubate at 50°C for 15 min. Vortex regularly.
4. Repeat step 2.
5. Resuspend pellet in 200 µl 0.01% Tween 20 solution and incubate at 50°C for 15 min.

6. Centrifuge at 12000 g for 5 min and resuspend pellet in 200 μ l 0.01% Tween 20 solution; add 300 μ l acetone and 1200 μ l petroleum ether.
7. Agitate on a rocking platform for 15 min.
8. Centrifuge at 12000 g for 15 min.
9. Discard both layers of the supernatant and repeat step 2 with the pellet.
10. Suspend pellet in 50 μ l double distilled water and add 50 μ l of 1 N NaOH.
11. Incubate at room temperature for 30 min.
12. Neutralize by addition of 100 μ l of 1 M Tris buffer, pH 7.5.
13. Make up the volume to 1 ml and use 5 μ l as template for PCR.

3.11.3 Polymerase Chain Reaction (PCR) Amplification of DNA

A volume of 5 μ l of extracted DNA was used as the template in the PCR-reaction mixtures that contained 2.5 μ L 10x PCR buffer, 4.0 μ L deoxyribonucleotide triphosphate (dNTP), 1.5 μ L $MgCl_2$, 0.4 μ L GMSF-GC forward primer, 0.4 μ L 534R reverse primer, 5 μ L dimethyl sulphoxide (DMSO), 0.5 μ L *Taq* DNA polymerase, and 5.7 μ l deionized water to produce a final volume of 25 μ l. All of the materials used in PCR amplification were obtained from Amersham Pharmacia, Biotech, USA. PCR amplification was performed on a PTC-225 Peltier Thermal Cycler (MJ, Research, Weltham, MA, USA). The amplification temperature program was carried out with a denaturation step at 95°C for 1 min, followed by 33 cycles of denaturation at 95°C for 40 sec, annealing at 54°C for 45 sec, and elongation at 64 for 45 sec. The final amplification was conducted for 2 min at 72°C. Amplification of DNA was verified by electrophoresis of 2 μ l of the PCR product through a 1% agarose gel in 1x TAE buffer (0.04 M Tris base, 0.02 M Sodium Acetate, 1.0 mM EDTA, pH 8.0). All reagents were of molecular biology grade.

3.11.4 Denaturing Gradient Gel Electrophoresis (DGGE)

Denaturing gradient gel electrophoresis (DGGE) can be used to resolve PCR-amplified 16S rDNA fragments by electrophoresis through an acrylamide gel that contains an increasing linear gradient of denaturants (Muyzer *et al.*, 1993). After

staining, each band on the denaturing gradient gel represents a specific 16S rDNA sequence from a specific microbial species. Thus, the species can be identified by the band positions on the gel, and their concentrations correspond to the band intensities.

DGGE was performed using a D-Gene system (Bio-Rad) according to the manufacturer's instructions. Each PCR-amplified sample (10 μ l) was added to 3 μ l of loading buffer (0.5 g/L bromophenol blue, 0.5 g/L xylene cyanol, 70 % glycerol in dH₂O) and loaded onto a 6% acrylamide gel. All DGGE gels were poured with 6% acrylamide in 1X TAE buffer, mixed with 40% denaturant (2.8 M urea and 18.7% (w/v) formamide) and 60% denaturant (4.2 M urea, and 24% formamide). The gels were polymerized by addition of 1% ammonium persulfate and 0.1% N,N,N',N'-Tetramethylethylenediamine (TEMED). Gel was run in a 1X TAE buffer solution for 5 minutes at 220 V and 14 hours at 37V. The gels were developed by silver staining (Blument *et al.*, 1987) and scanned using a Hewlett Packard ScanJet 11CX.

3.11.5 Image Analyses

The DGGE gels were scanned and analyzed by LabImage software version 2.7. The LabImage software was used to digitize those bands that were too faint to be recognized visually. The band position and intensities in the DGGE profile were acquired and corresponded to individual microbial identities and relative quantities. The similarity among various samples was calculated using similarity coefficients of bands common to two samples. The working definition was that two bands were common if they migrated the same distance on a gel. The total number of bands was determined for each of the samples that were compared. Then each sample was scored based on the presence or absence of each band in its profile when compared to the profile of each of the other

samples. For each sample, the presence of a band was marked as 1 and its absence was marked by 0. The data were input as a binary matrix and Sorensen's index of similarity (Krebs, 1978) were calculated using Equation 3-1 in a spreadsheet analysis.

$$C_s = 2j/(a + b) \quad (3-1)$$

Where C_s is Sorensen's index of similarity, a is the number of bands in Sample A, b is the number of bands in Sample B, and j is the number of bands common to A and B.

CHAPTER 4

PERFORMANCE EVALUATION OF SINGLE AND STAGED ANAEROBIC DIGESTION OPERATION

The following chapter presents and discusses the results of the laboratory experiments described in chapter 3. This chapter describes the feed sludge characteristics, followed by performance results for the following investigations;

- **Single Stage Temperature-HRT studies;** evaluation of the effect of elevated operating temperatures and HRT on digester performance and pathogen reduction.
- **Staging of Digesters;** evaluation of digester performance in terms of conventional parameters and pathogen reduction in digesters that are in a series configuration.

4.1 Feed Characteristics

As mentioned in section 3.1, a combination of primary sludge and TWAS with volumetric ratio of 1:1 that was collected from the Robert O. Pickard Environmental Centre (ROPEC) in Ottawa was used throughout this study to feed the laboratory digesters. The feed sludge was collected every 2-5 days from a sampling port off the primary sludge and TWAS feed lines to the blending tank and stored in 20-L containers at 4°C. The general composition of the feed sludge over the steady state period of each phase of this experimentation for the two year period that started March 10, 2003 and ended March 1, 2005 is shown in Table 4-1. A summary of the operating phases of this study will be subsequently described. The average feed TS concentration was 4.9% and the average VS concentration was 3.7%.

The variation in solid contents was relatively small as indicated by relatively low standard deviation of about 7 % to 10 % of the mean average for both TS and VS values. However, some variability was introduced into the data due to the inherent variability in the sludges that were generated at the plant.

Table 4-1: Feed Sludge Characteristics.

Parameter	Average	Standard Deviation	# of Observation
TS (mg/L)	48812	4702	88
VS (mg/L)	36670	2442	88
TKN (mg/L)	2583	333	54
SCOD (mg/L)	3609	1547	58
Ammonia-N (mg/L)	116	5.68	40
Average microbial density in feed sludge			
FC (log CFU/gTS)	6.43	0.45 ¹	74
<i>E.coli</i> (log CFU/gTS)	6.36	0.76	74
FS (log CFU/gTS)	6.79	0.33	66
<i>Salmonella</i> (log CFU/gTS)	5.00	0.80	74
<i>C. perfringens</i> (log CFU/gTS)	6.86	1.75	74

¹Standard deviation of log CFU/gTS of the bacterial concentration data

The ammonia nitrogen, SCOD, and TKN of the feed sludge was measured to obtain the organic nitrogen concentration as well as to assess biodegradability of the feed sludge. The ammonia nitrogen of the feed sludge was measured and subtracted from the feed sludge TKN to obtain the organic nitrogen concentration. From the Table 4-1 it can be concluded that a large portion of the total nitrogen was organic and could be considered as an estimate of protein concentration. For each operating condition, the feed and effluent from each digester was analyzed for microbial density on seven different days and were reported as log CFU /g TS of sludge samples. From Table 4-1 it can be seen that concentrations of FC, *E.coli*, FS, and *C. perfringens* were similar in feed samples.

However, the *Salmonella* spp. concentrations were somewhat smaller as compared to the other microorganisms in this study.

4.2 Performance Evaluation of Single, Two and Three Stage Anaerobic Digestion Process

In the following section, the performance of the single, two and three stage anaerobic digesters at steady state are compared. Operation after a time period of 3 HRTs is a conventional protocol to achieve steady state after any changes in operational parameters for completely-mixed reactors, since approximately 95% of the original solids will be removed by 3 HRTs. For operation at HRT's of 20 and 15 days, 3 HRT's of operating time was used before collecting steady state data, but for HRT's of 10 and 5 days, the steady state period was achieved between 2 and 3 HRTs. In this study, the stability of pH, gas production, methane biogas content and VFA concentration were used as indicators of steady state performance (i.e. a variation less than approximately 10%). The periods classified as steady state are shown in Table 4-2, and 4-3. Any computations that required feed sludge concentrations employed the values that were measured during the specific phase of testing that was being studied.

Table 4-2: Summary of Operating Phases of Single Stage Temperature-HRT Studies.

HRT, (d)	Single -stage system (°C)					Steady state data period (days)	Start date for steady state	End date for steady state
	I	II	III	IV	V			
20	35	42	49	55	62	36	March 1, 2003	April 5, 2003
15	35	42	49	55	62	17	Aug. 12, 2003	Aug. 28, 2003
5	35	42	49	55	62	35	Nov. 24, 2003	Dec. 28, 2003

Table 4-3: Summary of Operating Phases of Multistage Studies.

Operating Phase	Multistage system (°C)				Steady state data period (days)	Start date for steady state	End date for steady state
	SI	SII	SIII	SIV			
I	35-35-35	42-35-35	35-35	-	58	March 30, 2004	May 26, 2004
II	42-42-35	49-35-35	42-35	-	52	Oct. 10, 2004	Nov.29, 2004
III	49-35-35	42-42	49-35	35	30	Jan. 27, 2005	Feb. 25, 2005

Notes;

Phase I included two parallel three stage set ups and one two stage setup.

Phase II included two parallel three stage set ups and one two stage setup.

Phase III included one three stage set up and two parallel two stages set ups versus a single stage setup.

4.2.1 Digester Solid Analysis

Solids reduction in each of the systems was calculated based on a material balance of the amount of solids entering and leaving the system per day at steady state condition. This assumes that daily flows are steady, reasonably uniform, and that the digester volume and composition does not vary substantially on a daily basis (U.S EPA, 1999). These were reasonable assumptions to apply to this experiment.

4.2.1.1 Single Stage Temperature- HRT Studies

Table 4-4 shows the concentrations of TS and TSS present in the feed sludge and the effluent from the single stage anaerobic digester for the various temperatures and retention times after reaching steady state.

Table 4-4: Average Total Solids (TS) and Total Suspended Solids (TSS) Concentration. (Standard deviations in parentheses)

HRT (d)	Feed	Temperature				
		35°C	42°C	49°C	55°C	62°C
20						
TS (mg/l)	49000 (2200)	30000 (400)	29000 (300)	29000 (500)	30000 (700)	29000 (600)
TSS (mg/l)	47000 (1500)	29000 (300)	28000 (300)	27000 (700)	27000 (800)	27000 (800)
15						
TS (mg/l)	48000 (1700)	35000 (2300)	31000 (3000)	31000 (2200)	33000 (1200)	39000 (1600)
TSS (mg/l)	44000 (1500)	33000 (3100)	30000 (2500)	30000 (2400)	32000 (1100)	38000 (1400)
5						
TS (mg/l)	50000 (1800)	34000 (1100)	34000 (900)	31000 (1800)	32000 (420)	34000 (860)
TSS (mg/l)	49000 (1100)	33000 (1400)	33000 (1000)	31000 (1300)	30000 (850)	30000 (800)

The TS concentrations in the feed sludge were about 4.9% for all the tested HRT's. The feed TSS concentration averaged 4.7% and varied from 4.4 to 4.8 %. From Table 4-4 it can be seen that as the temperature was increased from 35°C to 49°C for the 15 and 20 day HRT's, there was a gradual decrease in solids concentration. This was followed by an increase from 55°C to 62°C. As the retention time was decreased to 5

days, different trends were observed for TS and TSS concentrations. The TS concentrations increased from 3.3% to 3.4% with an increase in temperature from 35°C to 42°C. Operation of an anaerobic digester at 49°C resulted in an 8.8% decrease in the TS concentration and a further increase of 3.3% for the digester that was operated at 62°C. As the temperature increased from 35°C to 62°C, the TSS concentration gradually decreased over the course of the study. The variation in the feed solids content was attributed to seasonal variations in wastewater quality as well as variability in the WWTP operation.

The VS and VSS level in the investigated digesters were further evaluated as two principal factors in design and sizing of the anaerobic digesters as well as to predict the mass of solids for disposal. The average VS and VSS removal efficiencies of the five lab scale digesters at steady state are summarised in Figure 4-1. The error bars in this study were calculated by dividing the standard deviation by the square root of number of measurements that make up the mean ($N=14$). From Figure 4-1 it can be seen that the VS and VSS removals varied with temperature and HRT. With the exception of the digester operated at 55°C, the VS and VSS removals for the 20 day HRT were higher than that of the 5 and 15 day HRT' by an average of 20 and 22 %, respectively. The reduced removal of VS solids was expected for the shorter HRTs.

(a). Volatile Solids

(b). Volatile Suspended Solids

Figure 4-1: Impact of HRT and Temperature on Solids Removal (a) Volatile Solids and (b) Volatile Suspended Solids.

The VS and VSS removals for the 55°C digester were essentially independent of HRT. The absence of an impact of HRT on VS reduction in this digester would suggest that its performance was not kinetically limited. Kinetics are generally considered to be highest

for thermophilic digestion and 55°C is in the range for optimal thermophilic digestion. The solids destruction kinetics were evaluated in this study and will be subsequently described.

At the 15 day HRT, the digesters that achieved the greatest VS and VSS reduction were operating at 35-55°C. An operating temperature of 49°C is considered to be near the lower extreme for thermophilic operation while 42°C is at the upper extreme for mesophilic operation. The successful operation of the digesters at 42 and 49°C at an HRT of 15 days is interesting in that these temperatures are rarely observed in practice and are often believed to be unstable. At the 15 day HRT the VS and VSS destruction efficiencies ranged from 30% at the 62°C to 50% at the 35°C and 42°C, VS and VSS destruction efficiencies appear to be similar for the 49°C digester.

The VS reduction for the single stage thermophilic and mesophilic digester with a 15 day HRT ranged from 48% to 52% which were similar to what was reported by Inman *et al.* (2005) and were lower than the 63% VSR reported by Moen *et al.* (2003). However, the feed total solids concentration was lower than the Moen *et al.* work (4.8% versus 5.9%). The difference could also be due to a higher proportion of waste activated sludge in the present study than in the Moen *et al.* study.

The successful operation of the digesters in this study might be attributed to the rapid start-up procedure that was employed. This start-up procedure forces the development of microbial cultures that are able to thrive at the operating temperature.

At the 5 day HRT the digesters that operated at 49° and 55°C achieved superior solids reduction. These temperatures span the range from low-thermophilic to mid-thermophilic operation.

The results suggest that at this reduced HRT traditional thermophilic operation is more effective in achieving reasonable VS reduction of about 48% which were similar to that reported by Inman *et al.* (2005). For the 15 day HRT the digester that was operating at 62°C was clearly stressed with respect to removal of VS and VSS when compared to the other operating temperatures. This operating temperature is generally considered to be an extreme value for thermophilic operation.

The performance of anaerobic digestion of municipal sewage sludge at elevated temperature versus mesophilic operation was evaluated in this study using a first order VS destruction model. The measured VSS destruction data were used to calculate equivalent first order rate coefficient for each digestion stage based on the CSTR mass balance as follows: The derivation of equation 4-1 is presented in Appendix E.

$$k\left(\frac{C}{C_0} - \frac{C^*}{C_0}\right) = \left(1 - \frac{C}{C_0}\right) / \theta \quad (4-1)$$

Where C is VSS concentration (mg/L) in the digester, C_0 is VSS concentration (mg/L) in the digester influent, C^* is nonbiodegradable VSS concentration (mg/L), and k and θ are the VSS utilization rate coefficient (d^{-1}) and the hydraulic retention time (day) of the single stage digester. First order rate coefficients were determined and listed in Table 4-5.

Table 4-5: Average First Order Rate Constant (d^{-1}). (Standard deviations in parentheses)

HRT (d)	Temperature				
	35°C	42°C	49°C	55°C	62°C
20	0.07 (0.01)	0.08 (0.01)	0.09 (0.01)	0.08 (0.01)	0.10 (0.04)
15	0.05 (0.02)	0.09 (0.02)	0.09 (0.04)	0.08 (0.01)	0.04 (0.02)
5	0.20 (0.06)	0.22 (0.07)	0.23 (0.08)	0.30 (0.04)	0.26 (0.09)

From Table 4-5 it can be seen that at 35°C and 62°C for 15 and 20 days HRT the decay coefficient of the single stage digester decreases with decreasing HRT, while for intermediate temperatures from 42°C to 55°C similar rate constants (approximately 0.09 d⁻¹) were observed. The fact that this coefficient varied with HRT challenges the notion that a first order equation adequately models the system. At the 5 day HRT a different trend was observed. Firstly the decay coefficients were higher when compared to decay coefficient for the previously described HRT's. Secondly as temperature increased from 35°C to 55°C, an increase in VSS destruction rate was observed from 0.20 d⁻¹ to 0.30 d⁻¹. Further temperature increase to 62°C reduced the destruction rate constant to 0.26 d⁻¹ as compared with 0.30 d⁻¹ at 55°C. In summary, based on these results it can be concluded that the first order model may be valid at HRT's of above 5 days and temperatures below 55°C. A two way ANOVA considering temperature and HRT as factors was performed to determine the significant impact of these two factors on decay coefficient. The results of ANOVA are presented in Appendix D, and show the main effects of both factors were statistically significant at the 95% confidence level.

4.2.1.2 Effect of Staging on Solids Reduction

In this portion of the study various temperature profiles in 2 and 3 stage digestion were evaluated with respect to solids reduction. The VS values presented in the two and three stage study results reflect the cumulative VS destruction that was observed as the sludge progressed through the individual reactors in each system.

Two Stage Operation

Table 4-6 shows the TS and TSS removal for the two stage system for the different temperature configurations. On average all the two stage systems with the first stage digester operated at either 42 or 49°C achieved relatively similar TS, and TSS removal. Operation of the first stage of the two stage system at 35°C resulted in 26% TS and TSS removal.

Table 4-6: Average Steady State TS and TSS Removal Efficiency. (Standard deviations in parentheses)

% of Removal	Temperature configuration			
	35°C-35°C	42°C-35°C	42°C-42°C	49°C-35°C
TS	26-39 (11-5)	37-41 (5-4)	36-42 (7-6)	36-43 (8-8)
TSS	26-39 (14-7)	36-41 (5-4)	34-41 (5-5)	39-45 (5-5)

A temperature increase in the first stage digester to 42°C and further to 49°C enhanced the rate of TS, and TSS removal by 27% and 36% respectively as compared to the 35°C digester in the first stage. Operation of the second stage digester at 35°C or 42°C resulted in average fractional removal of 42% in all the tested two stage systems in this study.

Figure 4-2 shows overall VS and VSS removal efficiencies during two stage operation of anaerobic digesters included in this experiment. From Figure 4-2, it can be seen that VS and VSS removal of all four system increased at elevated temperatures as compared to mesophilic (35°C) operation.

(a). Volatile Solids

(b). Volatile Suspended Solids

Figure 4-2: Solids Removal Versus Two Stage Temperature Configuration (a) Volatile Solids and (b) Volatile Suspended Solids.

At 35°C the first stage of the two stage system resulted in approximately 40% removal efficiency for both VS and VSS. As the temperature of the first stage digester increased to 42°C and 49°C the volatile solids destruction was 20% greater when compared to the

first stage digester that was operated at 35°C. Operation of the first stage at 42°C, or 49°C appears to show relatively similar VS and VSS removal in the first stage digester, therefore is possible that VS and VSS reduction was not affected by temperature increase above 42°C. Comparison of the tested systems showed that solids destruction were higher in the first stage than the second stage and this can be attributed to the reduction of available nutrient (substrate) in the second stage. Operation of the first stage at 42°C resulted in a lower VS destruction in the second stage of 5%. Temperature increase of the second stage digester from 35°C to 42°C increases the VS destruction rate by 4% as compared to the 35°C digester in the second stage. Performance of two stage system with the first stage at 49°C yielded VS destruction of 45% in the first stage followed by 15 % in the second stage. Similar VS removals were reported in a TPAD system that was fed with a mixture of primary and waste activated sludge at a 7.5 day thermophilic HRT and a 12.6 day mesophilic HRT (Vandenburg and Ellis, 2002).

At 49°C the volatile solids removal of 49% was achieved in the first digester and performance of the second stage was observed to be higher by 5% as compared to the second stage in the 42°C-35°C system. Performance of the second stage digester was observed to be function of both operational temperatures in the first and second stage, and availability of the nutrient in second stage digester. The results indicate that enhanced VS and VSS destruction could be achieved when the first stage is operated at an elevated temperature.

One way ANOVA and Tukey tests were applied on VS removal efficiency to evaluate the performance of the investigated treatment trends in this study. The results reveal that there were significant differences ($P=0.008$) in the VS removals between the

investigated treatment trains. The results of the statistical analysis are presented in Appendix D. The measured VSS destruction data were then used to calculate equivalent first order rate coefficient by employing equation (4-1) for each digester individually. First order rate coefficients are listed in Table 4-7.

Table 4-7: Calculated First Order Reaction Rate Coefficients' (d^{-1}). (Standard deviations in parentheses)

Digester	HRT, (d)	Temperature Configuration			
		35°C-35°C	42°C-35°C	42°C-42°C	49°C-35°C
1 st Series digester	5	0.27 (0.08)	0.24 (0.03)	0.27 (0.03)	0.27 (0.03)
2 nd Series digester	10	0.03 (0.01)	0.04 (0.02)	0.03 (0.01)	0.03 (0.01)
Control	15	0.11 (0.01)	0.11 (0.01)	0.11 (0.01)	0.11 (0.01)

¹Based on observed data

In this study temperature did not have a significant impact on the reaction rate coefficients. Mesophilic (35°C) operation of the first stage digester resulted reaction rate of $0.27 d^{-1}$, and temperature increase to 42°C and 49°C showed no significant changes in the reaction rate. From Table 4-7 it can be seen that the first order reaction rate coefficient drops significantly from the first series digester to the second series digester. This is contrary to the assumption made by Moen *et al*, (2003) which assumed that there is a mesophilic reaction rate coefficient and a thermophilic reaction rate coefficient which could be applied to either reactor stage. It is not surprising to see the reaction rate coefficient decrease through two stage operation, and this can be attributed to the reduction in nutrient availability levels and development of unique bacterial populations in the second stage digester. In this study 70% ultimate sludge biodegradability, was calculated based on the performance of best treatment trends with respect to VSS removal.

The detailed calculations for ultimate sludge biodegradability are presented in Appendix E. The results obtained in this study are comparable with what was reported by McCarthy *et al*, (2004).

McCarthy investigated two stage systems that were operated at 35°C with 18 days of HRT in each stage by assuming 80% ultimate sludge biodegradability. Most significantly, the coefficient of the first series reactor in both studies was about 0.2 d⁻¹ and decreases in the second stage to 0.03 d⁻¹ with increasing HRT. In both studies variation in HRT showed no impact on the first order coefficient. In conclusion, mesophilic operation of a two stage anaerobic digester (Figure 4-2) in series resulted a similar VS reduction to the single stage digester presented in Figure 4-1. However, operation of first stage of two stage system at elevated temperature enhanced the average VS destruction rate by 7 to 21%.

Three Stage Operation

The average steady state TS and TSS removal efficiencies of the three stage digester systems are summarized in Table 4-8 with standard deviations shown in parenthesis. On average operation of two and three stage anaerobic digester in series at 35°C showed similar overall TS and TSS removals of 40%. Operation of the first and the second stage of the three stage system at elevated temperature revealed relatively small improvement in overall TS and TSS removal as compared to the two stage system presented in Table 4-6 (i.e. 43 % for 42°C-35°C-35°C versus 40% for 42°C-35°C).

Table 4-8: Average Steady State TS and TSS Removal Efficiency. (Standard deviations in parentheses).

% of Removal	Temperature configuration				
	35°C-35°C-35°C	42°C-35°C-35°C	42°C-42°C-35°C	49°C-35°C-35°C	49°C-42°C-35°C
TS	30-34-40 (2-3-2)	32-37-42 (2-4-2)	37-39-44 (3-3-3)	36-40-47 (2-2-3)	35-43-48 (5-4-4)
TSS	29-34-39 (4-3-2)	33-39-41 (3-2-3)	31-38-43 (4-4-5)	36-40-45 (4-4-3)	35-41-47 (1-2-1)

Figure 4-3 shows VS and VSS removal efficiencies of the three stage digestion systems.

The values presented in Figure 4-3 reflect the cumulative VS destruction that was observed as the sludge progressed through the individual reactors in each system.

From Figure 4-3 it can be observed that each of the three stage systems demonstrated VS reductions that were greater than the single stage digester that achieved a VS reduction of 51%.

In the three stage systems, the overall removal appeared to increase with the temperature of the first stage of the system. System 35°C-35°C-35°C had an overall VS removal of approximately 55% while Systems 42°C-35°C-35°C and 42°C-42°C-35°C had VS removal efficiencies of approximately 59% and Systems 49°C-35°C-35°C and 49°C-42°C-35°C demonstrated removal efficiencies of approximately 66%.

(a). Volatile Solids

(b). Volatile Suspended Solids

Figure 4-3: Solids Removal Versus Two Stage Temperature Configuration (a) Volatile Solids and (b) Volatile Suspended Solids.

For Systems 42°C-35°C-35°C and 42°C-42°C-35°C and Systems 49°C-35°C-35°C and 49°C-42°C-35°C, the removal efficiencies in the first stages of these systems were calculated based on replicate samples from the same digesters collected approximately 3

months apart. Hence, the data presented for these reactors gives an indication of the reproducibility of the results over an extended period of time.

From Figure 4-3 it can be seen that the removal efficiencies in the first stage reactors of Systems 42°C-35°C-35°C and 42°C-42°C-35°C were virtually identical while the removal efficiencies in the first stage of System 49°C-35°C-35°C were slightly higher than that of System 49°C-42°C-35°C. The results suggest that the removal efficiencies that were observed in this study were sustainable for a significant period. The VS reduction results indicate that the impacts of an increased temperature in the first stage were not necessarily observed in the first stage of the three stage systems. Rather increased incremental reductions in VS were observed in the second and third stages of the systems. For example, the VS reductions observed in the first stage of Systems 42°C-35°C-35°C, 42°C-42°C-35°C and 49°C-35°C-35°C were virtually identical however, increased VS reductions were observed in the second and third stages of System 49°C-42°C-35°C despite the fact that these reactors were operated at similar temperatures to the second and third stages of Systems 42°C-35°C-35°C and 42°C-42°C-35°C. The results suggest that, while not responsible for reducing VS directly in the first stage, the elevated temperatures likely resulted in solids transformations (i.e. hydrolysis) that made the degradation products more amenable for digestion in subsequent stages.

Comparing Systems 42°C-35°C-35°C and 42°C-42°C-35°C and Systems 49°C-35°C-35°C and 49°C-42°C-35°C allowed an evaluation of the impact of an elevated temperature in the second stage on the performance of the three stage systems. From Figure 4-3 it can be seen that there was little difference between the overall performance of systems 42°C-35°C-35°C and 42°C-42°C-35°C or systems 49°C-35°C-35°C and

49°C-42°C-35°C with respect to VS reduction. The results suggest that if additional heat were available for elevating the temperature of digester systems it would be preferable to concentrate the heat input into the first stage digester and hence operate at a higher temperature (i.e. system 49°C-35°C-35°C) instead of distributing the heat over two digesters in series that each operated at lower temperatures (i.e. system 42°C-42°C-35°C). The underlying cause for the lack of effect of heating the second stage digesters is not apparent at this time. It may be due to differing microbial ecologies in the first and second stage digesters. The consortia that are present in the first stage of the three stage systems may be able to benefit from elevated temperature while those in the second stage are unable to do so. Additional research will be required to elucidate this response.

A one way ANOVA, in conjunction with a Tukey test was used to compare the VS removals of the five treatment trains in order, to determine the significance of the effect of temperature. The statistical analysis showed that there was a significant difference ($P=0.00$) in VS removals among the tested three stage treatment trains and the statistical results are presented in Appendix D. More detailed information on the performance of each individual stage in three stage operation can be achieved through performance evaluation of first order reduction rates calculated by employing equation (4-1) for each digester individually.

Table 4-9: Calculated First Order Reaction Rate Coefficients¹ (d⁻¹). (Standard deviations in parentheses)

Digester	HRT (d)	Temperature Configuration				
		35°C-35°C-35°C	42°C-35°C-35°C	42°C-42°C-35°C	49°C-35°C-35°C	49°C-42°C-35°C
1 st Series digester	5	0.25 (0.09)	0.24 (0.04)	0.24 (0.03)	0.26 (0.04)	0.25 (0.02)
2 nd Series digester	5	0.05 (0.01)	0.06 (0.02)	0.06 (0.02)	0.05 (0.02)	0.06 (0.02)
3 rd Series digester	5	0.02 (0.01)	0.03 (0.02)	0.04 (0.01)	0.04 (0.01)	0.05 (0.01)
Control	15	0.11 (0.01)	0.11 (0.01)	0.11 (0.01)	0.11 (0.01)	0.11 (0.01)

¹ Based on observed data

In general, from Table 4-9 it can be seen that the first order reaction rate coefficient drops significantly from the first series digester to the second series digester followed by further drops through third series digester of the tested system. Mesophilic (35°C) operation of the first stage digester exhibited reduction rate of 0.25 d⁻¹, and temperature increase to 42°C or 49°C showed no significant improvement in destruction rate coefficient for the tested three stage systems. Similar destruction rate coefficient was obtained over the course of two stage operation as reported in Table 4-7. Thorough review of the second stage digester rate reveals coefficients that the lowest reaction rate value of 0.05 d⁻¹ was achieved by systems that the second stage was operated at 35°C. Temperature increase in the second stage to 42°C enhanced the reaction rate to 0.06 d⁻¹ as compared to 0.05 d⁻¹ in second stage 35°C digester. Operation of the first stage of three stage digester in series at 49°C showed no improvement in decay rate coefficient as compared to the first stage of other three stage systems. From the results it can be concluded that operating the first stage and the second stage at increased temperatures did not have a significant effect on reaction rates. Therefore, it can be concluded that at HRT of 5 days, digesters were

overloaded and achieved similar VSS removal efficiencies which can be attributed to the similar reaction rate coefficients.

In summary, single stage operation at a HRT of 20 days showed minimum VS destruction at 55°C and was independent of changing HRT. For the temperature range of 35°C to 49°C increased HRT resulted in an increase in VS removal. The VS reduction results followed similar trends to those for VSS reduction. In addition, the first stages of two and three stage systems that was operated at HRT of 5 days represented replicate samplings of the same digesters that were collected over an extended period of time. Hence, the data presented for these reactors gives 40 to 45 % VS and VSS removal which is an indication of the reproducibility of the results over an extended period of time. The results suggest that the removal efficiencies that were observed in this study were sustainable for a significant period of time. This study suggests that overall two and three stage operation increases the destruction efficiency of the VS and possibly provide more efficient kinetics than single stage digestion.

4.2.2 Digesters Volatile Fatty Acid Analysis

Volatile fatty acids are a concern because their presence at high concentrations in the effluent from digesters can cause odour problems (WEF, 1987). They are also indicative of an imbalance between acidogens and methanogens bacteria and can give an insight into the overall state of the digesters. The results presented here show the impact of HRT, temperature, and staging on the quality of the digester effluent with respect to VFA concentrations.

4.2.2.1 Effect of Single Stage Temperature-HRT Studies on VFA Concentration

This portion of the study investigated the effect of HRT and temperature on the acidogenesis of the mixture of primary and thickened waste activated sludge. Temperature and HRT were reported to be responsible for the maintenance of the acidogenic phase by allowing the growth and enhancement of acidogenic bacteria while suppressing that of the methanogens (Elefsiniotis, 1993). Cha and Noike (1997) found evidence of species selection related to HRT and temperature during operation of two phase anaerobic digestion of municipal sewage sludge process. Figure 4-4 presents the TVFA that were measured in this study. The results indicate that the data that was collected in reactors over extended time intervals was consistent. On Figure 4-4 the error bars on the charts, which represent one standard deviation about the mean values, were relatively narrow and hence there was good replication between the duplicate samples, with the exception of the 42°C digester at the 5 day HRT. From Figure 4-4 it can be seen that at the 20 day HRT the TFVA concentrations increased with temperature. After the 20 day HRT at 35 and 42°C, the TVFA concentrations were less than 100 mg/L and these values are indicative of a well-stabilized product. The TVFA data at these temperatures was in agreement with the substantial reduction in VS that was previously described in section 4.2.1.1.

At 62°C and HRT of 20 days, the TVFA concentrations approached 3000 mg/L. These values are considered quite high for anaerobic digesters and would contribute to odors and indicate an imbalance between acidogens and methanogens in the anaerobic digester.

With the exception of the digester operated at 55°C the TVFA concentrations were higher when the digesters were operated at HRT's of 5 and 15 days as compared to the 20 day HRT. The lowest TVFA concentrations at HRT's of 15 and 5 day were observed for 42°C (220 mg/L) and 35°C (1519 mg/L) respectively, while the maximum values were observed at 62°C (4935-5000 mg/L) for both operational HRTs.

Figure 4-4: Impact of HRT and Temperature on TVFA.

Other researchers have demonstrated similar volatile fatty acid concentrations for thermophilic and mesophilic digestion at 20 and 15 days HRTs. Moen *et al.* (2003) found that thermophilic digestion for a 15 day HRT produced 450 mg/L of VFA. By reducing the HRT to 4 days the TVFA level went up to 2800 mg/L. The difference between this study and Moen's work may not be significant in view of the high standard deviations of Moen's data (about 40% of the mean value). In general the TVFA concentrations paralleled the effluent VS concentrations.

Hence, digesters that had reduced solids reductions tended to have increased TVFA concentrations. At the 5 day HRT, with an increase in temperature from 35°C to 42°C, there was increase in the VFA concentration, but when temperature further increased to 49°C and 55°C, VFA concentration went down from 3000 mg/L at 42°C to 1500 mg/L for 49 and 55°C, respectively. A similar trend was observed for VS values, which suggests that perhaps there is an optimum temperature of around 42°C for acidogenesis of this type of sludge.

In addition, over the 5 day steady state sampling period analysis which was started November 2003 and ended in December, 2003 the feed sludge had a high concentration of approximately 1000 mg/L of TVFA, which is considered unusual for municipal sewage sludge. The cause of the high VFA in the feed sludge could not be explained by the plant operators at ROPEC.

It is interesting to note that the TVFA concentrations at HRTs of 15 and 5 day were lower at 55°C than those that were observed at either 49 or 62°C. The results would suggest that the methanogens were most effective at 55°C and their efficiency decreased at higher temperatures that are considered to be relatively extreme for thermophilic operation.

Effect of Single Stage Temperature-HRT Studies on IVFA Formation and Distribution

The VFAs identified during the course of this study were acetic, propionic, and butyric acids. The identification of the individual VFAs provides valuable information about biochemical pathways of VFA formation. Figure 4-5 shows the effect of HRT and temperature on the distribution of individual VFAs represented as a mass fraction of total

VFA produced over the course of this study. At 35°C, acetic acid was the dominant species of VFA over the 5, 15, and 20 day HRT and ranging from 44 to 65% of the total VFA production. Operation of the anaerobic digester at 55°C resulted in 15 to 30% of the total amount of total VFA present as acetic acid. The results indicate that operation of anaerobic digestion at elevated temperatures resulted in a decrease in the acetic acid fraction. At the 20 day HRT and 55°C, an increase in acetic acid concentration was observed which revealed that longer HRT at 55°C improves the production rate of acetic acid by enhancing the reaction rate of anaerobic oxidation and fermentation of the anaerobic digestion process. Figure 4-5 shows that with the exception of the digester operated at 62°C the acetic acid fraction decreased with a decrease in HRT.

At 62°C the acetic acid fraction exhibited a different trend when compared to the rest of digesters investigated in this study. This indicates that at 62°C or extreme thermophilic temperature operation moves toward the thermal treatment which resulted in different trends as compared to the biological treatment that was investigated over the between temperatures of 35 to 55°C. Propionic acid was the most predominant acid during this study. An increase in temperature and a decrease in HRT led to an increase in the propionic acid fraction and a decrease in the acetic acid fraction. With the exception of the digester that was operated at 62°C the propionic acid fraction continuously increased as temperature increased from 35°C to 55°C.

There was also continuous decrease in the propionic acid fraction as the HRT decreased at 62°C when compared with other investigated digesters. There were no remarkable changes in propionic acid distribution over the digesters that were operated at 49°C and 55°C. Once again there is a relationship between the distribution of propionic

acid, HRT and the temperature of the systems. From Figure 4-5 it can be seen that the acetic acid concentration decreased as either the temperature increased or HRT decreased, and the percentage of propionic acid increased and resulted in an unstable process performance which resulted in less methane production and odour issue in the digester effluent. In addition, the digesters performed well over the period of the study.

Figure 4-5: Individual Fatty Acid Distribution Versus Temperature and HRT.

Figure 4-5 shows the effect of HRT and temperature on distribution of butyric acid over course of this study. Butyrate fractions were significantly higher in the digesters that were operated at 35°C and 42°C for 15 or 20 day HRT. However, with the exception of the 42°C digester butyric acid was responsible for less than 15% of the total VFA. Variations in HRT and temperature did not seem to play a role in the formation of butyric acid as experienced in this study.

The above trends indicated that temperature and HRT play a important role in the distribution of acetic and propionic acids while the formation pathway of butyric acids was noticed to be different.

4.2.2.2 Effect of Temperature Staging on VFA Concentration

In this portion of the study the TVFA concentrations were compared for two and three stage systems.

Two Stage Operation

Figures 4-6 illustrates the total volatile fatty acid concentrations for the two stage systems. The total volatile acids concentration in the first stage of the two stage operations ranged from 350 mg/l to 430 mg/l as compared to 340 mg/l in single stage mesophilic digester at 15 days SRT which indicates a 12% increase in TVFA concentration when the first digester was operated at 5 days HRT. The first stage of the tested systems in this phase of the study were operated under conditions identical to that of the single stage at 5 days HRT over temperature ranged from 35°C to 62°C. Comparing the TVFA concentrations presented in Figure 4-4 and Figure 4-6 it can be seen that over the period of the single stage study at 5 days, TVFA concentration were

50% greater in those systems that were operated in the 35°C to 49°C temperature range as compared to the first stage of two stage system. The difference in TVFA concentrations may be due to difference in feed compositions over the period of this study.

Figure 4-6: TVFA Concentration Versus Two Stage Temperature Configuration.

The volatile fatty acids concentration in the effluent of all the two stage systems was maintained at approximately 100 mg/l. An increase in the temperature from 35°C to 42°C or 49°C in the first stage and from 35°C to 42°C in the second stage digester didn't have a significant impact on the TVFA concentrations in the treatment train effluents. By applying mesophilic treatment in the second stage of the two stage operation, VFAs were reduced by 80-85%. These results indicate that the mesophilic digester can be applied to increase the stability of the effluent from digesters that were operated at elevated temperature and decrease the odor potential of digested biosolids for these temperatures (Han and Dague, 1997)

Effect of The Two Stage Temperature Configuration On IVFA Formation and Distribution

Figure 4-7 represents the distribution of the various acids measured in this study within two stage systems. In the first stage digesters acetic acid had the highest fraction of 44% in the digester that was kept at 35°C, and acetic acid declined by 24% as the temperature increased to 49°C. The acetic acid fraction was increased in the second stage of the two stage systems.

Operation of the second stage at 35°C resulted in a high acetic acid percentage of 89%. The temperature increase in the first stage from 35°C to 42°C did not significantly change the composition of the final effluent. Operation of the second stage at 42°C, resulted in a 10 % decrease in the acetic acid fraction. An increase in the temperature to 49°C in the first stage decreased the final acetic acid fraction to 60%.

Interestingly, the propionate fractions were higher than the acetic acid fractions in the first stage of the systems. It is believed that propionate is metabolized within a narrow range of hydrogen partial pressure therefore it can be concluded that propionate concentrations in these digesters are indicative of the balance between hydrogen producing and hydrogen consuming microorganisms.

Figure 4-7: Individual Fatty Acid Distribution Versus Two Stage Temperature Configuration.

This research has demonstrated that when anaerobic digesters are operated at elevated temperature and reduced HRT the propionate fraction increases in the digesters. On the other hand an increase in the fraction of acetic acid and simultaneous decrease in propionate fraction in the second stages indicates better overall performance of the two stage systems. It is notable that in all the systems except for the 49°C-35°C system that had a butyrate fraction of 15% the butyrate fraction was zero.

Results of this study reveal that temperature and HRT have an effect on the selection of specific VFA formation pathways especially in the case of two stage operations where there is the potential to produce a stable product sludge that has a low concentrations of VFA's and fewer potential problem associated with odors in the digester effluents.

Three Stage Operation

The concentrations of volatile fatty acids (VFA's) were measured as indicators of the relative rates of acidogenesis and methanogenesis in the digesters. In single stage digester systems the concentrations of VFA's are considered to be indicators of the stability of the product sludge. In staged digester systems the presence of elevated VFA concentrations in upstream digesters can be considered an indicator of the state of the digester, however are not necessarily of concern if there is a subsequent digester that is capable of converting of the VFA's to CH₄.

Figure 4-8: TVFA Concentration Versus Three Stage Temperature Configuration.

Figure 4-8 presents the total VFA concentrations in each of the stages of the three stage systems. From Figure 4-8 it can be seen that the concentrations of VFA's were higher in the first stage of all of the three stage systems as compared to the control single stage digester presented in Figure 4-4. The first stage of the three stage systems was operated under identical conditions to that of the first stage of the two stage systems and produced relatively similar TVFA concentration. These results represent replicate sampling of the same digesters and provide an indication of the reproducibility of the results over the period of this study.

The highest concentrations of VFA's were observed in the first stages of systems that were operated at 35°C and 42°C as compared with those system that the first stage was operated at 49°C. It would appear that despite the elevated rates of hydrolysis in the systems that first stage were operated at 49°C, the methanogens in these systems were more capable and able to maintain slightly lower VFA concentrations in the first stage. Despite the elevated concentrations of VFA's in the first stages of the three stage

systems, in all systems the VFA concentrations in the second stages were all less than those observed in the single stage system. In the third stage of the three stage systems, the total VFA concentrations were all less than 100 mg/L. These results indicate that three stage digester systems can be operated over a range of temperatures and still produce a stable product sludge that has low concentrations of VFA's.

Effect of Three Stage Temperature Configuration on IVFA Formation and Distribution

As previously noted in section 4.2.2.1 the conventional method uses mesophilic bacteria produce a better quality effluent than thermophilic bacteria.. Figure 4-9 presents distribution and formation of IVFA's within three stage systems. Figure 4-9 indicates that fraction of acetic acid varied from approximately 45 % in the first stage of the 35°C digester to approximately 20% in the digester that was operated at 49°. In all of the three stage systems, the acetic acid fraction increased in the second stage and further increased in the third stage, whilst the percentage of propionate was observed to continuously decline through three stage operation similar to what, was observed in the two stage systems. With the exception of the three stage system where the first stage operated at 49°C none of the investigated systems had detectable concentrations of butyrate in their effluents.

Figure 4-9: Individual Fatty Acid Distribution Versus Three Stage Temperature Configuration.

In summary, from the results it can be concluded that operation of two and three stage anaerobic digesters in series resulted in a low concentration of TVFA in the system effluent, which would reduced odor problems associated with high level of TVFA and resulted in better effluent quality when compared to a single stage digester that was operated at 35°C and 15 HRT.

With the exception of single stage digesters that were operated at 5 days HRT over the course of tested temperature, longer HRT resulted lower TVFA concentrations in the digester effluents than shorter HRT's. Operation of a single stage study at 55°C resulted in lower TVFA concentrations than those that were observed at 49°C and 62°C. This can be attributed to the effective performance of methanogens. Multi stage operation of anaerobic digesters in series resulted in high concentrations of VFA in the first stage (390 mg/l) when compared to single stage 35°C digester (340 mg/l) and reduced significantly to below 100 mg/L as sludge progressed through each individual stage of two and three stage systems. This indicates that effluent quality in terms of TVFA was improved in staged operation as compared to a single stage digester.

4.2.3 Ammonia Analysis

The concentration of ammonia in anaerobic digesters is indicative of the degradation of protein in the process. Ammonia is rapidly formed in a digester by deamination of protein constituents and reacts with carbon dioxide and water resulting in the production of ammonium bicarbonate (Speece, 1996). This ultimately influences the alkalinity level in the anaerobic digester which will be subsequently discussed in section 4.4.4. Ammonia toxicity is often a problem in anaerobic digestion processes. Free ammonia has been found to be much more toxic than the ammonium ion, and thus

ammonia toxicity thresholds are very sensitive to pH in basic conditions (Speece, 1996). This section describes the ammonia concentrations under single and multistage operation of anaerobic digesters.

4.2.3.1 Single Stage Temperature-HRT Studies

The concentrations of $\text{NH}_4^+\text{-N}$ in the digesters is indicative of the degradation of protein in the process. Figure 4-10 presents the concentrations of $\text{NH}_4^+\text{-N}$ that were measured in this study. From Figure 4-10 it can be seen that at an HRT of 20 day similar concentrations of ammonium were observed for all conditions (1050-1150 mg/L) with the exception of the digester operating at 55°C (900 mg/L). The lower ammonia concentrations at this temperature were in agreement with the slightly higher concentrations of VS that were observed in the digester effluent (Figure 4-1). Hence, these results confirm that there was less VS destruction in the 55°C digester operating at a 20 day HRT.

Figure 4-10: Impact of HRT and Temperature on Ammonium.

The NH_4^+ -N concentrations increased as the temperature increased for 15 and 5 day HRT (Figure 4-10). For the HRT of 15 days the mean concentration increased from 370 mg/L to 890 mg/L over the range of temperatures examined. The NH_4^+ -N data at this HRT was not in complete agreement with the VS data that was previously described in section 4.2.1.1. The VS removal data in Figure 4-1 would suggest that the lowest NH_4^+ -N concentrations should be observed at 62°C and that the highest values should be observed at 42 and 49°C.

In a study conducted by Ghosh (1987) the impact of operation of anaerobic digesters at 35°C and 55°C and a 15 day HRT on ammonia concentrations was investigated. In that study, ammonia concentrations of 779 mg/L and 1132 mg/L were observed in the effluent of the 35°C and 55°C digester where both digesters were fed with 1:1 volume ratio of primary and was activated sludge. These values were relatively high as compared to our work and that might be attributed to the higher VS reduction that was observed in Ghosh's work. At 5 days operational HRT NH_4^+ -N concentrations increased from 323 mg/L to 546 mg/L as temperature increased from 35°C to 62°C. From Figure 4-10 it can be seen that with exception of 62°C digester, NH_4^+ -N data was somewhat in agreement with VS concentration. In addition, mesophilic temperatures appeared to have the lowest utilization of protein (and production of NH_4^+ -N) and thermophilic temperatures resulted in the highest utilization of protein.

4.2.3.2 Effect of Staging on Ammonia Concentration

Two Stage Operation

The ammonia values measured during the two stage experiments are shown in Figure 4-11. From Figure 4-11 it can be seen that in all of the tested systems the ammonia concentration increased as the sludge progressed through the individual reactors in each system.

Figure 4-11: Ammonium Concentration Versus Two Stage Temperature Configuration.

Ammonia concentrations in the first stage of the two stage system were relatively similar to the tested single stage digesters that were operated at 5 days HRT at a similar operating temperature. This provides an indication of the reproducibility of the results over the period of this study.

From Figure 4-11 it can be observed that the final ammonia concentrations during two stage operation ranged from 550 to 720 mg/L as compared to 390 mg/L in the control 35°C digester. Operation of two stage system at 35°C resulted in ammonia concentrations of 420 mg/L in first stage and 550 mg/L in the second stage, with a final concentration

that was similar to what was reported by Ghosh (1987). Ghosh operated the first stage at 2 days HRT followed by 13 days in second stage, both digesters were operated at 35°C with 1:1 volume feed ratio of raw sludge to waste activated sludge. A temperature increase from 35°C to 42°C in the first and second stages of two stage systems had no impact on the final ammonia concentrations in the system effluents. Further increase in temperature to 49°C in first stage resulted in a 25 % increase in the final ammonia concentration to a value of 710 mg/L. Side by side comparison of the ammonia data with VS removal efficiency that is presented in Figure 4-2 a reveal that higher VS destruction was achieved in the first stage of two stage system followed by lower VS destruction in the downstream digester. Hence, opposite ammonia concentrations profile were observed in two stage study. Despite the higher VS removal in the first stage it is expected that partial protein hydrolysis was achieved in the first stage and the majority was converted to ammonium in the second stage that was operated at a longer HRT.

Three Stage Operation

Figure 4-12 presents the ammonium concentrations that were observed in each stage of the three stage systems. From Figure 4-12 it can be seen that there was considerable variability associated with many of the ammonium data sets.

The final ammonium concentrations in the effluent of all the tested systems were approximately twice that measured in the conventional single stage mesophilic digester (approx 400 mg/L). The first stage of the three stage systems was operated under identical conditions to that of the first stage of the two stage systems and the single stage system that was operated at 5 days HRT. Relatively similar ammonia concentration was produced in the first stages of these systems which represented replicate samplings of the

same digesters that were collected 7-15 months apart. Operation of a three stage digester at 35°C resulted in 410 mg/L of ammonium in the first stage and a further increase through the system to 710 mg/L. The ammonium concentration reached 840 mg/L in the three stage digester where the first stage was kept at 42°C. A further increase in the temperature to 42°C in the second stage resulted in similar ammonium concentrations in the effluents as compared to 42°C-35°C-35°C system.

Figure 4-12: Ammonium Concentration Versus Three Stage Temperature Configuration.

The results suggest that an increase in temperature in the first stage of three stage system to 49°C enhanced the rate of protein breakdown and resulted ammonium concentration of 900 mg/l in the final system effluent. Temperature increase in the second stage of three stage systems showed no significant impact on final ammonium concentration. It is interesting to note that the concentrations of $\text{NH}_4\text{-N}$ in the first stage of the three stage systems were generally higher than what was measured in the single stage digester. This response differed from that observed for VS reduction where at least

two stages of the three stage systems were required before the performance of the single stage digester was exceeded. These results suggest that considerable hydrolysis of protein was occurring in the first stage of the three stage systems, but complete conversion of the solids was not occurring. The results suggest that in the early stages of the three stage system hydrolysis was not the limiting factor.

The results presented in Figure 4-12 do however suggest that the hydrolysis of proteins was substantially impacted by temperature. In both the first and second stage digesters where direct temperature comparisons could be made, the reactor operating at the higher temperature appeared to generate a greater increment in the concentration of $\text{NH}_4\text{-N}$. The results suggest that in three stage digester systems there is an uncoupling between the rate of hydrolysis and the rate of VS destruction.

4.2.4 Alkalinity Analysis

As a result of anaerobic digestion of protein, ammonia is produced and its reaction with CO_2 and H_2O results in the production of ammonium bicarbonate (Speece, 1996). A good understanding of the interrelationships between VS destruction, pH, volatile fatty acid, and alkalinity are essential to assess the performance of the anaerobic digestion process. The alkalinity of the anaerobically digested sludge is important because it indicates the buffer in the digestion system; therefore, sufficient alkalinity is essential for proper pH control.

4.2.4.1 Single Stage Temperature-HRT Studies

Figure 4-13 presents the average alkalinity concentration for the five-bench scale digester that were operated at different steady-state HRT's. The high standard deviation for these data is indicative of the relatively large variability between replicate samples

Figure 4-13: Impact of HRT and Temperature on Alkalinity.

At the 20 day HRT, with the exception of the 62°C digester, similar alkalinity concentrations were observed for the digesters. Similar results were obtained by Bucher *et al.* (2001), who reported that thermophilic and mesophilic operation of pilot scale anaerobic digester at a 20 day HRT produced alkalinities of 6634 mg/L and 6464 mg/L, respectively. However, in their study the feed sludge was a 60:40 primary/ secondary blend as compared to a 50:50 ratio in this study. This would indicate that at 20 days HRT, temperature did not play a significant effect on alkalinity. As the digester HRT's decreased to the lower values different trends in alkalinity concentrations were observed. In all cases the alkalinity levels decreased as HRT decreased which would indicate that at a lower HRT's, greater VFA accumulation and lower extent of degradation was expected which resulted in the release of less NH_4 . In addition, from the results it can be seen that by increasing temperatures from 35°C to 55°C, the alkalinity generally increased for

HRTs of 5 and 15 days. This suggests that the degradation of protein was higher at temperature below 55°C, and the ammonia data supports this observation. At 62°C similar trends in alkalinity concentration was observed as compared to the rest of the digester over the range of investigated HRTs. At this temperature as it can be seen from Figure 4-4 high VFA concentration of 3000 mg/l to 5000 mg/l was observed which resulted in lower alkalinity concentration.

4.2.4.2 Effect of Staging on Alkalinity Concentrations

Two Stage Operation

Average alkalinity concentrations for the various two stage systems are shown in Figure 4-14. Operation of two stage digester system at 35°C resulted in an average alkalinity concentration of 4500 mg/L in the first stage. As the temperature in the first stage of two stage system increased from 35°C to 42°C and further to 49°C the alkalinity concentration in the first stage increased by 20% to approximately 5500 mg/l. Operation of the second stage at 42°C resulted in alkalinity of 6300 mg/l in the final effluent of the digester when the first stage was operated at 42°C. A further increase in the temperature in the first stage to 49°C resulted in 13% increase in final alkalinity as compared to the 6000mg/l of the 35°C-35°C system. These results are consistent with those of Bucher *et al.* (2003) who reported that operation of a two stage mesophilic digester at pilot scale at an HRT of 4 days in the first stage followed by 8 days in the second stage resulted in a alkalinity concentration of 6700 mg/l.

Figure 4-14: Alkalinity Concentrations Versus Two Stage Temperature Configuration.

When results for the single stage data presented in Figure 4-13 and two stage system are compared, similar alkalinity levels of about 4200 mg/L were observed for the first stage digester that was operated at 35°C as compared with alkalinity concentration of 4700 mg/L that was recorded for 35°C digester at 15 days HRT. However, with the exception of the digester that was operated at 42°C and 5 days HRT over the single stage study relatively similar alkalinity levels were observed for the first stage of two stage operations for the range of tested temperature. Operation of two stage systems produced higher alkalinity concentration in the final effluent as compared to the single stage digester at similar HRT.

Three Stage Operation

Figure 4-15 presents the concentrations of alkalinity that were measured in each stage of the three stage systems. From Figure 4-15 it can be seen that all of the final effluent of each of the three stage systems had alkalinities that were substantially higher than those observed in the single stage digester (Figure 4-13).

The alkalinity of the sludges increased as they progressed through the stages in the three stage systems. In general, the alkalinity increased with the digester temperatures although in several instances the impact of an increase in temperature appeared to be observed in downstream digesters. The alkalinity concentrations where all three stages were at 35°C increased from 4200 mg/L in the first stage to 5300 mg/L in the final effluent (Figure 4-15). An increase in the temperature from 35°C to 42°C in the first stage resulted in an increase in the final alkalinity concentration from 5300 mg/L to 6000 mg/L. A further increase in the temperature in the second stage to 42°C enhanced the alkalinity concentration in the final effluent by 6% as compared to the 42°C-35°C-35°C system. An increase in the temperature to 49°C in the first stage resulted similar alkalinity concentrations in the effluent as compared with 42°C-35°C-35°C and 42°C-42°C-35°C systems. Increasing the temperature in the second stage of the three stage system to 42°C while keeping the first stage at 49°C resulted in an increase of the final alkalinity concentration to 7000 mg/L.

Figure 4-15: Alkalinity Concentrations Versus Three Stage Temperature Configuration.

An increase in alkalinity concentration, as the digester temperatures increased was observed, although in several instances the impact of an increase in temperature appeared in downstream digesters. As was previously discussed, this effect was likely due to enhanced hydrolysis in the higher temperature digesters. By comparing alkalinity concentration, ammonia and TVFA concentrations over two and three stage anaerobic digester we observed that both alkalinity and ammonia levels were increased as sludge progressed through each unit of a two or three stage system. At the same time, accumulation of TVFAs was observed in the first stage of two and three stage system, but the TVFA concentration decreased through each individual unit. Increases in ammonia concentration and decreases in the TVFA concentrations through the digester contributed to high alkalinity concentration in downstream digesters.

4.2.5 pH Analysis

The pH that a sludge digester operates at is a function of the concentrations of volatile fatty acids and buffers, such as HCO_3^- and NH_4^+ that are present. Stable methanogenic digestion is typically associated with digesters operating in the pH range of 7 to 8.

4.2.5.1 Single Stage Temperature-HRT Study

Figure 4-16 presents the pH values for the investigated digesters in this study. From Figure 4-16 it can be seen that the pH values were all in the range of 7 to 8 and hence it would appear that the digesters were operated at a stable mode. With the exception of the 55°C digester the pH values for the 20 day HRT were higher than for the 5 and 15 day HRTs.

Figure 4-16: Impact of HRT and Temperature on pH.

TVFA data presented in Figure 4-4 indicates that lower TVFA concentrations in the digester that was operated at 55°C and 15 days HRT as compared to the both digesters that were operated at 5 and 20 days HRT. At the same time alkalinity results presented in Figure 4-13 reveal that as HRT decreases from 20 day to 5 day decrease in alkalinity

concentration was observed for the 55°C digester. Therefore it can be concluded that the VFA and alkalinity data tends to support the pH data that was achieved at 55°C.

4.2.5.2 Effect of Temperature Staging on pH Values

Two Stage Operation

The average digester pH values for the various temperature configurations in two stage operation are shown in Figure 4-17. Operation of two stage mesophilic digesters in series resulted in an increase in pH values from 7.7 to 8.0 as compared to 35°C single stage digester that was operated at 15 days HRT.

Figure 4-17: pH Values Versus Two Stage Temperature Configuration.

The error bars on the charts, for the mesophilic two stage system were relatively wide due to high standard deviations of about 20% to 30% of the mean average. As the temperature increased from 35°C to 42°C and 49°C in the first stage and from 35°C to 42°C in the second stage of two stage system there were no significant changes in the pH values in the digesters. The pH values throughout this experimentation were maintained in the optimum pH range that would results in stable operation of the anaerobic digestion. An increase in pH values for the digesters that were operated at elevated temperatures

was most likely related to the high ammonia concentrations and low VFA concentrations that were observed in two stage systems, in addition to the effect of alkalinity concentration.

Three Stage Operation

The pH values shown in Figure 4-18 are averages for each digester of the three stage systems. Figure 4-18 shows no significant changes in pH (7.8) in the three stage system that was operated at 35°C. An increase in temperature from 35°C to 42°C in the first of three stage system resulted in a increase in pH to a value of 8.0 and pH then remained steady as the sludge progressed through the stages in the three stage systems.

It is interesting to note that three stage operation of anaerobic digesters at temperature above 42°C did not impact the pH values of the system and resulted in stable and steady pH profiles throughout the system. High pH values through two and three stage operations were supported by alkalinity data that increased through the stages in the multistage systems.

Figure 4-18: pH Values Versus Three Stage Temperature Configuration.

The results of this study reveal that pH values were maintained well within the range of stable operation for anaerobic digesters, even though the TVFA values were very high under some conditions. The neutral pH's were likely maintained by the substantial release of NH_3 that resulted from the solids destruction that occurred at these elevated temperatures. The neutral pH allowed CH_4 production to be sustained despite a high level of VFA's in some of the digester contents.

4.2.6 Methane Yield Analysis

Methane yield in this study was defined as the amount of methane produced for a given quantity of VS added to the system, and characterizes the metabolic activity of a methanogenic ecosystem. Theoretically, the methane yield value reflects a balance between anabolic (biological synthesis) and catabolic processes and is also employed as an indicator of digester stability. In this part of the research work, the impact of single and multistage operation of the anaerobic digesters on methane yield values was evaluated.

4.2.6.1 Single Stage Temperature-HRT Studies

Figure 4-19 presents the average observed yield of methane as a fraction of the VS entering the digesters. From Figure 4-19 it can be seen that at a 20 day HRT the methane yield steadily decreased as the temperature of the digesters increased. These results disagreed with the VS reduction and TVFA accumulation that were observed in the digesters at this HRT. As was previously noted, the VS reduction was essentially constant for the different digester temperatures however the TVFA concentrations

increased as digester temperature increased. Hence, the reduced conversion of VFA's at the higher temperatures reduced the yield of methane under these conditions.

Figure 4-19: Methane Yield Versus HRT and Temperature.

A considerably different relationship between temperature and methane yield was observed at the 15 day HRT. From Figure 4-19 it can be seen that the yield of methane was lowest for the 35°C digester and reached a maximum value of 0.33 L/gVS at 55°C similar to that which was reported by Ghosh (1987) for a single stage thermophilic digester. A similar methane yield of 0.15 L Methane /gVS was reported by Moen *et al.* (2003) and Vandeburgh and Ellis (2002) for mesophilic anaerobic digesters that were operated at a 15 day HRT. The methane yields at this HRT also agree with the VS and TVFA data. At 35°C the reduction in VS was less than that of the 20 day HRT digester and some accumulation of VFA's was observed. Hence the yield at this temperature decreased. As the temperature increased between 42 and 55°C the reduction in VS increased relative to 35°C and the VFA concentrations were reduced and hence the yields

increased. At 62°C the reduction in VS decreased substantially, the TVFA concentration increased significantly and there was a concurrent decrease in the yield. At the 5 day HRT the methane yield increased to a maximum value at 55°C and decreased at 62°C. With the exception of the 42°C reactor the methane yield concurred with the VS and TVFA data. At 35°C the lowest methane yield was observed as compared to the other HRT's due to the higher volatile acid accumulation and reduced solids reduction. The digester operated at 42°C did not support the VS and TVFA data in that the yield increased relative to the 35°C operation while the VS destruction decreased and the TVFA concentrations increased. The methane yield increased at this HRT for digesters that were operated at 49 and 55°C as VS reduction reached a maximum value and TVFA concentrations were similar to the 35°C digester. The methane yield decreased at 62°C due to accumulation of TVFA's and reduced solids destruction. A two way ANOVA considering temperature and HRT as factors was performed to determine the significant impact of these two factors on methane yield.

The results of ANOVA presented in Appendix D, shows the main effects of all two factors were statistically significant at the 95% confidence level. The results of this study indicate that there is a considerable interaction between temperature and HRT over the range of temperatures and HRT's that were examined. The results suggest that at extended HRT's (20 days) increasing the temperature above mesophilic values tended to result in minimal increase in VS destruction and a decrease in methane yield due to the accumulation of VFA's. At shorter HRT's (15 days) the operation of the digesters was better at the intermediate temperatures of 42, 49 and 55°C as compared to the mesophilic digester.

These temperatures improved VS reduction and reduced TVFA concentrations and hence had higher methane yields. At an HRT of 5 days better digester performance was observed at 49 and 55°C. Higher TVFA concentrations were achieved at this HRT in comparison with the longer HRT's.

4.2.6.2 Effect of Temperature Staging on Methane Yield

Two Stage Operation

Average methane yield values obtained from the investigation of two stage systems in this study are shown in Figure 4-20. Operation of the first stage of the two-stage digester at 35°C resulted in a methane yield of about 0.15 L/gVS added to the system. An increase in temperature from 35°C to 42°C in the first stage of the two-stage system resulted in a 46 % increase in the methane yield values in the first stage. The methane yields were 50% higher in the first stage of the digester that was operated at 49°C than in the 35°C digester. This is reflected in the greater solid destruction rate for the higher temperature.

The methane yield followed the same trend as VS reduction and TVFA concentration for each of the four systems. Operation of the second stage at 35°C resulted in a lower methane yields. An increase in temperature from 35°C to 42°C in the second stage enhanced the methane yield by 15%. Overall, two-stage operation had a higher methane yield in the first stage and significant drops in the second stage. This might be due to reduced substrate availability in second stage and a decrease in the biological conversion for methane production.

Figure 4-20: Methane Yield Versus Two Stage Temperature Configuration.

A one way ANOVA and a Tukey test were applied on methane yield data that were obtained in the first and second stage of two stage systems to evaluate the performance of the investigated treatment trends in this study. The results reveal that there was significant difference in methane yield between investigated treatment trains. The results of statistical analysis are presented in Appendix D.

Three Stage Operation

The methane yield increased rapidly with increase in temperature in the first stage of three-stage operation. Figure 4-21 shows the methane yield per gram of VS fed to the tested systems. As it can be seen from Figure 4-21 the methane yield was 38-45% and 50% higher in the first stage of the digesters that were operated at 42°C and 49°C respectively as compared to the digester that was operated at 35°C. The methane yield in the first stage of the two and three stage systems and the single stage digester that were operated at 5 days HRT represented replicate samplings of the same digesters that were collected over a period of 15 months apart. Hence, the data presented for these reactors gives an indication of the variability of the results over an extended period of time.

Figure 4-21: Methane Yield Versus Three Stage Temperature Configuration.

The most significant variation was observed between single stage and multistage performance rather than between two and three stage systems. This might be attributed to the high concentrations of TFVA that were observed during operation of single stage digester at 5 days HRT for the tested temperature range.

The methane yield in the first stage increased as the temperature increased from 35°C to 42°C and was essentially the same for the 42°C and 49°C digesters. The 42°C-35°C-35°C system showed lower methane yield than the 42°C-42°C-35°C system in the first stage however this might be due to the analytical errors that were experienced during this study.

Operation of the first stage at elevated temperature resulted in higher destruction rate of substrate in the first stage and subsequently improved the performance of the second and third stage. This subsequently resulted in methane yields to be higher in the first and second stages than the third stage in all the investigated systems. This was likely due to the higher VS loading which was added to the first stage when compared to the

second stage and the third stages. Therefore, there is a potential to reduce the size of the digester in second and third stage without significant changes in the overall performance of the systems. This phenomenon would require further studies. A one way ANOVA in conjunction with a Tukey test was used to analyze the differences in the methane yields of the all treatment trains. The statistical analysis showed that there was significant difference in methane yield among tested three stage treatment trains and the statistical results are presented in Appendix D.

In conclusion, gas production results were generally in agreement with what would be predicted from volatile solids results. In addition two and three stage operation of anaerobic digesters can achieve a significant increase in methane production, as compared to the single stage systems. More energy can be recovered from multistage operation, which may compensate for any added energy requirements for operation at elevated temperatures.

4.2.7 Dewaterability Analysis

The dewaterability of digested sludge is an important parameter in plant operations. Improvements in dewaterability of digested sludge can result in significant savings in polymer and hauling costs. In this study the dewaterability was assessed by the Capillary Suction Time (CST) method. The values that are presented are for the unconditioned sludges and it is assumed that they would improve if the sludges were dosed with conditioning agents such as polymers.

4.2.7.1 Single Stage Temperature-HRT Studies

Figure 4-22 presents the CST values that were measured in this study. From Figure 4-22 it can be seen that there was considerable variability amongst replicate values that were measured in this study. However, certain trends in the data can be identified. The results indicate that sludges produced at an HRT of 15 days dewatered more effectively (lower CST) as compared to those produced by operation at an HRT of 20 and 5 days.

Figure 4-22: Dewaterability Versus HRT and Temperature.

For digesters that were operated at an HRT of the 20 days the CST increased over the temperature range from 35°C to 49°C and the CST decreased for digestion at 55 and 62°C. Such changes suggest that longer treatment at higher temperatures destroyed some of the substances initially and formed finer particles which caused poor dewaterability, or changed the formation, stability, or relevant functional groups of the substances affecting CST (Zhou *et al.* 2002). The occurrence of such substances directly correlated to the operating temperatures. At the 15 day operational HRT the CST increased from 35 to

42°C and remained similar at 49°C. The CST then increased by 25% as temperature increased to 55°C and then decreased by 50% at 62°C to a CST value of 1100 Sec. The increase in CST at 55°C indicated a gradual build-up of factors such as Extracellular Cellular Polymeric Substances (ECPS) that resulted in poor dewaterability (Novak *et al.*, 2003).

For the 5 day HRT similar CST values were observed over the temperature range from 35 to 49°C. The CST then increased by 8 and 19% for the 55 and 62°C digesters. The results of this study indicate that temperature had a significant effect on dewaterability. This may have been due to the reduction of particles at elevated temperatures.

4.2.7.2 Effect of Staging on Dewaterability

This section provides the results of two and three stage operation of anaerobic digesters with respect to dewaterability.

Two Stage Operation

The effect of two stage operation on the dewaterability of digested sludge is illustrated in Figure 4-23. From Figure 4-23 it can be seen that sludges produced during two stage operation dewatered more effectively (low CST) as compared to control 35°C digester that was operated at 15 days HRT(Figure 4-22). This study showed no significant difference between the final CST values of two stage systems (900-1000 sec) where the second stage was kept at 35°C, which resulted more efficient dewaterability as compared to the single stage system (1300 sec). The CST values increased with an increase in temperature from 35°C to 42°C in the first stage of the investigated systems.

A further temperature increase to 49°C resulted in CST values of 1500 seconds and poor dewaterability in the effluent of the first stage digester. In addition as the temperature was increased to 42°C in the second stage of the two stage system a 30% increase in the CST values was observed. It is important to note that two different Capillary Suction Time units were used in the single and multistage operations and this may have caused some of the variability amongst the reported values.

Figure 4-23: Dewaterability Versus Two Stage Temperature Configuration

In summary, results of this study suggest that, to minimize deterioration in dewaterability, digestion temperatures of 35°C are desirable in the second stage of two stage systems. It seems that elevated temperature in the first and second stage creates or releases colloidal materials that cause poor dewatering. Downstream mesophilic operation in the second stage could significantly improve the dewaterability during two stage operation of anaerobic digesters.

Three Stage Operation

The CST values obtained in the three stage digestion are illustrated in Figure 4-24. For the two and three stage systems, the CST values in the first stages of these systems represented replicate samplings of the same digesters that were collected approximately 12 months apart. Hence, the data presented for these reactors gives an indication of the reproducibility of the results over an extended period of time.

Figure 4-24: Dewaterability Versus Three Stage Temperature Configuration.

In this study the CST values were observed to improve as the sludge progressed through the individual reactors in each of three stage systems. From Figure 4-24 it can be seen that the CST values increased in the first stage of the examined systems as the operating temperature increased. However, there was large variability between replicated samples as well as investigated systems. This might be due to the sensitivity of the analytical techniques that were used in this experimentation. Analysis of dewaterability is mainly affected by the degree of sludge homogenization and temperature, which is expected to be a major cause of variability for this analysis.

An increase in temperature in the second stage from 35°C to 42°C demonstrated higher CST values that suggested poor dewaterability. Operation of the three-stage digester in series with the second and third stages at 35°C improved dewaterability of the digested sludge as compared to the single stage 35°C digester operated at a HRT of 15 days (Figure 4-22). In this case operation of the first stage at an elevated temperature showed no significant impact on the final CST values. In this study, single stage operation of anaerobic digesters at elevated temperatures enhanced the rate of VS destruction. However, improved volatile solids reduction may come at the cost of poor dewatering in the digesters that operated at elevated temperatures. This problem can be solved through staged operation by keeping the down stream digester in the mesophilic temperature range.

The results of this study suggest that prolonged treatment at a thermophilic operating temperature in single and multi stage systems produced greater amounts of extracellular polymeric substances than mesophilic digestion, which can result in poor dewaterability and high conditioner demand (Novak *et al*, 2003).

4.2.8 COD Analysis

Particulate organic matter first undergoes liquifaction by extracellular enzymes before being taken up by microorganisms. Since most of the organic matter in the feed sludge is in a particulate form (VSS), solubilisation is a crucial step in the digestion process.

4.2.8.1 Effect of Single Stage Temperature-HRT Studies on COD Concentration

The average digester COD concentrations are illustrated in figure 4-25. The general trend for all of the investigated digesters was an increase in TCOD and SCOD concentrations with decreasing HRT's.

With the exception of 62°C digester that was operated at HRT of 15 days, TCOD concentrations were increased as HRT decreased from 20 days to 5 days. From Figure 4-25a it can be seen that the higher concentrations of TCOD observed in the digesters that were operated at shorter HRT. With the exception of the 55 and 62°C digester, volatile solids data presented in Figure 4-1 shows increase in VS reduction as HRT increased and resulted in lower TCOD concentration in the digester effluent. However, variability in the TCOD data observed in this study may be attributed to the analytical testing method that was used for the TCOD, and the level of sludge homogenization prior to the analysis.

Although the 55°C and 62°C digester showed slightly less volatile solids removal as compared to mesophilic operations SCOD concentrations were higher in the digesters operated at 55°C and 62°C than in the mesophilic (35°C)digester for HRTs of 20, 15, and 5 day. At 35°C the soluble COD concentration were around 2000 mg/L for all the HRTs and remained below 2800 mg/L in all digesters that were operated at 42°C and 49°C. An increase in the SCOD concentration in both the 55°C and 62°C digesters to about 6000mg/ L and 8000mg/L at 5 day HRT was attributed to the accumulation of TVFAs which make up a large portion of the SCOD concentration.

SCOD concentrations in the thermophilic digesters were significantly higher than mesophilic digester for all the HRT's, and followed similar trends to the TVFA concentration.

(a). TCOD

(b). SCOD

Figure 4-25: Average COD Concentrations Versus HRT and Temperature (a) TCOD and (b) SCOD.

It should be noted that there were some significant differences between the SCOD concentration presented in Figure 4-25 and the TVFA concentrations previously presented in Figure 4-4. For the 42°C digester, accumulation of TVFA's was observed at reduced HRT and can be translated to the gradual increase in SCOD concentration at shorter HRT. VFA is considered small portion of SCOD, therefore VFA concentration for 42°C digester does not corresponded to the estimated SCOD concentration at a HRT of 5 days and this may be attributed to the analytical testing and the level of sludge homogenization prior to the analysis. In addition operation of the 55°C digester at a 15 day HRT resulted in minimal TVFA accumulation in the digester when compared with the digesters that were operated at the same operating temperature for the HRTs of 20 and 5 days. The 55°C operating temperature represents optimal conditions for methanogenic activity. At 55°C, degradation rates for lipids, proteins and carbohydrates were expected to be optimal which resulted in an increase in SCOD concentration at a reduced HRT.

4.2.8.2 Effect of Staging on COD

Two Stage Operation

Total and soluble COD concentrations for the two stage systems are shown in Figure 4-26. At steady state, average TCOD concentrations in the first stage and the effluent of the 35°C-35°C and the 42°C-35°C systems were 20500 to 22000 mg/L, respectively. Increasing the temperature of the second stage of the 42°C- 35°C system from 35°C to 42°C increased the TCOD concentration in the digester effluent quite substantially from 22000 mg/L to 30000 mg/L. The TCOD concentration increase in the second stage of 42°C- 42°C system is considered to be unusual and can be attributed to

analytical error such as the degree of sludge homogenization which is usually the cause of error in TCOD analysis of sewage sludge. Further temperature increase in the first stage to 49°C resulted in a 33% increase in TCOD, and a gradual decrease to a concentration of 26000 mg/L in the effluent of the 49°C- 35°C system.

(a). TCOD

(b). SCOD

Figure 4-26: Average COD Concentrations Versus Two Stage Temperature Configuration (a) TCOD and (b) SCOD.

As can be seen in the results, continuous increase in temperature in the first stage of a two stage system resulted in a continuous increase in TCOD concentration. As can be seen in the results, continuous increase in temperature in the first stage of a two stage system resulted in a continuous increase in TCOD concentration. Side by side comparison of Figure 4-2 and Figure 4-26 reveals consistent increase in VS removal as the temperature of the two stage systems are raised. This would translate to reduction in TCOD concentration as a result of increase in VS removal for the tested systems. However, both 42°C-42°C and 49°C-35°C systems showed increase in TCOD concentration as a result of increase in VS removal which is considered to be incorrect and can be attributed to the complexity of the COD analysis of the sludge sample.

SCOD was tested in this study as a parameter, that represents the extent of hydrolysis and solubilisation carried out by acidogenic bacteria (Maharaj and Elefsiniotis, 2001). Figure 4-26 shows the concentrations of SCOD were about 3000 mg/L in the first stage of all four systems. The results indicate that at an HRT of 5 days an increase in temperature from 35°C to 49°C resulted in similar net result of hydrolysis and methanogenesis as demonstrated by similar SCOD concentration in the digesters. SCOD concentrations of 1500 mg/L to 1800mg/L were observed in the effluent from the systems when the second stage was kept at 35°C. A temperature increase in the second stage of the two stage system resulted in a 30 to 40% reduction in SCOD concentration to a values of approximately 1000 mg/L. This is due to the fact that operation of the second stage of two stage system at 42°C enhanced the hydrolysis rate and methanogenesis activity in the 42°C-42°C system. Overall two stage systems improve the hydrolysis rate

and methanogenesis activity as compared with single stage operation of anaerobic digesters at 35°C and 15 days HRT (2000 mg/L).

Three Stage Operation

Figure 4-27 presents the total and soluble COD concentrations in the three stage digesters. As can be seen from the wide error bars in this study there was a large variability between the replicated analyses. The first stage of the three stage systems was operated under identical conditions to that of the first stage of the two stage systems and the single stage that was operated at 5 days HRT and produced relatively similar COD concentrations. A result for the first stages of these systems represented replicate sampling of the same digesters and provides an indication of the reproducibility of the results over the period of this study.

As shown in Figure 4-27 the performances of three stage systems with the first stages kept at 35°C and 42°C were identical in terms of TCOD concentrations. However, TCOD results did not agree with VS removal data that were observed through operation of three stage systems. The VS removal data presented in Figure 4-3 showed greater reductions as temperature increased in the first stage of three stage system followed by relatively similar VS removal rate for the downstream digesters. TCOD concentrations of 22500 mg/L were attained in the first stage followed by gradual decline in the second stage and a further decline at third stage to the value of 21000 mg/L.

(a). TCOD

(b). SCOD

Figure 4-27: COD Versus Three Stage Temperature Configurations (a) TCOD and (b) SCOD.

Figure 4-27 shows that during operation of the three stage systems, the effluent TCOD from the first stage of the systems that were operated at 49°C were higher than the 35°C digester as can be seen from the higher VS removal at 49°C (Figure 4-3). The average

TCOD in the first stage of 49°C-35°C-35°C system was 30000 mg/L and quickly decreased to the concentration of 23000mg/L through downstream digesters. A temperature increase in the second stage of 49°C-35°C-35°C to 42°C increased the concentration of TCOD to 32500 mg/L in the effluent from the second stage, followed by a gradual decrease in the third stage to 27000mg/L. This can be explained by comparing the TCOD and VS removal data for the both 49°C-35°C-35°C and 49°C-42°C-35°C systems. The Higher TCOD concentration in the second stage of the 49°C-42°C-35°C system when compared with the first stage of the similar system appears to be due to the complication of TCOD analysis of the sewage sludge.

As noted previously, changes in the temperature in the first stage of the two stage systems didn't have significant effects on SCOD concentration, and similar trends were observed for the three stage systems. The data presented in Figure 4-26 and Figure 4-27 illustrates that two and three stage operation of anaerobic digesters was very effective in terms of SCOD removal as compared with the control 35°C digester that was operated at a HRT of 15 days .

In general increasing the temperature of the first stage resulted in poor effluent quality for the first stage digester when compared to the 35°C digester. However, based on the results obtained in this study, the poor effluent quality from high temperature digesters can be improved by operation of second and third stages at mesophilic temperatures. Overall, the results indicate that the good effluent quality in terms of SCOD was mainly attributed to the low VFA in the effluent of the systems. In addition, temperature staging of the anaerobic digesters was observed to enhance the hydrolysis rate and methanogenesis activity of the tested systems.

4.3 Evaluation of Single, Two and Three Stage Anaerobic Digestion for Pathogen Reduction

A number of microbial indicators were measured in this study to assess the impact of HRT, temperature and temperature staging on pathogen inactivation. The bacterial indicators that were evaluated in this study consisted of FC, FS, *Salmonella*, *E.coli*, and *C. perfringens*.

ANOVA procedure was employed to determine whether the impacts of process parameters on bacterial indicators were statistically significant. The computation of ANOVA was accomplished using the Minitab software program.

To explore the rate of microbial reduction during conventional and staged operation, first order kinetic decay constants were calculated using the formula of Farrel *et al.* (1988) for each digester separately:

$$\frac{v}{V} \frac{C_f}{C_d} = \left(1 + \frac{v}{V}\right) e^{k\theta} - 1 \quad (4-2)$$

Where:

v = volume withdrawn and added,
 V = digester volume before volume v is fed,
 k = first order rate constant (d^{-1}),
 θ = HRT,
 C_f = microbial density in feed,
 C_d = microbial density in sludge withdrawn.

The experimental data describing pathogen behaviour in the various digesters consisted of 7 replicate measurements of pathogen concentrations in the feed and effluent of each digester. As is typical of data of this nature, there was considerable variability between the concentrations measured in the replicate data points. Hence a procedure that

addressed variability in both feed and effluent concentrations was developed to estimate the mean and standard deviations of the biokinetic coefficients. In this procedure the probability distribution of each set of replicate organism concentrations was determined. The statistical software package STATISTICA was employed for this purposes. For all of the organisms the concentrations were determined to be lognormally distributed. Hence, the mean and standard deviation of the underlying normal distributions were determined.

A Monte Carlo approach that employed these probability distributions was then developed to estimate the mean and standard deviations of the decay coefficients. In the Monte Carlo analysis, Equation 4-2 was rearranged to solve for the decay coefficient. For each organism in each digester a total of 5000 pairs of influent and effluent concentrations were generated by randomly selecting them from the previously described probability distributions. Equation 4-2 was then solved to obtain 5000 values of the decay coefficients. The estimated values were then used to calculate the mean and standard deviations of the decay coefficients.

4.3.1 Fecal Coliform Analysis

4.3.1.1 Effect of Single Stage Temperature and HRT on FC reduction

Traditionally temperature is known as the principal agent responsible for FC destruction. However the effect of operation at intermediate temperatures over a range of HRTs has not been fully investigated. Figure 4-28 presents FC concentration and log reductions for HRTs of 5, 15 and 20 day for the digesters that were operated over temperatures that ranged from 35°C to 62°C.

(a). Log FC densities

(b). FC log reduction

Figure 4-28: Effect of Temperature and HRT on (a) FC Densities, and (b) FC Log Reduction in Digesters.

The data in Figure 4-28 shows the reduction of FC at all operating conditions was greater than 2-logs, except for 35°C, and 42°C. These experiments reveal that the reduction of FC

increased with temperature leading to a characteristic heat inactivation profile. In addition, it was difficult to directly assess the impact of digester HRT on the reduction of FC because of the substantial increase in FC concentrations that was observed during the testing at a 5 day HRT. The results presented in Figure 4-28 indicate that at the 20 day HRT conventional mesophilic operation of the digesters resulted in approximately 1 log reduction in FC. This is in agreement with values that are reported by Berg and Berman (1980). For HRTs of 15 and 20 days operation at a “high-mesophilic” temperature (42°C) resulted in approximately 1.5-2 log reduction of FC while operation at a “low-thermophilic” temperature (49°C) resulted in approximately 2.6-2.8 log reduction of FC. There is limited data available in the literature for operation at these temperatures. The results suggest that operation at these intermediate temperatures can result in reduced concentration of this indicator organism however “disinfection” is not achievable.

At 55°C, and 62°C the maximum FC reduction of 3.9 logs, and 4.3 logs occurred at the 5 day HRT and the minimum FC reduction of 2.8 logs and 3.2 logs were observed for the 20 day HRT. It was anticipated that the longer HRT would result in higher FC log reduction than that of the short HRT. This might be due to the higher feed FC concentration that was experienced during the 5 day HRT experiment.

The results indicate that at temperatures of 55 and 62°C the outlet concentrations of FC did not substantially differ between HRT's. Hence, at these temperatures, the rates of inactivation were sufficiently high such that HRT was not a factor. At the lower temperatures the rates of inactivation were lower and hence digester effluent concentrations were influenced by fluctuations of inlet concentrations.

•

A two way (ANOVA) was carried out on FC log reduction in order to evaluate the significance of temperature and hydraulic retention time on FC destruction. In this analysis temperature and hydraulic retention time were evaluated at (35°C, 42°C, 49°C, 55°C, and 62°C) and (5, 15, and 20 days) respectively. The results of ANOVA are summarized in Appendix D.

In summary the temperature and HRT interaction suggest that the effect of increasing the temperature from 35°C to 62°C on the log reduction of FC was greater at 5 days than that at 15 and 20 days. The effect of increasing the temperature on the FC reduction was minimum at 20 days.

Figure 4-29: FC Decay Rate Constant.

To provide quantitative information on the temperature and HRT effects on pathogen destruction, decay rate constants were calculated as previously described. The decay values for FC over the temperature range from 35°C to 62°C for HRT's of 5, 15, and 20 days are presented in Figure 4-29. The results presented in Figure 4-29 show that

calculated decay rates were consistently lower for the shorter HRT as well as at lower temperatures. At 35°C the decrease in HRT from 20 days to 5 days results in a decrease in the decay rate constant from 0.8 d^{-1} to 0.3 d^{-1} . Similarly a decay rate constant of 1.4 d^{-1} was observed for the 42°C digester that was operated at HRT's of 20 and 15 day and decay rate was dropped to 0.7 d^{-1} when HRT reduced to 5 days. As temperature in the digester increased to 49°C, and 55°C, and further to 62°C, the highest decay rate was observed for a HRT of 20 days followed by gradual reduction for a HRT on 15 days and further reduction at 5 days HRT. From the results it can be seen that at higher temperature and longer HRT's FC would be inactivated quickly when compared to the lower temperature and shorter HRT's. It is anticipated that at reduced HRT high concentrations of different FC species with different susceptibility rates were introduced to the investigated digester systems and lower die-off rates were expected at operating temperature of 35°C and 42°C. Therefore, the decay rate constants represent an average value for all FC species.

4.3.1.2 Effect of The Temperature Staging on FC Destruction

In this part of the study the impact of two and three stage operation of anaerobic digesters on inactivation of FC was studied.

Two Stage Operation

Figure 4-30 presents the log reductions of FC for two stage operation of an anaerobic digestion process. The two stage system at 35°C resulted in approximately 1 log unit reduction in each of the first and second stages. Operation of the first stage of a two stage system at 42°C resulted in an additional reduction of 0.88 log units when compared to operation at 35°C for the first stage. A temperature increase in the second

stage of two stage systems from 35°C to 42°C enhanced log reduction of FC by 0.5 log unit.

Figure 4-30: FC Destruction Versus Two Stage Temperature Configuration.

An increase in temperature from 42°C to 49°C in the first stage of 49°C-35°C system resulted in an additional 1.5 log reduction in FC when compared to operating the first stage digester at 35°C. From the results it can be seen that two stage operation enhanced the reduction of FC by 0.89 log units for 35°C-35°C, 2 log units for 42°C-35°C, and 2.5 log units for both the 42°C-42°C and the 49°C-35°C systems as compared to 1.35 log unit reduction for the single stage conventional digesters. A one way ANOVA and a Tukey test were applied on FC log reduction to assess the significance of temperature variations in the first and second stage of the digesters that were operated in series operation on overall FC reduction of the investigated systems. The results reveal that there was significant difference ($P=0.002$) in FC log reduction between first and second stage. The results of statistical analyses are presented in Appendix D.

The susceptibility of FC in two stage operations was determined by calculating decay rate constants. Figure 4-31 summarizes the decay rate values obtained from two stage operation. The average decay values of the 35°C-35°C system were 1.5, and 1.1 day⁻¹ in the first and second stages, respectively. The values of the decay rate under 42°C-35°C conditions were found to be 3 and 1.9 day⁻¹ for the first and second stages, respectively. An increase in the decay rate in the second stage of 42°C-42°C system to 3 day⁻¹ was observed and may be due to the exposure of FC to the 42°C operating temperature for a longer period which caused more FC microorganisms to die or to lose their viability.

Figure 4-31: FC Decay Rate Constant Versus Two Stage Temperature Configuration.

The average decay values in the first stage of 49°C-35°C system was found to be 5.2 day⁻¹ while a value of 0.82 d⁻¹ was observed in the second stage that was operated at 35°C. From the results it can be concluded that in addition to the temperature effect,

susceptibility of the different FC species plays a significant role in the decay rate calculation.

Three Stage Operation

Figure 4-32 (a) presents the incremental log reductions of fecal coliform that were observed in the individual digesters as well as the cumulative reductions that were observed in the staged systems. From Figure 4-32 (a) it can be observed that three stage operations resulted in a substantially greater reduction in FC when compared to the single stage control that demonstrated a log reduction of approximately 1.5. The three stage system that had all digesters operating at 35°C demonstrated almost 3 log reductions, while the 49°C-42°C-35°C system demonstrated approximately 4.5 log reduction of FC and the rest of the tested three stage systems demonstrated approximately 4 log reduction of FC.

The results presented in Figure 4-32(a) indicate that increasing the temperature of the first stage digester had a substantial impact upon FC in this stage. An increase from 35°C (35°C-35°C-35°C) to 42°C (Systems 42°C-35°C-35°C and 42°C-42°C-35°C) resulted in an increase in FC reduction by approximately 1 log and an increase from 42°C to 49°C (Systems 49°C-35°C-35°C and 49°C-42°C-35°C) resulted in almost an additional 1 log increment in log reduction. In addition, there was significant variation for the systems that had their first stage operated at 42°C as they were run approximately 4 months apart.

(a). Log Reductions in Digesters

(b). Log Reductions Versus Temperature

Figure 4-32: FC in Three Stage Digester (a) Log Reductions in Digesters (b) Log Reductions Versus Temperature.

To further explore the impact of temperature on FC reduction, it was assumed that each individual digester operated at similar temperature resulted in a similar degree of reduction, despite of their order of arrangement in the investigated systems. If the temperature considered to be the main factor in FC log reduction obtained incremental log reductions are expected to be condensed in one point other wise if the data points are scattered it can be concluded that some other parameter other than temperature affected reduction rate in these digesters. Therefore, the incremental log reductions that were observed in each of the stages were plotted against the temperatures of the stages (Figure 4-32b).

From Figure 4-32b it can be seen that there was a strong relationship between temperature and log reductions of FC in the three stage systems. There was however considerable variability in the reductions that were observed at the individual temperatures. In general the greater log reductions were observed in the earlier stages of the 3 stage systems while the lower reductions were observed in the latter stages. The results suggest that factors other than temperature had an impact upon the reduction of FC in the staged digester systems. It is hypothesized that the elevated concentrations of VFA's in the first stages may have contributed towards inhibition of FC while in latter stages the concentrations of VFA's were lower and hence the contribution of this mechanism to VFA reduction was limited.

In summary, maximum reduction was achieved in the systems with the first and second stages kept at 49°C and 42°C respectively. Side by side comparison of single, two and three stage operation which are presented in Figure 4-28, Figure 4-30, and Figure 4-32, respectively, show that higher FC reduction was observed for three stage operation

when compared to two and single stage systems. This can be attributed to the reduced likelihood of FC bleeding through that might be experienced when compared to two and single stage systems. In addition, higher operating temperatures in the first and second stage significantly enhanced the log reduction of FC. Higher FC log reduction in the first stage of the two and three stage systems can be due to changes in availability of the substrates and the inactivation of more susceptible species of FC in the first stage while less inactivation of more resistant species was observed in the latter stages.

Figure 4-33 shows the decay rate constant values for FC under three stage operation of the anaerobic digesters. In general, a gradual decrease in decay rate constants was observed as the sludge progressed through the individual reactors in each system.

Figure 4-33: FC Decay Rate Constant Versus Three Stage Temperature Configurations.

The decay rates in the first stage of three stage system increased from 1.4 day^{-1} to 5.5 day^{-1} as the temperature increased from 35°C to 49°C . Temperature increase

from 35°C to 42°C in the second stage of the 42°C-42°C-35°C and 49°C-42°C-35°C systems reveal that decay rates in this stages were influenced by operational temperature in the second stage as well as operating temperature in the first stage. The similar trend was observed for the third stage. These results may be due to the fact that different species of FC had different removal rates. The most temperature resistance ones would decline more slowly than the less resistant and this resulted in the lower rate constants.

4.3.2 *E.coli* Analysis

E.coli. has been proposed as indicator organism for pathogens in biosolids (European Union, 2000). Mesophilic anaerobic digesters that were considered to be well operated demonstrated *E.coli* reductions between 1.35 and 2.36 log units (Harrison *et al*, 1997). Operation of thermophilic anaerobic digestion has been found to result in greater than 4 log reduction in *E.coli* concentrations (Harrison *et al*, 1997). This section will subsequently present the impacts of temperature, HRT, and staging on *E.coli* destruction

4.3.2. Effect of Temperature and HRT on *E.coli* Inactivation

The inactivation of *E.coli* during single stage anaerobic digestion of municipal sewage sludge was investigated at steady state over a range of temperatures and HRT's. In general the reduction of *E.coli* was relatively lower as compared to the observed for FC that presented in Figure 4-28. Figure 4-34 presents the concentrations of *E. coli.* that were measured in the inlet and outlets of the digesters in this portion of the study. From Figure 4-34 it can be seen that there was substantial variability in the inlet concentrations between the runs that were conducted at HRT's of 5, 15 and 20 day. The inlet concentration of *E.coli.* varied from 4.5 to 7.8 log CFU/gTS. The cause of high *E.coli* concentration in the feed sludge at 5 days HRT is unknown. The trend in the responses of

E. coli as a function of temperature were similar to those observed for FC. The outlet concentrations decreased as temperature increased until a temperature of 55°C was attained. There was little difference between the responses obtained for 55°C and 62°C. The results indicate that for conventional HRT's there is little additional benefit to operate at temperatures above 55°C.

(a). *E. coli* Densities

(b). *E. coli* Log Reduction

Figure 4-34: Effect of Temperature and HRT on (a) *E. coli* Densities, and (b) *E. coli* Log Reduction in Digesters.

The outlet concentrations of *E. coli* increased as the HRT decreased from 20 days to 5 days at operating temperatures of 35°C, 42°C and 49°C. However, it must be noted that the inlet concentrations for the 5 and 15 day HRT were also higher and the elevated outlet concentrations of *E. coli*. At this HRT may have at least partially resulted from the higher loading of the organisms during these period. The concentrations of *E. coli* were below the method detection limit in almost all of the effluent samples that were collected from the effluents of the digesters operating at 55 and 62°C. At these temperatures there was no clear HRT influence on the *E.coli* reduction. In all cases the effluent samples had concentrations that were less than 1000 CFU/gTS. Hence, it is clear that operation at these temperatures and HRT was able to produce a high quality biosolids product. Operation at temperatures of 42°C and 49°C resulted in incremental reductions of approximately 1-1.4 log for each increase in temperature as compared to conventional mesophilic operation for HRT's of 5, 15 and 20 day.

The results of two ways (ANOVA) on *E.coli* log reduction are presented in Appendix D. The statistical analysis revealed that the effect of temperature and HRT and the interaction of both two parameters on log reduction of *E.coli* were statistically significant at 95% confidence interval. However, p-values indicated that the interaction of temperature and HRT were less significant than temperature and HRT individually.

Figure 4-35: *E.coli* Decay Rate Constant Versus Temperature and HRT.

Performance of single stage anaerobic digester for HRT's of 5, 15 and 20 days over the range of temperatures was evaluated in terms of decay rate constant. From Figure 4-35 it can be seen that the error bars on the charts, which represent one standard deviation about the mean values, are relatively wide and hence there was wide variation between the duplicate samples. From Figure 4-35 it can be seen that 35°C and 42°C operation over the HRT ranged from 5 to 20 days resulted in a similar decay rate constant of 0.25 d^{-1} and 0.52 d^{-1} respectively. For 49°C decay rate constant of 1.6 d^{-1} was observed at HRT of 20 days and decay rate reduced at reduced HRT's. For the digesters that were operated at 55°C and 66°C and HRT of 15 day greater decay rate was observed as compared to the 20 day HRT. This might be explained that the decay rate either not follow first order or, more likely reflect the presence of more susceptible *E.coli* species in the digesters that were operated at HRT of 15 day over tested temperature range of 55°C to 62°C.

4.3.2.2 Effect of Temperature Staging on *E.coli* Destruction

This section reports the results for *E.coli* under two and three stage operation of anaerobic digester in series.

Two Stage Operation

The effect of the staged operation of anaerobic digestion on inactivation of *E.coli* in two stage operation is depicted in Figure 4-36. Operation of two stage systems with both stages at 35°C showed average *E.coli* log reduction of 0.5 and 1.3 for the first and the second stage, respectively. This demonstrates that staged operation at 35°C enhanced the log reduction of *E.coli* by almost 0.8 log unit as compared to 1 log reduction in control digester that was operated at 35°C.

Figure 4-36: *E. coli* Log Reductions Versus Two Stage Temperature Configuration.

Performance of systems operated at 42°C-35°C and 42°C-42°C consistently gave log reduction of *E.coli* by 2.4 and 2.7 units as compared to 1, and 1.8 log units during single stage and mesophilic two stage operation. The largest *E.coli* log reduction of 3.5 units was observed for the two stage system that the first stage was operated at 49°C.

From the results appears that the temperature appears to be the decisive factor in the *E.coli* removal.

Figure 4-37: *E.coli* Decay Rate Constant Versus Two Stage Temperature Configuration.

The fate of *E.coli* in the two stage configurations was investigated by calculation of decay rate constants. Figure 4-37 shows that 35°C-35°C system demonstrated decay rate constants values of 0.52 day^{-1} in the first stage and 1.80 d^{-1} in the second stage. By comparison the 42°C-35°C and 42°C-42°C systems, relatively similar decay rate constant of 2.8 d^{-1} and 1.2 d^{-1} were observed for the *E.coli* in the first and second stage of the two stage system. Change in temperature from 42°C to 49°C resulted in an increase in the rate constant by 50% as compared to 42°C operation of the first stage. .

The results suggest that the temperature in the first stage was the main factor in destruction of more susceptible *E.coli* species and this can be seen by high decay rate constant in the first stage as compared to the second stage. Overall, from the results it can be seen that temperature is considered to be the prime responsible for *E.coli* destruction rate.

Three Stage Operation

The effects of three stage temperature configurations on *E.coli* log reductions are shown in Figure 4-38. Figure 4-38 presents the log reductions that were observed for *E. coli* in the individual stages (a) and versus digester temperature (b). The patterns in log reductions of *E. coli* were similar to those observed for FC with substantial increases in reduction observed in the staged digesters.

From Figure 4-38 (a) it can be seen that when the three stage system was operated at 35°C *E.coli* were reduced by 2.5 log units that was about 0.9 more log reductions as compared to two stage system that was operated at 35°C. Operation at 42°C-35°C-35°C and 42°C-42°C-35°C configurations resulted in different *E.coli* reduction in the first stage and similar log reductions by 2.9 units in final system effluents. However, variation in *E.coli* log reduction in the first stage of the three stage systems can be attributed to the variability of *E.coli* concentration in the feed sludge that was obtained seven months apart. Operation of three stage system with the first stage at 49°C resulted in 3.5 log reductions. A temperature increase to 42°C in the second stage demonstrated 3.8 *E.coli* log reductions in final system effluent which was about 0.3 log unit higher than the system with the first and second stages operated at 49°C and 35°C respectively.

(a) Log Concentrations

(b) Log Reductions

Figure 4-38: *E.coli* in Three Stage Digester (a) Log Concentrations (b) Log Reductions Versus Three Stage Temperature Configuration.

Overall, increases in temperature resulted in greater reductions of *E. coli* and degree of reductions were somewhat lower than those observed for FC (Figure 4-32), suggesting that *E. coli* was somewhat more resistant to inhibition in the digesters.

To further explore the impact of temperature on *E.coli* reduction the incremental log reductions that were observed in each of the stages were plotted against the temperatures of the stages (Figure 4-38b). From Figure 4-38b it can be observed that there was a strong relationship between log reductions of *E.coli* and temperature. However as with FC, there was considerable variability at each of the temperatures suggesting that factors other than temperature were impacting on reduction of *E. coli* and was observed to be higher at elevated temperature.

The susceptibility of the *E.coli* in this study was investigated in terms of decay rate constants. Figure 4-39 presents the results of decay rate constants obtained in this study after exposure to Monte Carlo approach.

Figure 4-39: *E.coli* Decay Rate Constant Versus Three Stage Temperature Configuration.

Operation of three stage anaerobic digester at 35°C resulted in a value of decay rate constants of 1.4 day^{-1} in the first stage of the 35°C-35°C-35°C system followed by a

value of 0.8 day^{-1} in the second stage and a value of 0.51 day^{-1} in the final stage. Operation of 42°C - 35°C - 35°C system resulted in decay rate constants of 1.6 day^{-1} in the first and second stage and a value of 0.56 day^{-1} in the third digester. The 42°C - 42°C - 35°C system demonstrated significant difference in the decay rate constants in the first stage as compared to the of the 42°C - 35°C - 35°C system. This can be attributed to the high *E.coli* concentrations that were observed in feed sample over the study period that was performed in the summer of 2004 (Table 4-3).

During operation of the three stage configuration with the first stage at 49°C a decay rate constants of 4.3 day^{-1} was observed in the first stage and it decreased to 0.4 day^{-1} and 0.5 day^{-1} in the second and third stages. During this study operation of the second stage at 42°C show significant improvement in decay rate constant in the second (0.7 day^{-1}) and further in the third stage (0.9 day^{-1}). However the error bars on the charts, give values were relatively wide about 20 to 75% of mean average and hence there was variation between the replicate samples. In summary, the results of this study stated that the temperature appears to be the decisive factor in the *E.coli* removal.

4.3.3 *Salmonella* spp. Analysis

Samonella spp. are the most common pathogens that are present in municipal wastewater treatment sludges and have major concern in beneficial reuse of biosolids (Bitton, 1994). In this study the survival of *Salmonella* at different operating conditions of HRT, temperature, and staging was investigated.

4.3.3.1 Effect of Single Stage Temperature and HRT on *Salmonella* spp.

Figure 4-40 presents the effect of retention time and temperature on concentrations and log reduction of *Salmonella*. The data presented in Figures 4-40 indicate that the effects of HRT, and temperature on *Salmonella* were more similar to the effects on FC (Figure 4-28) than to *E.coli* (Figure 4-33). Therefore using FC as an indicator of the presence of *Salmonella* spp. is reasonable. The maximum reduction in *Salmonella* densities occurred at 49°C, 55°C, and 62°C for all HRT's. The effluent concentrations measured at 5 and 15 day HRT appeared to be somewhat higher than those observed at a 20 day HRT for these temperatures.

From Figure 4-40 it can be seen that the feed sludge concentrations were in the range of 5-6.2 log₁₀ CFU/g TS and these concentrations were reduced to below the detection limit of 2 log₁₀ CFU/g TS in all of the samples collected from the digesters operating at 49, 55 and 62°C with the exception of 49°C digester at 5 days HRT. The detection limits of the analytical method employed for *Salmonella* spp. in this study were considerably higher than those that have been used elsewhere (U.S EPA, 1999). Hence it was not possible to evaluate the impact of temperature on *Salmonella* spp. reduction at these higher temperatures.

(a). *Salmonella* Densities (b). *Salmonella* Log Reduction

Figure 4-40: Effect of Temperature and HRT on (a) *Salmonella* Densities, and (b) *Salmonella* Log Reduction in Digesters.

Operation of the digesters at 42°C appeared to reduce the effluent concentrations of *Salmonella* somewhat as compared to operation at 35°. However, the substantial variability in concentrations that were observed in these digesters prevented making definitive conclusions in this regard.

The results of two way ANOVA on *Salmonella* log reduction are presented in appendix D. The F-value and P-value indicate that the effect of temperature, were more significant than the HRT and the interaction between these two factors at the 95% confidence interval.

In this phase of experimentation decay rate constant were calculated in order to study the fate of *Salmonella* spp. under various temperatures and HRT's.

Figure 4-41: *Salmonella* spp. Decay Rate Constant Versus Temperature and HRT.

The results presented in Figure 4-41 show a consistent trend in decay rate constant at the 5 and 20 day HRT's. At the 5 and 20 day HRT's the increase in the temperature resulted in an increase in decay rate constant. Similar decay rates were observed for those digester that they were operated at temperature higher than 49°C for both the 5 and 20 day HRT's. The decay rate constants at the 15 day HRT were lower than the decay rate constants which were calculated at the 5 and 20 days HRT. This result was not expected and can be attributed to the large variation in decay rate constant over

the range of analysis and this can contribute significantly to the fact that there is still lack of standard analysis for *Salmonella* spp.

4.3.3.2 Effect of Temperature Staging on *Salmonella* pp. Inactivation

In this study *Salmonella* concentrations were examined under two and three stage operation of anaerobic digester in series.

Two Stage Operation

The average efficiencies of *Salmonella* removal in the two stage digestion system are presented in Figure 4-42. Side by side comparison between *Salmonella* removal rate and *E.coli* (Figure 4-36) and FC (Figure 4-30) it was evident that *Salmonella* were the more resistant group of bacteria.

Figure 4-42: *Salmonella* spp. Log Reductions Versus Two Stage Temperature Configuration.

From Figure 4-42 it can be seen that the lowest *Salmonella* spp. reduction of 1.3 log units occurred in the two stage system where both stages were operated at 35°C. Operation of the first stage of the two stage system at 42°C enhanced the log reduction of

Salmonella spp. by 1.2 log units. Increasing the temperature in the second stage of the two stage operation to 42°C resulted in greater log reduction of *Salmonella* spp. by 0.5 log unit in the second stage as compared to the system where the second stage was operated at 35°C. When the first stage was operated at 49°C the reduction of *Salmonella* spp. was enhanced by 1.3 log units as compared to the 42°C-35°C. The results of this study demonstrate the significance of multistage operation at elevated temperature on *Salmonella* spp. destruction as compared to conventional single stage operation. A one way ANOVA and a Tukey test were applied on *Salmonella* log reduction to assess the significant of temperature variations of the first and second stage of the digesters that were operated in series operation on overall *Salmonella* reduction. Statistical results are presented in Appendix D and reveal that the impact of temperature variation on log reduction in the first and second stage at 95% confident interval was significant.

Figure 4-43: *Salmonella* Decay Rate Constant Versus Two Stage Temperature Configuration.

The effect of two stage temperature operation on the destruction rate as stated for *Salmonella* spp. was studied. The decay rate constant values were plotted in Figure 4-43. Operation of two stage system at 35°C demonstrated two different rate constants in first and second stages. This trend was observed for all the investigated system in this study. Temperature increases in the first stage to 42°C and 49°C resulted in increase in decay rate constants of 2.5 d⁻¹ to 3 d⁻¹ and 5.4 d⁻¹ respectively. From the results it can be seen that the rapid die-off of the individual *Salmonella* cells occurred in the first stage followed by slow death of the resistant or protectively clumped bacteria in second stage. These fast and slow decay rate are effectively dependent.

Three Stage Operation

The data of *Salmonella* in three stage configuration is summarised in Figure 4-44. Figure 4-44 presents the log reductions of *Salmonella* spp. that were observed in the individual reactors (Figure 4-44 a) and versus temperature (Figure 4-44 b). From Figure 4-44a it can be observed that the log reductions for *Salmonella* spp. in the first stage digesters were strongly affected by temperature. All treatment systems showed more than 2 log units reduction and up to 4.5 log unit reductions in *Salmonella* concentration was observed. Increasing the temperature from 35 to 42°C resulted in approximately 1 log reduction while increasing the temperature from 42 to 49°C resulted in an additional 1.5 log reductions in the first stage digester.

(a) Log Reductions in Digesters

(b) Log Reductions Versus Temperature

Figure 4-44: *Salmonella* spp. in Three Stage Digester (a) Log Reductions in Digesters (b) Log Reductions Versus Temperature.

The overall system reductions of *Salmonella* spp. were strongly impacted by temperature and increasing the temperature in the second stage digesters (Systems 42°C-42°C-35°C and 49°C-42°C-35°C) resulted in a substantial increase in overall reduction as compared to the comparable systems where the second stages were maintained at 35°C (Systems 42°C-35°C-35°C and 49°C-35°C-35°C).

Figure 4-44 b presents the log reductions of *Salmonella* spp. in all of the reactors as a function of temperature. From Figure 4-44 b it can be seen that, as with FC (Figure 4-32b) and *E. coli*. (Figure 4-38b), there was a strong relationship between log reductions and temperature at elevated temperature than 35°C. From the results it can be seen that at 35°C and 42°C other factors rather than temperature affecting the log reductions and for 49°C digester, temperature is mainly the inactivation factor. However, there was also considerable variability which was also observed with the other organisms.

Salmonella die-off was investigated by determination of decay rate constants. The results presented in Figure 4-45 demonstrate that the *Salmonella* decay rate constants were influenced by the temperature in the first stage of three stage operation. The average level of the *Salmonella* decay rate constants in the first stage of investigated system shows operation of anaerobic digesters at elevated temperatures would enhance the decay rate of susceptible *Salmonella* spp. A further increase in the temperature in the second stage of tested system resulted in an increase in the decay rate constant of more resistant species of *Salmonella*. Overall from this portion of the study it can be seen that decay rate coefficients is significantly affected by the temperature.

Figure 4-45: *Salmonella* Decay Rate Constant Versus Three Stage Temperature Configuration

But the response of *Salmonella* to temperature was considerably different from the previously described organisms.

4.3.4 Fecal Streptococci Analysis

In this part of the study the fate of fecal streptococci in single and multistage operation of anaerobic digesters was investigated.

4.3.4.1 Effect of Temperature and HRT on FS Inactivation

FS are more resistant to inactivation in conventional sludge treatment systems than FC (Bitton, 1994). The effects of retention time and temperature on the densities and log reduction of FS are illustrated in Figure 4-46. The data presented in Figure 4-46 shows that the log reduction of FS increased rapidly at 20 and 15 days retention time, and at temperature higher than 49°C as compared to the lower temperatures. At HRTs of 15 and 20 days, the FS reduction increased at 49°C and higher and slower reduction rate was

observed at 5 days HRT. At the HRT of 5 day no significant reduction was observed for digesters that were operated at 35°C and 42°C.

(a). FS Densities,

(b). FS Log Reduction in Digesters

Figure 4-46: Effect of Temperature and HRT on (a) FS Densities and (b) FS log Reduction in Digesters.

The maximum FS reductions of 3-4.3 logs units were observed for the digesters that were operated at 49°C, 55°C, and 62°C for HRT's of 15 and 20 day. At the 5 day HRT as temperature increased from 35°C to 42°C gradual improvement in FS log

reduction was observed. Further temperature increase from 49°C to 62°C enhanced FS log reduction rapidly with the exception of the 62°C digester that showed similar reductions for all the tested HRT's. FS concentrations were relatively higher in the digesters that were operated at the 5 day HRT. Overall, lower FS log reduction was obtained for lower HRT and at mesophilic operating temperature of 35°C and 42°C.

The results presented in Figure 4-46 indicate that temperature was more significant than retention time in reducing the FS densities, yet the inactivation of FS was lower than FC data presented in Figure (4-28) at the same operating conditions which reveals that FS were more resistant to inactivation than FC during anaerobic digestion.

A two way ANOVA test was performed to statistically compare the FS log reductions. The ANOVA results showed that the effects of temperature and HRT and the interaction between HRT and temperature were statistically significant at the 95% confidence level. The results of the ANOVA were summarized in Appendix D.

In this study destruction rate constants for FS were investigated. Monte Carlo simulation was performed on obtained results to verify the variability of the results. Figure 4-47 shows the decay rate constants for temperature that ranged from 35° to 62°C over the HRT's of 5, 15, and 20 days. The digesters that were operated at 35°C and 42°C show the lowest decay rate constants that were reflective of the low FS destruction. On the contrary, at 49°C and higher for HRT of 15 and 20 days constant decay rate were observed and the values declined at the HRT of 5 days. For the 5 day HRT as temperature increased from 35°C to 62°C gradual increase in the decay rate was observed. From the results it can be concluded that effective FS destruction rate was achieved at

HRT of 15 and 20 day for temperature higher than 49°C as indicated by higher decay rate constants.

Figure 4-47: FS Decay Rate Constant Versus Temperature and HRT.

4.3.4.2 Effect of The Temperature Staging on FS Inactivation

This section presents the results of FS inactivation observed in the temperature staging study.

Two Stage Operation

In this study the average concentration of FS in the feed sludge was 6.89 log units which was similar to the concentrations of FC and *E.coli* as indicated in Table 4-1. Figure 4-48 presents the effects of two stage operation on FS log reductions. Figure 4-48 revealed that the single stage mesophilic digester resulted in a 0.52 log reduction. Overall the FS concentrations decreased in the first stage of two stage systems as the temperature increased from 35°C to 49°C. Operation of the second stage at elevated temperature improved the reduction of FS significantly. For the systems investigated in this study a gradual decrease in FS concentrations was observed as the sludge passed through each

individual digester. Operation of the two stage digester systems at 35°C resulted in 1.6 log unit reductions of FS concentrations. A reduction of 2.56 log concentration units of FS was observed during two stage operation when the temperature in the first and second digesters was maintained at 42°C. The concentration of FS declined up to 2.5 log units in the first stage digester that was kept at 49°C and further declined to 3.2 log units in the digester effluent of the downstream mesophilic digester. From the results it can be seen that the temperature increase was largely responsible for FS destruction in two stage systems.

Figure 4-48: FS Log Reductions Versus Two Stage Temperature Configuration.

The trends in removal of FS were similar to FC and *E.coli*, however the FS concentrations in the effluents of the digesters were higher in each system, demonstrating their greater survival in the digesters. A one way ANOVA and a Tukey test were applied on FS log reduction to assess the significant of temperature variations of the first and second stage of the digesters that were operated in series operation on overall FS reduction of the investigated systems. The results reveal that there was significant

difference in FS log reduction between first and second stage. The results of statistical analysis are presented in Appendix D. To further investigate the die-off rate of FS in the two stage study decay rate constants were determined.

Figure 4-49: FS Decay Rate Constant Versus Two Stage Temperature Configuration.

Figure 4-49 presents the decay rate constants for FS in the two stage digesters. Figure 4-49 shows that the FS decay rates for the temperatures below 49°C were relatively equal. This suggests that most FS species could survive temperatures between 35°C and 42°C. An increase in temperature to 49°C enhanced the decay rate of FS in the first digester by 55% as compared to 35°C digester. The results of this study show that effective treatment of FS was achieved at temperatures higher than 49°C. With the exception of 49°C-35°C system, relatively high decay rates constant was observed for the second stage of two stage system than the first stage. This indicates that FS destruction rate through staging operation did not follow first order decay coefficient. In addition, high standard error for these data is indicative of the relatively large variability between replicate samples. Further research needs to be done to study the fate of certain FS species at higher temperatures.

Three stage operation

The log reductions of FS observed in the three stage anaerobic digesters are shown in Figure 4-50. From Figure 4-50a it can be seen that operation of three stage system at 35°C reduced the log concentration of FS by 2.3 units.

(a) Log Reductions in Digesters

(b) Log Reductions Versus temperature

Figure 4-50: FS in The Three Stage Digester (a) Log Reductions in Digesters (b) Log Reductions Versus Temperature.

An increase in temperature to 42°C in the first stage of the three stage system resulted in a log reduction of 1.2 and a further incremental log reduction of 0.9 and 0.7 in

the second and third stage of 42°C-35°C-35°C and 42°C-35°C-35°C systems. Operation of the first stage of the three stage system at 49°C resulted in average FS log reduction of 1.6 units as compared to 0.5 and 1.1 log in the first stage of 35°C and 42°C digester. Temperature increase from 35°C to 42°C in the second stage of 49°C-35°C-35°C system resulted in higher log reduction of FS by 0.4 log unit. Overall, 49°C-35°C-35°C and 49°C-42°C-35°C systems showed similar log reduction of 3 units in the system effluent. Overall, as with FC, *E. coli* and *Salmonella* operation of the first stage of two stage system at elevated temperature enhanced log reduction rate of these micro-organisms significantly as compared to 35°C operation of the two stage system. However, each microorganism showed different respond to the similar operating conditions.

To further explore the impact of temperature on FS reduction the incremental log reductions that were observed in each of the stages were plotted against the temperatures of the stages (Figure 4-50b). From Figure 4-50b it can be seen that there was a strong relationship between log reductions of FS and temperature. However as with FS, there was considerable variability at each of the temperatures suggesting that factors other than temperature were impacting on reduction of FS and was observed to be higher at elevated temperature. The decay rate constants were determined to obtain die-off rate of FS through three stage operation of anaerobic digester and presented in Figure 4-51.

Figure 4-51: FS Decay Rate Constant Versus Two Stage Temperature Configuration.

Operation of three stage system at 35°C resulted in the decay rate constants of 0.55 d⁻¹ in the first stage digester and stayed relatively the same in the down stream digesters. With the temperature increase to 42°C, decay rate constant of 1.29 d⁻¹ was observed in the first stage with gradual decrease to 0.85 d⁻¹ and 0.65 d⁻¹ in the second and the third stage digester respectively. Temperature increase in the second stage to 42°C in the 42°C-42°C-35°C system improve decay rate constants by 20% as compared to the 42°C-35°C-35°C. The overall decay rate in any combination of treatment at 49°C was significantly higher in the first stage than second and third stage. Temperature increase in the second stage of the 49°C-35°C-35°C system to 42°C enhanced the decay rate constant from 1 d⁻¹ to 1.4 d⁻¹ and significant drops in the last stage to 0.4 d⁻¹. However, the error bars on the charts were relatively wide about 50 to 85% of mean values and hence there was variation between replicates samples.

4.3.5 *Clostridium perfringens* Analysis

This portion of the study investigated the effects of single and multistage operation of anaerobic digesters on *C. perfringens* inactivation.

4.3.5.1 Effect of Single Stage Temperature and HRT on *Clostridium perfringens*

Figure 4-51 presents the concentrations of *C. perfringens* that were measured in the digester inlets and outlets. From Figure 4-59 it can be seen that the response of this organism to temperature was considerably different from the previously described organisms. With the exception of the digesters operated at 55 and 62°C the concentrations of *C. perfringens* appeared to increase in the digesters. In these digesters the concentrations leaving the digester were somewhat similar to the inlet concentrations. *C. perfringens* is an obligate anaerobe with optimal growth in the thermophilic temperature range hence due to the availability of nutrients considerable growth of this organism can occur over a range of operating temperatures.

Figure 4-51: *C. Perfringens* Concentrations Versus HRT and Temperature.

C. perfringens has been proposed as an indicator for the effectiveness of wastewater and sludge treatment processes (Hirata *et al.*, 1991). The results suggest that *C. perfringens* is not a good indicator of pathogen reduction in anaerobic digestion of municipal sludges.

The results of two way ANOVA on the log concentration of *C. perfringens* is presented in Appendix D. The statistical analysis revealed that the effect of temperature on log concentration of *C. perfringens* was not statistically significant at the 95% confidence interval. However, P-values for both HRT and interaction of temperature and HRT indicate that their impact on log *C. perfringens* concentrations were not statistically significant. The effectiveness of anaerobic digestion process on removal of *C. perfringens* from theoretical view point was investigated in this study by calculation of decay rate constant for different operational conditions and presented in Figure 4-52.

Figure 4-52: *C. perfringens* Decay Rate Constant Versus Temperature and HRT

C. perfringens decay rates in each system were determined by using equation 4-2. Overall *C. perfringens* were more resistant to inactivation that was caused by temperature exposure than the previously described microorganisms.

From Figure 4-52 it can be seen that the decay rates for *C. perfringens* did not show any particular pattern over the course of this study. At 35°C decay rate constant of 0.4 d^{-1} was observed for the digester that was operated at the HRT of 20 days and growth of this microorganism was experienced at reduced HRT's.

Operation of anaerobic digester at 42°C resulted in growth of *C. perfringens* at 20 days HRT and optimal inactivation rate at the HRT of 15 day with the minimum decay rate at 5 day digester. At 49°C *C. perfringens* decay rate was maximum at HRT of 20 and 5 days and minimum at 15 days HRT. Operation of anaerobic digester at 55°C and 62°C resulted substantially improvement in decay rate in reduced HRT digesters. In summary, temperature is generally acknowledged to be the most important factor determining *C. perfringens* survival, however, large variability among the obtained data unable us to draw any conclusion on the significant effect of the HRT, temperature and their combination on *C. perfringens* deactivation rate.

4.3.5.2 Effect of Temperature Staging on *C. perfringens* Inactivation

Two Stage Operation

Figure 4-53 presents the concentration of *C. perfringens* that were measured during two stage operation of the anaerobic digester. From Figure 4-53 it can be seen that the response of this organism was considerably different from the previously described organisms.

Figure 4-53: *C. perfringens* Removal Versus Two Stage Temperature Configuration.

Operation of conventional mesophilic digester resulted in concentrations of *C. perfringens* that were often higher than the feed sludge by more than 0.4-1 log unit (Figure 4-51). *C. perfringens* is an obligate anaerobe with optimal growth and proliferation in the temperature range of 35°C to 49°C. Figure 4-53 shows that the log concentration of *C. perfringens* declined from 6.36 in the first stage through the second stage to a final log concentration of 5.22 for the both 35°C-35°C and 42°C-35°C systems.

For both 42°C-35°C and 49°C-35°C systems growth of *C. perfringens* by 0.33 log units was observed in the second stage as compared to the first stage. However, the enumeration technique that was used in this study does not provide any indication regarding viability of *C. perfringens* spore.

Decay rate coefficients of the *C. perfringens* was determined in order to provide additional information on inactivation of this organism under two stage operation of anaerobic digestion (Figure 4-54).

Figure 4-54: *C. perfringens* Decay Rate Constant Versus Two Stage Temperature Configuration.

In this study, significant increase in the decay rate constants was observed for the two stage system that was operated at 35°C as compared with the rest of the two stage systems. From Figure 4-54 it can be seen that decay rates was increase from 0.7 d⁻¹ to 1.1 d⁻¹ as sludge progressed through each individual digester unit. During operation of two stage systems at elevated temperature decay rate of less than 0.1 d⁻¹ was observed in the first stage followed by re growth in the second stage digester. From the results it can be concluded that operation of two stage digesters at elevated temperature was ineffective in reducing *C. perfringens*.

Three Stage Operation

The performance of the three stage anaerobic digesters, that were operated at the different temperature configurations is shown in Figure 4-55. Figure 4-55 presents the log concentrations of *C. perfringens* that were observed in the various stages of the three stage digester systems. It is seen from Figure 4-55 that there was variation in the concentration of *C. perfringens* in feed sludge over the duration of the study.

Figure 4-55: *C. Perfringens* Removal Versus Three Stage Temperature Configuration.

From Figure 4-55 it can be seen that a variety of responses were observed in the differing systems. In some cases the concentrations of *C. perfringens* remained the same through the digesters while in other cases they were reduced. The first stage of the three stage systems was operated under identical conditions to that of the first stage of the two stage systems and single stage that was operated at 5 days HRT and produced an average similar *C. perfringens* reduction in the first stages of these systems represented replicate sampling of the same digesters and provide an indication of the reproducibility of the results over the period of this study. There appeared to be little relationship between the behaviour of *C. perfringens* in the digesters and temperature. Operation of the three stage anaerobic digesters at 35°C showed that the log concentration of *C. perfringens* declined from 6.4 log units in the first stage to 5.8 in second stage and further declined to 5.0 log units in the system effluent. However, a temperature increase in the first stage of three stage system to 42°C improved the inactivation of *C. perfringens* in the this stage by 0.5 log units. The system with first and second stage operated at 42°C exhibited constant *C.*

perfringens concentrations about 6.8 log units in their effluent. A similar trend was observed for those treatment systems with the first stage operated at 49°C. The results were expected as *C. perfringens* is an obligate anaerobe with optimal growth in the temperature range from 42°C to 49°C. The results suggest that there was limited removal of this organism through anaerobic digesters. However, the variable responses obtained suggest that there may have been other variables impacting upon the results that could not be explained at this time.

In this study decay rate constants for *C.perfringens* were investigated using equation 4-2. Monte Carlo simulation was applied on obtained results to verify the variability of the results. The decay rate constants for *C.perfringens* in this study were influenced by increasing the temperature to 42°C and 49°C in the first and second stages of the tested systems (Figure 4-56).

Figure 4-56: *C. Perfringens* Decay Rate Constant Versus Three Stage Temperature Configuration.

Values of 0.20, 0.18, and 0.16 day⁻¹ were observed for the system that was operated at 35°C and values of 0.29, 0.24, and 0.14 day⁻¹ were observed for the system that had a temperature of 42°C in the first stage. The decay rate for the inactivation of *C. perfringens* for the rest of the treatment systems were found to be below 0.05 day⁻¹. Although temperature is considered as a principal agent for pathogen reduction, but in terms of *C. perfringens* operation of two and three stage anaerobic digestions in series at elevated temperature exhibited growth in population. From the results of single stage study that is presented in Figure 4-51 it can be seen that inactivation of *C. perfringens* in digested sludge requires a temperature higher than 55°C for the longer HRT period. From the results it can be seen that operation of the anaerobic digester at elevated temperature improve the population of temperature resistant *C. perfringens* and consequently resulted lower decay rate values.

CHAPTER 5

IMPACT OF VOLATILE FATTY ACIDS ON THE INACTIVATION OF *SALMONELLA* SPP. AND *CLOSTRIDIUM PERFRINGENS*

The increased interest in producing biosolids that have reduced pathogen concentrations has resulted in the development of advanced digestion systems. A variety of advanced digestion systems are now available and include temperature phased, two phase and three phase configurations. The reduction in pathogens in advanced digestion technologies is generally attributed to the impact of their exposure to elevated temperatures. However, many of the advanced digestion technologies also expose the pathogenic bacteria to high concentrations of organic acids. The effectiveness of organic acids as pathogen inhibitors depends on their concentration, pH, temperature, exposure time and the sensitivity of specific microorganisms (Goepfert and Hicks 1969; Abdul and Lloyd, 1985; Fukushi *et al.* 2003). At lower pH's the organic acids are protonated to a greater extent and this form of the acids is generally believed to be more toxic to bacteria than the dissociated form (Taherzadeh *et al.* 1997). While the impact of organic acids on pathogens has been examined at mostly mesophilic temperatures (Abdul and Lloyd. 1985; Kunte *et al.* 2000) their impact at elevated temperatures has received less attention. This study was conducted to investigate the influence of volatile fatty acids on the inactivation of viable and culture able forms of *Salmonella* spp. and *C. perfringens* in digesters that operate at elevated temperatures as compared to mesophilic temperatures. Viable and culture able forms of *Salmonella* spp. and *C. perfringens* refers to bacteria that are capable of dividing and forming colonies on specific microbiological media. In this study digesters that were

treating municipal wastewater treatment plant sludges were operated at temperatures that ranged from 35-55°C and had a solids residence time of 15 days. Samples collected from the effluent of the digesters were dosed with solutions containing acetic, propionic and butyric acids alone and in mixtures and the dosed effluents were analyzed for *Salmonella* spp. and *C. perfringens* over time. In the first round of testing the digester effluents were dosed with individual organic acids and also the mixture containing all three VFA's over a range of concentrations from 750-6000 mg/L while the pH of the samples was fixed at a value of 5.5. Due to experimental constraints, the impact of individual fatty acids on inactivation of *C. perfringens* was not evaluated in this study. In the second round of testing the sludges were spiked with a fixed concentration (750 mg/L) of the organic acid mixture, while the pH was varied from 4.5 to 7.5.

5.1 Characteristics of Digester Effluent

The digester effluents were characterized for conventional parameters such as pH, total and volatile solids, and volatile fatty acid concentrations. The results of this characterization are shown in Table 5-1. With the exception of the sludge samples that were exposed to 55°C the most significant between the digester effluents was the increase in organic acid concentration and decrease in volatile solids concentrations with increasing temperature.

Table 5-1: Characterization of Feed Sludge and Digester Effluents (No of observations =14).

Parameter	Feed	35°C	42°C	49°C	55°C
pH	7.2 (0.20)	7.8 (0.40)	7.8 (0.04)	8.0 (0.07)	8.0 (0.10)
Total solids,%	4.82 (0.50)	3.24 (0.70)	3.17 (0.50)	3.15 (0.20)	3.6 (0.70)
Volatile Solids, %	3.62 (0.20)	2.27 (0.20)	1.92 (0.20)	1.88 (0.130)	2.1 (0.10)
Total volatile fatty acids (mg/L)	168 (0.20)	139 (25.00)	192 (19.70)	853 (107.30)	590 (16.30)

** Values in parentheses are standard deviations*

The increased temperature appeared to enhance the hydrolysis of the sludge in the digesters. This variation occurred while the pH remained constant or increased slightly. Due to the elevated background concentrations of organic acids in the 49°C digester effluent, these samples were not dosed with acids to assess the lowest acid concentration (i.e. 750 mg/L). Total organic acid concentrations and pH were measured during the 24 hour incubation period. These measurements indicated that there were no significant changes in organic acid concentrations and pH over the experimental period.

5.2 Inactivation of *Salmonella* spp. by Volatile Fatty Acids: Effects of Concentration, Temperature and pH

In this study the concentrations of *Salmonella* spp. that were measured under the various exposure conditions will be initially presented. While statistical tests were not performed directly on the concentration data, they were employed to assess differences in decay coefficients that were estimated from the concentration data. The calculation and comparison of the decay coefficients will be subsequently described. Conclusions regarding the impact of the exposure conditions on *Salmonella* spp. inactivation that are stated during the presentation of the concentration data are based upon the statistical assessment of the decay coefficients. Figure 5-1 presents the concentrations of

Salmonella spp. that were observed in the flasks that were maintained at 35°C. From Figure 5-1 it can be seen that the concentrations of *Salmonella* spp. were essentially constant with time in the control flasks. This was expected since *Salmonella* spp. are mesophilic organisms. These results do however validate the sampling and analytical techniques that were employed in these tests. In addition it can be seen that the error bars on the charts, that represent one standard deviation about the mean values, are relatively narrow. In this study standard deviation was observed to be within 5 to 10 % of the mean value which indicates good replication between the duplicate flasks.

Figure 5-1a presents the concentrations of *Salmonella* spp. when the digested sludges were exposed to a range of concentrations of the organic acid mixture. From Figure 5-1a it can be seen that the greatest decrease in *Salmonella* spp. occurred at the highest dosage of 6000 mg/L and the reductions in *Salmonella* spp. were less for the lower dosages. After 24 hours at this dosage the concentrations decreased from approximately 11,000 CFU/100 mL to approximately 3000 CFU/100 mL.

Figure 5-1: Average Reduction of *Salmonella* spp. in Different Concentration of Acids (a = Mixed Acids, b = Acetic Acid, c = Propionic Acid, d = Butyric Acid) at 35°C.

At the lowest dosage of 750 mg/L of mixed acids the *Salmonella* spp. concentrations only decreased to approximately 9000 CFU/100 mL. From Figure 5-1a it can be seen that the concentrations of *Salmonella* spp. decreased with increasing time of exposure and that the onset of measurable reduction occurred more rapidly for the higher dosages of acids. At a dosage of 6000 mg/L, reductions in *Salmonella* spp. were observed after 4 hours of exposure while at 750 mg/L reductions were not observed until after 24 hours. Examination of Figure 5-1a would suggest that the maximum reduction in *Salmonella* spp. had not been achieved by the 24 hour sampling time. Additional reductions might

be expected if the tests had been conducted for a longer period. Fukushi *et al*, (2003) reported that it took more than 8 days in a mesophilic acid-phase digester to lower the concentration of *Salmonella* spp. by 5 orders of magnitude. In this study the pH value of the sludge samples was adjusted to 5.5 to 6.0 while they were exposed to organic acid concentrations that ranged between 4000 mg/L and 9000 mg/L. In Fukushi's study the organic acid were dosed in to the feed sludge prior to digestion while in this study the organic acid was dosed into the digester effluents.

Figures 5-1b, 5-1c and 5-1d present the concentrations of *Salmonella* spp. that were measured for flasks that were dosed with only acetic, propionic and butyric acids respectively. From these figures it can be observed that similar trends in *Salmonella* spp. concentrations with respect to dosage concentration and exposure time were obtained for these other acids. In all cases greater reductions were observed for higher acid dosages and increased exposure times. However, the extent of reduction was substantially different for the different acids. For a dosage of 6000 mg/L of acid the *Salmonella* spp. concentrations after 24 hours of exposure at 35°C were 4700, 6300 and 8700 CFU/100 mL for acetic, propionic and butyric acids respectively.

Hence, it would appear that the different organic acids exerted different degrees of inhibition towards *Salmonella* spp.. Acetic acid was the most inhibitory while butyric acid was the least inhibitory. The results suggest that acids with increased chain lengths induced less reduction of *Salmonella* spp. than shorter chained acids. Goepfert and Hicks, (1969) investigated the inhibitory effects of volatile fatty acids on a pure culture of *Salmonella typhimurium* at pH of 5.0 to 6.0 with organic acid concentrations between 1000 to 5000 mg/L. They observed similar trends of decreasing inhibitory effects of

organic acids with increasing chain length of the fatty acids. This can be explained that under passive processes, the net movement of a substance from an area of high concentration to an area of low concentration is influenced by concentration gradient, cross-sectional area, temperature and the molecular weight of a substance. Smaller, lighter molecules such as acetic acid diffuse faster than larger, heavier ones. As a result the toxicity level created by the low weight organic acids can be expected to be greater than the larger acids.

It is also interesting to note that the inhibitory affects of the mixed acids were greater than any of the individual acids that made up the mixture. Hence, it would appear that the effects of the different acids are synergistic with respect to their ability to inhibit *Salmonella* spp.

Figure 5-2 presents the concentrations of *Salmonella* spp. that were measured in the flasks that were maintained at 42°C. From Figure 5-2 it can be seen that the concentrations of *Salmonella* spp. at the beginning of the tests were lower than those observed at 35°C. The initial concentrations at 42°C were approximately 8000 CFU/100 mL as compared to 11000 CFU/100 mL that were observed at 35°C. The reduced concentrations of *Salmonella* spp. in these tests were due to the increased reduction that was achieved in the digester that was operating at 42°C.

Figure 5-2: Average Reduction of *Salmonella* spp. in Different Concentration of Acids (a = Mixed Acids, b = Acetic Acid, c = Propionic Acid, d = Butyric Acid) at 42°C.

The reduced concentrations of *Salmonella* spp. were expected since this temperature is above the optimal temperature range for survival of these organisms and were statistically significant. It is likely that the *Salmonella* spp. that were present in this sludge were in a stressed state due to the extended exposure to the elevated temperature. In addition to the reduced concentrations of *Salmonella* spp. in the initial samples, the concentrations in the controls decreased with time in these tests. The reduction in the controls indicates that the effect of temperature on the activity of these organisms was ongoing as the batch tests

preceded. Hence, the reductions of *Salmonella* spp. that were observed in the test bottles were likely due to a combination of temperature effects as well as the effects of the acids.

From Figure 5-2 it can be seen that the trends in reduction in *Salmonella* spp. concentrations at the exposure temperature of 42°C were similar to those that were observed at 35°C, however, the extent of reduction was greater in most cases. For example, when a dosage of 6000 mg/L of the mixed acid solution was employed, the concentrations of *Salmonella* spp. decreased to 1900 CFU/100 mL after 24 hours of exposure at 42°C as compared to 3000 CFU/100 mL for the 35°C exposure. The results would suggest that the stress of the elevated temperature enhanced the inhibitory effects of the organic acids. Higher temperature may cause fluidity of cell membrane thereby allowing more rapid diffusion of organic acids into the cytoplasm. Accumulation of fatty acids in the cells would result in a high level of toxicity that enhanced the destruction rate of *Salmonella* spp.

Figure 5-3 presents the concentrations of *Salmonella* spp. that were measured in the flasks that were maintained at 49°C. From Figure 5-3 it can be seen that, with a concentration of approximately 5000 CFU/100 mL, the initial concentrations of *Salmonella* spp. were again lower in these tests than those of the previously described tests. At these temperatures the *Salmonella* spp. were clearly stressed. There was also additional reduction of *Salmonella* spp. in the control bottles in these tests indicating that temperature had an effect on the results of this study.

The trends with respect to dosage and time that were observed at the exposure temperature of 49°C were similar to those previously described for 35 and 42°C. However, in a number of cases the samples had *Salmonella* spp. concentrations that were

below the analytical detection limit of 1000 CFU/100 mL. After 24 hours of exposure all concentrations of the mixed acids and acetic acid resulted in concentrations that were below the method detection limit

Figure 5-3: Average Reduction of *Salmonella* spp. in Different Concentration of Acids (a = Mixed Acids, b = Acetic Acid, c = Propionic Acid, d = Butyric Acid) at 49°C.

Figure 5-4 presents a summary of the removal efficiencies that were observed after 24 hours of exposure to the differing organic acid species and dosages. From Figure 5-4 it can be seen that the reduction efficiencies increased with temperature for all of the acid types and dosages. In addition, the extent of reduction observed in the acid-dosed flasks were consistently higher than those observed in the controls. The results presented

in this Figure clearly indicate that the impact of organic acids on *Salmonella* spp. is affected by temperature.

Figure 5-4: *Salmonella* spp. Removal Efficiencies After 24 Hrs Exposure to Organic Acids.

From Figure 5-4a it can be seen that the reduction efficiencies in the control flasks increased from 10 % at 35°C to 22% at 42°C and to almost 50 % at 49°C. At 35°C as the concentration of the organic acid mixture increased from 750 to 6000 ppm the removal efficiency increased from 22 % to 72 %. At 42°C, for the same range of acid mixture concentrations, the removal efficiency increased from 55 % to 75 %. From the results it can be seen that the presence of the organic acid mixture enhanced reduction of

Salmonella spp. at the elevated temperature. For the samples at 49°C removal efficiencies of 80% were observed over the range of acid concentration. The consistent removal efficiencies at this temperature were due to the fact that the *Salmonella* spp. concentrations were consistently below the method detection limit. In summary, the extent of reductions observed in the acid-dosed flasks were consistently higher than those observed in the controls.

Figure 5-4b, 5-4c and 5-4d present a summary of the removal efficiencies that were observed after 24 hours of exposure to the various concentrations of the different acids. Figure 5-4 shows the interaction between VFA type and concentrations and temperature to achieve *Salmonella* spp. inactivation. There was a significant effect of temperature on the inhibitory action of acetic acid. At 49°C, the removal efficiency was 80% over a range of concentrations of acetic acid, while for propionic acid, the maximum reduction efficiency of 60% was achieved at 3000 ppm and higher. Butyric acid showed maximum inactivation at 6000 ppm of dosed acid and efficiency dropped as acid concentration decreased to 750 ppm. The results clearly indicate that the impact of organic acids on *Salmonella* spp. was affected by temperature.

To explore the rate of microbial reduction in this study it was assumed that the reduction followed first order kinetics. Hence, equation 5-1 was used to describe the decay and could be employed to estimate the decay coefficient from the experimental data.

$$C/C_0 = e^{-kt} \quad (5-1)$$

In equation 5-1, C is concentration of *Salmonella* spp., C_0 is the initial concentration, t is time and k is the first order decay rate coefficient. In this study Curve

Expert 1.3 software was used to estimate the best fit decay coefficient from the observed data. The decay coefficients describe the susceptibility of the *Salmonella* spp. to inactivation.

Figure 5-5 presents, as an example, the observed data and the best fit model predictions for a dose of 6000 mg/L of mixed acids at the three temperatures investigated in this study. From Figure 5-5 it can be seen that the first order decay model was able to adequately describe the observed data. An exception to this good fit was for the data point at 24 hours at a temperature of 49°C. This data point represented a value where the observed value was below the method detection limit and hence the method detection limit was employed in the data set.

Figure 5-5: Observed and Model Predicted Fraction Remaining Versus Time.

The decay coefficients that were estimated for the various exposure conditions are presented in Table 5-2. The values presented in Table 5-2 represent the average of coefficients that were separately estimated from the duplicate bottles that were tested for each exposure condition.

From Table 5-2 it can be seen that the decay coefficients spanned approximately 2 orders of magnitude and generally increased with temperature and acid dosage. The acid mixture had the highest decay coefficients while butyric acid had the lowest values. The trends in decay coefficients were in agreement with the trends that were identified in the previous discussion of *Salmonella* spp. concentrations. Statistical testing of the significance of the differences between the decay coefficients will be subsequently described

A three way ANOVA considering temperature, acid type and acid concentration as factors was performed to determine if the decay coefficients were significantly impacted by these factors. The individual decay coefficients that were estimated from each of the test bottles were employed in the ANOVA. A 5% level of significance was used in the test. The results of the ANOVA test revealed that all of the factors had a significant impact upon the estimated decay coefficients.

Table 5-2: Average Decay Coefficients (d^{-1})

Temp	Acid mixture (mg/L)				Acetic acid (mg/L)			
	750	1500	3000	6000	750	1500	3000	6000
35°C	0.01	0.01	0.04	0.07	0.007	0.011	0.03	0.043
42°C	0.03	0.04	0.05	0.07	0.016	0.02	0.04	0.05
49°C	0.11	0.11	0.14	0.20	0.09	0.09	0.11	0.14
	Propionic acid (mg/L)				Butyric acid (mg/L)			
	750	1500	3000	6000	750	1500	3000	6000
35°C	0.01	0.01	0.018	0.027	0.001	0.01	0.02	0.02
42°C	-0.01	0.011	0.015	0.04	0.001	0.11	0.11	0.03
49°C	0.01	0.03	0.04	0.10	-0.01	0.02	0.04	0.10

Figure 5-6 presents the concentrations of *Salmonella* spp. that were observed in Phase 2 of this study where a constant concentration (750 mg/L) of mixed organics acids was dosed into the digester effluents but the pH of the samples was adjusted over a range of values. From Figure 5-6 it can be seen that pH had a substantial impact on the response of *Salmonella* spp. and that different responses with respect to pH were observed at different temperatures. Figure 5-6a presents *Salmonella* spp. concentrations that were observed at an exposure temperature of 35°C. The provision of organic acids at relatively low concentrations and mesophilic temperatures appeared to stimulate the growth of *Salmonella* spp. From Figure 5-6a it can be seen that for relatively neutral pH's of 6.5 and 7.5 the concentrations of *Salmonella* spp. increased with time indicating that injured cells of *Salmonella* spp at neutral pH's and low concentration of organic acid could

recover and regrowth occurred. The flasks that were operated at pH values of 4.5 and 5.5 demonstrated reductions in *Salmonella* spp. with greater reductions at a pH of 4.5 as compared to a pH of 5.5. The results indicate that the more acidic conditions were inhibitory to *Salmonella* spp in this temperature range. This is likely attributable to the protonation of the organic acids over this pH range. The protonated forms of the organic acids are generally believed to be more toxic to *Salmonella* spp than the dissociated acids (Taherzadeh *et al.* 1997).

Figure 5-6b presents the concentrations of *Salmonella* spp. that were observed at an exposure temperature of 42°C. From Figure 5-6b it can be seen that the response of *Salmonella* at a pH of 6.5 and 42°C was similar to that observed at 35°C in that regrowth was observed. The behaviour observed at pH values of 4.5 and 5.5 had similar trends to those that were observed at 35°C, however, the extent of reduction was greater and there was little difference in the degree of reduction at the two pH values.

Figure 5-6: Impact of pH and Temperature on *Salmonella* spp. Concentrations (a = 35°C, b = 42°C, c = 49°C)

The response that was observed at a pH of 7.5 was dramatically different than that observed at 35°C. At this pH the concentrations of *Salmonella* spp. were found to be below the detection limit of 1000 CFU/100 mL within the first 12 hours of the test. This response could not be readily explained with the data that was available in this study, however, a proposed cause will be discussed subsequently.

Figure 5-6c presents the concentrations of *Salmonella* spp. that were observed at an exposure temperature of 49°C. From Figure 5-6c it can be seen that reductions in *Salmonella* spp. were observed at all pH values. The trends in reduction at the acidic pH values of 4.5 and 5.5 were similar to those observed at the other temperatures. However, at this temperature, the reductions were greatest for both of the neutral pH exposures. The results would suggest that as temperature increases, there is an increasing inhibition of *Salmonella* spp. at neutral pH values.

The increased inhibition of *Salmonella* spp. at neutral pH's for elevated temperatures can not be explained with certainty with the data that was obtained in this study. However, it is interesting to note that the tests that were conducted at a pH of 7.5 and 42°C and pH's of 6.5 and 7.5 at 49°C were found to stimulate the growth of considerable quantities of *Klebsiella* spp. Confirmation that the observed colonies were *Klebsiella* spp. was achieved by examining typical colonies using test method API 20E (Biomérieux 2010). *Klebsiella* spp. are known to ferment long-chain organic acids to generate low molecular weight acids such as formic and acetic acid (Goepfert and Hicks, 1969). In this study total organic acid concentrations were measured at the beginning and the end of the tests and were found to have minimal changes. However, the analytical method that was employed was not able to differentiate between the species of the acids that were present. It is hypothesized that at the elevated temperatures and neutral pH's *Klebsiella* spp. were actively fermenting longer chained organic acids to the more inhibitory low-molecular weight acids and hence causing a reduction in the *Salmonella* spp. Further testing would be required to verify this hypothesis.

To further explore if the nonionized forms of VFA's that are responsible for inactivation of *Salmonella* spp. the Henderson-Hasselbalch equation (5-2) was used. The proportion of ionized (e.g., acetate) to nonionized (e.g., acetic acid) form of a VFA is dependent upon the pH of the solution. The concentration of the nonionized form of VFA's in digested sludge sample is estimated using the Henderson-Hasselbalch equation as follows:

$$\text{mM nonionized VFA} = \frac{\text{mM (nonionized plus ionized VFA)}}{\left[(10^{-\text{pKa}}/10^{-\text{pH}})+1 \right]} \quad (5-2)$$

Where mM (nonionized plus ionized VFA) = concentration of a VFA in solution (millimolar), pKa (24°C) = equilibrium constant. Table 5-3 present characteristics of short-chain VFA's present in the digested sludge sample used in this study.

Table 5-3: Characteristics of Short-Chain VFA's.

VFA	Formula	Molecular Weight (g/M)	Solubility (mM) ¹	pKa ²
Acetic	CH ₃ COOH	60.1	>5,000	4.76
Propionic	CH ₃ CH ₂ COOH	74.1	5,000	4.86
<i>n</i> -Butyric	CH ₃ (CH ₂) ₂ COOH	88.1	1,100	4.83

¹Values obtained from (Khan *et al.* 1995).

²pH-dependent equilibrium constant obtained from (Perrin and Dempsey. 1974).

The ability of compounds to penetrate cell membranes is reduced by their size and the solubility in water (Alberts *et al.* 1994). A tendency for slightly reduced toxicity with increasing size of VFA's from the acetic acid to the butyric acids suggests that it is the ability to penetrate cells that determines toxicity. From Table 5-3 it can be seen that the acetic acid ability to penetrate to the cell membrane is higher than other tested VFA's, which would ultimately shows higher inhibition effect to the *Salmonella* spp. as compare

to the Propionic and *n*-Butyric acids. As previously noted, in the first phase of the study consisted of dosing the digester effluents with either 750, 1500, 3000 or 6000 mg/L of either acetic, propionic or butyric acids or a mixture of the three fatty acids while pH of the samples was adjusted to 5.5. The concentration of the nonionized form of an individual VFA in sludge samples is estimated using equation 5-2 and presented in Table 5-4.

Table 5-4: Concentration of the Nonionized Form IVFA's at pH=5.5.

Concentration (ppm)	Nonionized concentration (ppm)		
	Acetic	Propionic	<i>n</i> -Butyric
6000	924	1118	1057
3000	462	559	528
1500	231	280	264
750	115	140	132

Toxicity of the VFA's to *Salmonella* spp. can be attributed to the concentrations of nonionized form of VFA's that is higher following exposure to increasing concentration of investigated VFA's. In the second phase of the study the impact of varying pH from 4.5 to 7.5 while dosing with a mixture containing 750 mg/L of an equimolar mixture of acetic, propionic, and butyric acids on inactivation of *Salmonella* spp. was examined.

Figure 5-7: Impact of varying pH on Nonionized Concentration of VFA's.

From Figure 5-7 it can be seen that at pH of 4.5 optimal nonionized concentration of about 500 ppm was observed for all three acids tested in this study. A nonionized portion of the investigated VFA's declined rapidly with an increase in pH value from 4.5 to 7.5.

It is the protonation of VFA's in acidic solution that generates nonionized forms toxic to the *Salmonella* spp. It is important to note that pKa value is a function of temperature, since it is related to the Gibbs free energy of reaction. It is anticipated that temperature increase would result in a decrease in pKa value (an increase in Ka), which means that the reaction favors dissociation more as temperature increase. However, verification of this assumption require further study.

In summary, the reduction of *Salmonella* spp. in digester effluents, when dosed with volatile organic acids, was found to depend on pH, temperature, the chain length of the acids and the concentration and composition of the acids present. A mixture of organic acids was found to be more inhibitory than the individual organic acids. It was found that the inhibitory effects of the organic acids decreased as their chain length increased. Increases in temperature appeared to increase the inhibitory effects of the volatile organic acids. An interaction between temperature and pH on inhibition of *Salmonella* spp. was observed. At mesophilic temperatures acidic pH's resulted in a greater inhibition of *Salmonella* spp. while at higher temperatures neutral pH's were found to be more inhibitory. The results that were observed in the batch tests suggest that exposure times beyond 24 hours would result in greater reductions of *Salmonella* spp. than those determined in this study.

5.3 Inactivation of *Clostridium perfringens* by Volatile Fatty Acids Mixture: Effects of Concentration, Temperature and pH

C. perfringens is an anaerobic, Gram-positive, spore-forming, and heat-resistant bacterium. Municipal sludges contain high levels of this organism and hence they have been proposed as indicators for the survival of pathogens in sludge treatment processes. The objective of this part of study was to assess the impact of a fatty acid mixture on the inactivation of *C. perfringens* over a range of digestion temperatures. The interaction between temperature and pH on survival of *C. perfringens* was evaluated.

The digester effluents were characterized for conventional parameters such as pH, volatile solids, and volatile fatty acids. The details of the feed sludge analyses were previously reported in Table 5-1. In this study the reported values of *C. perfringens* concentrations include both spore and vegetative cells of *C. perfringens*. Figure 5-8 presents the concentrations of *C. perfringens* that were measured as a function of time for the various temperatures and acid dosages. Figure 5-8 reveals that the standard deviations were less than 4% of the mean value which indicate good replication between the duplicate samples.

From Figure 5-8 it can be seen that at 35°C the *C. perfringens* concentrations did not significantly change in the control and when the digested sludges were exposed to 750 and 1500 mg/L of the organic acid mixture. With 3000 and 6000 mg/L of mixed acids the concentrations of *C. perfringens* were reduced by approximately 18% relative to the initial concentrations.

At 42°C the *C. perfringens* concentration in the control and the sample dosed with 1500 mg/L of mixed acid showed a 13% increase in concentration. With a dosage of 750

mg/L of mixed acids, the *C. perfringens* concentrations were higher than the control at the 24 hour sampling time. The samples dosed with 3000 and 6000 mg/L of the organic acid mixture showed reductions relative to the initial concentration of 12 and 20% respectively.

From Figure 5-8 it can be seen that at 49°C the control increased by 27 % over the sampling period. With 750 mg/L of mixed acid *C. perfringens* concentrations increased relative to the initial concentration but less than the control after the 24 hour incubation time. For dosages of 1500, 3000 and 6000 mg/L of mixed acid the *C. perfringens* concentrations after 24 hours of exposure decreased by 14, 31 and 60% relative to their initial concentration. At 55°C, the initial concentrations of *C. perfringens* were lower than those of the previously described tests (Figure 5-8).

At this temperature the *C. perfringens* were clearly stressed with temperature. After 24 hours of exposure all concentrations of the mixed acids resulted in a reduction of *C. perfringens*. There was also a reduction of *C. perfringens* in the control indicating that at this temperature *C. perfringens* were inactivated without acid addition. The results indicate that as organic acid concentrations increased more reduction was observed at 55°C. Puchajda and Oleszkiewicz (2003) evaluated the impact of retention time on *C. perfringens* removal in a thermophilic acid phased anaerobic digester. They investigated HRT's of 5, 3, and 1 days with volatile fatty acid concentrations of 3480, 4168 and 5398 mg/L respectively. *C. perfringens* destruction of less than 1 log was achieved for all the tested HRTs. Regrowth of *C. perfringens* in mesophilic conditions was observed in both studies. In addition, in Puchajda and Oleszkiewicz's study organic acids were produced in the acid phased digester which enhanced the adaptability of the *C. perfringens* to

organic acids at concentrations higher than 3500 mg/L and lower reduction while in this study the organic acid was dosed into the digester effluents to avoid cell adaptability and to enhance *C. perfringens* reduction. To explore the rate of *C. perfringens* reduction during this study first order kinetic decay constants were calculated using equation 5-1 for each exposure condition. In this study the data were subjected to Curve Expert 1.3 software to estimate the best fit decay coefficient from the observed data.

Figure 5-8: *Clostridium perfringens* Concentrations at (a) 35°C, (b) 42°C, (c) 49°C and (d) 55°C Versus Acid Concentration.

Figure 5-9 presents, as an example, the observed data and the best fit model predictions for a dose of 6000 mg/L of mixed acids at the four temperatures investigated in this study. From Figure 5-9 it can be seen that the first order decay model was able to adequately describe the observed data.

Figure 5-9: Observed and Model Predicted Fraction Remaining Versus Time.

The estimated average decay constants from duplicate bottle that were tested for each experimental conditions are summarized in Table 5-3. From the Table 5-5 it can be seen that the decay coefficients for *C. perfringens* ranged from -0.007 to 0.041 hr^{-1} and were generally lower than those of *Salmonella* spp. *C. perfringens* is a spore-forming obligate anaerobe and hence its improved survival in anaerobic conditions was expected.

Table 5-5: Average Decay Coefficients for *C. perfringens* (d⁻¹).

Temperature	Acid mixture concentration (mg/L)			
	750	1500	3000	6000
35°C	0.001	0.003	0.009	0.009
42°C	-0.010	-0.006	0.006	0.010
49°C	-0.007	0.006	0.016	0.020
55°C	0.021	0.024	0.032	0.041

In general, the average decay coefficients for the 35°C experiment were somewhat higher than those observed in the 42°C digester over the range of organic acid tested. Decreased decay rates of *C. perfringens* at 42°C digester for all the exposure condition and for 49°C for those samples that were exposed to the mixed acid concentration of 750 mg/L and 1500 mg/L were expected as *C. perfringens* which is a moderate thermophilic organism could potentially grow under these conditions. For those samples that were exposed to the mixed acid concentrations of 3000 mg/L and 6000 mg/L at 49°C and 55°C gradual increase in decay coefficient were observed. From the results it can be concluded that operation of acid phased digesters at the thermophilic temperature range can significantly reduce *C. perfringens* concentration.

A two way ANOVA considering temperature and acid concentration as factors was performed to evaluate the impact of these two factors on decay coefficient. At a 5% level of significance the ANOVA test indicated that both temperature and mixed acid concentration had a significant impacts on the decay rate coefficients.

Figure 5-10 presents the ratio of the final to initial concentrations of *C. perfringens* that were observed in Phase 2 of this study where a constant concentration (750 mg/L) of mixed organics acids was dosed into digester effluents that were incubated for a period of 24 hours over range of pH values from 4.5 to 7.5.

From Figure 5-10 it can be seen that pH had a substantial impact on the response of *C. perfringens* and that different responses with respect to pH were observed at different temperatures.

Figure 5-10: Ratio of Final to Initial Concentrations of *C. perfringens*.

From Figure 5-10 it can be seen that at 35°C for pH's ranging from 4.5 to 7.5 the concentrations of *C. perfringens* increased by 50% over the 24 hour exposure period. At 42°C it can be seen that concentrations of *C. perfringens* increased by 380 % relative to the initial concentration at a pH of 6.5. The provision of relatively low concentrations of organic acids at these temperatures appeared to stimulate the growth of *C. perfringens*.

At 49°C *Clostridium perfringens* concentrations were highest at a pH of 7.5 and were similar for the pH values of 5.5, 6.5 and 8.0. For 55°C it can be seen that a substantial reduction in *C. perfringens* was observed at a pH value of 4.5 and

concentrations did not significantly change over the remaining range of pH. At a pH value of 5.5, that is of an typical acid phased digester pH, *C. perfringens* concentrations did not significantly change over a range of temperatures from 35° to 55°C. The results would suggest that as temperature increases, there is an increasing inhibition of *C. perfringens* at the acidic pH value of 4.5. This is likely attributable to the protonation of the organic acids over this pH range.

The protonated forms of the organic acids are generally believed to be more toxic to *Clostridium perfringens* than the dissociated acids (Taherzadeh *et al.* 1997). *C. perfringens* were relatively resistant to the effect of temperature and organic acids. This observation suggests that *C. perfringens* might be a good test organism for future studies. Treatment processes capable of a large decrease in the *C. perfringens* population should reduce a number of other bacteria by an equal or larger amount.

This study showed that the presence of volatile organic acids in anaerobic digesters impacted upon the growth of *C. perfringens*. At the highest dosages, the concentrations of *C. perfringens* were reduced. Lower concentrations of acids appeared to have neutral, or in one case, stimulatory effects. The impact of volatile organic acids on *C. perfringens* concentrations was observed to be a function of temperature and pH.

In conclusion, acid treatment at elevated temperature can significantly injure the bacterial pathogen tested in this study. However the degree of injury varies with organic acid concentration, type of organic acid, temperature, and type of microorganism. From the results of this study it can be seen that acid treatment at elevated temperatures caused significantly more injury to *Salmonella* spp. than at mesophilic temperature. The injury of *C. perfringens* was observed to be less than the injury of *Salmonella* spp. under similar

exposure conditions. For *C. perfringens* at operational temperatures of 42°C and 49°C low mixed acid concentration stimulate the growth of this microorganism while under the similar conditions 50% to 80% *Salmonella* inactivation was observed. This may have been related to the strong tendency for *Salmonella* spp. to be injured by organic acids. Statistical analysis reveals that there was significant effect of organic acid concentration and temperature on *Salmonella* and *C. perfringens*. Further study is required to evaluate the impact of acid treatment and temperature on germination rate of *C. perfringens*.

CHAPTER 6

MICROBIAL DIVERSITY OF STAGED OPERATION OF ANAEROBIC DIGESTION OF MUNICIPAL SEWAGE SLUDGE

More recently the development of molecular and genetic fingerprinting techniques to complement the more classical techniques has allowed us to better monitor and investigate the complex microbial communities, and overcome the problems associated with the traditional cultivation dependent methods. A number of molecular genetic techniques, such as Polymerase Chain Reaction (PCR)-based Terminal-Restriction Fragment Length Polymorphism (T-RFLP), Length Heterogeneity PCR (LH-PCR), Denaturing Gradient Gel Electrophoresis (DGGE) and Fluorescence In Situ Hybridization (FISH), have been applied to study microbial communities in a non-cultivation based way (Muyzer, 1998).

The analysis of 16S rRNA genes (rDNA) is typically used to characterize and monitor microbial communities in various ecological systems. 16S rDNA-based methods can provide information on the phylogenetic structure of microbial communities (Muyzer *et al.*, 1993). Denaturing gradient gel electrophoresis (DGGE) can be used to resolve PCR-amplified 16S rDNA fragments by electrophoresis through an acrylamide gel that contains an increasing linear gradient of denaturants (Muyzer *et al.*, 1993). After staining, each band on the denaturing gradient gel represents a specific 16S rDNA sequence from a specific microbial species. Thus, the species can be identified by the band positions on the gel, and their concentrations correspond to the band intensities. The number and intensity of resolved fragments gives an approximate estimate of the

diversity of the predominant species. This study was conducted to evaluate the impact of temperature staging on the diversity of the microbial population during series operation of anaerobic digesters. The main objective of this study was to compare bacterial communities of the two and three stage digester systems and compare them with feed sludge and single stage control digester.

After each system reported in Table 3-4 reached steady state, samples were taken from the influent and effluent of each reactor for DGGE testing. The DNA was extracted and amplified using the PCR technique. The amplicons were then separated by DGGE and the band patterns that were generated in each lane were compared.

DNA was extracted from samples of the influent and effluent of the single, two and three stage anaerobic digestion systems on two separate sampling events which were seven to eight days apart. The DGGE profiles were observed to be similar and indicated the reproducibility of the DGGE results over an extended period of time.

6.1 Microbial Diversity in Two Stage Study

Figure 6-1 presents the DGGE band patterns of the two parallel two stage anaerobic digester systems as compared to the single stage mesophilic digester and the digester feed sludge. From Figure 6-1 it can be seen that in the feed sample there were a large number of bands, with each corresponding to a different microbial species. Image analysis was required to identify some of the faint bands. The DGGE fingerprint of the raw sludge was reflective of the mixed primary and secondary sludges that clearly had considerable microbial diversity.

From Figure 6-1 it can be seen that effluent of the single stage mesophilic digester operated at an HRT of 15 days had considerably fewer bands than the feed sludge with two bands that appeared to increase in intensity as compared with the feed sludge. The culture that develops in a single stage sludge digester will be a function of the die-off of organisms that are present in the feed which can not survive in the anaerobic environment and the growth of cultures of competent organisms. The results indicate that in the long residence time digesters there was considerable die-off of the organisms that were present in the feed sludge. In addition there were relatively few types of organisms that proliferated in the digester. This might be attributed to the relatively low availability of substrates in the extended HRT digesters.

The samples from System III (R1-35°C-5 followed by R1-35°C-10) had a greater number of intense bands than the single stage digester. In addition, there were a number of intense bands that appeared to persist in the second stage digester of this system. These results suggest a significant difference in microbial populations between single and multi-stage digesters that were operated at the same temperature. It would appear that the higher concentrations of nutrients that would be present in the first stage of the two stage system stimulated the growth of a more diverse population. The persistence of these organisms in the second stage would suggest that, once established, they could survive under conditions of reduced nutrient availability.

Figure 6-1 shows that the profile for the first stage of system IV that was operated at 42°C had a reduced number of bands as compared to the feed sludge profile and also as compared to the first stage of System III that was operated at 35°C. It would appear that the elevated temperature may have increased the decay of organisms that were present in

the feed and also stimulated the growth of a less diverse population as compared to when the first stage was operated at 35°C. As with System III the band patterns of the two stages of System IV were similar profiles suggesting a persistence of the populations that were established in the first stage digesters.

Similarity coefficients were calculated to more quantitatively compare the band patterns of the various sludge samples. The similarity coefficients comparing the feed sample, the two-stage digesters, and the single stage digester are presented in Table 6-1. The indices of similarity ranged from 0.08 to 0.78.

Figure 6-1: DGGE Images From Two Stage Study.

Table 6-1: Similarity Coefficients (C_s) in Two-Stage Study.

Lanes compared	1	2	3	4	5	6
1	1.00					
2	0.43	1.00				
3	0.25	0.78	1.00			
4	0.10	0.29	0.20	1.00		
5	0.08	0.30	0.25	0.50	1.00	
6	0.33	0.33	0.14	0.20	0.13	1.00

The similarity coefficients generally confirmed the previous visual examination of the band profiles. The similarity coefficient for the comparison of the single stage digester versus the feed sludge (Lane 6 vs Lane 1) was less than the similarity coefficient of the first stage of System III and the feed (Lane 2 vs Lane 1). The difference in similarity coefficients was attributed to the increased die-off of feed organisms in the single stage digester and the differing nutrient availability in the first stage of System III that apparently stimulated the growth of a more diverse population as compared to the single stage digester. The comparison of the first stage of System IV with the feed sludge (Lane 4 vs Lane 1) resulted in a smaller value than that obtained for the System III with the feed sludge (Lane 2 vs Lane 1). This was attributed to the differing rates of die-off and growth at the elevated temperature (42°C) in the first stage of System IV.

The similarity coefficients comparing the first and second stages of both Systems III (Lane 2 vs Lane 3) and IV (Lane 4 vs Lane 5) were the highest values observed in this portion of the study. This is indicative of the persistence of the populations as the sludges progressed through the staged digesters.

6.2 Microbial Diversity in Three Stage Study

The DGGE fingerprints of the influent feed, the three stage anaerobic digesters and the single stage digester were compared in Figure 6-2. As observed in the two stage study the DGGE profiles of the various samples differed from each other and from the feed as indicated by the differences in the band location and intensities. The band pattern for the feed sample in the three stage study (Figure 6-2) had a large number of bands of relatively low intensity. This was indicative of a diverse bacterial population and was consistent with that observed in the two stage study (Figure 6-1). The single stage digester that was operated as a control in the three stage study had relatively few bands that were of increased intensity as compared to the raw sludge. This pattern was consistent with that observed in the single stage digester that was operated in the two stage study. The results demonstrate that the populations present in the extended residence time digesters are relatively simple in composition.

The first stage of system I that was operated at 35°C retained a number of the bands from the feed sludge, but the bands were lower in intensity suggesting that the bacteria were present in lower concentrations (Figure 6-2). The first stage of System I was operated under identical conditions to that of the first stage of System III. It is therefore interesting to compare the profiles of these two digesters. Comparing the bands presented in Figure 6-1 (System III) and Figure 6-2 (System I) it can be seen that both band patterns had a large number of bands however the bands were considerably darker for System III than System I. The differences in the intensities may be due to differences in concentrations of the organisms in the digesters. As previously mentioned the digesters operated in this study had been previously operated under differing temperature

profiles. It is possible that this prior operation may have had an impact upon the populations that were observed in this study. A review of the previous temperature profiles of the reactors did not reveal any patterns between the historical practices and the current populations.

Figure 6-2: DGGE Images from Three Stage Study.

The differing results may also have been an artifact of the relatively complex analytical techniques that were employed. With the information that was available it was not possible to identify which of these explanations may have been responsible for the observed response. Regardless, it would appear that both first-stage digesters that were operated at 35°C and a residence time of 5 days retained some of the microbial diversity

of the feed sludge. The band patterns of second and third stage digesters in System I that were operated at 35°C had five identical intense bands and two that varied in intensity. The results suggest that as the sludge progressed through the second and third stages of System I the bacterial populations that were developed in the first stage persisted in the downstream digesters with an increase in their concentration. In addition it would appear that additional populations developed in the second stage digester and persisted in stage three.

The results from the three stage System I where all of the reactors were operated at 35°C (Figure 6-2) were generally consistent with those of the previously described 2 stage digester (System III) that had both reactors operated at 35°C (Figure 6-1). In both systems, there was a transition in diversity and intensity of the band patterns from the feed sludge. In addition, populations that were developed in the first stage persisted in the downstream digesters. The final populations leaving the staged digester systems differed considerably from those of the single stage digesters that were operated at the same overall HRT and temperature.

The pattern generated by the first stage digester of System II that was operated at 42°C was considerably different from the feed sludge and from the first stage of System I (Figure 6-2). The differing responses between the 42°C and the 35°C first stage digesters were consistent with that which was observed in the two stage study (Figure 6-1). However, as with the 35°C systems, the intensity of the bands in System II appeared to be greater than that of the System IV. The patterns observed in the samples from the second and third stage digesters of System II indicated an increase in the number of intense bands as compared to the first stage digester with a few of the initial bands decreasing in

intensity (Figure 6-2). The trend in patterns in System II where the first stage was operated at 42°C was similar to that of System IV that also had the first stage at 42°C (Figure 6-1). The collective results suggest that the organisms that proliferate at 42°C in the first stage reactors can persist in downstream digesters of both two and three stage systems. As observed in the other staged systems the pattern observed in the final effluent from System II was considerably different from that of the single stage digester.

Similarity indices were calculated to quantitatively compare the responses in the various samples generated in the three stage study. The similarity coefficients comparing the feed sample, the three stage reactors and the single stage digester are presented in Table 6-2. As observed in the two stage system analysis the similarity coefficients for the comparison between the single stage digester and the feed sludge was low and confirms the substantial die-off of feed sludge populations in this type of operation. The similarity coefficients comparing the first stage digesters of the three stage systems and the feed sludge were higher than that of the single stage digester suggesting some carry over of feed sludge populations in these digesters. The similarity coefficients comparing the sequential stages of the three-digester systems were the highest values observed and were indicative of the persistence of populations as they progress through the digester systems.

Table 6-2: Similarity Coefficient (C_s) from Three Stage Study.

Lanes compared	1	2	3	4	5	6	7	8
1	1.00							
2	0.18	1.00						
3	0.24	0.58	1.00					
4	0.33	0.44	0.86	1.00				
5	0.40	0.26	0.51	0.62	1.00			
6	0.23	0.32	0.48	0.56	0.75	1.00		
7	0.19	0.31	0.53	0.54	0.73	0.91	1.00	
8	0.10	0.17	0.10	0.17	0.10	0.18	0.19	1.00

In conclusion, the similarity analysis based on the DGGE pattern matrix indicated that the microbial communities in the single, two and three stage digester systems were different. Populations that were developed in the first stage of staged digester systems tended to persist in downstream digesters despite differences in operating temperatures. In addition, the populations present in the staged systems tended to be more diverse than those observed in a single stage digester that was operated at the same HRT.

Further study is required to monitor and to assess population dynamics in both the bacterial and archaeal communities in multistage operation of anaerobic digesters. In addition, this study has shown that the analysis of sludge samples with DGGE can generate information that can be employed for visual comparison of the populations that are present in sludge digestion systems. This is beneficial in ecological studies where a large number of samples often need to be screened.

CHAPTER 7

CONCLUSIONS AND ENGINEERING SIGNIFICANCE

The primary objective of this research was the development of a better understanding of the operation of anaerobic digesters in single and multi stage configurations, to improve process performance and pathogen destruction rate. This section presents overall conclusions and engineering significance of this research study.

7.1 Conclusions

7.1.1 Single Stage Temperature-HRT studies

Successful anaerobic digestion of mixed primary and secondary sludges was achieved over a range of conventional and extreme mesophilic and thermophilic temperatures. The success that was achieved at the extreme temperatures was attributed to the rapid start-up technique that was employed. Substantially different responses were observed between operation at HRT's of 5, 15 and 20 days. Operation at a 20 day HRT generally had higher VSR, reduced accumulation of TVFA's, an increased release of NH_3 , higher methane yields and decreased dewaterability than operation at a 5 and 15 day HRT. At the 20 day HRT there was little impact of temperature on VS reduction, however TVFA accumulation increased with temperature and methane yield decreased with temperature. At the 15 day HRT, TVFA concentrations were lowest at 42°C and 55°C and NH_3 concentrations increased with temperature. At this HRT the highest CH_4 yields were observed at temperatures of 42°C and 55°C and NH_3 concentrations increased with temperature. During operation at 5 day HRT, TVFA concentration were highest at 42°C and 62°C and VS reduction was reached to the minimum values at this

temperatures. However increase in NH_3 concentrations was achieved with increase in temperature. With the exception of *Clostridium perfringens* the concentrations of the microbial species in the digester effluents decreased as the temperature of operation increased. Operation at 55°C and 62°C resulted in essentially non-detectable concentrations of fecal coliforms, *E. coli* and *Salmonella* spp.. Operation at 42°C and 49°C resulted in incremental decreases in effluent concentrations as compared to operation at 35°C. With the exception of operation at 62°C, the effluent concentrations of *Clostridium perfringens* were observed to increase relative to the feed concentrations. The results suggest that *Clostridium perfringens* is not a useful indicator organism for the fate of pathogens in anaerobic digestion.

7.1.2 Staging of Digesters

In this study, two and three stage operation were able to achieve VS reduction greater than 38% which is required for vector attraction requirement. Implementation of stage operation of the digesters generally improved VS destruction and biogas generation. Three stage operation improved performance with respect to VS destruction and biogas generation as compared to two stage operation. Operation of the first stage of the two and three stage system at elevated temperature resulted in an improvement in VS reduction and biogas production as compared to operation at 35°C. The process stability and effluent quality in terms of TVFA and alkalinity were improved in staged operation as compared to a single stage digester. In addition, staged operation of the digesters resulted in substantial improvements in pathogen destruction as compared to single stage digestion for all of the indicators. With the exception of *E. coli*., all of the indicators were reduced to a greater extent in three stage digestion as compared to two stage

digestion. With the exception of *Salmonella* spp. all of the indicators were removed to a greater extent when the first stage of the three stage system was operated at elevated temperature as compared to the three stage system that was operated at 35°C. This study revealed that fecal streptococci and *Clostridium perfringens* were more resistant to inactivation in the digesters as compared to Fecal coliform, *E. Coli.* and *Salmonella* spp.

7.1.3 Impact of Volatile Fatty Acids on the Inactivation of *Salmonella* Spp. and *Clostridium perfringens*

The main significant finding in this portion of study was that acid treatment at elevated temperature can significantly injure tested bacterial pathogens. However the degree of injury varies with organic acid concentration, type of organic acid, temperature, and type of microorganism. From the results of this study it can be seen that acid treatment at elevated temperatures caused significantly more injury to *Salmonella* spp. than at mesophilic temperature. The injury of *C. perfringens* was observed to be less than the injury of *Salmonella* spp. under similar exposure conditions. For *C. perfringens* at operational temperatures of 42°C and 49°C low mixed acid concentration stimulate the growth of this microorganism while under the similar conditions 50% to 80% *Salmonella* inactivation was observed. This may have been related to the strong tendency for *Salmonella* spp. to be injured by organic acids. Statistical analysis reveals that there was significant effect of organic acid concentration and temperature on *Salmonella* and *C. perfringens*. Further study is required to evaluate the impact of acid treatment and temperature on germination rate of *C. perfringens*.

7.1.4 Microbial Diversity of Staged Operation of Anaerobic Digestion of Municipal Sewage Sludge

The similarity analysis based on the DGGE pattern matrix indicated that the microbial communities in the single, two and three stage digester systems were different. Populations that were developed in the first stage of staged digester systems tended to persist in downstream digesters despite differences in operating temperatures. In addition, the populations present in the staged systems tended to be more diverse than those observed in a single stage digester that was operated at the same HRT.

7.2 Engineering Significance

The results can be useful for operators, design engineers researchers, and regulatory officials to better understand the limits of various sludge treatment processes, individually or as part of a processing train, for optimizing anaerobic digestion process that meet the regulatory requirements for stabilizing volatile solids and disinfecting municipal sludge to protect the public health and the environment.

CHAPTER 8

ASPECTS FOR FUTURE RESEARCH

The experimental results presented in this study provides insight into the performance difference between thermophilic, and mesophilic digestion. This section lists some area of research that would further enhanced and clarify issues in this research.

- Assessment of the effect of temperature fluctuations on digester performance and stability to determine, operating temperatures that are more sensitive to temperature fluctuation than mesophilic digesters.
- Characterizations of anaerobic digestion for carbohydrate, protein, and lipids during single and staged operation at elevated temperature. Knowledge of the digester composition could provide insight into the differences between the digestion systems.
- Assessment of possible population changes with temperature and HRT should be studied to determine differences in biomass populations in each type of system to advance the understanding of anaerobic digestion performance issues.

CHAPTER 9

REFERENCES

Abdul P. and Lloyd D. (1985). Pathogen survival during anaerobic digestion: Fatty acids inhibit anaerobic growth of *Escherichia coli*. *Biotechnology Letters*, 7, 2, 125-128.

Ahring, B.K. (1994) Status of science and application of thermophilic anaerobic digestion source. *Water Science and Technology*, 30, 12, 241-249.

Aitken, M.D. and Mullenix, R.W. (1992). Another look at thermophilic anaerobic digestion of wastewater sludge. *Water Environment Research*, 64, 7, 915-919.

Aitken, M.D, Sobsey, M.D., Walters, G.W., Hill, V., Shehee, M., Blauth, K., Crunk, P.E., Sharon P., and Nicole A. (2003) Evaluation of thermophilic anaerobic digestion to meet class A biosolids criteria. Proceedings of WEFTEC 2003, October 12-15, Los Angeles, California, LA, USA.

Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., and Watson, J. D. (1994) *Molecular Biology of the Cell*. 3rd ed. Garland Publishing, New York.

American Public Health Association, American Water Works Association, Water Environment Federation, (1995) Standard methods for examination of water and wastewater, 19th ed., Washington DC.

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*, 64, 1, 53-59.

Berg, G., and Berman, D. (1980) Destruction by anaerobic mesophilic and thermophilic digestion of viruses and indicator bacteria indigenous to domestic sludges. *Applied and Environmental Microbiology*, 39, 361-368.

Bean and Griffin (1990) www.fao.org/DOCREP/003/T1768E/T1768E02.htm.

Bej, A.K., McCarty, S.C., and Atlas, R.M. (1991) Detection of coliform bacteria and *Escherichia coli* by multiplex polymerase chain reaction: comparison with defined substrate and plating methods for water quality monitoring. *Applied and Environmental Microbiology*, 57, 2429-2432.

Bitton, G. (1994) *Wastewater Microbiology*. Wiley-Liss, Inc., New York, N.Y.

Blum, H, Beier H, and Gross HJ (1987) Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. *Electrophoresis*, 8, 93-99.

Bucher, B., Newman., Moore, R., Lepisto, R., Stensel. H.D., and Ferguson, J.F. (2001) Pilot Plant Investigation of Thermophilic-Mesophilic Digestion for a Full-Scale Retrofit, Biosolids. Proceedings Joint WEF/AWWA Biosolids and Plant Residuals Conference, February 13, San Diego, USA.

Burlingame, G.A., McElhaney, J., Bennett, M., and Pipes, W.O.(1984) Bacterial interference with coliform colony sheen production on membrane filters. *Applied and Environmental Microbiology*, 47, 56-60.

Carrington E.G., Harman S.A, and Pike E.B. (1982) Inactivation of *Salmonella* during anaerobic digestion of sewage sludge. *Journal of Applied Bacteriology*, 53, 331.

Carrington E.G. (1984) *Pasteurization: Effect upon ascaris eggs.*, in inactivation of microorganisms in sewage sludge by stabilization processes, edited by Strauch D., Havelaar A.H., L'Hermite P ., Elsevier Applied Science Publishers.

Carrington, E.G., and Harman, S. A. (1984) The effect of anaerobic digestion temperature and retention time on the survival of *salmonella* and ascaris ova. in: *Sewage Sludge Stabilization and Disinfection*. Ed. A. M. Bruce, Chichester, Ellis Horwood, 546-549.

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] WRc plc, Medmenham.

Carrington, E G. (2001) Evaluation of sludge treatments for pathogen reduction – FINAL REPORT Study Contract No B4-3040/2001/322179/MAR/A2 for the European Commission Directorate-General Environment.

Cha, G.C., and Noike, T. (1997) Effect of rapid temperature change and HRT on anaerobic acidogenesis. *Water Science and Technology*, 36, 6-7, 247-253.

Chauret C, Springthorpe S, and Sattar S.(1999) Fate of Cryptosporidium oocysts, Giardia cysts, and microbial indicators during wastewater treatment and anaerobic sludge digestion. *Canadian Journal of Microbiology*, 45, 3, 257-262.

Cheunbern T. and Pagilla K.R. (2000) Anaerobic thermophilic/mesophilic dual-stage sludge treatment. *Journal of Environmental Engineering*, 126, 9, 796-801.

Chyi, Y.T., and Dague, R.R. (1994) Effects of particle size in anaerobic acidogenesis using cellulose as a sole carbon source. *Water Environment Research*, 66, 5, 670-678.

De Leon C, and Jenkins D. (2002) Removal of fecal coliforms by thermophilic anaerobic digestion processes. *Water Science and Technology*, 46, 10, 147-52.

Demeyer, D.I. and Henderickx, H.K. (1967) The effects of unsaturated fatty acids on methane production in vitro by mixed rumen bacteria. *Biochimica Et Biophysica Acta*, 137, 484-497.

Drury D., Lee S., and Baker C. (2002) Comparing pathogen reduction in three different anaerobic thermophilic processes. Proceedings of WEFTEC, 28th September -2nd October, Chicago, IL, USA.

Elefsiniotis, P (1993) The effect of operational and environmental parameters on the acid phase anaerobic digestion of primary sludge. A thesis submitted at the University of British Columbia.

Farrah, S. R., and Bitton, G. (1984) Enteric bacteria in aerobically digested sludge. *Applied and Environmental Microbiology*, 47, 831-834.

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 Water Pollution Control Federation*, 60, 5, 635-644.

Feachem, R.G., Bradley, D.J. Garelick, H. and Mara, D. D. (1983) *Sanitation and Disease: Health aspect of excreta and wastewater management*. John Wiley and Sons.

Fenters J., Reed j. Lue-Hing C., Bertucci J. (1979) Inactivation of viruses by digested sludge components, *Journal of the Water Pollution Control Federation*, 51, 689-692.

Ferran B., Huyard A., Adamik J. (2002) Two-phase anaerobic digestion of municipal sewage sludge optimization of the pathogen destruction. Proceedings of WEFTEC, 28th September -2nd October, Chicago, IL, USA.

Fox, P., and Pohland, F.G. (1994) Anaerobic treatment applications and fundamentals: substrate specificity during phase separation. *Water Environment Research*, 66, 5, 716-724.

Fukushi K., Babel S., and Burakrai S. (2003) Survival of *salmonella* spp. in a simulated acid-phase anaerobic digester treating sewage sludge, *Bioresource Technology*, 86, 177-181.

Gabb DMD, Jenkins D, Gosh S, Hake J, De Leon C, and Williams D. (2000) Pathogen destruction efficiency in high temperature anaerobic digestion. In Proceedings of the 14th Annual Residuals and Biosolids Management Conference, Feb 27–Mar 1, Boston, Massachusetts, USA.

Ginnivan, M. J., Woods, J. L., and O' Callaghan, J. R. (1980) Survival of *salmonella dublin* in pig slurry during thermophilic treatment in batch, cyclic and continuous systems. *Journal of Applied Bacteriology*, 49, 13-18.

Goepfert J.M. and Hicks R. (1969) Effect of volatile fatty acids on *salmonella typhimurium*. *Journal of Bacteriology*, 70, 956-958.

Ghosh, S. (1987) Improved sludge gasification by two-phase anaerobic digestion. *Journal of Environmental Engineering*, 113, 6, 1265-1284.

Hanai, K., Satake, M., Nakanishi, H., and Venkatsewaran, K. (1997) Comparison of commercially available kits with standard methods for detection of *Salmonella* strains in foods. *Applied and Environmental Microbiology*, 63, 775-778.

Han, Y., and Dague, R. R. (1996) Laboratory studies on the temperature-phased anaerobic digestion of domestic primary sludge. *Water Environment Research* 69,6, 1139-1143.

Han V., Sung S., and Dague R.R. (1997) Temperature-Phased Anaerobic Digestion of Wastewater Sludges. *Water Science and Technology*, 36, 6-7, 367-374.

Haug, R. T., Kuchenrither, R., Malina, J. F. Jr., Oerke, D., Tata, P., Sawyer, B., Soszinski, S., and Zenz, D. (1998) Sludge processing technology, in *municipal sewage sludge management: A Reference Text on Processing, Utilization and Disposal*, Second Edition, Water Quality Management Library, Vol. 4. Technomic Publishing Co., Inc., Lancaster, PA.

Harrison, J. (1997) <http://cff.wsu.edu/Publications/sunnyside/harrison.pdf>

Hirata, T., K. Kawamura, S. Sonoki, K. Hirata, M. Kaneko and K. Taguchi. (1991) *Clostridium perfringens* as an indicator microorganism for the evaluation of the effect of wastewater and sludge treatment systems. *Water Science and Technology*, 24, 2, 367-372.

Hu'bner, I., Steinmetz, I., Obst, U., Giebel, D., and Bitter-Suermann, D (1992) Rapid determination of members of the family Enterobacteriaceae in drinking water by an immunological assay using a monoclonal antibody against enterobacterial common antigen. *Applied and Environmental Microbiology*, 58, 3187- 3191.

Huyard, A., Ferran, B., and Audic, J.M. (2000) The two phase anaerobic digestion process: sludge stabilization and pathogens reduction. *Water Science and Technology*, 42, 9, 41-47.

Inman, D.C., Murthy, S., Schafer, P., Schlegel, P., Webb, J., and Novak, J.T. (2005) A Comparative Study of Two-Stage Thermophilic, Single Stage Mesophilic, and Temperature Phased Anaerobic Digestion. *Proceedings WEF Residual and Biosolids Management Conference*, April 17-20, Nashville, Tennessee, United States.

Iranpour R., Cox H.H.J., Oh S., Soung T., Alatraste F., Ardent T., Chang S., Moghaddam O., Fan S., Mundine J.E., and Kearney R.J. (2002) Full-Scale thermophilic anaerobic digestion at the hyperion treatment plant: Proceedings of WEFTEC, 28th September -2nd October, Chicago, IL, USA.

Kennedy M., Wurpts J., Johnson L., and Stack G. (1997) Reduction of wastewater pathogens using second stage thermophilic digestion. Proceedings of WEFTEC 1997 70th Annual Conference and Exposition of the Water Environment Federation, Chicago, IL, USA.

Kfir, R., and Genthe, B. (1993) Advantages and disadvantages of the use of immunodetection techniques for the enumeration of microorganisms and toxins in water. *Water Science and Technology*, 27, 243–252.

Kowal, N. E. (1983) An overview of public health effects. In: *Utilization of Municipal Wastewater and Sludge on Land*. Ed. A. L. Page, T. L. Gleason, J.E. Smith, I. K. Iskander, and L. E. Sommers, 329-394, University of California, Riverside, CA.

Khan, I., Brimblecombe, P., and Clegg, S. L. (1995) Solubilities of pyruvic acid and the lower (C1–C6) carboxylic acids. Experimental determination of equilibrium vapour pressures above pure aqueous and salt solutions. *Journal of Atmospheric Chemistry*, 22, 285-302.

Krebs, C. J (1978) *Ecology: the experimental analysis of distribution and abundance* Harper & Row. New York.

Krugel, S., Nemeth, L., and Peddie, C. (1998) Extended thermophilic anaerobic digestion for producing class A biosolids at the Greater Vancouver Regional Districts Annacis Island wastewater treatment plant. *Water Science and Technology*, 38, 8, 409-416.

Kunte, D.P., Yeole, T.Y., and Ranade, D.R. (2000) Inactivation of *Vibrio cholerae* During Anaerobic Digestion of Human Night Soil, *Bioresource Technology*, 75, 2, 149-151.

Lee K.M., Brunner C.A., Farrell J.B., and Erarp A.E. (1989) Destruction of enteric bacteria and viruses during two-phase digestion. *Journal of Water Pollution Control Federation*, 61, 8, 1421-1429.

Lewis-Jones, R., and Winkler, M. (1991) *Sludge parasites and other pathogens*. Ellis Horwood Limited, England.

Lin, J.S., Lee, I.S., Frey, J., Slonczewski, J.L., and Foster, J.W. (1995) Comparative-Analysis of Extreme Acid Survival in *Salmonella typhimurium*, *Shigella flexneri*, and *Escherichia coli*. *Journal of Bacteriology*, 177,14, 4097–4104.

Malina, J. F., Jr., (1992) Anaerobic Sludge Digestion, in *Design of Anaerobic Processes for the Treatment of Industrial and Municipal Wastes*, Eds. J.F.Malina, Jr. and F. G. Pohland, Water Quality Management Library, Vol. 7. Technomic Publishing Co., Inc., Lancaster, PA.

Massa, S., Brocchi, G.F., Peri, G., Altieri, C., and Mammina, C.(2001) Evaluation of recovery methods to detect faecal streptococci in polluted waters. *Letters in Applied Microbiology*, 32, 298-302

McCarthy, C.W., and Vandenburg, S.R. (2004) Improved digester performance without the acronyms: full-scale evaluation of series mesophilic digestion at king county's south treatment plant.. Proceedings WEF/WEAU Joint Residual and Biosolids Management Conference, Feb 22–25, Salt Lake City, UT, USA.

McFarland, M.J.(2000) *Biosolids engineering*. New York, McGraw-Hill.

McFeters, G.A., Cameron, S.C., and LeChevallier, M.W., (1982) Influence of diluents, media, and membrane filters on detection of injured waterborne coliform bacteria. *Applied and Environmental Microbiology*, 43, 97– 103.

Means, E.G., Olson, B.H. (1981) Coliform inhibition by bacteriocin-like substances in drinking water distribution systems. *Applied and Environmental Microbiology*, 42, 506–512.

Merck index. (2003) <http://pb.merck.de/servlet/PB/menu/1001723/index.html>

Metcalf and Eddy (2003) *Wastewater Engineering: Treatment Disposal and Reuse*, Fourth Edition, McGraw Hill, New York, NY.

Mocé-Llivina, L., Muniesa, M., Pimenta-Vale, H., Lucena, F., and Jofre, J. (2003) Survival of bacterial indicator species and bacteriophages after thermal treatment of sludge and sewage. *Applied and Environmental Microbiology*, 69, 1452-1456.

Moen, G., Stensel, H.D., Lepisto, R., and Ferguson, J.F.(2003) Effect of solids retention time on the performance of thermophilic and mesophilic digestion of combined municipal wastewater sludges. *Water Environment Research*, 75, 6, 539-548.

Muyzer G, de Waal EC, and Uitterlinden AG (1993) Profiling of complex microbial population by DGGE analysis of polymerase chain reaction amplified genes encoding for 16S rRNA. *Applied and Environmental Microbiology*, 62, 2676–2680.

Muyzer G (1998) Structure, function and dynamics of microbial community: the molecular biological approach. *Advanced Molecular Ecology*. New York: IOS Press, pp. 157–184.

National Research Council (1996) *Use of reclaimed water and sludge in food crop production*. National Academy Press. Washington, D.C.

Nielsen B. and Petersen G. (2000) Thermophilic anaerobic digestion and pasteurisation. practical experience from Danish wastewater treatment plants. *Water Science and Technology*, 42, 9, 65-72.

Novak, J.T., Sadler, M.E., and Murthy, S.N. (2003) Mechanisms of floc destruction during anaerobic and aerobic digestion and the effect on conditioning and dewatering of biosolids. *Water Research*, 37, 13, 3136–3144.

Oropeza M., Cabirol N., Ortega S., Castro Ortiz L.P., and Noyola A. (2001) Removal of fecal indicator organisms and parasites (fecal coliforms and helminth eggs) from municipal biologic sludge by anaerobic mesophilic and thermophilic digestion. *Water Science and Technology*, 44, 4, 97-101.

Parkin, G. F., and Owen, W. F. (1986) Fundamentals of anaerobic digestion of wastewater sludges. *Journal of Environmental Engineering*, 112, 867-920.

Pecson B. and K.L. Nelson,. (2003) The effects of exposure time, temperature, pH, and ammonia concentration on the inactivation rate of ascaris eggs. Proceedings of WEFTEC 2003, October 12-15, Los Angeles, California, LA, USA.

Pedersen, D.C. (1981) Density levels of pathogenic organisms in municipal wastewater sludge: *A Literature Review*. EPA-600/2-81-170. Cincinnati, OH.

Perrin, D. D., and Dempsey, B. (1974) *Buffers for pH and Metal Ion Control*. Chapman & Hall, New York.

Pike, E.B., Carrington, E. G., and Hartman, S. A. (1988) Destruction of *salmonellas*, enteroviruses and ova of parasites in wastewater sludge by pasteurization and anaerobic digestion. *Water Science and Technology*, 20, 337-340.

Pohland FG and Ghosh S. (1971) Developments in anaerobic stabilization of organic wastes-the two-phase concept. *Environmental Letters* 1, 255-266.

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*, 69, 1195-1206.

Puchajda B and Oleszkiewicz J. (2003) Single and two-stage anaerobic digestion: hydrolysis, acidification and pathogen inactivation. Proceedings of WEFTEC 03, October 12-15, Los Angeles, California, LA, USA.

Puchajda and Oleszkiewicz (2004) Pathogen inactivation through organic acids in anaerobic digestion. Proceedings of WEFTEC 04, October 2-6, New Orleans, LA, USA.

Purohit HJ, Kapley A, Moharikar AA, and Narde G. (2003) A novel approach for extraction of PCR-compatible DNA from activated sludge samples collected from different biological effluent treatment plants. *Journal of Microbiology Methods*, 52, 3, 315-323

Rice, E.W., Fox, K.R., Nash, H.D., Read, E.J., and Smith, A.P.(1987) Comparison of media for recovery of total coliform bacteria from chemically treated water. *Applied Environmental Microbiology*, 53, 1571–1573.

Sanders, D. A., Malina, J. F. Jr., Moore, B. E., Sagik, B. P., and Sorber, C. A. (1979). Fate of *poliovirus* during anaerobic digestion. *Journal Water Pollution Control Federation*, 51, 333-343.

Santha, H., Sandino, J., and Shimp, G.F.(2003) Laboratory studies on a sequencing-batch temperature-phased anaerobic digestion (TPAD) system. Proceedings WEF/AWWA/CWEAJoin Residual and Biosolids Management Conference, Feb 19–21, Baltimore, Maryland, USA.

Schafer P.L. and Farrell J.B. (2000) Turn up the Heat. *Water Environment and Technology*, 12, 11, 26-34.

Schafer P., Farrell J.B., Newman G., and Vandenburg S. (2002) advanced anaerobic digester performance comparison, Proceedings of WEFTEC, 28th September -2nd October, Chicago, IL, USA.

Seidler, R.J., Evans, T.M., Kaufman, J.R., Warwick, C.E., and LeChevalier, M.W. (1981) Limitations of standard coliform enumeration techniques. AWWA 73, 538–542.

Sidhu, J., Gibbs, R .A., Ho, G.E ., and Unkovich, I. (2001) The role of indigenous microorganisms in suppression of *Salmonella* regrowth in composted biosolids. *Water Research*, 35, 4, 913-920.

Singleton, P. and Sainsbury, D., (1987) *Dictionary of microbiology*. Wiley and Sons, Chichester.

Speece, R.E. (1996) *Anaerobic biotechnology for industrial wastewaters*. Archae, Nashville, Tenn.

Stadterman K.L., Sninsky A.M., Sykora J.L., Jakubowski W. (1995) Removal and inactivation of *cryptosporidium* oocysts by activated sludge treatment and anaerobic digestion. *Water Science and Technology*, 31, 5-6, 97-104.

Strauch, D., (1998). Pathogenic microorganisms in sludge. Anaerobic digestion and disinfection methods to make sludge usable as a fertilizer. *European water Management*, 1, 2, 12-26.

Stryer, L.(1988) *Biochemistry*, 3rd edn. W.H. Freeman, New York, USA.

Stukenberg, J. R., Shimp, G. Sr., Sandino, J., Clark, J. H., and Crosse, J. T (1994) Compliance outlook. Meeting 40 CFR part 503, class B pathogen reduction criteria with anaerobic digestion. *Water Environment Research*, 66, 255-263.

Taherzadeh, M.J., Niklasson, C., Linden, G. (1997) Acetic acid-friend or foe in anaerobic batch conversion of glucose to ethanol by *Saccharomyces cerevisiae*. *Chemical Engineering Sciences*, 52, 15, 2653-2659.

Toranzos, G.A., Alvarez, A.J., and Dvorsky, E.A. (1993) Application of the polymerase chain reaction technique to the detection of pathogens in water. *Water Science and Technology*, 27, 207– 210.

European Union (2000) Working Document on Sludge, 3rd Draft, Brussels.

U.S. EPA. (1992) Environmental regulations and technology. Control of pathogens and vector attraction reduction in sewage sludge. EPA/625/R-92/013.

U.S EPA. (1999) Biosolids generation, use, and disposal in the United States. EPA530-R-99-009, September.

U.S EPA. (1999) Control of Pathogens and Vector Attraction in Sewage Sludge, U.S. Environmental Protection Agency, Office of Research and Development, National Risk Management Research Laboratory, Center for Environmental Research Information, EPA/625/R-92-013, Cincinnati, OH.

Vandenburgh, S. R., and Ellis, T. G. (2002) Effect of varying solids concentrations and organic loading on the performance of temperature phased anaerobic digestion. *Water Environment Research*, 74, 142-148.

Waage, A.S., Vardund, T., Lund, V., and Kapperud, G.(1999) Detection of low numbers of Salmonella in environmental water, sewage and food samples by a nested polymerase chain reaction assay. *Journal of Applied Microbiology*, 87, 3, 418–428.

Ward R.L. and Ashley C.S., and Moseley R.H.(1978) Heat inactivation of poliovirus in wastewater sludge, *Applied and Environmental Microbiology*, 32, 3, 339–346.

Ward R.L. and Ashley C.S.(1977) Identification of a virucidal agent in wastewater sludge. *Applied and Environmental Microbiology*, 33, 4, 860-864.

Watanabe, H., Kitamura, T., Ochi, S., and Ozaki, M. (1997) Inactivation of pathogenic bacteria under mesophilic and thermophilic conditions. *Water Science and Technology*, 36, 25-32.

WEF. (1995) Wastewater residuals stabilization. *Manual of Practice FD-9*. Water Environment Federation.

Zachar, V., Thomas, R.A., and Goustin, A.S. (1993) Absolute quantification of target DNA: a simple competitive PCR for efficient analysis of multiple samples. *Nucleic Acids Research*, 21, 2017–2018.

Zhou J, Mavinic D.S , Harlan G. K, and William D. R. (2002) Effects of temperatures and extracellular proteins on dewaterability of thermophilically digested biosolids. *Journal of Environmental Engineering Science*, 1, 409–415.

APPENDIX A: RAW DATA FOR SINGLE STAGE TEMPERATURE-HRT STUDY

20 day HRT steady state data (March 1, 2003 - April 5, 2003)

TS						
	Feed	35°C	42°C	49°C	55°C	62°C
10-Mar	49700	31000	30000	29550	28250	29300
12-Mar	49700	30250	28750	27250	30250	28150
14-Mar	46420	28350	29250	27500	29750	30000
17-Mar	46420	28250	27700	26800	29250	28050
19-Mar	48150	28240	30500	28250	27810	29263
21-Mar	48150	29300	27550	26550	33050	25800
24-Mar	52050	29550	28200	28700	26850	27850
26-Mar	59550	31000	29750	31750	29100	32650
28-Mar	40100	30750	29150	28800	29190	30050
31-Mar	47650	30100	28900	29950	33450	30050

VS						
	Feed	35°C	42°C	49°C	55°C	62°C
10-Mar	36250	17250	17250	16650	17350	18500
12-Mar	36250	17750	16250	18250	21750	16500
14-Mar	36250	17750	18000	16150	20500	18150
17-Mar	36250	16750	16400	16500	18211	17850
19-Mar	35920	17320	17110	17420	18590	18222
21-Mar	34810	16950	16100	15500	22850	15600
24-Mar	36100	18500	19200	18000	17750	17500
26-Mar	38900	18100	17500	18450	18350	19950
28-Mar	37950	17900	17300	16600	17450	19500
31-Mar	34550	17250	16550	16900	21000	18000

TSS						
	Feed	35°C	42°C	49°C	55°C	62°C
10-Mar	46500	29550	28200	29500	27260	28750
12-Mar	46500	29400	28550	26250	28800	24500
14-Mar	42310	27750	28850	25500	28450	23000
17-Mar	42310	27900	27500	26250	23750	26000
19-Mar	48000	27220	27860	24750	27456	27680
21-Mar	48000	28250	26000	23750	22250	23310
24-Mar	48250	28750	27250	28500	23750	27000
26-Mar	53000	30250	28250	30250	27500	30250
28-Mar	53000	30250	27000	26500	28220	30250
31-Mar	45250	29500	26250	25250	28500	26750

VSS						
	Feed	35°C	42°C	49°C	55°C	62°C
10-Mar	34250	16850	16400	15500	15750	17000
12-Mar	34250	17500	14500	15750	17950	15000
14-Mar	34250	17250	16550	15750	19050	14000
17-Mar	34250	16500	15500	16340	15000	15250
19-Mar	35220	17213	16890	16810	15930	16250
21-Mar	34500	15750	15000	14000	15000	9750
24-Mar	35750	17450	18500	16850	16750	17400
26-Mar	36250	18000	16500	17000	16750	18500
28-Mar	34500	17750	15750	15250	16380	18600
31-Mar	34500	17100	16250	16750	18750	16500

TVFA mg/L					
	35°C	42°C	49°C	55°C	62°C
10-Mar	12.86143	28.91084	105.8787	91.27806	1959.516
12-Mar	8.279729	26.59521	380.2646	1728.886	2926.383
14-Mar	14.0095	27.3623	313.2837	1867.009	2214.531
17-Mar	14.80453	23.44796	398.9409	2468.261	2689.903
19-Mar	11.32272	12.05647	309.1983	2013.311	3158.872
21-Mar	8.612564	9.709654	9.090844	957.0117	2998.756
24-Mar	8.891017	17.96184	56.62298	1581.692	3096.179
26-Mar	11.76872	85.08793	24.21031	1783.532	3193.341
28-Mar	4.918967	252.6329	96.24145	1970.681	2861.307
31-Mar	11.53424	2.109224	112.8756	162.8529	2883.212

ACETIC Acid mg/L					
	35°C	42°C	49°C	55°C	62°C
10-Mar	66.5	25.4	112	66	43.8
12-Mar	35	72	58	45.5	79
14-Mar	32	15.3	100	743	264
17-Mar	45	65	77	1137	517
19-Mar	54	51	46	141	618
21-Mar	54	20.2	39	210	657
24-Mar	41	46	67	146	363
26-Mar	66.5	25.4	112	66	43.8

Propionic Acid mg/L					
	35°C	42°C	49°C	55°C	62°C
10-Mar	25.4	1.4	7.6	64	1627.5
12-Mar	15	9.3		1664	1460
14-Mar	3	10	249.6	403.5	1563
17-Mar	12.11	26	1177	965.6	1037
19-Mar	19	44	42.4	750	1260.2
21-Mar	19.4	37.4	15.9	810	1624
24-Mar	0	0	12	1144.7	1521
26-Mar	25.4	1.4	7.6	64	1627.5

Butyric Acid mg/L					
	35°C	42°C	49°C	55°C	62°C
10-Mar	76	0	0	5.5	0
12-Mar	0	0	0	51	0
14-Mar	0	0	9.5	57.5	54
17-Mar	0	0	7.9	145.6	0
19-Mar	0	0	0	0	47
21-Mar	0	27	0	0	52.3
24-Mar	0	0	0	22.3	24
26-Mar	76	0	0	5.5	0

Ammonia-N (mg/L)					
	35°C	42°C	49°C	55°C	62°C
10-Mar	1158.463	1121.53	1211.239	977.2567	651.8076
12-Mar	1292.327	1324.115	1334.884	748.0347	1266.42
14-Mar	1373.269	1367.719	1329.488	769.545	1163.164
17-Mar	1261.301	1373.269	1139.847	837.8586	667.8405
19-Mar	1459.281	1297.571	1226.046	811.1471	831.0994
24-Mar	1034.268	1186.958	1149.117	1059.709	1395.697
26-Mar	643.936	567.9591	1005.358	1121.53	1395.697
28-Mar	858.468	897.5774	876.0292	977.2567	1094.606
31-Mar	848.1007	1139.847	854.9982	745.0113	1064.009

Alkalinity (mg/L)					
	35°C	42°C	49°C	55°C	62°C
10-Mar	6596.04	6496.821	6605.515	8340.748	4518.636
12-Mar	6251.717	6499.018	6350.045	6542.892	3895.088
14-Mar	6252.456	6412.205	6292.25	6828.033	4281.223
17-Mar	5993.202	6442.011	6272.792	5659.55	3900.129
19-Mar	6188.835	6572.805	6296.168	6893.688	3644.15
21-Mar	6600.069	7092.033	7152.618	6934.042	4032.245
24-Mar	6900.476	6771.882	7012.227	6145.888	4242.699
26-Mar	6645.417	6685.266	6825.95	5996.477	3851.462
28-Mar	6254.902	6397.215	6614.684	6042.499	4281.239
31-Mar	6874.997	6906.904	6899.939	8385.76	4236.926

pH					
	35°C	42°C	49°C	55°C	62°C
10-Mar	7.92	8.13	8.18	8.32	8.27
12-Mar	7.71	8.03	8.14	8.1	8.16
14-Mar	7.65	7.87	8	7.8	8.23
17-Mar	7.61	7.74	7.84	7.25	7.76
19-Mar	7.63	7.82	7.87	7.68	7.74
21-Mar	7.81	7.9	8.13	7.98	8.11
24-Mar	7.78	7.67	7.99	7.95	7.94
26-Mar	7.62	7.76	7.98	7.92	7.98
28-Mar	7.65	7.64	7.84	7.77	7.87
31-Mar	7.2	7.66	7.74	8.03	7.84

CH ₄ Fraction						CO ₂ Fraction				
	35°C	42°C	49°C	55°C	62°C	35°C	42°C	49°C	55°C	62°C
14-Mar	69.7	67	56	62	30	31	28	28	32	24
17-Mar	65	66	68	67.5	59	27	30	31	33	34
19-Mar	67	68	65	71	57	32	32	31	28	40
24-Mar	65	65	69	66.5	63	30.5	31	31	27	36
26-Mar	64.5	66	65	67	62	31	31.5	34	26.8	29
28-Mar	66	67	66.4	66.5	59	34	33	35	31.5	39
31-Mar	67.5	66	64	70	63	32.5	33	31	26.4	34

Dewatering (Sec)					
	35°C	42°C	49°C	55°C	62°C
10-Mar	2188.8	2426.4	2504.3	2356.7	2157
12-Mar	1930.8	2417.7	2705.4	2142	2099.2
14-Mar	1459.2	2078.1	2609.9	2204	2105.7
17-Mar	1757.1	1963.5	2574.4	2235.2	2288.2
19-Mar	1850	2241	2519	2275	2079
24-Mar	2381.55	2260	2572.75	2221.9	2983.5
28-Mar	2299.4	2778.35	3005.6	3112.4	2128.5
31-Mar	2142.5	2297.9	2601.8	2375.05	2251.15

TCOD					
	35°C	42°C	49°C	55°C	62°C
21-Mar	10767.86	10640.09	11193.76	11534.47	12017.16
24-Mar	11080.19	11733.23	11733.23	11562.87	12386.26
26-Mar	12187.51	11648.05	10909.83	11392.51	12499.84
28-Mar	13152.88	12187.51	5245.425	14146.63	11037.6
31-Mar	10299.38	10995.01	11846.8	11335.72	13550.38
2-Apr	13181.27	12698.59	14004.67	15623.07	13493.59
4-Apr	70669.08	69902.47	64933.7	75595.27	74984.82
	SCOD				
	35°C	42°C	49°C	55°C	62°C
21-Mar	986.4745	1685.226	3414.076	5827.481	5841.678
24-Mar	688.348	1718.448	1707.664	6168.197	7680.978
26-Mar	902.095	1874.609	2505.5	6133.559	7638.388
28-Mar	801.92	2477.107	2264.16	5950.711	8311.869
31-Mar	5018.281	1972.278	1156.833	1895.051	5950.711
2-Apr	461.204	2426.566	2390.679	1625.317	6666.781
4-Apr	858.322	1854.233	1638.911	1600.316	4090.404

	12-Mar	14-Mar	24-Mar	26-Mar	28-Mar
	CFU/100mL				
	FC	FC	FC	FC	FC
Feed	2.40E+06	3.10E+06	2.80E+06	4.20E+06	2.60E+06
35°C	1.40E+05	1.10E+05	7.30E+04	1.50E+05	9.50E+04
42°C	4.90E+04	3.60E+04	2.70E+04	4.30E+04	3.20E+04
49°C	5.00E+03	6.20E+03	4.00E+03	8.00E+03	7.00E+03
55°C	2.00E+03	4.00E+03	1.00E+03	3.00E+03	3.00E+03
62°C	1.00E+03	1.00E+03	1.00E+03	1.00E+03	2.00E+03
	Salmonella	Salmonella	Salmonella	Salmonella	Salmonella
Feed	2.00E+05	2.30E+05	2.30E+05	1.60E+05	1.60E+05
35°C	8.00E+02	1.20E+04	2.00E+03	1.90E+04	1.50E+04
42°C	1.00E+02	1.00E+02	1.00E+02	5.00E+03	2.00E+03
49°C	1.00E+02	1.00E+02	1.00E+02	1.00E+02	1.00E+02
55°C	1.00E+02	1.00E+02	1.00E+02	1.00E+02	1.00E+02
62°C	1.00E+02	1.00E+02	1.00E+02	1.00E+02	1.00E+02
	E-coli	E-coli	E-coli	E-coli	E-coli
Feed	1.20E+06	9.00E+04	1.00E+05	1.40E+05	1.40E+05
35°C	1.10E+05	1.00E+04	4.00E+03	1.00E+05	2.00E+04
42°C	1.00E+04	9.00E+03	2.00E+03	5.90E+04	1.80E+04
49°C	1.00E+03	2.50E+03	1.00E+03	4.00E+03	6.00E+03
55°C	1.00E+03	2.00E+03	1.00E+03	2.00E+03	1.00E+03
62°C	1.00E+03	1.00E+03	1.00E+03	2.00E+03	1.00E+03
	C.perfingens	C.perfingens	C.perfingens	C.perfingens	C.perfingens
Feed	7.60E+04	7.00E+06	7.00E+06	1.40E+06	1.40E+06
35°C	1.50E+06	5.00E+05	5.00E+05	6.70E+06	5.00E+06
42°C	2.00E+06	9.70E+06	1.60E+06	1.33E+07	1.63E+07
49°C	6.50E+04	4.50E+06	4.00E+06	1.40E+07	1.46E+07
55°C	5.20E+04	3.20E+06	5.40E+06	6.00E+06	1.10E+07
62°C	1.70E+04	1.00E+06	1.00E+06	1.20E+06	3.70E+06
	FS	FS	FS	FS	FS
Feed	3.00E+07	3.60E+07	1.90E+07	2.00E+07	1.60E+07
35°C	8.00E+06	2.20E+06	2.00E+06	1.90E+06	2.50E+06
42°C	2.40E+05	8.40E+05	9.90E+05	5.00E+05	7.50E+05
49°C	2.50E+03	1.00E+03	1.80E+03	2.40E+04	1.00E+03
55°C	1.00E+03	1.00E+03	1.00E+03	1.00E+03	1.00E+03
62°C	1.00E+03	1.00E+03	1.00E+03	1.00E+03	1.00E+03

15 day HRT steady state data (August 12, 2003 - August 28, 2003)

TS						
	Feed	35°C	42°C	49°C	55°C	62°C
12-Aug	53290	42965	42995	33550	37415	41950
14-Aug	53290	43065	38765	29190	36075	42160
18-Aug	53290	34700	33975	22015	32975	41165
22-Aug	44515	27280	23680	27700	30885	41375
24-Aug	44515	33730	23920	40205	30975	38915
26-Aug	44515	30460	24770	33135	29335	33475
28-Aug	45580	31800	26620	33800	30385	31700

VS						
	Feed	35°C	42°C	49°C	55°C	62°C
12-Aug	40230	16650	24940	19230	22000	27120
14-Aug	40230	15735	22640	17470	20755	26675
18-Aug	40230	19685	20855	14380	19530	27310
22-Aug	34210	18460	14875	17565	18370	28410
24-Aug	34210	18905	15495	23275	18615	26630
26-Aug	34210	20010	15995	19215	17380	22260
28-Aug	34400	18450	17400	20900	18200	21150

TSS						
	Feed	35°C	42°C	49°C	55°C	62°C
12-Aug	47050	46450	39100	32950	35575	39275
14-Aug	47050	38950	36800	28900	37075	38950
18-Aug	47050	30875	32350	20975	32750	38400
22-Aug	41850	27325	27975	24650	31500	39475
24-Aug	41850	20775	22325	27625	29375	40800
26-Aug	43850	33375	23425	40300	29550	37000
28-Aug	43850	33000	24950	32000	29250	29500

VSS						
	Feed	35°C	42°C	49°C	55°C	62°C
12-Aug	36350	16425	22900	19100	19625	24800
14-Aug	36350	15500	21075	16775	21475	24325
18-Aug	36350	19625	19750	13500	18775	24850
22-Aug	32425	17775	17525	17000	18275	26100
24-Aug	32425	17500	14150	17175	17350	27250
26-Aug	33000	19575	15225	23375	17075	25250
28-Aug	33000	17750	16500	19500	17250	19250

TVFA mg/L					
	35°C	42°C	49°C	55°C	62°C
12-Aug	498.82	146.52	655.49	354.23	5941.08
14-Aug	491.69	41.13	700.96	589.81	5777.46
18-Aug	479.7	82.03	1323.63	494.71	4343.26
22-Aug	416	277.68	876.82	352.6	3812.68
24-Aug	482	226.8	262.57	316.11	3990.83
26-Aug	578	432.64	726.72	148.87	4967.47
28-Aug	546	334.96	534.46	294.64	5924.88

ACETIC Acid mg/L					
	35°C	42°C	49°C	55°C	62°C
12-Aug	66.5	25.4	112	66	43.8
14-Aug	35	72	58	45.5	79
18-Aug	32	15.3	100	743	264
22-Aug	45	65	77	1137	517
24-Aug	54	51	46	141	618
26-Aug	54	20.2	39	210	657
28-Aug	41	46	67	146	363

Propionic Acid mg/L					
	35°C	42°C	49°C	55°C	62°C
12-Aug	25.4	1.4	7.6	64	1627.5
14-Aug	15	9.3		1664	1460
18-Aug	3	10	249.6	403.5	1563
22-Aug	12.11	26	1177	965.6	1037
24-Aug	19	44	42.4	750	1260.2
26-Aug	19.4	37.4	15.9	810	1624
28-Aug	0	0	12	1144.7	1521

Butyric Acid mg/L					
	35°C	42°C	49°C	55°C	62°C
12-Aug	76	0	0	5.5	0
14-Aug	0	0	0	51	0
18-Aug	0	0	9.5	57.5	54
22-Aug	0	0	7.9	145.6	0
24-Aug	0	0	0	0	47
26-Aug	0	27	0	0	52.3
28-Aug	0	0	0	22.3	24

Ammonia-N (mg/L)					
	35°C	42°C	49°C	55°C	62°C
12-Aug	460.488	357.3397	835.6809	960.7621	998.0135
14-Aug	460.488	896.0416	936.7035	936.7035	1056.616
18-Aug	388.0394	578.5511	779.3863	857.1447	1043.303
22-Aug	400.5373	582.2307	713.1892	1043.303	1070.099
24-Aug	352.8372	608.6521	582.2307	669.3766	1030.157
26-Aug	331.1617	593.4107	582.2307	699.7526	884.7515
28-Aug	263.5826	446.1195	567.651	291.7236	496.8891

Alkalinity (mg/L)					
	35°C	42°C	49°C	55°C	62°C
12-Aug	5435.46	5193.41	6839.59	7372.41	2201.82
14-Aug	5252.87	5195.27	6592.81	6972.81	2759.28
18-Aug	5127.86	5156.5	5556.49	7049.25	3490.65
22-Aug	3803.31	5532.18	4938.49	6400	3781.4
24-Aug	4113.71	5519.37	6231.37	6590.24	3904.72
26-Aug	3904.81	5144.43	5835.56	6673.77	2763.08
28-Aug	3953.35	5175.37	5954.48	6598.29	1893.46

pH					
	35°C	42°C	49°C	55°C	62°C
12-Aug	7.33	7.67	7.92	8.1	7.44
14-Aug	7.34	7.68	7.93	7.99	7.66
18-Aug	7.54	7.73	7.79	8.05	7.93
22-Aug	7.33	7.59	7.65	7.94	7.72
24-Aug	7.24	7.6	7.84	8.03	7.66
26-Aug	7.22	7.55	7.75	8.06	7.24
28-Aug	7.16	7.46	7.69	7.92	7.05

CH ₄ Fraction						CO ₂ Fraction				
	35°C	42°C	49°C	55°C	62°C	35°C	42°C	49°C	55°C	62°C
12-Aug	59.16	63.75	63.22	53.37	49.15	34.14	31.01	31.19	39.39	45.45
14-Aug	54.82	58.82	59.74	60.39	60.6	30.55	29.43	29.71	30.51	30.32
18-Aug	56.32	59.2	59.19	62.4	46.09	32.87	29.47	29.46	26.27	41.93
22-Aug	52.74	54.79	54.02	60.06	54.28	31.61	25.31	29.64	26.93	30.66
24-Aug	50.48	53.59	50.38	56.72	56.94	34.1	33.69	34.46	30.65	30.17
26-Aug	50.46	50.56	53.45	50.77	51.01	34.57	35.78	33.24	31.7	31.91
28-Aug	53.2	59.3	60.1	59.32	51.5	32.5	31	31	31.3	49.85

Gas Production (L/day)					
	35°C	42°C	49°C	55°C	62°C
12-Aug	18.785	25.256	27.72	30.225	55.104
13/8/03	14.62	28.864	30.712	34.255	18.528
14/8/03	0.935	30.832	33.088	32.395	3.614
15/8/03	15.64	38.704	25.623	36.58	18.348
16/8/03	29.155	51.414	32.058	51.615	33.221
17/8/03	16.32	1	16.614	29.14	18.626
18/8/03	20.485	33.374	28.08	38.595	13.205
19/8/03	5.1	7.79	9.36	10.23	9.174
20/8/03	3.145	3.444	5.733	4.2625	8.896
21/8/03	23.8	34.604	28.782	34.3325	8.201
22/8/03	19.72	24.272	25.038	26.1175	8.201
23/8/03	18.615	21.976	26.676	26.2725	10.564
24/8/03	23.885	25.01	27.495	26.5825	6.394
25/8/03	10.455	16.728	18.72	20.2275	6.533
26/8/03	6.77	41.492	24.57	27.125	18.348
27/8/03	5.44	15.006	16.029	17.67	14.456
28/8/03	5.865	21.238	24.219	25.42	20.989
29/8/03	4.25	23.698	19.188	26.66	23.63
30/8/03	8.245	27.306	27.261	28.055	33.638

Dewatering (Sec)					
	35°C	42°C	49°C	55°C	62°C
12-Aug	2188.8	2426.4	2504.3	2356.7	2157
14-Aug	1930.8	2417.7	2705.4	2142	2099.2
18-Aug	1459.2	2078.1	2609.9	2204	2105.7
22-Aug	1757.1	1963.5	2574.4	2235.2	2288.2
24-Aug	1850	2241	2519	2275	2079
26-Aug	2381.55	2260	2572.75	2221.9	2983.5
28-Aug	2299.4	2778.35	3005.6	3112.4	2128.5

			TCOD		
	35°C	42°C	49°C	55°C	62°C
12-Aug	22521.84	20119.6	19368.9	18618.2	24623.8
14-Aug	21621	19368.9	19368.9	19368.9	26125.2
18-Aug	11111.2	9384.59	8108.4	10660.78	11861.9
22-Aug	14864.7	16366.1	15765.54	14114	16741.45
24-Aug	12612.6	11111.2	12987.95	13588.51	15540.33
26-Aug	12612.6	11861.9	12237.25	12987.95	14864.7
28-Aug	8859.1	9609.8	11861.9	8859.1	11861.9
			SCOD		
	35°C	42°C	49°C	55°C	62°C
12-Aug	976.75	1352.1	7357.7	8859.1	9309.52
14-Aug	1126.89	2853.5	150.98	2628.29	4505.04
18-Aug	1502.24	1727.45	376.19	3829.41	10360.5
22-Aug	4354.9	3228.85	5706.16	6531.93	9985.15
24-Aug	3979.55	1277.03	4204.76	5706.16	9534.73
26-Aug	2177.87	2252.94	3003.64	4880.39	9009.24
28-Aug	676.47	2102.8	376.19	2252.94	4505.04

	12-Agust	20-Agust	25-Agust	29-Agust	
	CFU/100mL				
	FC	FC	FC	FC	
Feed	2.40E+06	2.80E+06	2.50E+06	3.10E+06	
35°C	1.30E+05	2.10E+05	6.40E+05	1.60E+06	
42°C	3.30E+04	2.80E+04	1.60E+04	3.10E+04	
49°C	1.00E+03	8.00E+03	2.00E+03	8.00E+03	
55°C	1.00E+03	1.00E+03	1.00E+03	1.00E+03	
62°C	1.00E+03	1.00E+03	1.00E+03	1.00E+03	
	<i>Salmonella</i>	<i>Salmonella</i>	<i>Salmonella</i>	<i>Salmonella</i>	
Feed	2.30E+05	2.00E+04	1.20E+05	1.10E+05	
35°C	1.20E+05	1.00E+04	2.00E+04	1.40E+04	
42°C	9.00E+04	100	100	5.00E+03	
49°C	100	100	100	100	
55°C	100	100	100	100	
62°C	100	100	100	100	
	E-coli	E-coli	E-coli	E-coli	
Feed	1.20E+06	7.00E+05	4.20E+06	2.80E+05	
35°C	4.00E+05	2.40E+05	9.00E+04	1.20E+05	
42°C	1.90E+05	6.00E+04	5.00E+04	4.60E+04	
49°C	1000	3.00E+04	2.00E+04	4.00E+03	
55°C	1000	2.00E+03	1000	2.00E+03	
62°C	1000	1000	1000	1000	
	<i>C.perfingens</i>	<i>C.perfingens</i>	<i>C.perfingens</i>	<i>C.perfingens</i>	
Feed	5.50E+04	4.00E+06	1.40E+06	2.00E+06	
35°C	5.60E+07	2.30E+07	2.20E+06	7.60E+06	
42°C	1.20E+07	5.00E+06	4.00E+06	1.00E+07	
49°C	3.00E+06	4.80E+06	2.30E+07	1.40E+07	
55°C	8.00E+05	5.00E+06	1.00E+06	4.00E+06	
62°C	6.00E+05	2.00E+06	2.20E+06	1.00E+06	
	FS	FS	FS	FS	
Feed	2.10E+07	9.00E+06	3.70E+07	4.10E+07	
35°C	1.10E+07	7.60E+06	2.40E+07	5.90E+06	
42°C	2.00E+06	2.80E+05	1.40E+07	2.20E+06	
49°C	3.00E+04	2.00E+04	1.00E+03	7.00E+03	
55°C	1.00E+03	1.00E+03	1.00E+03	1.00E+03	
62°C	1.00E+03	1.00E+03	1.00E+03	1.00E+03	

5 day HRT steady state data (November 24, 2003 – December 28, 2003)

TS						
	Feed	35°C	42°C	49°C	55°C	62°C
24-Nov	46000	29050	30150	30550	32100	34600
26-Nov	56250	33850	35700	35350	33800	34750
13-Dec	56250	33900	33750	30300	30800	30650
15-Dec	47150	30950	33700	26850	32300	33850
18-Dec	47150	34050	34100	29050	32800	33700
21-Dec	49750	38250	38250	39900	33400	37950
28-Dec	48802	35212	34421	27779	31150	32293

VS						
	Feed	35°C	42°C	49°C	55°C	62°C
24-Nov	33550	18550	18800	18650	19450	22850
26-Nov	40750	21600	21750	21450	20450	22250
13-Dec	40750	22150	22750	19200	18500	19850
15-Dec	35000	20500	23100	17000	19750	23200
18-Dec	35000	22350	22900	18300	20250	20950
21-Dec	37850	25300	25900	25400	21450	25400
28-Dec	36600	22530	23657	20201	19840	21308

TSS						
	Feed	35°C	42°C	49°C	55°C	62°C
24-Nov	45750	30500	27750	32500	26750	31250
26-Nov	52750	39750	33000	33500	33750	31000
13-Dec	48750	30250	32250	28000	29500	28250
15-Dec	48750	33000	31250	27250	30250	26750
18-Dec	48750	30750	34250	31000	30750	28250
21-Dec	48000	36500	36000	37250	27750	32250
28-Dec	47812	32229	33449	29907	30080	30749

VSS						
	Feed	35°C	42°C	49°C	55°C	62°C
24-Nov	34250	19750	17500	22750	16250	20000
26-Nov	39750	20000	20500	20500	19750	19750
13-Dec	39750	19750	19000	17750	17500	15500
15-Dec	33250	22500	21500	16500	16500	17750
18-Dec	33250	20250	24000	20500	18250	18500
21-Dec	37250	24000	23250	23250	17500	21500
28-Dec	34609	22905	18771	19491	18237	21933

TVFA mg/L					
	35°C	42°C	49°C	55°C	62°C
24-Nov	1992.41	2768.97	1872.66	1890.51	6474.45
26-Nov	1794.90	2827.06	1252.47	1438.23	5531.52
13-Dec	1645.99	3233.41	1313.30	1101.95	4421.15
15-Dec	1821.93	2911.21	1118.99	1045.03	4284.24
18-Dec	1004.19	2948.95	1448.33	1297.76	4106.98
21-Dec	856.35	2907.73	2422.65	2782.77	5049.35
28-Dec	1520.00	2750.00	1292.11	1152.69	4680.45

Acetic Acid mg/L					
	35°C	42°C	49°C	55°C	62°C
24-Nov	885	1163	215	0	2469
26-Nov	915	958	194	0	1899
13-Dec	182	1438	39	0	1578
15-Dec	979	1709	506	0	1677
18-Dec	146	433	147	327	1012
21-Dec	394	1424	288	2739	1450
28-Dec	563	1244	341	245	1681

Propionic Acid mg/L					
	35°C	42°C	49°C	55°C	62°C
24-Nov	1713	2084	1487	1860	2988
26-Nov	1932	2105	1434	1819	2693
13-Dec	1261	3068	1714	1758	2631
15-Dec	2166	2598	222	1622	2662
18-Dec	958	1821	1563	5471	1962
21-Dec	559	2862	565	6393	2979
28-Dec	904	2265	1321	1642	2417

Butyric Acid mg/L					
	35°C	42°C	49°C	55°C	62°C
24-Nov	0	34	38	0	1157
26-Nov	0	50	0	0	453
13-Dec	0	649	0	0	553
15-Dec	0	2737	37	0	756
18-Dec	0	297	0	0	339
21-Dec	0	144	66	511	281
28-Dec	0	333	0	0	401

Ammonia-N (mg/L)					
	35°C	42°C	49°C	55°C	62°C
24-Nov	194.4251	273.8024	211.1285	255.3581	272.072
26-Nov	163.8361	191.9753	255.3581	239.6709	263.5826
13-Dec	361.8996	493.7488	546.4631	660.9425	660.9425
15-Dec	346.1897	512.8928	546.4631	546.4631	556.9563
18-Dec	405.6484	481.3847	469.3303	481.3847	620.3394
21-Dec	410.8248	539.5777	582.2307	632.2512	830.3994
28-Dec	380.7286	457.5777	546.4631	612.5232	620.3394

Alkalinity (mg/L)					
	35°C	42°C	49°C	55°C	62°C
24-Nov	4133.53	4261.47	4841.87	5545.79	1422.54
26-Nov	3774.91	3563.62	4096.05	4043.10	2044.79
13-Dec	3256.78	860.09	3558.45	3934.78	1753.23
15-Dec	3687.30	1834.78	3798.50	4468.57	2237.29
18-Dec	4747.81	3305.80	5596.27	5157.35	3061.98
21-Dec	6027.98	3913.04	5531.26	5436.96	4108.21
28-Dec	3356.97	2750.58	4391.50	4002.00	2531.33

pH					
	35°C	42°C	49°C	55°C	62°C
24-Nov	7.2	7.4	7.6	7.8	7
26-Nov	7.2	7.3	7.6	7.7	7.3
13-Dec	7.5	7.3	8	8	7.6
15-Dec	6.9	6.4	7.6	7.9	7.3
18-Dec	7.7	7.5	8	8	7.8
21-Dec	7.6	7.4	7.9	7.8	7.9
28-Dec	7.5	7.4	7.7	7.9	7.8

CH ₄ Fraction						CO ₂ Fraction				
	35°C	42°C	49°C	55°C	62°C	35°C	42°C	49°C	55°C	62°C
24-Nov	67	47	53	57	40	57	51	47	41	61
26-Nov	46	54	52	57	48	53	41	48	45	50
13-Dec	50	40	53	53	48	47	56	42	42	51
15-Dec	46	46	32	32	53	47	46	62	61	44
18-Dec	53	43	48	50	41	45	43	48	46	56
21-Dec	44	49	50	56	40	53	53	47	40	60
28-Dec	51	43	49	52	47	49	47	51	42	43

Dewatering (Sec)					
	35°C	42°C	49°C	55°C	62°C
24-Nov	2225	1920	1823	2800	2310
26-Nov	1970	1905	1924	2118	2434
13-Dec	1757	2290	2094	2342	2749
15-Dec	1750	2249	1814	1976	2318
18-Dec	2009	2050	1832	1873	2542
21-Dec	1824	2050	2035	1754	2268
28-Dec	2212	1825	1800	2065	2399

			TCOD		
	35°C	42°C	49°C	55°C	62°C
24-Nov	15015.68	13214	13664.42	14565.26	13814.56
26-Nov	14264.98	14415.12	14565.26	13514.28	14715.4
13-Dec	13964.7	26876.74	19670.02	13514.28	15766.38
15-Dec	14715.4	13214	12463.3	12463.3	13364.14
18-Dec	15165.82	15616.24	13514.28	13063.86	14114.84
21-Dec	14415.12	13964.7	15766.38	15015.68	14114.84
28-Dec	14114.84	15015.68	15466.1	13664.42	15165.82
			SCOD		
	35°C	42°C	49°C	55°C	62°C
24-Nov	2554.06	2403.92	1653.22	1803.36	6757.98
26-Nov	2704.2	5707	8709.8	5707	9460.5
13-Dec	3755.18	1052.66	1052.66	2253.78	8259.38
15-Dec	1202.8	1503.08	2554.06	1052.66	6007.28
18-Dec	2704.2	1352.94	1352.94	28228	6307.56
21-Dec	2554.06	752.38	1052.66	602.24	9310.36
28-Dec	3304.76	3154.62	2403.92	1052.66	10811.76

CFU/100 mL						
	13-Dec	15-Dec	18-Dec	21-Dec	25-Dec	28-Dec
	FC	FC	FC	FC	FC	FC
Feed	3.10E+07	2.40E+07	5.20E+07	1.44E+07	1.55E+07	6.30E+07
35°C	2.75E+06	1.70E+06	2.60E+06	4.30E+06	3.70E+06	4.20E+05
42°C	1.15E+06	1.10E+06	1.00E+05	8.30E+05	3.13E+06	3.60E+05
49°C	1.10E+04	1.30E+05	1.80E+04	6.30E+04	1.80E+05	8.60E+04
55°C	1.00E+03	1.00E+03	1.00E+03	1.00E+04	1.00E+03	1.40E+04
62°C	1.00E+03	1.00E+03	1.00E+03	1.00E+03	1.00E+03	1.00E+03
	FS	FS	FS	FS	FS	FS
Feed	2.30E+07	3.48E+07	2.19E+07	1.78E+07	3.50E+07	2.74E+07
35°C	1.01E+06	2.20E+07	1.16E+07	1.33E+07	1.50E+06	7.30E+06
42°C	5.45E+05	1.92E+07	6.14E+06	1.00E+07	3.66E+06	6.10E+06
49°C	4.95E+04	4.50E+06	1.50E+05	1.80E+05	1.65E+05	3.10E+05
55°C	1.00E+03	1.00E+03	2.00E+05	7.00E+04	2.50E+04	1.90E+04
62°C	1.00E+03	1.00E+03	1.00E+03	1.00E+03	1.00E+03	1.00E+03
	Salmonella	Salmonella	Salmonella	Salmonella	Salmonella	Salmonella
Feed	2.00E+06	8.00E+05	5.50E+06	4.00E+06	3.50E+06	4.20E+06
35°C	4.00E+05	1.09E+05	1.60E+06	6.20E+05	4.50E+05	3.20E+05
42°C	2.00E+04	2.50E+03	8.00E+03	7.00E+03	4.00E+03	5.00E+03
49°C	2.30E+03	5.00E+02	2.00E+03	5.00E+03	1.00E+02	1.00E+02
55°C	100	100	100	100	100	100
62°C	100	100	100	100	100	100
	E-coli	E-coli	E-coli	E-coli	E-coli	E-coli
Feed	3.41E+08	2.23E+08	7.21E+08	3.54E+08	1.80E+08	3.12E+08
35°C	4.00E+06	3.60E+06	5.90E+06	9.27E+07	5.15E+07	4.23E+07
42°C	1.20E+06	9.40E+05	1.80E+06	2.53E+07	9.50E+06	2.70E+07
49°C	3.00E+05	1.00E+04	1.85E+05	1.00E+03	1.00E+05	2.10E+05
55°C	2.00E+03	1000	1000	1.00E+03	7.00E+03	1.00E+03
62°C	1000	1000	1000	1000	1000	1000
	C.perfingens	C.perfingens	C.perfingens	C.perfingens	C.perfingens	C.perfingens
Feed	6.25E+06	1.00E+06	4.15E+07	3.00E+06	7.25E+06	4.56E+06
35°C	4.13E+07	9.00E+07	1.33E+07	7.00E+06	8.75E+06	1.20E+07
42°C	9.50E+07	1.13E+07	1.28E+07	4.75E+06	1.03E+07	3.10E+07
49°C	1.03E+07	8.00E+06	8.25E+06	3.60E+06	4.50E+06	2.90E+07
55°C	2.50E+06	6.25E+06	4.25E+06	2.00E+06	4.00E+06	3.82E+06
62°C	9.80E+05	4.75E+06	1.50E+06	1.50E+06	3.25E+06	4.79E+05

APPENDIX B: RAW DATA FOR STAGING OF DIGESTERS

Phase I -steady state data (March 30, 2004– May 26, 2004)

TS	30-Mar	2-Apr	7-Apr	12-Apr	1-May	6-May	11-May
Feed	40150	45200	41650	43570	46400	48750	46300
S1-35	25600	32750	34850	30950	32650	33000	32250
S1-35	25350	29350	30050	29070	29300	30450	31500
S1-35	24950	25200	25650	26850	27050	28000	29900
S2-42	25650	29650	31050	32300	31600	33150	29450
S2-35	23950	29750	26600	33850	27150	27300	28050
S2-35	24250	26000	27350	26848	24050	24950	25300
S3-35	25000	29850	30150	29147	34750	35250	49000
S3-35	23350	24050	25050	24990	27700	30450	36600

VS	30-Mar	2-Apr	7-Apr	12-Apr	1-May	6-May	11-May
Feed	33500	34800	32100	33260	32800	36950	36700
S1-35	19500	20900	20900	20500	21000	21100	20100
S1-35	15600	17450	17050	17000	17250	18150	17100
S1-35	15050	14800	14100	15541	15300	16150	15850
S2-42	17600	18500	18600	18750	18400	20450	18550
S2-35	15450	15050	15600	16470	16750	17950	15650
S2-35	14050	14550	14700	14310	14350	12150	13900
S3-35	19800	18300	19050	19500	20550	22300	24800
S3-35	15900	16650	15325	15390	15650	18250	21850

TSS	30-Mar	2-Apr	7-Apr	12-Apr	1-May	6-May	11-May
Feed	42500	42500	37750	37750	45500	43250	45000
S1-35	25750	26250	33500	27950	32000	32750	30750
S1-35	23500	26500	28500	26700	28250	30250	30250
S1-35	24250	24250	26000	23870	25500	27750	28000
S2-42	24500	27750	30000	28260	29250	28000	27000
S2-35	22000	27250	24250	25850	25750	26250	27000
S2-35	22500	24250	27250	26750	23250	24250	23000
S3-35	29000	27250	32500	27100	33500	33250	33500
S3-35	21000	24250	27250	25450	26000	28750	25750

VSS	30-Mar	2-Apr	7-Apr	12-Apr	1-May	6-May	11-May
Feed	33000	33000	32220	32220	34750	32750	32250
S1-35	18000	17500	14220	13910	20750	22000	18750
S1-35	15250	14750	12600	11970	17000	18750	17250
S1-35	14250	13500	12500	11000	16000	17750	16250
S2-42	16500	17500	19000	18750	18750	18000	16000
S2-35	14000	14500	14250	15200	15500	15750	14000
S2-35	13500	13000	12970	13200	13750	13750	13000
S3-35	14250	16750	14360	15450	21250	20750	17500
S3-35	11250	14000	12440	14710	15750	17250	14750

TVFA mg/L							
	30-Mar	2-Apr	7-Apr	12-Apr	1-May	6-May	11-May
S1-35	421.3474	339.3098	1087.85	335.3237	248.2046	491.3326	219.0437
S1-35	90.65335	119.9568	93.83441	151.0034	45.75747	411.6371	295.1322
S1-35	109.7106	90.41721	42.81232	80.83379	54.32965	37.18528	3.395322
S2-42	90.65335	511.7678	1344.305	620.8062	183.3459	218.4118	424.5515
S2-35	148.4975	136.4835	77.19477	136.4835	117.6689	132.4615	52.16473
S2-35	92.09992	53.09337	78.94661	95.95895	21.34406	13.75551	20.5844
S3-35	398.2545	344.631	437.6294	344.631	294.2708	463.5615	536.6966
S3-35	75.2953	149.2635	73.26355	104.7226	68.71637	127.4926	64.75701

Ammonia- N (mg/L)							
	30-Mar	2-Apr	7-Apr	12-Apr	1-May	6-May	11-May
S1-35	451.8123	105.1162	451.8123	346.1897	398.0059	373.5556	628.2554
S1-35	851.7276	174.5597	500.0494	512.8928	726.8839	481.3847	475.3193
S1-35	879.1599	239.6709	612.5232	546.4631	907.4757	660.9425	660.9425
S2-42	620.3394	248.9636	632.2512	546.4631	308.8534	546.4631	582.2307
S2-35	789.3318	308.8534	385.587	432.1993	873.6037	289.8799	704.2031
S2-35	446.1195	408.2284	1140.141	1214.766	434.9481	516.1548	1857.686
S3-35	574.8946	277.2964	434.9481	750.2953	299.2163	255.3581	273.8024
S3-35	948.6565	337.5207	398.0059	308.8534	722.2901	704.2031	799.4043

Alkalinity (mg/L)							
	30-Mar	2-Apr	7-Apr	12-Apr	1-May	6-May	11-May
S1-35	3666.679	3682.553	4068.093	3746.262	5090.324	4623.656	4696.657
S1-35	5056.649	3946.795	4331.908	4122.381	6180.881	5239.377	5587.895
S1-35	4918.57	4335.164	4800.287	4404.259	5331.086	7030.677	6701.014
S2-42	4456.74	3702.46	3407.705	3673.108	5692.252	5734.589	5286.776
S2-35	6145.155	4713.166	4467.693	4713.166	6636.963	6698.135	7076.448
S2-35	6738.576	4670.549	5247.706	4786.901	6444.006	6511.197	7021.722
S3-35	5049.413	3617.554	3591.016	3617.554	4933.27	4776.621	5002.342
S3-35	5575.846	5510.304	5505.412	5175.601	5979.13	6517.134	7004.498

pH							
	30-Mar	2-Apr	7-Apr	12-Apr	1-May	6-May	11-May
S1-35	7.64	8	7.9	8	7.8	7.7	7.71
S1-35	7.72	8.4	8.32	7.79	7.6	7.5	7.72
S1-35	7.83	8	8.24	7.6	7.6	7.6	8
S2-42	8.31	7.9	8.9	7.9	7.5	7.6	7.97
S2-35	7.83	8	8.1	7.9	7.9	7.8	8.1
S2-35	8.24	8	8.11	8.2	7.7	7.7	8
S3-35	7.96	7.88	8	8	7.5	7.2	7.7
S3-35	7.98	8.12	8.7	8.14	7.6	7.6	8.4

CH4 Fraction								
	S1-35	S1-35	S1-35	S2-42	S2-35	S2-35	S3-35	S3-35
30-Mar	55.7	59	60	60	58	58	51	61
2-Apr	54	62	48	53	60	45	60	59
7-Apr	53	60	51	53	60	42	57	58
12-Apr	54	61	57	54	57	50	58	59
1-May	54	61	59	54	61	51	61	60
6-May	58	61	50	61	59	47	61	61
11-May	63	61	50	62	58	47	58	61
26-May	56	60	49	59	59	44	58	60

CO2 Fraction								
	S1-35	S1-35	S1-35	S2-42	S2-35	S2-35	S3-35	S3-35
30-Mar	44.3	41	40	40	42	38	39	41
2-Apr	46	38	35	47	40	38	40	41
7-Apr	47	40	36	47	40	34	43	42
12-Apr	46	39	43	46	43	50	42	41
1-May	46	39	41	46	39	34	39	40
6-May	42	39	36	39	41	35	39	39
11-May	37	39	36	38	42	35	42	39
26-May	44	40	36	41	41	34	42	40

CST (Sec)							
	30-Mar	2-Apr	7-Apr	12-Apr	1-May	6-May	11-May
S1-35	1479	1062	1371	1277	1547	1128	1325
S1-35	1129	805	1211	1097	1235	805	1170
S1-35	1009	811	1004	853	841	811	1107
S2-42	1200	1614	1212	1225	1522	1614	1212
S2-35	1352	1075	934	883	863	1075	934
S2-35	1061	942	837	685	901	837	675
S3-35	1232	998	1088	1318	1810	1088	998
S3-35	1210	961	902	858	1006	961	902

TCOD	S1-35	S1-35	S1-35	S2-42	S2-35	S2-35	S3-35	S3-35
30-Mar	23949.01	22522.68	19820.16	20946.21	21771.98	21997.19	21321.56	23648.73
2-Apr	22522.68	20270.58	19519.88	22973.1	21321.56	16517.08	21771.98	17117.64
7-Apr	23723.8	23573.66	18994.39	21133.89	21471.7	22485.15	21171.42	24136.69
12-Apr	22973.1	22672.82	22522.68	24324.36	21021.28	20721	22672.82	19820.16
1-May	22222.4	21321.56	20270.58	21621.84	21321.56	20420.72	21171.42	19820.16
6-May	22072.26	21021.28	21471.7	22822.96	22222.4	21922.12	22822.96	20420.72
11-May	22372.54	22372.54	22522.68	23723.8	22522.68	21771.98	22822.96	20721

SCOD	S1-35	S1-35	S1-35	S2-42	S2-35	S2-35	S3-35	S3-35
30-Mar	3423.864	120.784	90.756	1832.38	901.512	-59.384	3333.78	721.344
2-Apr	3363.808	180.84	24.6944	2583.08	481.12	120.784	2943.416	2823.304
7-Apr	3513.948	0.672	30.7	7207.392	751.372	60.728	2733.22	2553.052
12-Apr	2643.136	1622.184	361.008	1682.24	1081.68	901.512	2883.36	1321.904
1-May	1622.184	901.512	361.008	2763.248	1862.408	1261.848	2402.912	1562.128
6-May	2583.08	2102.632	1502.072	2763.248	3063.528	2943.416	2883.36	601.232
11-May	3423.864	2763.248	601.232	4745.096	1742.296	1862.408	3604.032	721.344

CFU/100 mL							
	30-Mar	2-Apr	7-Apr	12-Apr	1-May	6-May	11-May
	FC	FC	FC	FC	FC	FC	FC
Feed	1.30E+07	5.00E+06	3.50E+07	4.00E+07	7.20E+07	2.00E+08	6.66E+07
S1-35	2.40E+06	9.00E+05	4.20E+06	6.60E+06	6.00E+06	2.00E+06	2.00E+06
S1-35	5.00E+05	1.30E+05	2.10E+05	1.40E+06	1.20E+06	4.00E+04	1.60E+05
S1-35	5.00E+04	5.00E+04	9.00E+04	6.00E+05	2.20E+05	2.80E+04	2.00E+04
S2-42	4.10E+05	3.00E+05	6.60E+05	3.20E+05	2.00E+05	5.60E+05	1.40E+06
S2-35	3.00E+04	2.00E+04	2.00E+04	2.00E+04	3.00E+04	1.00E+05	8.00E+04
S2-35	9.00E+03	6.00E+03	4.00E+03	2.00E+03	2.00E+03	4.00E+03	6.00E+03
S3-35	1.70E+06	2.00E+06	3.40E+06	2.10E+06	1.70E+06	1.90E+06	1.70E+06
S3-35	5.00E+05	4.00E+05	1.70E+05	4.00E+05	5.00E+04	1.80E+05	1.60E+04

	FS	FS	FS	FS	FS	FS	FS
Feed	2.30E+07	2.44E+07	2.00E+07	3.60E+08	1.10E+07	2.60E+07	1.90E+07
S1-35	3.80E+06	1.00E+07	5.70E+06	9.20E+07	1.00E+06	1.20E+07	1.34E+06
S1-35	1.04E+06	8.00E+05	7.50E+05	2.00E+06	1.92E+05	1.62E+06	1.50E+05
S1-35	1.40E+05	8.00E+04	1.50E+05	3.40E+05	3.80E+04	3.20E+05	4.40E+04
S2-42	3.20E+05	2.95E+06	7.10E+05	7.40E+05	8.40E+05	8.60E+06	9.20E+05
S2-35	5.60E+04	1.20E+04	6.70E+05	2.40E+04	4.20E+05	7.60E+05	6.00E+05
S2-35	6.00E+03	2.50E+03	1.40E+05	8.00E+03	5.80E+04	2.40E+05	1.84E+05
S3-35	2.72E+06	3.70E+06	5.20E+06	1.06E+07	1.18E+07	9.00E+06	8.20E+06
S3-35	3.40E+05	1.50E+05	5.60E+05	7.40E+05	6.00E+05	6.60E+05	5.44E+05

	Salmonela(cfu/100ml)	Salmonela(cfu/100ml)	Salmonela(cfu/100ml)	Salmonela	Salmonela	Salmonela	Salmonela
Feed	1.62E+06	3.12E+06	5.50E+05	3.20E+05	2.90E+05	1.80E+05	1.52E+05
S1-35	9.50E+04	7.80E+04	9.30E+04	1.12E+05	1.02E+05	7.20E+04	6.60E+04
S1-35	5.50E+04	1.20E+03	5.00E+03	4.00E+04	3.60E+04	8.00E+03	1.60E+04
S1-35	1.25E+03	1.90E+03	1.30E+03	9.00E+03	8.00E+03	5.00E+03	1.00E+04
S2-42	4.50E+03	2.00E+03	1.00E+04	1.40E+04	3.20E+04	4.40E+04	5.50E+04
S2-35	4.00E+03	1.50E+03	4.00E+03	9.00E+03	1.10E+04	1.20E+04	8.00E+03
S2-35	1.50E+03	1.25E+03	1.50E+03	1.67E+03	1.99E+03	6.00E+03	5.00E+03

S3-35	6.50E+04	7.20E+04	7.80E+04	1.34E+05	1.14E+05	9.00E+04	8.40E+04
S3-35	2.25E+04	2.50E+03	3.00E+04	2.80E+04	3.40E+04	4.40E+04	4.00E+04

	E-coli	E-coli	E-coli	E-coli	E-coli	E-coli	E-coli
Feed	2.00E+07	1.92E+07	3.20E+08	3.08E+07	2.20E+07	3.10E+07	2.50E+07
S1-35	8.00E+06	6.80E+06	1.00E+06	8.00E+05	3.40E+06	1.88E+06	1.64E+06
S1-35	4.60E+05	6.40E+05	7.40E+05	5.40E+05	2.30E+05	2.40E+05	3.40E+05
S1-35	2.00E+05	4.00E+04	1.60E+05	2.00E+04	7.50E+04	6.80E+04	1.42E+05
S2-42	3.60E+06	3.40E+06	8.00E+05	4.00E+05	4.40E+06	3.00E+06	2.40E+06
S2-35	5.00E+04	3.60E+05	4.00E+05	2.70E+05	2.20E+05	2.00E+04	1.00E+04
S2-35	2.00E+04	8.00E+04	1.84E+05	1.70E+05	2.56E+04	2.00E+03	2.00E+03
S3-35	4.16E+06	4.32E+06	7.40E+06	6.20E+06	3.08E+07	1.00E+07	1.20E+07
S3-35	3.20E+05	1.80E+05	1.02E+06	5.90E+05	2.16E+05	2.20E+05	1.60E+05

	C.perfingens	C.perfingens	C.perfingens	C.perfingens	C.perfingens	C.perfingens	C.perfingens
Feed	2.30E+07	2.44E+07	2.00E+07	3.60E+08	1.10E+07	2.60E+07	1.90E+07
S1-35	3.80E+06	1.00E+07	5.70E+06	9.20E+06	1.00E+07	1.20E+07	1.34E+07
S1-35	1.04E+06	8.00E+05	7.50E+05	2.00E+06	1.92E+06	1.62E+06	1.50E+06
S1-35	1.40E+05	8.00E+04	1.50E+05	3.40E+05	3.80E+05	3.20E+05	4.40E+05
S2-42	3.20E+06	2.95E+06	7.10E+06	7.40E+05	8.40E+05	8.60E+06	9.20E+06
S2-35	5.60E+05	1.20E+05	6.70E+05	2.40E+05	4.20E+05	7.60E+05	6.00E+05
S2-35	6.00E+04	2.50E+04	1.40E+05	8.00E+04	5.80E+04	2.40E+05	1.84E+05
S3-35	2.72E+06	3.70E+06	5.20E+06	1.06E+07	1.18E+07	9.00E+06	8.20E+06
S3-35	3.40E+05	1.50E+05	5.60E+05	7.40E+05	6.00E+05	6.60E+05	5.44E+05

Phase II -steady state data (Oct. 10, 2004– Nov.29, 2004)

TS	10-Oct	15-Oct	25-Oct	1-Nov	11-Nov	19-Nov	29-Nov
Feed	42500	42400	45000	41600	40550	43600	43667
S1-49	28000	27850	28400	27550	26350	27950	26417
S1-35	26400	25900	26200	25700	24500	25350	24667
S1-35	23550	22350	24000	23400	21100	22350	23033
S2-42	27150	26750	27800	27350	26200	26800	27300
S2-42	28050	26050	25500	26350	23400	26150	26133
S2-35	25600	23550	23450	24850	21450	23300	24667
S3-42	27050	27000	27300	26800	26050	27850	27367
S3-35	27200	25000	23950	24200	24350	25300	25383

VS	10-Oct	15-Oct	25-Oct	1-Nov	11-Nov	19-Nov	29-Nov
Feed	31800	32650	34050	30550	33900	34350	32417
S1-49	18750	18000	18500	17350	15800	18150	18283
S1-35	14450	13600	14500	14000	12850	15300	16533
S1-35	12100	11200	11750	12000	10200	11200	14650
S2-42	17850	17200	17050	17550	18600	17650	16600
S2-42	15200	13100	14500	15800	13750	14350	13133
S2-35	14150	12550	13650	14600	12400	13450	12267
S3-42	18500	17350	17250	15550	15450	17700	17050
S3-35	17050	15400	15900	15750	12850	15900	15683

TSS	10-Oct	15-Oct	25-Oct	1-Nov	11-Nov	19-Nov	29-Nov
Feed	40250	40500	42250	40000	41750	46750	40917
S1-49	28000	25750	26250	26750	27500	27250	26250
S1-35	25750	22750	27250	24000	25750	25000	25667
S1-35	22750	21750	25000	23250	21000	22750	24000
S2-42	29750	28750	28750	27750	29250	29000	28417
S2-42	25750	26000	26500	25750	26750	25500	26417
S2-35	22000	24250	25000	23500	25250	23000	24583
S3-42	27000	28750	26000	27250	27250	26250	26000
S3-35	24750	26250	24750	25000	25250	24500	24000

TSS	10-Oct	15-Oct	25-Oct	1-Nov	11-Nov	19-Nov	29-Nov
Feed	40250	40500	42250	40000	41750	46750	40917
S1-49	28000	25750	26250	26750	27500	27250	26250
S1-35	25750	22750	27250	24000	25750	25000	25667
S1-35	22750	21750	25000	23250	21000	22750	24000
S2-42	29750	28750	28750	27750	29250	29000	28417
S2-42	25750	26000	26500	25750	26750	25500	26417
S2-35	22000	24250	25000	23500	25250	23000	24583
S3-42	27000	28750	26000	27250	27250	26250	26000
S3-35	24750	26250	24750	25000	25250	24500	24000

VSS	10-Oct	15-Oct	25-Oct	1-Nov	11-Nov	19-Nov	29-Nov
Feed	30250	29500	32250	30000	26250	34500	30250
S1-49	16750	16500	16500	15250	14500	16000	16083
S1-35	14000	14500	14750	13750	11750	13000	15333
S1-35	13500	12500	13500	12250	10750	11250	12417
S2-42	17750	16250	16250	15500	15000	18000	16667
S2-42	15000	14500	13250	12250	12500	14750	12333
S2-35	13750	12000	12750	10750	11000	12000	11500
S3-42	16750	16750	16500	15750	15250	17500	16333
S3-35	12250	12250	13750	12500	13000	12000	14833

TVFA (mg/L)							
TVFA	10-Oct	15-Oct	25-Oct	1-Nov	11-Nov	19-Nov	29-Nov
S1-49	349.33	375.08	375.23	388.92	329.35	428.64	362.21
S1-35	99.23	169.77	112.41	184.02	85.51	96.65	134.50
S1-35	69.85	52.92	101.63	63.95	67.34	80.35	61.39
S2-42	335.19	436.82	325.15	364.07	594.96	414.22	386.00
S2-42	185.76	91.69	94.53	152.65	125.36	142.17	138.73
S2-35	63.95	70.58	83.89	25.29	30.09	45.66	67.26
S3-42	473.58	414.32	326.70	467.65	380.44	504.40	443.95
S3-35	51.24	133.25	88.75	145.61	50.59	99.71	92.25

Ammonia-N (mg/L)							
	10-Oct	15-Oct	25-Oct	1-Nov	11-Nov	19-Nov	29-Nov
S1-49	799.4043	851.7276	597.1849	481.3847	582.2307	907.4757	529.4119
S1-35	628.2554	704.2031	907.4757	799.4043	1030.157	966.8727	907.4757
S1-35	1140.141	851.7276	868.0825	840.9959	1327.519	966.8727	1056.616
S2-42	769.5661	424.0565	512.8928	469.3303	451.8123	660.9425	460.488
S2-42	620.3394	620.3394	500.0494	726.8839	799.4043	1605.622	762.2822
S2-35	1245.966	704.2031	660.9425	942.6611	1414.409	851.7276	1154.69
S3-42	481.3847	660.9425	597.1849	295.4462	451.8123	451.8123	365.3577
S3-35	620.3394	851.7276	750.2953	481.3847	546.4631	620.3394	512.8928

Alkalinity (mg/L)							
	10-Oct	15-Oct	25-Oct	1-Nov	11-Nov	19-Nov	29-Nov
S1-49	5176.402	5158.245	5260.962	4778.967	5375.311	6004.643	5077.139
S1-35	6611.841	6098.092	5637.713	5844.629	5963.168	6614.299	5903.898
S1-35	6579.015	6414.399	6767.904	6103.624	6160.385	6749.752	6132.004
S2-42	4714.405	4850.319	4855.88	4492.334	4957.014	6030.664	4724.674
S2-42	5962.928	5115.628	6616.315	6234.323	5625.378	6304.262	5929.851
S2-35	6103.624	6097.321	6554.063	6983.259	7277.376	6240.965	7130.318
S3-42	5219.271	5448.362	5636.816	4562.655	5673.22	6095.298	5117.937
S3-35	6235.66	6432.706	7138.791	5847.229	6296.268	6551.412	6071.748

pH							
	10-Oct	15-Oct	25-Oct	1-Nov	11-Nov	19-Nov	29-Nov
S1-49	8	8	7.8	7.8	8.12	7.83	7.9
S1-35	7.92	7.97	7.7	7.6	8	7.85	7.835
S1-35	8	8	7.8	7.8	8.2	7.82	7.9
S2-42	7.94	7.87	7.7	7	8	7.66	7.785
S2-42	8.01	8	7.8	7.9	8.12	7.82	7.9
S2-35	7.8	8	7.8	7.82	8.11	7.73	7.9
S3-42	8.1	8.14	7.72	7.7	8.1	7.71	7.93
S3-35	7.86	8	7.8	7.8	8.1	7.75	7.9

CH4 Fraction								
	S1-49	S1-35	S1-35	S2-42	S2-42	S2-35	S3-42	S3-35
10-Oct	55	60		60	55	34	60	48
15-Oct	57	59	39	55	56	39	52	58
25-Oct	55	61	45	58	56	46	58	55
1-Nov	58	63	42	55	58	50	56	49
11-Nov	60	55	55	60	57		59	
19-Nov	63	52	51	62	57	55	60	53
29-Nov	59	59	48.5	57.5	57.5	50	57.5	49

CO2 Fraction								
	S1-49	S1-35	S1-35	S2-42	S2-42	S2-35	S3-42	S3-35
10-Oct	45	40		40	45	30	40	32
15-Oct	43	41	45	45	44	31	48	42
25-Oct	45	39	36	42	44	34	42	45
1-Nov	42	37	36	45	42	34	44	39
11-Nov	40	35	35	40	43		41	
19-Nov	27	36	39	38	43	45	40	47
29-Nov	41	41	51.5	42.5	42.5	50	42.5	51

Gas Production (L/ day)								
	S1-49	S1-35	S1-35	S2-42	S2-42	S2-35	S3-42	S3-35
10-Oct	79.65	9.125	3.36	63.168	17.5	2.144	79.035	6.5
15-Oct	67.5	9.375	2.8	60.648	16.5	2.479	67.54	5.98
25-Oct	67.35	13.375	3.584	64.68	13.875	4.422	69.795	8.84
1-Nov	70.05	10.625	2.8	52.416	23.125	3.015	54.89	6.11
11-Nov	73.5	11.125	3.248	60.48	23.875	2.814	66.11	4.29
19-Nov	74.85	11.5	2.8	68.544	23	3.484	64.68	5.2
29-Nov	71.775	13.05	4.05	100.8	28.2	6.525	165	6

CST(Sec)								
CST	S1-49	S1-35	S1-35	S2-42	S2-42	S2-35	S3-42	S3-35
10-Oct	1676	830	832	1108	783	1194	1517	927
15-Oct	1570	1274	831	1120	942	931	1136	897
25-Oct	1520	1325	1034	1240	835	1395	1353	1037
1-Nov	1987	1428	1351	1353	1287	1133	1231.5	723
11-Nov	1698	1179	1128	1131	1365	1130	1769	1219
19-Nov	1700	1255	978	1070	1547	690	1838	1314
29-Nov	1970	998	1217	1089	1513	755.5	1234	690

TCOD (mg/L)								
	S1-49	S1-35	S1-35	S2-42	S2-42	S2-35	S3-42	S3-35
10-Oct	27552.37	23723.8	24549.57	25675.62	24999.99	24399.43	25900.83	24474.5
15-Oct	29579.26	24549.57	21021.28	23573.66	22672.82	20270.58	24024.08	23423.52
25-Oct	34383.74	22147.33	20270.58	20270.58	21621.84	19820.16	21321.56	20120.44
1-Nov	31230.8	20721	17117.64	19970.3	22973.1	19820.16	20420.72	18168.62
11-Nov	23123.24	20871.14	25075.06	23123.24	23123.24	22522.68	21922.12	23723.8
19-Nov	35735	24024.08	23423.52	22973.1	22672.82	24174.22	23273.38	21771.98
29-Nov	27177.02	26426.32	28678.42	25825.76	26576.46	26726.6	26276.18	24174.22

SCOD (mg/L)								
	S1-49	S1-35	S1-35	S2-42	S2-42	S2-35	S3-42	S3-35
10-Oct	3574.004	1742.296	1742.296	3213.668	1231.82	991.596	4144.536	1231.82
15-Oct	3574.004	2583.08	2222.744	3393.836	2042.576	541.176	2973.444	2553.052
25-Oct	2703.192	1922.464	1201.792	2853.332	661.288	361.008	2312.828	1562.128
1-Nov	4156.547	1021.624	481.12	2703.192	781.4	1141.736	2102.632	3604.032
11-Nov	3243.696	1682.24	1021.624	4084.48	901.512	361.008	3543.976	3003.472
19-Nov	2222.744	60.728	1201.792	2763.248	961.568	421.064	2943.416	1081.68
29-Nov	3123.584	2342.856	1141.736	3243.696	2162.688	2042.576	3423.864	961.568

CFU/100 mL									
	11-Oct	17-Oct	26-Oct	3-Nov	13-Nov	20-Nov	28-Nov		
	FC	FC	FC	FC	FC	FC	FC		
Feed	4.20E+07	5.00E+07	4.20E+07	6.00E+07	3.80E+07	3.20E+07	4.80E+07		
S1-49	6.90E+04	7.60E+04	9.00E+04	7.50E+04	8.00E+04	4.00E+04	7.00E+04		
S1-35	3.20E+04	4.60E+04	3.00E+04	6.00E+03	3.00E+04	2.60E+04	6.00E+03		
S1-35	7.00E+03	4.00E+03	4.20E+03	4.00E+03	6.00E+03	4.00E+03	4.00E+03		
S2-42	3.00E+05	1.80E+05	6.00E+04	8.00E+04	4.00E+05	3.80E+05	3.30E+05		
S2-42	1.60E+04	1.20E+04	7.00E+03	1.00E+04	8.00E+03	1.00E+04	9.00E+03		
S2-35	6.00E+03	8.00E+03	5.00E+03	7.00E+03	4.00E+03	7.00E+03	2.00E+03		
S3-42	1.90E+05	2.40E+05	8.00E+05	2.00E+05	9.40E+05	6.40E+05	2.00E+05		
S3-35	2.00E+03	4.00E+04	2.00E+03	4.00E+04	2.20E+04	2.40E+04	1.70E+04		
	FS	FS	FS	FS	FS	FS	FS		
Feed	2.28E+07	2.18E+07	2.88E+07	2.80E+07	1.32E+07	1.42E+07	1.40E+06		
S1-49	4.00E+05	3.40E+05	1.80E+05	1.40E+05	3.00E+05	2.50E+05	2.70E+05		
S1-35	1.20E+04	1.80E+04	8.00E+03	1.00E+04	2.00E+04	3.20E+05	3.40E+06		
S1-35	9.00E+03	8.00E+03	5.00E+03	8.00E+03	1.40E+04	1.60E+04	1.50E+04		
S2-42	2.80E+06	2.60E+06	3.80E+05	3.60E+05	3.00E+06	1.20E+06	6.00E+05		
S2-42	1.60E+05	8.00E+04	1.20E+05	1.40E+04	1.90E+05	1.80E+05	1.60E+05		
S2-35	4.00E+04	4.00E+04	4.00E+04	2.00E+03	1.40E+04	1.30E+04	1.50E+04		
S3-42	2.40E+06	2.54E+06	2.60E+05	3.00E+05	2.80E+06	1.40E+06	4.10E+06		
S3-35	2.20E+04	1.80E+04	3.20E+04	1.14E+05	2.70E+05	3.30E+05	2.90E+04		

	Salmonela	Salmonela	Salmonela	Salmonela	Salmonela	Salmonela	Salmonela
Feed	2.40E+07	1.84E+06	1.96E+06	1.60E+06	1.57E+06	1.71E+06	1.82E+06
S1-49	4.60E+03	9.40E+03	6.00E+02	1.70E+03	4.20E+03	3.20E+03	5.10E+03
S1-35	7.40E+03	1.90E+03	4.00E+02	1.49E+03	3.50E+03	2.70E+03	2.30E+03
S1-35	3.00E+03	5.20E+03	7.00E+02	9.90E+02	1.80E+03	2.20E+02	1.90E+03
S2-42	2.50E+04	1.38E+05	3.20E+05	4.00E+04	2.50E+05	2.70E+04	1.80E+04
S2-42	3.90E+03	3.60E+04	1.50E+03	2.30E+03	1.70E+03	1.60E+04	2.10E+03
S2-35	6.40E+03	8.40E+03	7.00E+02	1.00E+03	9.50E+02	1.20E+03	8.00E+02
S3-42	2.80E+04	6.00E+04	2.90E+04	1.70E+04	2.70E+04	8.60E+04	7.50E+04
S3-35	1.76E+04	1.24E+04	1.90E+04	1.10E+04	1.80E+03	1.60E+04	1.90E+04
	E-coli	E-coli	E-coli	E-coli	E-coli	E-coli	E-coli
Feed	4.40E+08	4.30E+08	7.60E+06	7.20E+07	1.12E+07	1.08E+08	1.60E+07
S1-49	6.00E+04	1.00E+05	5.20E+04	5.60E+04	3.60E+05	3.40E+04	4.00E+05
S1-35	4.00E+04	8.00E+04	3.80E+04	2.80E+04	4.00E+04	6.00E+04	2.80E+05
S1-35	5.60E+03	3.00E+04	1.40E+04	2.60E+04	1.20E+04	1.40E+04	9.00E+04
S2-42	6.00E+05	1.00E+06	7.00E+06	1.20E+05	1.40E+06	7.50E+05	6.80E+06
S2-42	6.00E+04	6.00E+04	4.00E+04	1.20E+05	2.60E+05	3.00E+05	3.20E+05
S2-35	3.60E+04	2.00E+04	2.00E+04	1.40E+04	2.00E+05	4.16E+05	6.60E+04
S3-42	2.00E+05	1.70E+06	1.40E+06	8.00E+05	5.60E+05	5.30E+06	8.00E+05
S3-35	1.76E+05	1.40E+05	5.00E+04	5.20E+04	4.40E+05	6.80E+04	3.40E+05

	C.perfingens	C.perfingens	C.perfingens	C.perfingens	C.perfingens	C.perfingens	C.perfingens
Feed	2.00E+07	1.50E+07	1.60E+07	1.40E+07	2.00E+06	1.50E+06	1.50E+06
S1-49	1.25E+07	6.00E+06	5.00E+06	6.50E+06	1.35E+07	1.00E+07	2.25E+07
S1-35	1.25E+07	1.00E+07	9.00E+06	1.00E+07	1.15E+07	9.50E+06	2.55E+07
S1-35	6.00E+06	6.20E+06	5.60E+06	7.50E+06	7.60E+06	6.00E+06	1.45E+07
S2-42	1.70E+07	1.30E+07	1.70E+07	1.25E+07	1.30E+07	9.00E+06	9.50E+06
S2-42	1.70E+07	1.20E+07	1.25E+07	1.40E+07	1.55E+07	1.20E+07	9.00E+06
S2-35	1.05E+07	1.20E+07	1.30E+07	1.20E+07	1.50E+06	2.00E+06	4.00E+06
S3-42	1.35E+07	1.30E+07	1.25E+07	1.45E+07	1.50E+06	2.50E+06	1.32E+06
S3-35	1.40E+07	1.35E+07	1.15E+07	1.55E+07	1.50E+07	1.75E+07	2.30E+07

Phase III -steady state data (Jan. 27, 2005– Feb. 25, 2005)

TS	Jan 27/05	Jan31/05	Feb 5/05	Feb 9/05	Feb 13/05	Feb 17/05	Feb 25/05
Feed	49500	48000	47950	46000	39850	56400	47317
S1-49-5	32500	30550	31000	30950	28900	31650	31167
S1-42-5	29250	27550	26500	27200	24150	28250	28417
S1-35-5	26750	25400	24000	23500	22250	25700	26633
S2-42-5	30550	31250	30300	30200	31150	29750	30583
S2-42-10	28750	26500	27900	27950	27150	27600	27783
S3-49-5	30550	30650	31250	30050	28800	29350	30650
S3-35-10	27650	27900	27500	26100	26800	26500	26167
S4-35-15	29050	28050	28450	28100	28850	26600	28200

VS	Jan 27/05	Jan31/05	Feb 5/05	Feb 9/05	Feb 13/05	Feb 17/05	Feb 25/05
Feed	37900	35650	35500	34100	30300	40950	35083
S1-49-5	20300	19400	19600	18550	17400	19850	18517
S1-42-5	16000	15100	16200	14250	14950	14350	14850
S1-35-5	12950	12700	12250	11950	11200	11600	12967
S2-42-5	18950	17700	18250	18300	17750	17200	18083
S2-42-10	17050	17050	16900	16200	17450	16050	16717
S3-49-5	19100	18600	19000	18500	17900	16350	18700
S3-35-10	15850	15450	12650	12950	14700	13200	13683
S4-35-15	17950	16800	17500	16750	17300	19000	17017

TSS	Jan 27/05	Jan31/05	Feb 5/05	Feb 9/05	Feb 13/05	Feb 17/05	Feb 25/05
Feed	47000	47500	45750	46250	44250	48250	46500
S1-49-5	31000	30000	30500	30000	29500	30500	30166.67
S1-42-5	26750	29250	27000	27750	26500	27000	27000
S1-35-5	24750	25500	24750	24000	23500	24500	24083.33
S2-42-5	32500	29750	29500	29500	32750	30750	29583.33
S2-42-10	30000	26750	25750	26000	30000	28500	25166.67
S3-49-5	30750	28500	27750	31750	26750	25000	29333.33
S3-35-10	26250	27000	23000	23250	26500	23250	24416.67
S4-35-15	27500	27250	26750	27000	30750	31000	27000

TSS	Jan 27/05	Jan31/05	Feb 5/05	Feb 9/05	Feb 13/05	Feb 17/05	Feb 25/05
Feed	47000	47500	45750	46250	44250	48250	46500
S1-49-5	31000	30000	30500	30000	29500	30500	30166.67
S1-42-5	26750	29250	27000	27750	26500	27000	27000
S1-35-5	24750	25500	24750	24000	23500	24500	24083.33
S2-42-5	32500	29750	29500	29500	32750	30750	29583.33
S2-42-10	30000	26750	25750	26000	30000	28500	25166.67
S3-49-5	30750	28500	27750	31750	26750	25000	29333.33
S3-35-10	26250	27000	23000	23250	26500	23250	24416.67
S4-35-15	27500	27250	26750	27000	30750	31000	27000

VSS	Jan 27/05	Jan31/05	Feb 5/05	Feb 9/05	Feb 13/05	Feb 17/05	Feb 25/05
Feed	36250	35250	36000	34750	33250	34250	35333.33
S1-49-5	18000	18000	19750	18750	18500	19000	18833.33
S1-42-5	15750	15750	16250	15250	15000	15000	14416.67
S1-35-5	13000	13000	14000	13750	12500	12000	12250
S2-42-5	18750	17250	18500	17750	17000	19750	17833.33
S2-42-10	15250	15500	15750	14500	14250	16250	15583.33
S3-49-5	17000	17750	18250	18500	17500	18900	19500
S3-35-10	14000	15000	15250	14500	14500	15000	14916.67
S4-35-15	16250	16250	17250	16000	15750	17750	16500

	TVFA (mg/L)							
	S1-49-5	S1-42-5	S1-35-5	S2-42-5	S2-42-10	S3-49-5	S3-35-10	S4-35-15
Jan 27/05	358.059	64.13862	59.51599	338.6802	117.2157	349.7885	99.02853	69.62087
Jan31/05	207.3181	169.2195	104.6574	463.7396	78.39291	304.5731	94.39271	175.3081
Feb 5/05	365.1127	124.7057	37.49018	406.4863	125.129	367.4783	72.77286	122.5389
Feb 9/05	301.0267	196.6615	40.43752	300.5548	97.53185	453.1885	159.4653	172.2359
Feb 13/05	481.9796	178.8112	78.86242	485.2274	115.3326	346.5035	169.8568	117.1385
Feb 17/05	285.9324	146.8487	50.61129	515.237	115.4745	254.6228	66.18561	253.543
Feb 25/05	286.2154	146.9626	71.07379	435.1129	101.761	336.0257	83.58278	148.9235

	Ammonia-N (mg/L)							
	S1-49-5	S1-42-5	S1-35-5	S2-42-5	S2-42-10	S3-49-5	S3-35-10	S4-35-15
Jan 27/05	582.2307	660.9425	966.8727	546.4631	546.4631	451.8123	907.4757	512.8928
Jan31/05	799.4043	907.4757	924.9011	582.2307	398.0059	350.6073	582.2307	481.3847
Feb 5/05	451.8123	660.9425	1030.157	512.8928	620.3394	546.4631	750.2953	582.2307
Feb 9/05	799.4043	704.2031	704.2031	451.8123	582.2307	546.4631	660.9425	329.0688
Feb 13/05	398.0059	750.2953	1097.584	350.6073	546.4631	424.0565	620.3394	424.0565
Feb 17/05	481.3847	851.7276	1030.157	512.8928	512.8928	512.8928	660.9425	272.072
Feb 25/05	437.7145	799.4043	1063.336	424.0565	529.4119	466.3642	640.3192	339.6674

	Alkalinty (mg/L)							
	S1-49-5	S1-42-5	S1-35-5	S2-42-5	S2-42-10	S3-49-5	S3-35-10	S4-35-15
Jan 27/05	5358.92	6108.129	6542.619	5316.337	6174.653	5666.045	7564.189	6434.642
Jan31/05	5425.819	6357.336	7108.908	6206.028	6898.827	6001.78	7567.861	6252.563
Feb 5/05	6205.345	6692.509	8012.819	6351.021	6692.173	6281.091	7083.544	6694.227
Feb 9/05	5353.202	6135.663	7309.097	5693.341	6463.156	5535.765	6415.052	6255.003
Feb 13/05	3810.764	5293.366	6263.603	4524.204	6699.94	5172.513	6384.467	5345.736
Feb 17/05	5465.13	5873.61	6599.658	4372.603	6098.408	4387.062	6056.52	4387.925
Feb 25/05	5779.273	6414.086	7660.958	6022.181	6577.664	5908.428	6749.298	6474.615

				pH				
	S1-49-5	S1-42-5	S1-35-5	S2-42-5	S2-42-10	S3-49-5	S3-35-10	S4-35-15
Jan 27/05	8.5	8.4	8.4	8.2	8.2	8.24	8.3	8.14
Jan31/05	8.4	8.2	8.2	8	8.14	8.2	8.11	7.9
Feb 5/05	8.17	8.2	8.2	8.1	8.22	8.2	8.2	8.11
Feb 9/05	8.11	8.1	8.1	7.9	8	8.12	8.1	7.83
Feb 13/05	7.4	7.6	7.5	7.9	7.9	8	7.91	7.8
Feb 17/05	8.1	8.3	8.3	8	8.2	8.2	8.1	8.13
Feb 25/05	8.14	8.15	8.15	8	8.11	8.16	8.15	7.97

CH4 Fraction								
	S1-49-5	S1-42-5	S1-35-5	S2-42-5	S2-42-10	S3-49-5	S3-35-10	S4-35-15
Jan 27/05	52	52	60	54	64	51	60	58
Jan31/05	55	60	60	59	58	55	54	57
Feb 5/05	56	62	60	61	58	55	61	55
Feb 9/05	59	57	59	59	57	54	60	55
Feb 13/05	53	58	55	57	58	55	59	54
Feb 17/05	60	57	57	58	57	54	60	55
Feb 25/05	57	60	60	60	58	55	58	56

CO2 Fraction								
	S1-49-5	S1-42-5	S1-35-5	S2-42-5	S2-42-10	S3-49-5	S3-35-10	S4-35-15
Jan 27/05	48	48	40	46	36	49	40	42
Jan31/05	45	40	40	41	42	45	46	43
Feb 5/05	44	38	40	39	42	45	39	45
Feb 9/05	41	43	41	41	43	46	40	45
Feb 13/05	47	42	45	43	42	45	41	46
Feb 17/05	40	43	43	42	43	46	40	45
Feb 25/05	43	40	40	40	42	45	42	44

Gas Production (L/day)								
	S1-49-5	S1-42-5	S1-35-5	S2-42-5	S2-42-10	S3-49-5	S3-35-10	S4-35-15
Jan 27/05	77.4	10.75	4.816	68.75	2.948	59.64	7.67	39.875
Jan31/05	75.75	10.625	10.528	105.375	0	81.48	5.265	47.245
Feb 5/05	73.35	21.375	6.944	89.125	9.944	59.43	4.29	39.655
Feb 9/05	71.7	10.5	6.496	57.875	8.588	50.96	4.355	48.62
Feb 13/05	77.55	11.625	6.608	75.5	13.447	38.99	5.135	36.96
Feb 17/05	60.6	11.625	8.288	93	13.56	56.35	4.55	42.46
Feb 25/05	72.525	15.9375	6.72	73.5	9.266	55.195	4.3225	44.1375

CST (S)								
CST	S1-49-5	S1-42-5	S1-35-5	S2-42-5	S2-42-10	S3-49-5	S3-35-10	S4-35-15
Jan 27/05	1611	1451	953	1495	1366	1735	660	1627
Jan31/05	1680	1380	956	1193	1320	1662	616	1700

Feb 5/05	1150.2	1255	1194	1421	1532	1407	965	1291
Feb 9/05	1203	1217	1075	1342	1640	1680	1351	1251
Feb 13/05	1638	1397	1348	1753	1323	1754	759	1199
Feb 17/05	1341.6	1421	1364	1636	1314	1385	802	1620
Feb 25/05	1510.8	1268	1416	1430	1191	2015	937	1203

TCOD (mg/L)								
T	S1-49-5	S1-42-5	S1-35-5	S2-42-5	S2-42-10	S3-49-5	S3-35-10	S4-35-15
Jan 27/05	31681.22	33933.32	30029.68	18243.69	30029.68	40689.62	27327.16	34533.88
Jan31/05	34233.6	38887.94	26426.32	20495.79	34083.46	37386.54	28678.42	33933.32
Feb 5/05	32281.78	32882.34	27627.44	25375.34	26426.32	31531.08	29429.12	28978.7
Feb 9/05	27777.58	36635.84	29579.26	29729.4	28528.28	36785.98	19670.02	31080.66
Feb 13/05	27177.02	39938.92	34684.02	25225.2	38287.38	37386.54	30780.38	32882.34
Feb 17/05	26576.46	35134.44	38437.52	19519.88	33332.76	33032.48	37236.4	34834.16
Feb 25/05	35735	27927.72	27627.44	29729.4	31080.66	33182.62	30329.96	35584.86

SCOD (mg/L)								
	S1-49-5	S1-42-5	S1-35-5	S2-42-5	S2-42-10	S3-49-5	S3-35-10	S4-35-15
Jan 27/05	3003.472	2282.8	2042.576	3664.088	1742.296	4985.32	601.232	601.232
Jan31/05	4324.704	1201.792	1562.128	1502.072	1201.792	4685.04	541.176	1381.96
Feb 5/05	2222.744	1381.96	3423.864	2282.8	1081.68	1201.792	300.952	1562.128
Feb 9/05	2703.192	1862.408	1261.848	3303.752	1802.352	1321.904	961.568	481.12
Feb 13/05	3183.64	1802.352	1562.128	3063.528	1201.792	3604.032	361.008	421.064
Feb 17/05	1922.464	1742.296	2342.856	2823.304	2342.856	1922.464	1201.792	3123.584
Feb 25/05	3784.2	2222.744	1081.68	3063.528	901.512	3183.64	4564.928	1922.464

CFU/100mL							
	Jan 28/05	Jan31/05	Feb 6/05	Feb 12/05	Feb 15/05	Feb 18/05	Feb 28/05
	FC	FC	FC	FC	FC	FC	FC
Feed	9.00E+07	6.40E+07	5.40E+07	6.40E+07	8.40E+07	6.80E+07	7.50E+07
S1-49-5	8.00E+04	6.00E+04	3.50E+04	4.00E+04	4.20E+04	4.30E+04	5.70E+04
S1-42-5	6.00E+03	4.00E+03	3.00E+03	2.50E+03	2.00E+03	8.00E+03	5.00E+03
S1-35-5	2.00E+03	2.00E+03	1.50E+03	1.00E+03	2.00E+03	2.00E+03	3.20E+03
S2-42-5	1.20E+05	1.04E+05	1.40E+06	1.80E+06	1.20E+06	2.30E+06	1.10E+06
S2-42-10	8.00E+03	6.00E+03	6.00E+03	4.00E+03	8.00E+03	7.00E+03	9.00E+03
S3-49-5	5.00E+04	9.60E+04	7.70E+04	5.50E+04	4.40E+04	6.50E+04	6.30E+04
S3-35-10	6.00E+03	4.00E+03	9.50E+03	1.10E+04	6.00E+03	9.00E+03	9.00E+03
S4-35-15	1.50E+06	1.10E+06	2.50E+06	2.40E+06	4.00E+06	2.00E+06	2.10E+06
	FS	FS	FS	FS	FS	FS	FS
Feed	1.60E+07	1.50E+07	1.20E+07	1.40E+07	1.14E+07	1.20E+07	1.40E+07
S1-49-5	3.20E+05	4.00E+05	3.80E+05	2.00E+05	6.00E+05	5.00E+05	4.40E+05
S1-42-5	1.60E+04	2.80E+04	2.60E+04	1.60E+04	4.40E+03	3.80E+04	1.80E+04
S1-35-5	6.00E+03	8.00E+03	9.00E+03	6.00E+03	9.00E+03	5.00E+03	1.00E+03
S2-42-5	1.00E+05	8.00E+05	1.80E+06	2.00E+05	1.44E+06	1.40E+06	8.40E+05
S2-42-10	4.00E+04	2.00E+04	2.40E+04	1.40E+04	3.00E+04	1.20E+04	1.40E+04
S3-49-5	3.00E+04	4.00E+04	3.00E+04	1.80E+04	3.40E+04	3.00E+04	1.20E+04
S3-35-10	2.00E+03	1.60E+04	1.80E+03	1.20E+04	3.20E+03	1.40E+04	6.00E+03
S4-35-15	6.00E+06	4.00E+06	1.20E+06	1.00E+06	2.00E+06	8.00E+06	1.00E+06
	Salmonela	Salmonela	Salmonela	Salmonela	Salmonela	Salmonela	Salmonela
Feed	5.40E+06	6.00E+06	4.40E+06	5.00E+06	3.60E+06	3.20E+07	3.40E+06
S1-49-5	4.50E+04	4.00E+03	4.00E+03	3.50E+03	3.50E+03	3.00E+03	5.40E+03
S1-42-5	2.00E+03	2.50E+02	1.00E+03	1.50E+03	1.00E+03	1.50E+03	2.50E+02
S1-35-5	2.00E+02	1.00E+02	3.00E+02	2.00E+02	2.00E+02	3.00E+02	1.60E+02
S2-42-5	2.30E+04	2.50E+05	3.00E+04	2.40E+05	2.00E+04	2.40E+05	2.80E+04
S2-42-10	8.00E+03	1.00E+03	4.00E+03	8.00E+04	1.00E+03	8.00E+04	7.00E+03
S3-49-5	8.00E+03	4.00E+04	1.50E+03	2.00E+03	2.00E+04	1.00E+03	1.50E+03

S3-35-10	8.00E+02	6.00E+03	9.00E+02	1.00E+03	9.90E+03	6.60E+02	5.00E+02
S4-35-15	8.00E+05	5.60E+05	8.20E+05	6.60E+05	3.35E+05	5.40E+06	8.10E+05
	E-coli	E-coli	E-coli	E-coli	E-coli	E-coli	E-coli
Feed	6.4E+08	50000000	19000000	16000000	1.22E+08	1.64E+08	1.44E+08
S1-49-5	3.80E+05	5.60E+05	4.40E+05	5.80E+05	1.06E+05	6.20E+05	5.20E+05
S1-42-5	4.80E+04	4.00E+04	5.40E+04	2.00E+05	4.60E+04	3.20E+05	4.00E+05
S1-35-5	4.40E+03	3.60E+04	2.00E+03	2.80E+04	6.00E+03	5.60E+04	2.40E+04
S2-42-5	1.30E+06	1.40E+06	9.20E+05	9.40E+05	9.70E+05	1.00E+06	1.04E+06
S2-42-10	6.00E+04	4.00E+05	8.40E+04	7.20E+04	2.00E+04	1.60E+05	2.00E+04
S3-49-5	5.00E+05	5.60E+04	5.60E+05	7.60E+05	4.60E+04	6.60E+04	1.60E+04
S3-35-10	2.40E+04	3.40E+04	2.00E+04	2.60E+04	7.80E+03	8.40E+03	2.20E+03
S4-35-15	4.00E+06	8.00E+06	4.00E+06	6.00E+06	1.00E+06	6.00E+06	4.00E+06
	C.perfingens	C.perfingens	C.perfingens	C.perfingens	C.perfingens	C.perfingens	C.perfingens
Feed	7.50E+06	9.50E+06	1.10E+07	8.50E+06	1.50E+07	1.95E+07	1.50E+07
S1-49-5	1.30E+07	1.35E+07	1.15E+07	1.30E+07	1.05E+07	1.10E+07	1.10E+07
S1-42-5	1.95E+07	1.05E+07	1.35E+07	1.30E+07	1.30E+07	1.10E+07	1.10E+07
S1-35-5	1.30E+07	1.65E+07	1.40E+07	1.35E+07	1.15E+07	1.05E+07	1.40E+07
S2-42-5	1.35E+07	8.50E+06	1.85E+07	9.00E+06	8.50E+06	9.50E+06	1.15E+07
S2-42-10	1.20E+07	1.10E+07	1.75E+07	1.60E+07	1.75E+07	1.25E+07	1.15E+07
S3-49-5	7.80E+06	7.00E+06	1.15E+07	1.25E+07	7.20E+06	7.00E+06	7.00E+06
S3-35-10	1.25E+07	1.15E+07	1.10E+07	1.05E+07	8.00E+06	7.50E+06	1.20E+07
S4-35-15	1.25E+07	1.00E+07	1.60E+07	1.05E+07	1.85E+07	1.65E+07	1.65E+07

APPENDIX C: RAW DATA FOR VOLATILE FATTY ACIDS STUDY

Salmonella Data

Mixed acid solution at 35 C & 6000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	11000	9000	6000	3000
Second data set (CFU/100 ml)	10600	8500	6300	2700
Control (CFU/100 ml)	11000	11000	10700	10000
Average (CFU/100 ml)	10800	8750	6150	2850

Mixed acid solution at 35 C & 3000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	11000	11000	8000	6000
Second data set (CFU/100 ml)	11000	10100	9000	4000
Control (CFU/100 ml)	11000	11000	10700	10000
Average (CFU/100 ml)	11000	10550	8500	5000

Mixed acid solution at 35 C & 1500 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	11000	11000	11000	8000
Second data set (CFU/100 ml)	12500	10800	11000	5000
Control (CFU/100 ml)	11000	11000	10700	10000
Average (CFU/100 ml)	11750	10900	11000	6500

Mixed acid solution at 35 C & 750 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	11000	11000	11000	9000
Second data set (CFU/100 ml)	12200	11000	10600	8600
Control (CFU/100 ml)	11000	11000	10700	10000
Average (CFU/100 ml)	11600	11000	10800	8800

Acetic acid solution at 35 C & 6000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	11000	11000	8000	5000
Second data set (CFU/100 ml)	10600	13000	6300	4300
Control (CFU/100 ml)	11000	11000	10700	10000
Average (CFU/100 ml)	10800	12000	7150	4650

Acetic acid solution at 35 C & 3000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	11000	11000	10000	7000
Second data set (CFU/100 ml)	10800	11500	7600	6200
Control (CFU/100 ml)	11000	11000	10700	10000
Average (CFU/100 ml)	10900	11250	8800	6600

Acetic acid solution at 35 C & 1500 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	11000	11000	11000	9000
Second data set (CFU/100 ml)	10700	10200	9800	8100
Control (CFU/100 ml)	11000	11000	10700	10000
Average (CFU/100 ml)	10850	10600	10400	8550

Acetic acid solution at 35 C & 750 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	11000	8900	11000	9000
Second data set (CFU/100 ml)	11800	10300	7500	8300
Control (CFU/100 ml)	11000	11000	10700	10000
Average (CFU/100 ml)	11400	9600	9250	8650

Propionic acid solution at 35 C & 6000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	11000	10500	9000	6000
Second data set (CFU/100 ml)	13000	10100	7600	6500
Control (CFU/100 ml)	11000	11000	10700	10000
Average (CFU/100 ml)	12000	10300	8300	6250

Propionic acid solution at 35 C & 3000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	12100	11000	11000	8000
Second data set (CFU/100 ml)	10900	10800	10100	6200
Control (CFU/100 ml)	11000	11000	10700	10000
Average (CFU/100 ml)	11500	10900	10550	7100

Propionic acid solution at 35 C & 1500 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	11000	9800	11000	10200
Second data set (CFU/100 ml)	14000	10400	10500	7300

Control (CFU/100 ml)	11000	11000	10700	10000
Average (CFU/100 ml)	12500	10100	10750	8750

Propionic acid solution at 35 C & 750 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	11000	10700	11000	11000
Second data set (CFU/100 ml)	10100	12000	9700	8700
Control (CFU/100 ml)	11000	11000	10700	10000
Average (CFU/100 ml)	10550	11350	10350	9850

Butyric acid solution at 35 C & 6000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	11000	10900	10000	9000
Second data set (CFU/100 ml)	10400	11500	9600	8400
Control (CFU/100 ml)	11000	11000	10700	10000
Average (CFU/100 ml)	10700	11200	9800	8700

Butyric acid solution at 35 C & 3000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	11000	12000	9000	8200
Second data set (CFU/100 ml)	13000	9700	10100	7400
Control (CFU/100 ml)	11000	11000	10700	10000
Average (CFU/100 ml)	12000	10850	9550	7800

Butyric acid solution at 35 C & 1500 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	11000	13600	11000	10000
Second data set (CFU/100 ml)	10300	12000	11200	10800
Control (CFU/100 ml)	11000	11000	10700	10000
Average (CFU/100 ml)	10650	12800	11100	10400

Butyric acid solution at 35 C & 750 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	11000	11500	10300	11000
Second data set (CFU/100 ml)	10700	11000	9800	10700
Control (CFU/100 ml)	11000	11000	10700	10000
Average (CFU/100 ml)	10850	11250	10050	10850

Mixed acid solution at 42 C & 6000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	8000	6000	4000	2000
Second data set (CFU/100 ml)	9100	5800	4300	1800

Control (CFU/100 ml)	8000	7000	7000	6000
Average (CFU/100 ml)	8550	5900	4150	1900

Mixed acid solution at 42 C & 3000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	8000	5400	6000	2000
Second data set (CFU/100 ml)	7900	6000	6100	1600
Control (CFU/100 ml)	8000	7000	7000	6000
Average (CFU/100 ml)	7950	5700	6050	1800

Mixed acid solution at 42 C & 1500 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	8000	8000	7000	3000
Second data set (CFU/100 ml)	7100	8300	5900	2900
Control (CFU/100 ml)	8000	7000	7000	6000
Average (CFU/100 ml)	7550	8150	6450	2950

Mixed acid solution at 42 C & 750 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	8000	7600	7100	4000
Second data set (CFU/100 ml)	9000	7200	7800	3700
Control (CFU/100 ml)	8000	7000	7000	6000
Average (CFU/100 ml)	8500	7400	7450	3850

Acetic acid solution at 42 C & 6000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	8000	6500	5000	2000
Second data set (CFU/100 ml)	7400	7000	5100	2600
Control (CFU/100 ml)	8000	7000	7000	6000
Average (CFU/100 ml)	7700	6750	5050	2300

Acetic acid solution at 42 C & 3000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	8700	8000	5600	4000
Second data set (CFU/100 ml)	8000	7300	6000	3700
Control (CFU/100 ml)	8000	7000	7000	6000
Average (CFU/100 ml)	8350	7650	5800	3850

Acetic acid solution at 42 C & 1500 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	8300	8000	7500	6000
Second data set (CFU/100 ml)	7900	8500	7900	5100

Control (CFU/100 ml)	8000	7000	7000	6000
Average (CFU/100 ml)	8100	8250	7700	5550

Acetic acid solution at 42 C & 750 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	9000	8400	8000	6700
Second data set (CFU/100 ml)	7600	7500	8000	7000
Control (CFU/100 ml)	8000	7000	7000	6000
Average (CFU/100 ml)	8300	7950	8000	6850

Propionic acid solution at 42 C & 6000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	7600	8000	5700	5000
Second data set (CFU/100 ml)	8100	7900	6100	4700
Control (CFU/100 ml)	8000	7000	7000	6000
Average (CFU/100 ml)	7850	7950	5900	4850

Propionic acid solution at 42 C & 3000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	8500	7700	8000	6000
Second data set (CFU/100 ml)	7700	8000	8200	4900
Control (CFU/100 ml)	8000	7000	7000	6000
Average (CFU/100 ml)	8100	7850	8100	5450

Propionic acid solution at 42 C & 1500 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	8000	8200	7800	6000
Second data set (CFU/100 ml)	8100	7900	8000	5500
Control (CFU/100 ml)	8000	7000	7000	6000
Average (CFU/100 ml)	8050	8050	7900	5750

Propionic acid solution at 42 C & 750 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	7600	7900	8000	8200
Second data set (CFU/100 ml)	8300	7600	8400	8800
Control (CFU/100 ml)	8000	7000	7000	6000
Average (CFU/100 ml)	7950	7750	8200	8500

Butyric acid solution at 42 C & 6000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	8000	8000	7100	3700
Second data set (CFU/100 ml)	8100	8000	6400	4000
Control (CFU/100 ml)	8000	7000	7000	6000

Average (CFU/100 ml)	8050	8000	6750	3850
-----------------------------	-------------	-------------	-------------	-------------

Butyric acid solution at 42 C & 3000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	7800	8000	8000	6000
Second data set (CFU/100 ml)	8000	7500	7700	5400
Control (CFU/100 ml)	8000	7000	7000	6000
Average (CFU/100 ml)	7900	7750	7850	5700

Butyric acid solution at 42 C & 1500 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	8000	7800	6900	8000
Second data set (CFU/100 ml)	7600	7900	7600	6800
Control (CFU/100 ml)	8000	7000	7000	6000
Average (CFU/100 ml)	7800	7850	7250	7400

Butyric acid solution at 42 C & 750 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	8000	7800	7600	8000
Second data set (CFU/100 ml)	8000	7500	8100	7400
Control (CFU/100 ml)	8000	7000	7000	6000
Average (CFU/100 ml)	8000	7650	7850	7700

Mixed acid solution at 49 C & 6000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	5000	2800	1000	1000
Second data set (CFU/100 ml)	4800	3000	1000	1000
Control (CFU/100 ml)	5000	5000	4000	3800
Average (CFU/100 ml)	4900	2900	1000	1000

Mixed acid solution at 49 C & 3000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	5000	4000	1000	1000
Second data set (CFU/100 ml)	5100	3800	1000	1000
Control (CFU/100 ml)	5000	5000	4000	3800
Average (CFU/100 ml)	5050	3900	1000	1000

Mixed acid solution at 49 C & 1500 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	5000	4600	1000	1000
Second data set (CFU/100 ml)	4800	5100	1000	1000
Control (CFU/100 ml)	5000	5000	4000	3800

Average (CFU/100 ml)	4900	4850	1000	1000
-----------------------------	-------------	-------------	-------------	-------------

Mixed acid solution at 49 C & 750 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	5300	5000	1000	1000
Second data set (CFU/100 ml)	5000	4900	1000	1000
Control (CFU/100 ml)	5000	5000	4000	3800
Average (CFU/100 ml)	5150	4950	1000	1000

Acetic acid solution at 49 C & 6000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	5000	3000	1000	1000
Second data set (CFU/100 ml)	4800	3100	1000	1000
Control (CFU/100 ml)	5000	5000	4000	3800
Average (CFU/100 ml)	4900	3050	1000	1000

Acetic acid solution at 49 C & 3000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	5000	4000	1000	1000
Second data set (CFU/100 ml)	4900	3700	1000	1000
Control (CFU/100 ml)	5000	5000	4000	3800
Average (CFU/100 ml)	4950	3850	1000	1000

Acetic acid solution at 49 C & 1500 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	5000	5000	1000	1000
Second data set (CFU/100 ml)	5000	4800	1000	1000
Control (CFU/100 ml)	5000	5000	4000	3800
Average (CFU/100 ml)	5000	4900	1000	1000

Acetic acid solution at 49 C & 750 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	5000	5000	2000	1000
Second data set (CFU/100 ml)	5600	4100	1000	1000
Control (CFU/100 ml)	5000	5000	4000	3800
Average (CFU/100 ml)	5300	4550	1500	1000

Propionic acid solution at 49 C & 6000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	5000	3800	3100	2000
Second data set (CFU/100 ml)	5200	4000	2800	1800
Control (CFU/100 ml)	5000	5000	4000	3800
Average (CFU/100 ml)	5100	3900	2950	1900

Propionic acid solution at 49 C & 3000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	5000	5000	3000	2000
Second data set (CFU/100 ml)	4900	4800	3300	2200
Control (CFU/100 ml)	5000	5000	4000	3800
Average (CFU/100 ml)	4950	4900	3150	2100

Propionic acid solution at 49 C & 1500 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	4700	5200	3000	3000
Second data set (CFU/100 ml)	5000	4600	2800	2800
Control (CFU/100 ml)	5000	5000	4000	3800
Average (CFU/100 ml)	4850	4900	2900	2900

Propionic acid solution at 49 C & 750 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	5000	5100	4900	3000
Second data set (CFU/100 ml)	5600	5300	4600	2800
Control (CFU/100 ml)	5000	5000	4000	3800
Average (CFU/100 ml)	5300	5200	4750	2900

Butyric acid solution at 49 C & 6000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	5000	4800	4000	1000
Second data set (CFU/100 ml)	5300	5000	3800	1000
Control (CFU/100 ml)	5000	5000	4000	3800
Average (CFU/100 ml)	5150	4900	3900	1000

Butyric acid solution at 49 C & 3000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	5000	5000	5000	2000
Second data set (CFU/100 ml)	5000	5700	4900	2200
Control (CFU/100 ml)	5000	5000	4000	3800
Average (CFU/100 ml)	5000	5350	4950	2100

Butyric acid solution at 49 C & 1500 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	4900	5000	4700	4000
Second data set (CFU/100 ml)	5100	4200	5100	3900
Control (CFU/100 ml)	5000	5000	4000	3800
Average (CFU/100 ml)	5000	4600	4900	3950

Butyric acid solution at 49 C & 750 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	5000	4500	4700	5000
Second data set (CFU/100 ml)	4600	4300	5400	4700
Control (CFU/100 ml)	5000	5000	4000	3800
Average (CFU/100 ml)	4800	4400	5050	4850

4.5 at 35 C		
Incubation period (h)	First data set (CFU/100 ml)	Second data set (CFU/100 ml)
0	11000	11000
12	9000	8000
24	5000	5000
pH 5.5 at 35 C		
Incubation period (h)	First data set (CFU/100 ml)	Second data set (CFU/100 ml)
0	13000	10000
12	11000	12000
24	9000	9000
pH 6.5 at 35 C		
Incubation period (h)	First data set (CFU/100 ml)	Second data set (CFU/100 ml)
0	11000	11500
12	12000	10800
24	14000	12500
pH 7.5 at 35 C		
Incubation period (h)	First data set (CFU/100 ml)	Second data set (CFU/100 ml)
0	11000	10500
12	13000	13000
24	17000	16000

	4.5 at 42 C	
Incubation period (h)	First data set (CFU/100 ml)	Second data set (CFU/100 ml)
0	8000	8100
12	6000	5000
24	3000	1000
	pH 5.5 at 42 C	
Incubation period (h)	First data set (CFU/100 ml)	Second data set (CFU/100 ml)
0	8000	8000
12	4000	4000
24	4000	2500
	pH 6.5 at 42 C	
Incubation period (h)	First data set (CFU/100 ml)	Second data set (CFU/100 ml)
0	8000	8000
12	9500	10000
24	12000	7500
	pH 7.5 at 42 C	
Incubation period (h)	First data set (CFU/100 ml)	Second data set (CFU/100 ml)
0	8000	7000
12	1000	1000
24	1000	1000

	4.5 at 49 C	
Incubation period (h)	First data set (CFU/100 ml)	Second data set (CFU/100 ml)
0	5000	5000
12	3000	4000
24	1000	1000
	pH 5.5 at 49 C	
Incubation period (h)	First data set (CFU/100 ml)	Second data set (CFU/100 ml)
0	5000	5000
12	4000	4000
24	3000	2000
	pH 6.5 at 49 C	
Incubation period (h)	First data set (CFU/100 ml)	Second data set (CFU/100 ml)
0	5000	5000
12	2000	1000
24	1000	1000
	pH 7.5 at 49C	
Incubation period (h)	First data set (CFU/100 ml)	Second data set (CFU/100 ml)
0	5000	5000
12	1000	2000
24	1000	1000

Clostridium perfringens Data

Mixed acid solution at 35 C & 6000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	15100000	14000000	12500000	12500000
Second data set (CFU/100 ml)	15000000	14500000	12200000	12200000
Control (CFU/100 ml)	15500000	15250000	15500000	14750000
Average (CFU/100 ml)	15050000	14250000	12350000	12350000

Mixed acid solution at 35 C & 3000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	15500000	15500000	14500000	13000000
Second data set (CFU/100 ml)	15500000	15000000	15000000	12000000
Control (CFU/100 ml)	15500000	15250000	15500000	14750000
Average (CFU/100 ml)	15500000	15250000	14750000	12500000

Mixed acid solution at 35 C & 1500 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	15500000	15500000	15000000	14500000
Second data set (CFU/100 ml)	15300000	15500000	15500000	14000000
Control (CFU/100 ml)	15500000	15250000	15500000	14750000
Average (CFU/100 ml)	15400000	15500000	15250000	14250000

Mixed acid solution at 35 C & 750 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	15100000	15500000	16000000	14500000
Second data set (CFU/100 ml)	15500000	15500000	15000000	15000000
Control (CFU/100 ml)	15500000	15250000	15500000	14750000
Average (CFU/100 ml)	15300000	15500000	15500000	14750000

Mixed acid solution at 42 C & 6000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	19000000	20000000	19000000	15000000
Second data set (CFU/100 ml)	20000000	19500000	18000000	16000000
Control (CFU/100 ml)	20000000	19500000	21750000	23000000
Average (CFU/100 ml)	19500000	19750000	18500000	15500000

Mixed acid solution at 42 C & 3000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	20000000	20000000	19000000	17500000
Second data set (CFU/100 ml)	18700000	21000000	19000000	16750000

Control (CFU/100 ml)	20000000	19500000	21750000	23000000
Average (CFU/100 ml)	19350000	20500000	19000000	17125000
Mixed acid solution at 42 C & 1500 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	20000000	21000000	22750000	24000000
Second data set (CFU/100 ml)	20000000	20500000	22000000	23000000
Control (CFU/100 ml)	20000000	19500000	21750000	23000000
Average (CFU/100 ml)	20000000	20750000	22375000	23500000

Mixed acid solution at 42 C & 750 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	21000000	22000000	24500000	27500000
Second data set (CFU/100 ml)	20000000	21000000	22500000	28500000
Control (CFU/100 ml)	20000000	19500000	21750000	23000000
Average (CFU/100 ml)	20500000	21500000	23500000	28000000

Mixed acid solution at 49C & 6000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	20700000	20500000	18500000	12500000
Second data set (CFU/100 ml)	20000000	20000000	18500000	13000000
Control (CFU/100 ml)	20250000	21000000	22750000	27250000
Average (CFU/100 ml)	20350000	20250000	18500000	12750000

Mixed acid solution at 49C & 3000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	20500000	20500000	19500000	15000000
Second data set (CFU/100 ml)	21000000	20500000	18000000	13500000
Control (CFU/100 ml)	20250000	21000000	22750000	27250000
Average (CFU/100 ml)	20750000	20500000	18750000	14250000

Mixed acid solution at 49C & 1500 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	19900000	20500000	19500000	17500000
Second data set (CFU/100 ml)	20000000	19000000	18000000	17000000
Control (CFU/100 ml)	20250000	21000000	22750000	27250000
Average (CFU/100 ml)	19950000	19750000	18750000	17250000

Mixed acid solution at 49C & 750 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	19700000	21000000	22500000	24500000
Second data set (CFU/100 ml)	20000000	21000000	22000000	23500000
Control (CFU/100 ml)	20250000	21000000	22750000	27250000
Average (CFU/100 ml)	19850000	21000000	22250000	24000000

Mixed acid solution at 55C & 6000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	16500000	17000000	12500000	5000000
Second data set (CFU/100 ml)	17000000	16500000	12000000	6500000
Control (CFU/100 ml)	17150000	17000000	14750000	11000000
Average (CFU/100 ml)	16750000	16750000	12250000	5750000

Mixed acid solution at 55C & 3000 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	16700000	17500000	13500000	9000000
Second data set (CFU/100 ml)	17000000	17000000	12000000	8000000
Control (CFU/100 ml)	17150000	17000000	14750000	11000000
Average (CFU/100 ml)	16850000	17250000	12750000	8500000

Mixed acid solution at 55C & 1500 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	17300000	17000000	14000000	9500000
Second data set (CFU/100 ml)	17200000	16000000	14500000	10000000
Control (CFU/100 ml)	17150000	17000000	14750000	11000000
Average (CFU/100 ml)	17250000	16500000	14250000	9750000

Mixed acid solution at 55C & 750 mg/L	Incubation period (h)			
	0	4	10	24
First data set (CFU/100 ml)	17500000	17000000	15000000	11000000
Second data set (CFU/100 ml)	16800000	17000000	14500000	11000000
Control (CFU/100 ml)	17150000	17000000	14750000	11000000
Average (CFU/100 ml)	17150000	17000000	14750000	11000000

	pH=4.5	pH=4.5				
35	1.90E+07	1.00E+07	1.45E+07	6363961	1.414214	4500000
42	2.15E+07	2.00E+07	2.08E+07	1060660	1.414214	750000
49	1.05E+07	9.50E+06	1.00E+07	707106.8	1.414214	500000
55	3.50E+06	2.50E+06	3.00E+06	707106.8	1.414214	500000
35	16500000	14500000	1.55E+07	1414214	1.414214	1000000
42	23000000	24000000	2.35E+07	707106.8	1.414214	500000
49	20600000	24000000	2.23E+07	2404163	1.414214	1700000
55	18000000	11500000	1.48E+07	4596194	1.414214	3250000
35	22000000	24500000	2.33E+07	1767767	1.414214	1250000
42	80000000	75000000	7.75E+07	3535534	1.414214	2500000
49	22500000	22500000	2.25E+07	0	1.414214	0
55	14000000	16000000	1.50E+07	1414214	1.414214	1000000
35	25500000	28500000	2.70E+07	2121320	1.414214	1500000
42	35000000	42500000	3.88E+07	5303301	1.414214	3750000
49	28000000	31900000	3.00E+07	2757716	1.414214	1950000
55	16500000	12500000	1.45E+07	2828427	1.414214	2000000
35	13800000	17000000	1.54E+07	2262742	1.414214	1600000
42	20000000	21000000	2.05E+07	707106.8	1.414214	500000
49	20000000	19700000	1.99E+07	212132	1.414214	150000
55	17000000	17400000	1.72E+07	282842.7	1.414214	200000

	pH				
	4.5	5.5	6.5	7.5	Con
35	14500000	15500000	23250000	27000000	15400000
42	20750000	23500000	77500000	38750000	20500000
49	10000000	22300000	22500000	29950000	19850000
55	3000000	14750000	15000000	14500000	17200000

APPENDIX D: RESULTS OF STATISTICAL ANALYSIS

MPN/100mL VS CFU/100 mL**One-Way Analysis of Variance**

Analysis of Variance for Salmonella density

Source	DF	SS	MS	F	P
Method	1	2.107E+11	2.107E+11	4.07	0.048
Error	58	3.000E+12	5.173E+10		
Total	59	3.211E+12			

Individual 95% CIs For Mean
Based on Pooled StDev

Level	N	Mean	StDev	+-----+-----+-----	
CFU	30	118557	321639	(-----*-----	
MPN	30	32	26	(-----*-----)	
Pooled StDev =				+-----+-----+-----	
160000				-80000 0 80000	

Two way analysis of variance on Solids decay rates

Factor	Levels	Values
C1	5	35 42 49 55 62
C2	3	5 15 20

Analysis of Variance for C3

Source	DF	Seq SS	Adj SS	Adj MS	F	P
C1	4	0.008503	0.010708	0.002677	5.53	0.000
C2	2	0.234871	0.234871	0.117436	242.50	0.000
C1*C2	8	0.012599	0.012599	0.001575	3.25	0.002
Error	105	0.050848	0.050848	0.000484		
Total	119	0.306821				

Performance of the investigated treatment two stage systems on trends volatile solids removal efficiency

Analysis of Variance for C2					
Source	DF	SS	MS	F	P
C1	3	473.6	157.9	4.99	0.008
Error	24	758.7	31.6		
Total	27	1232.2			

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev		
-----+				-----+-----+-----+---		
1	7	48.927	6.602	(-----*-----)		
2	7	52.627	4.980	(-----*-----)		
3	7	52.520	5.435	(-----*-----)		
4	7	60.211	5.340		(-----*-----)	
----)						
				-----+-----+-----+---		
-----+						
Pooled StDev =		5.622		48.0	54.0	60.0
66.0						

Tukey's pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.0109

VS removals among the tested three stage treatment trains

Analysis of Variance for C2					
Source	DF	SS	MS	F	P
C1	4	570.0	142.5	9.28	0.000
Error	30	460.8	15.4		
Total	34	1030.8			

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev		
+-----				-----+-----+-----		
1	7	53.780	2.793	(-----*-----)		
2	7	59.434	3.881		(-----*-----)	
3	7	60.660	4.496		(-----*-----)	
4	7	63.710	5.008		(-----*-----)	
--)						
5	7	65.486	2.941			(-----
*-----)						
				-----+-----+-----		

+-----
Pooled StDev = 3.919 55.0 60.0 65.0

Tukey's pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.00691

Two way analysis of variance on TVFA concentrations in the digesters effluent

Factor	Levels	Values					
C1	5	35	42	49	55	62	
C2	3	5	15	20			

Analysis of Variance for C3

Source	DF	Seq SS	Adj SS	Adj MS	F	P
C1	4	41290293	31363699	7840925	5371.75	0.000
C2	2	8357372	8468043	4234022	2900.69	0.000
C1*C2	8	5053187	5053187	631648	432.74	0.000
Error	5	7298	7298	1460		
Total	19	54708150				

Two way analysis of variance on Methane Yield

Factor	Levels	Values					
C1	5	35	42	49	55	62	
C2	3	5	15	20			

Analysis of Variance for C3

Source	DF	Seq SS	Adj SS	Adj MS	F	P
C1	4	0.057791	0.048963	0.012241	6.86	0.020
C2	2	0.086056	0.066097	0.033049	18.53	0.003
C1*C2	8	0.081438	0.081438	0.010180	5.71	0.024
Error	6	0.010701	0.010701	0.001783		
Total	20	0.235985				

Results of two way analysis of variance on fecal coliform log reductions

Factor	Levels	Values					
Temp	5	35	42	49	55	62	
HRT	3	5	15	20			

Analysis of Variance for log reductions						
Source	DF	Seq SS	Adj SS	Adj MS	F	P
Temp	4	87.2611	83.8766	20.9692	117.30	0.000
HRT	2	3.2373	3.2373	1.6186	9.05	0.000
Temp*HRT	8	6.9707	6.9707	0.8713	4.87	0.000
Error	60	10.7255	10.7255	0.1788		
Total	74	108.1946				

FC log reduction between first and second stage of two stage system

Analysis of Variance for FC log reduction

Source	DF	SS	MS	F	P
Stage	2	5.051	5.051	10.01	0.002
Error	62	31.277	0.504		
Total	63	36.328			

Tukey's pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.0500

Two way analysis of variance on *E.coli* log reductions

Factor Levels Values

C1	3	5	15	20		
C2	5	35	42	49	55	62

Analysis of Variance for C3

Source	DF	Seq SS	Adj SS	Adj MS	F	P
C1	2	58.275	58.275	29.138	71.50	0.000
C2	4	92.525	81.562	20.390	50.03	0.000
C1*C2	8	20.975	20.975	2.622	6.43	0.000
Error	60	24.452	24.452	0.408		
Total	74	196.227				

Two way analysis of variance on *Salmonella* spp log reductions

Factor	Levels	Values
Temp	5	35 42 49 55 62
HRT	3	5 15 20

Analysis of Variance for C3

Source	DF	Seq SS	Adj SS	Adj MS	F	P
HRT	2	10.4895	10.4895	5.2447	20.39	0.000
Temp	4	77.1288	71.2700	17.8175	69.27	0.000
HRT*Temp	8	7.6613	7.6613	0.9577	3.72	0.001
Error	60	15.4334	15.4334	0.2572		
Total	74	110.7129				

Salmonella log reduction between first and second stage of two stage system

One-Way Analysis of Variance

Analysis of Variance for Salmonella log reductions

Source	DF	SS	MS	F	P
stage	2	21.754	10.877	37.91	0.000
Error	29	8.320	0.287		
Total	31	30.075			

Individual 95% CIs For Mean Based on Pooled StDev

Level	N	Mean	StDev	-----+-----+-----+-----		
35	8	0.6136	0.4887	(---*---)		
42	16	1.6923	0.4516		(--*--)	
49	8	2.9426	0.7160			(--*---)
				-----+-----+-----+-----		
Pooled StDev =		0.5356		1.0	2.0	3.0

Tukey's pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.0197

Two way analysis of variance on Fecal Streptococci log reductions

Factor	Levels	Values				
Temp	5	35	42	49	55	62
HRT	3	5	15	20		

Analysis of Variance for FS log reduction						
Source	DF	Seq SS	Adj SS	Adj MS	F	P
Temp	4	167.876	167.900	41.975	156.49	0.000
HRT	2	8.432	8.432	4.216	15.72	0.000
Temp*HRT	8	9.208	9.208	1.151	4.29	0.000
Error	60	16.093	16.093	0.268		
Total	74	201.610				

One-Way Analysis of Variance on FS log reduction among two stage treatment trains

Analysis of Variance for C2					
Source	DF	SS	MS	F	P
C1	3	9.458	3.153	18.39	0.000
Error	28	4.801	0.171		
Total	31	14.258			

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev	
-				-----+-----+-----+-----	
35°C-35°	8	1.6065	0.4233	(-----*-----)	
42°C-35°	8	2.2501	0.5740		(-----*-----)
42°C-42°	8	2.5860	0.1592		(-----*-----)
49°C-35°	8	3.1044	0.3897		(-----*-----)
)				-----+-----+-----+-----	
-					

Pooled StDev = 0.4141

1.80 2.40 3.00

Tukey's pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.0108

Critical value = 3.86

Two way analysis of variance on *C. perfringens* densities

Factor	Levels	Values				
Temp	5	35	42	49	55	62
HRT	3	5	15	20		

Analysis of Variance for Density

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Temp	4	2.7291	2.5781	0.6445	6.11	0.084
HRT	2	0.6929	0.6804	0.3402	3.23	0.179
Temp*HRT	8	0.4884	0.4884	0.0611	0.58	0.762
Error	3	0.3164	0.3164	0.1055		
Total	17	4.2268				

Three way analysis of variance on *Salmonella* decay rate constants

Factor	Levels	Values			
Temp	3	35	42	49	
Acid	3	1	2	3	
concentr	4	750	1500	3000	6000

Analysis of Variance for K-values

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Temp	2	0.0301983	0.0127125	0.0063562	58.72	0.000
Acid	2	0.0139416	0.0139416	0.0069708	64.40	0.000
concentr	3	0.0207321	0.0192770	0.0064257	59.36	0.000
Temp*Acid	4	0.0432755	0.0432755	0.0108189	99.94	0.000
Temp*concentr	6	0.0104410	0.0108898	0.0018150	16.77	0.000
Acid*concentr	6	0.0081286	0.0081286	0.0013548	12.52	0.000
Temp*Acid*concentr	12	0.0043738	0.0043738	0.0003645	3.37	0.023
Error	12	0.0012990	0.0012990	0.0001083		
Total	47	0.1323899				

Two way analysis of variance on *C. perfringens* decay rate constant

Factor	Levels	Values			
C1	4	35	42	49	55
C2	4	750	1500	3000	6000

Analysis of Variance for C3

Source	DF	Seq SS	Adj SS	Adj MS	F	P
C1	3	0.00373137	0.00373137	0.00124379	710.74	0.000
C2	3	0.00177062	0.00177062	0.00059021	337.26	0.000
C1*C2	9	0.00031988	0.00031988	0.00003554	20.31	0.000

APPENDIX E: OTHERS

Mass Balance for the CSTR

$$V \cdot \frac{dc}{dt} = F(C_0 - C) - V \left(-\frac{dc}{dt} \right)_{\text{deg}}$$

In a steady state

$$\frac{(C_0 - C)}{\theta} = \left(-\frac{dc}{dt} \right)_{\text{deg}}$$

where

 C_0 = VSS concentration in influent C = VSS concentration in digester θ = retention time (d)

If we assume that VSS destruction rate to be first order reaction, the following is derived

$$\left(-\frac{dc}{dt} \right)_{\text{deg}} = k(C - C^*)$$

where

 C^* = non biodegradable organic substance concentration k = destruction rate constant (1/day)

Therefore

$$k \left(\frac{C}{C_0} - \frac{C^*}{C_0} \right) = \left(1 - \frac{C}{C_0} \right) / \theta$$

The best performed 3 stage system in terms of VSS destruction was chosen and pro-rated to the high HRT to obtain Ultimate Biodegradability

S1-49-5	18000	18000	19750	18750	18500	19000	18833.33
S1-42-5	15750	15750	16250	15250	15000	15000	14416.67
S1-35-5	13000	13000	14000	13750	12500	12000	12250
F	36250	35250	36000	34750	33250	34250	35333.33
S1-49-5	50.34483	48.93617	45.13889	46.04317	44.3609	44.52555	46.69811
S1-42-5	56.55172	55.31915	54.86111	56.11511	54.88722	56.20438	59.19811
S1-35-5	64.13793	63.12057	61.11111	60.43165	62.40602	64.9635	65.33019

5	46.13359
10	57.63348
15	62.07176
20	64.41747
25	65.86724
30	66.85183
35	67.56411
40	68.10329
45	68.52563
50	68.86538
55	69.14462
60	69.37818
70	69.7468
80	70.02454
90	70.24134
100	70.41525